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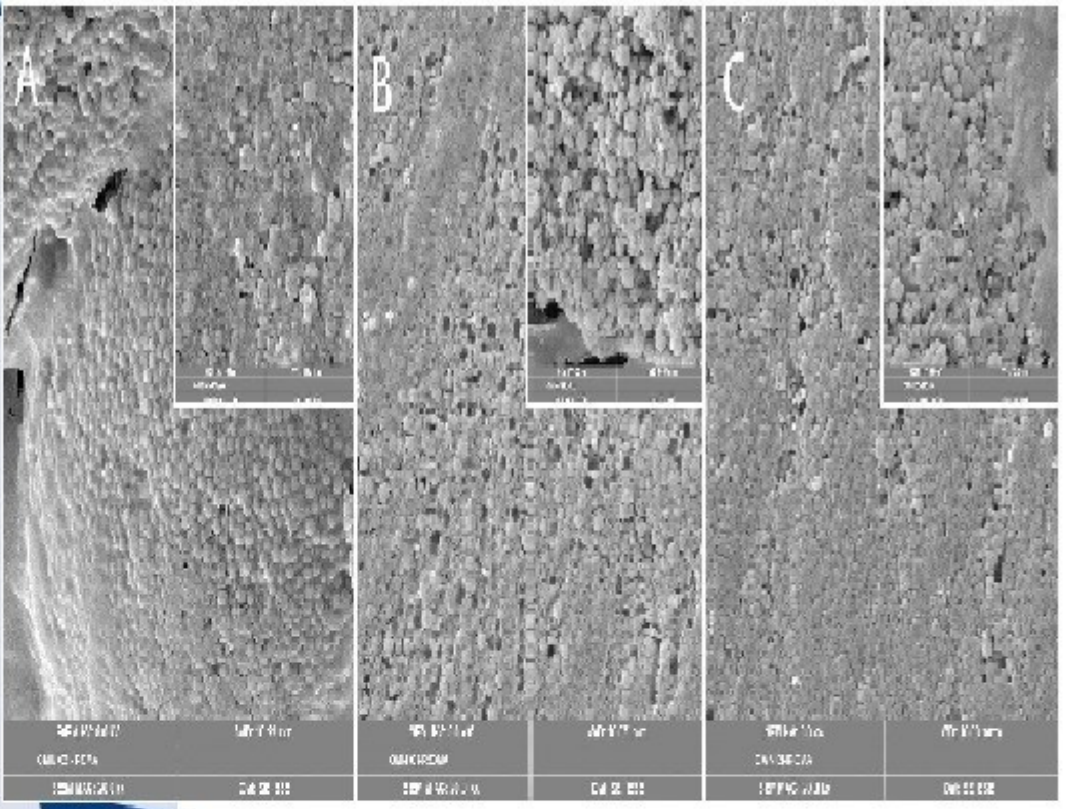

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Probiotic Chewing Gums for Adjuctive Treatment of Periodontitis in Diabetics

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ABSTRACT

Introduction: The treatment of periodontal disease in diabetic subjects should also focus on lowering blood glucose levels, which might act as an adjuvant to conventional periodontal treatment. In the form of probiotics, bacterial therapy offers a dual role in controlling blood glycemic levels and reducing colonization of oral bacteria.
Aim: To evaluate the efficacy of probiotics in managing periodontitis among diabetic and non-diabetic subjects.
Methodology*:* This study was designed as a randomized, double-blinded clinical trial among diabetic and non-diabetic subjects with periodontitis. Twenty-four subjects in each diabetic and non-diabetic group were randomly assigned into two probiotic test sub-groups and one placebo sub-group. *Lactobacillus fermentum* MCC2760 and *Bifidobacterium longum* NCIM5684 probiotic chewing gums were provided to subjects in test groups to use twice a day for 30 days. Supragingival plaque samples were collected at baseline and 30 days to analyze total bacterial count and subgingival plaque for *P.gingivalis, A.actinomycetemcomitans* through quantitative polymerase chain reaction(qPCR). Clinical parameters were recorded at baseline, 30, 45, and 90 days.
Results: After 30 days, a significant reduction in plaque index, gingival Index, probing pocket depth, and gingival bleeding index was observed in scaling and root planing group(SRP) and SRP+probiotic groups. There was a significant reduction in total bacterial count among probiotic groups compared to placebo. qPCR analysis revealed non-significant reduction of *p.gingivalis* and *A.actinomycetemcomitans* in test groups. Intergroup comparison between diabetic and non-diabetic groups did not show any significant differences either in clinical or microbial parameters.
Conclusions*:* probiotic functional foods can be delivered as an adjunct to SRP to manage periodontitis in systemically compromised subjects. Long-time use of probiotics is recommended to maintain the recolonization of bacteria in periodontal tissues.

Key words*:* Probiotics, Periodontitis, Lactobacillus fermentum, Bifidobacterium longum, Aggregatibacter actinomycetemcomitans.

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Introduction

The prevalence of periodontal disease in India is high (51%) as half of the adults suffer from some form of periodontal disease.[1] Recent epidemiology studies have stated that periodontitis does not follow a linear progression and is not age-dependent. In the 2018 EFP/AAP case definition, a participant was a periodontitis case if: interdental CAL ≥ 2 non-adjacent teeth, or Buccal or Oral CAL ≥ 3 mm with PPD > 3 mm is detectable at ≥2 teeth.[2] However, its initiation and progression are strongly influenced by host susceptibility, local and systemic risk factors.[3]

Diabetes mellitus and periodontitis are polygenic disorders with some grade of immuno-regulatory dysfunction.[4] Diagnostic criteria by the American Diabetes Association (ADA) for type 2 diabetes include the following:[5]

- A fasting plasma glucose(FPG) level of 126 mg/dL or higher, *or*
- A 2-hour plasma glucose level of 200 mg/dL or higher during a 75-g oral glucose tolerance test (OGTT), *or*
- A random plasma glucose of 200 mg/dL or higher in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, *or*
- A hemoglobin A1c (HbA1c) level of 6.5% or higher

There is emerging evidence that supports the existence of a two-way relationship between diabetes and periodontitis. Diabetes increases the risk for periodontitis, and periodontal inflammation negatively affects glycemic control.[6] However, the mechanism that underpins the link between these conditions is limited to the aspects of immune functioning, neutrophil activity, and cytokine biology.[6]

Scaling and root planning(SRP), the conventional treatment for periodontitis, will not entirely eliminate the pathogenic bacteria as they may reside at sites inaccessible for instrumentation. So, mechanical therapy combined with antibiotics or antibiotic combinations

offered satisfactory results as they significantly suppressed the growth of periodontal pathogens. But, in the recent era, antimicrobial resistance(AMR) has been the current global issue due to its overuse and misuse. Drug resistance of bacterial dental biofilm has unlatched newer approaches to non-surgical periodontal therapy(NSPT). In this process, probiotics evolved as a trending bacteria as they might provide an opportunity for replacement therapy in bacterial-mediated oral diseases.[7]

The FAO/WHO defines probiotics as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host."[8] probiotics combat infections by displacing pathogenic bacteria and replacing them with harmless beneficial microorganisms. Research highlighted the success of probiotics in many areas of medicine, such as treating gastrointestinal tract and oropharyngeal infections.[9] The immunomodulatory and anti-inflammatory properties of probiotics help treat periodontitis. Probiotics also reported having a favorable impact on the metabolic control of subjects with type 2 diabetes.[10]

Lactobacillus and Bifidobacterium are the most commonly used probiotic strains. Current probiotic delivery systems include mouth rinses, probiotic drops, lozenges, and dentifrice. They are also available as "functional food," which, apart from their nutritional value, apparently improves the health and well-being of consumers.[11]

In the present study, chewing gums of two different probiotic strains were delivered as an adjuvant to SRP to diabetic and non-diabetic subjects with periodontitis to assess their potency on clinical and microbial parameters.

This study aimed to evaluate the efficacy of probiotics in managing periodontitis among diabetic and non-diabetic subjects.

Material and Methods

This randomized, double-blinded (patients and examiner blinded), placebo-controlled trial was approved by the Institutional Ethics committee, JSS Dental College and Hospital (44/2019). Systemically healthy and diabetic subjects with periodontitis fulfilling the inclusion criteria were divided into three sub-groups. GROUP; A. periodontitis subjects with diabetes(A1, A2, A3) GROUP; B. periodontitis subjects without diabetes(B1, B2, B3). Subjects were allotted to *L.f* test groups(A2, B2), *B.l* test groups(A3, B3), and control groups(A1, B1) based on a Computer-generated random allocation sequence. Informed consent was taken from all the patients. This trial was registered at clinical trials.gov as CTRI/2020/10/028466.

Inclusion criteria; Age; 35 years-75 years, periodontal pocket depth ≥ 5mm, Type II diabetic subjects with glycated hemoglobin range between 7% –10 %.

Exclusion criteria; Smokers, pregnant and lactating mothers, people with a compromised immune system, antibiotic therapy during the previous six months, systemic diseases other than diabetes, subjects taking medications that could interfere with gingival tissue responses.

Plaque Index (Silness & Loe-1964), Gingival Index (Loe & Silness-1963), Probing Pocket Depth, Gingival Bleeding Index (Ainamo & Bay-1975) were recorded. Supragingival plaque samples from the buccal surface of anterior maxillary teeth and subgingival plaque samples from the pocket sites were collected by using sterile curettes. After sample collection, scaling and root planning were performed for all the subjects. In the diabetic and non-diabetic groups, subjects in the placebo subgroup were given plain chewing gums, and subjects in test subgroups were given *Lactobacillus fermentum* MCC2760 and *Bifidobacterium longum* NCIM5684 probiotic chewing gums. Each chewing gum contains $1*10^8$ CFU of probiotic bacteria. They were asked to chew it for 10 minutes, twice a day, morning 1 hour after breakfast, and at night, 1-hour post-dinner for 30 days. These chewing gums were prepared freshly every week to maintain the viability of probiotic cells. They were packed, coded, and distributed to the appropriate groups regularly once a week. Supra and subgingival plaque samples were collected after 30 days. Clinical parameters were recorded after 30, 45, and 90 days. Subjects were evaluated, and oral hygiene instructions were reinforced at each visit.

Preparation of probiotic culture & chewing gum

This probiotic culture was developed at Central Food Technological Research Institute (CSIR-CFTRI) Mysuru. The probiotic strains were activated and then passaged twice in MRS broth (pH.4 for *Lactobacillus* and pH.5 for *Bifidobacterium*). They were incubated at 37 °C for 24 hours. The strains were centrifuged at 15,000 rpm for 5 minutes, and then the supernatant was discarded. The obtained biomass of the strains was washed with 0.1M phosphate-buffered saline(PBS). The cells were suspended in PBS, and optical density was adjusted to correspond to colony-forming units per milliliter (CFU/mL). The culture was lyophilized by centrifugal methods to obtain freeze-dried cells. All chemicals were supplied by HiMedia Pvt.Ldt., India.

The chewing gum base was placed for softening in the oven for 5 hours at 50 – 60° C. In the softened gum base, sodium alginate and pectin were added in batches and blended adequately for 4 to 5 minutes. Essence was also added to the preparation. The temperature was cooled to 37° C, and freeze-dry probiotic powder was added, stirred, and allowed to cool. After cooling, they were molded into required shapes and packed. 1 gram of freeze-dried powder contains $1*10^8$ CFU. Freeze-dried powder was added based on the number of chewing gums required.

Microbiological Parameters

In the course of initiation and progression of periodontal disease, the subgingival bacteria multiply in numbers and invade the cells of pocket epithelium and underlying tissues. *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* can invade the

gingival tissues and cause severe chronic periodontitis and aggressive periodontitis.

The supragingival plaque was analyzed for the total bacterial count and subgingival plaque for *P.gingivalis* and *A.actinomycetemcomitans* through quantitative PCR. The collected samples were transferred to the laboratory (Faculty of Life sciences, JSSAHER) in 2 hours. For total bacterial count, the pore plate technique was used. For qPCR, DNA extraction was done according to the modified method by Wilson *et al*. PCR amplification was performed in a total reaction mixture volume of 25 µl. The sequence of primers and probes for *P.g* and *A.a*[12] are

F: GCGCTCAACGTTCAGCC,

R: CACGAATTCCGCCTGC,

6FAMCACTGAACTCAAGCCCGGCAGTTTCAA-TAMRA

F: GAACCTTACCTACTCTTGACATCCGAA,

R: TGCAGCACCTGTCTCAAAGC

6FAM-AGAACTCAGAGATGGGTTTGTGCCTTAGGG-TAMRA

The samples were subjected to an initial amplification cycle of 50°C for 2 min

and 95°C for 10 min, followed by 45 cycles at 95°C for 15 s and 60°C for 1 min.

The data were analyzed with ABI 7000 Sequence Detection System software.

Statistical Analysis

The sample size was calculated based on hypothesis testing between the two means using nMaster software. The sample size was computed to be 7 per group at an assumed mean difference of 0.45 with 5% alpha error and 80% power and an effect size of 1.67. However, the sample size was rounded off to 8 per group anticipating a 10% dropout.

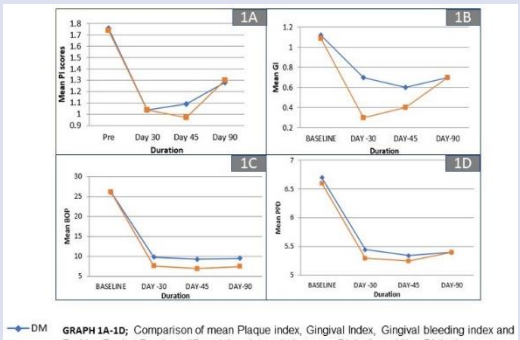
All the clinical and microbiological parameters were analyzed using SPPS version 21 software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). Descriptive statistics like mean and standard deviation were applied. Repeated measures ANOVA was used to know the significance in a mean difference of groups versus sessions for PI, GI, Bleeding Index, PPD, and bacterial count. Unpaired 't-test was applied for intragroup comparison at different time intervals. Statistical significance was set at $p<0.05$

Results

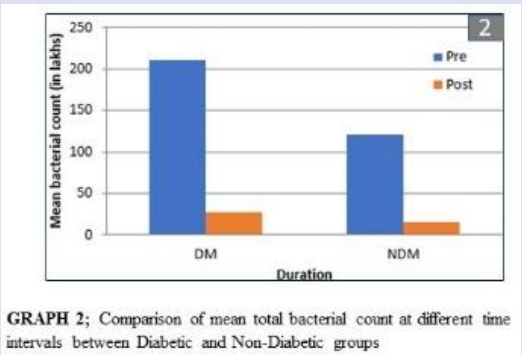
48 subjects were included in the study, and only 40 subjects were considered for final analysis, 18 females and 22 males. The mean age group of subjects in the diabetic group is 50.0 ± 11.75, and the non-diabetic group is 43.4 ± 11.75. There is no statistically significant difference between groups($F= 2.905$, $p= 0.097$) and subgroups($F= .227$, $p= 0.798$) with respect to age of subjects.

Intergroup comparison for PI, GI, Gingival Bleeding Index, PPD, and total bacterial count, from baseline to days 30, 45, and 90 showed a non-significant difference between diabetic and non-diabetic groups(graphs 1&2). *P.g* and *A.a* are slightly higher in the diabetic group, which is non-significant (graph-3 &4).

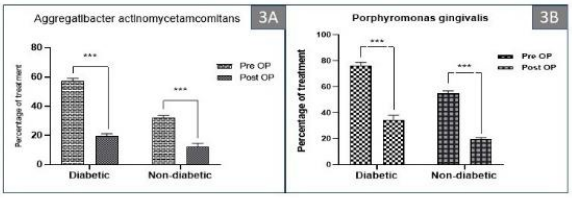
On intra-group comparison, a statistically significant reduction was observed for PI, GI, Gingival Bleeding Index, and PPD from baseline to day 30 for test and control sub-groups of both diabetic and non-diabetic groups(Tables 1-4). The total bacterial count was significantly reduced in probiotic groups(table-5). Non-significant reduction of *P.g* and *A.a* was observed in all sub-groups after 30 days (Table-6).



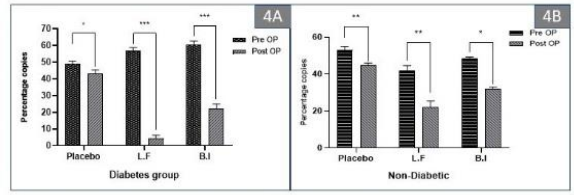
GRAPH 1A-1D; Comparison of mean Plaque index, Gingival Index, Gingival bleeding index and Probing Pocket Depth at different time intervals between Diabetic and Non-Diabetic groups



GRAPH 2; Comparison of mean total bacterial count at different time intervals between Diabetic and Non-Diabetic groups



GRAPH-3A & 3B; Comparison of *A.a* and *P.g* at baseline and 30 days between Diabetic and Non-Diabetic groups



GRAPH-4A & 4B; Intragroup comparison of *A.a+P.g* at baseline and 30 days in Diabetic and non-diabetic groups.

Table 1. Intragroup comparison of plaque index from baseline to 90 days.

Plaque İndex		Diabetic Group			Non-Diabetic Group		
		Paired diff		.sig	Paired diff		.sig
		Mean	S.D		Mean	SD	
Placebo	BL -DAY 30	0.64	0.23	0.000	0.64500	0.22474	0.001
	BL -DAY 45	0.54	0.3	0.003	0.69500	0.21843	0.001
	BL -DAY 90	0.35	0.26	0.012	0.36167	0.30195	0.032
L.f	B.L -DAY 30	0.70333	0.44189	0.011	0.79857	0.32231	0.001
	B.L -DAY 45	0.73667	0.51465	0.017	0.87000	0.42249	0.002
	B.L -DAY 90	0.65333	0.44212	0.015	0.72143	0.38255	0.002
B.l	B.L -DAY 30	0.81714	0.21685	0.000	0.64571	0.20895	0.000
	B.L -DAY 45	0.73143	0.24876	0.000	0.71714	0.24985	0.000
	B.L -DAY 90	0.44571	0.26757	0.005	0.37429	0.23734	0.006

*BL- Baseline *L.f – *Lactobacillus fermentum* *B.l – *Bifidobacterium longum*

Table 2. Intragroup comparison of Gingival index from baseline to 90 days.

Gingival İndex		Diabetic Group			Non-Diabetic Group		
		Paired diff		.sig	Paired diff		.sig
		Mean	S.D		Mean	SD	
Placebo	B.L -DAY 30	0.83571	0.23187	0.000	0.60333	0.40033	0.014
	B.L -DAY 45	0.83571	0.23187	0.000	0.60333	0.40033	0.014
	B.L -DAY 90	0.82000	0.23144	0.000	0.45333	0.54080	0.095
L.f	B.L -DAY 30	0.45667	0.39808	0.038	0.85000	0.43768	0.002
	B.L -DAY 45	0.45667	0.39808	0.038	0.85857	0.42928	0.002
	B.L -DAY 90	0.45667	0.39808	0.038	0.70000	0.54708	0.015
B.l	B.L -DAY 30	0.47571	0.38043	0.016	0.65429	0.43749	0.007
	B.L -DAY 45	0.31286	0.59930	0.216	0.61857	0.41875	0.008
	B.L -DAY 90	0.47571	0.38043	0.016	0.27000	0.31596	0.064

*BL- Baseline *L.f – Lactobacillus fermentum *B.l – Bifidobacterium longum

Table 3. Intragroup comparison of Gingival Bleeding index from baseline to 90 days.

Gingival Bleeding İndex		Diabetic Group			Non-Diabetic Group		
		Paired diff		.sig	Paired diff		.sig
		Mean	S.D		Mean	SD	
Placebo	B.L -DAY 30	13.85714	5.04739	0.000	17.66667	9.75021	0.007
	B.L -DAY 45	14.42857	5.28700	0.000	18.66667	11.14750	0.009
	B.L -DAY 90	14.71429	5.43796	0.000	18.33333	11.27239	0.010
L.f	B.L -DAY 30	16.50000	6.18870	0.001	17.14286	7.19788	0.001
	B.L -DAY 45	16.83333	6.70572	0.002	18.00000	8.48528	0.001
	B.L -DAY 90	17.50000	6.97854	0.002	17.71429	8.82637	0.002
B.l	B.L -DAY 30	18.57143	13.83061	0.012	20.57143	6.50275	0.000
	B.L -DAY 45	18.85714	13.81338	0.011	20.57143	6.50275	0.000
	B.L -DAY 90	17.28571	14.93000	0.022	19.71429	7.52140	0.000

*BL- Baseline *L.f – *Lactobacillus fermentum* *B.l – *Bifidobacterium longum*

Table 4. Intragroup comparison of Probing pocket depth from baseline to 90 days.

Probing Pocket Depth		Diabetic Group			Non-Diabetic Group		
		Paired diff		.sig	Paired diff		.sig
		Mean	S.D		Mean	SD	
Placebo	B.L -DAY 30	1.14286	0.69007	0.005	1.33333	0.81650	0.010
	B.L -DAY 45	1.28571	0.48795	0.000	1.66667	0.51640	0.001
	B.L -DAY 90	1.14286	0.69007	0.005	1.33333	0.81650	0.010
L.f	B.L -DAY 30	1.50000	0.54772	0.001	1.42857	0.78680	0.003
	B.L -DAY 45	1.66667	0.51640	0.001	1.28571	0.75593	0.004
	B.L -DAY 90	1.66667	0.51640	0.001	1.28571	0.75593	0.004
B.l	B.L -DAY 30	1.14286	0.69007	0.005	1.14286	0.69007	0.005
	B.L -DAY 45	1.14286	0.69007	0.005	1.14286	0.69007	0.005
	B.L -DAY 90	1.14286	0.69007	0.005	1.00000	0.81650	0.018

*BL- Baseline *L.f – *Lactobacillus fermentum* *B.l – *Bifidobacterium longum*

Table 5. Intragroup comparison of total bacterial count from baseline to 30 days.

Total Bacterial Count		Diabetic Group			Non-Diabetic Group		
		Paired diff		.sig	Paired diff		.sig
		Mean	S.D		Mean	SD	
Placebo	B.L -DAY 30	31.57482	52.66532	0.17	8.56667	11.00430	0.115
L.f	B.L -DAY 30	182.37350	347.73634	0.045	145.96357	166.88299	0.040
B.l	B.L -DAY 30	335.10929	357.65829	0.048	149.77657	174.71266	0.046

*BL- Baseline *L.f – Lactobacillus fermentum *B.l – Bifidobacterium longum

Table 6. Inter and intragroup comparison of P.g and A.a from baseline to 30 days

	Diabetic		Non-Diabetic	
	Mean ± SEM	P- value	Mean ± SEM	P- value
P.g	-41.67 ± 2.494	0.679	-35.00 ± 1.291	0.40
A.a	-37.33 ± 1.453	0.737	-20.00 ± 1.826	0.60
Sub-Groups (*P.g + A.a*)				
placebo	-11.67 ± 2.848	0.329	-8.000 ± 1.291	0.4
L.f	-50.67 ± 2.603	0.426	-22.67 ± 2.963	0.785
B.l	-33.00 ± 2.887	0.720	-12.00 ± 2.582	0.4

*P.g – Porphyromonas gingivalis * A.a – Aggregatibacter actinomycetemcomitans
*BL- Baseline *L.f – Lactobacillus fermentum *B.l – Bifidobacterium longum

Discussion

Studies have revealed a possible link between systemic diseases and periodontitis. It was accepted that people with diabetes are more prone to establish periodontal diseases. Similarly, periodontal disease might be a risk factor for diabetes.[13] There is also evidence indicating that oral bacteria play an essential role in diabetes and obesity. Direct association between *A.actinomycetamcomitans, P.gingivalis,* and glycemic control was reported in a few studies.[14] These pathogens were also believed to cause dysbiosis in gut microbiota,[15] altering glucose metabolism. So, the treatment of periodontal disease in diabetic subjects should also focus on lowering blood glucose levels, which might act as an adjuvant to conventional periodontal therapy.

Bacterial therapy, in the form of probiotics, offers a dual role in maintaining gut health as well as reducing the colonization of oral bacteria. Anti-diabetic effects of probiotics are due to their competitive inhibition, immunomodulation, antioxidant, and anti-inflammatory properties.[16] An analogous mechanism occurs in the oral cavity and intestine when probiotics are consumed. In the oral cavity, probiotics directly engage in the metabolism of bacterial substrates and inhibit bacterial colonization. They compete and intervene with bacterial attachments and prevent plaque formation.[17]

Lactobacillus fermentum is a ubiquitous, gram-positive, fermentative bacteria and helps in the production of enzymes that metabolize carbohydrates proteins and break down bile salts.[18] *Bifidobacterium longum* is an anaerobe, predominates in the large intestine[19], and is also present in the oral cavity of healthy subjects. They metabolize lactose and ferment indigestible carbohydrates.[19]

To our knowledge, this is the first study comparing the efficacy of *Lactobacillus* and *Bifidobacterium* species among diabetic and non-diabetic subjects with periodontitis. After screening, 48 subjects were selected for the study, and informed consent was taken. Due to the COVID-19 pandemic, 8 subjects were dropped out of the study, and 40 subjects (20-diabetic, 20-non-diabetic) were analyzed for final results. Chewing gum was selected for carrying probiotics with the aim that 'functional foods' which have better compliance should become a part of the treatment of periodontal diseases.

When considering changes in mean PI, a significant reduction was observed in both groups and also within the subgroups. From day 30 to day 90, there is a trend of a mild increase in PI in all the groups and subgroups, which was not statistically significant. For PI, the mean difference from baseline to 90 days in placebo, L.f, B.l are 0.36±0.27, 0.7±0.4, 0.41±0.25, respectively, which shows statistically significant difference within the subgroups(p=.028). The above mean difference stated that *Lactobacillus fermentum* is more effective in controlling plaque at the end of 90 days, followed by *Bifidobacterium longum*.

On intergroup and intragroup comparison of mean GI, Gingival bleeding Index, and PPD, a significant reduction was observed from baseline to 30 and 45 days. At the end of day 90, the *Bifidobacterium longum* subgroup showed an increase in mean GI (Diabetic and Non-diabetic groups) and gingival bleeding (Diabetic group), which is statistically significant.

A study by Sabatini *et al*. assessed the efficacy of *L. reuteri* tablets on gingivitis subjects with diabetes.[20] SRP was not performed, and subjects were asked to use probiotic pills twice a day for 30 days. A significant change

was observed in only GI. So, this study proved that probiotics act better when used as an adjuvant to SRP. Szkaradkiewicz *et al*. provided *L. reuteri* lozenge to subjects with Periodontitis two times/day for 14 days after SRP.[21] A significant reduction was observed only in BOP, PPD, CAL, and GCF biomarkers in the probiotic group. PI and GI were not reduced significantly. In our present study, when probiotics are given two times/day after SRP for 30 days, all clinical parameters were significantly reduced. There is no evidence showing the exact dosage of probiotics required for maintaining oral health. Most of the studies used 10^8 CFU/ml of probiotics for different time periods. Each chewing gum contained 10^8 CFU in this study and was taken twice daily for 30 days.

Microbial analysis showed no significant difference in the total bacterial count for intergroup comparison but a considerable reduction within the subgroups(p=0.048). Probiotic subgroups of diabetic and non-diabetic groups revealed a statistically significant decrease in the bacterial count, and control groups non-significant reduction.

It was believed that supragingival plaque control affects the subgingival microbial environment by reducing pocket depth in advanced supragingival lesions but not in the case of angular bone defects and deep pockets.[22] However, the mean PPD of the present study at baseline is 6.7±0.98 in the diabetic group and 6.6±1.05 in the non—diabetic group, which indicates that subjects with moderate periodontitis were included in the study.

In the PCR analysis of subgingival plaque samples, it was observed that the prevalence of P.g and A.a before treatment is slightly more remarkable in the diabetic group, but the difference is not significant(graphs 3A and 3B). Intragroup analysis of PCR revealed that L.f and B.l in the diabetic group showed a three-fold decrease of *P.g* and *A.a* while placebo showed only a one-fold decrease. In the non-diabetic group, placebo and *L.f* showed a two-fold decrease of *P.g* and *A.a* while *B.l* showed only a one-fold decrease. However, the reduction is not significant in any of the subgroups for both *P.g* and *A.a* when evaluated 30 days after using probiotics.

Randomized control trial by Invernici *et al.* evaluated the effect of *Bifidobacterium lactis* lozenges on chronic periodontitis when taken twice/day for 30 days as an adjunct to SRP. At the end of 30 days, no difference was observed between test and control groups for red-complex bacteria, whereas this percentage has reduced significantly after 90 days in the test group. This could be explained by probiotics that might have acted delaying in the recolonization of pathogens in periodontal pockets.[23] In the present study, after 30 days, there is no significant reduction in *P.g* and *A.a,* which might be explained by the same.

Chen *et al*. reported that *Lactobacillus fermentum* showed more potent inhibitory effects on *Porphyromonas gingivalis.*[24] The mechanism can be explained that probiotics produce organic acids, which decrease the pH oxidation-reduction potential and inhibits the growth of pathogenic bacteria. Hojo *et al*. explained that *B.longum* competes with *P.gingivalis* for salivary vitamin K, which is their mutual growth factor.[25]

In the present study, there is no significant difference in clinical or microbial parameters between diabetic and non-diabetic groups. The intragroup comparison significantly reduced all clinical parameters from baseline to 30,45 and 90 days. A substantial decrease in the total bacterial count was observed in probiotic groups but not in placebo. PCR analysis showed a non-significant reduction of *P.g* and *A.a* in all the subgroups.

This study has certain limitations, such as a small sample size and less follow-up, and HbA1C levels of diabetic subjects were not evaluated post-treatment.

Conclusions

Probiotic functional foods can be delivered as an adjunct to SRP for the management of periodontitis in systemically compromised subjects. Long term use or inclusion of probiotics in the diet is recommended to maintain recolonization of bacteria.

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Conflicts of Interest Statement

No conflicts of interest.

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Effects of Surface Characteristics of Conventionally Manufactured, CAD/CAM Milled, and 3D-Printed Interim Materials on Adherence of Streptococcus Mutans and Candida Albicans

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ABSTRACT

Objectives: The purpose of this *in vitro* study was to compare conventionally manufactured, CAD/CAM milled, and 3D-printed interim materials based on their susceptibility to adherence of *Streptococcus mutans* and *Candida albicans*, and examine the influence of surface roughness and hydrophobicity.

Materials and Methods: Eighty disc-shaped specimens fabricated from autopolymerized polymethyl methacrylate (A-PMMA), bis-acryl composite (Bis-acrylate), CAD/CAM PMMA-based polymer (Milled-PMMA), and 3D-printed resin (Printed) were subjected to 10,000 thermal cycles (5-55 °C) and divided into two groups (n=10) according to microbial suspension used: *Streptococcus mutans* and *Candida albicans*. Surface roughness (Ra) and hydrophobicity (WCA) of specimens were measured. An adhesion test was performed by incubating the specimens in *Streptococcus mutans* and *Candida albicans* suspensions at 37 °C for 24 hours, and the adherent cells were evaluated by counting colony-forming units (CFU/ml). Scanning electron microscopy (SEM) was performed to analyze the surfaces (n=2). Data were analyzed with Kruskal-Wallis and Mann-Whitney U tests. Spearman's correlation analysis was used to determine correlation among the measurements (α=.05).

Results: Type of restorative material significantly influenced Ra and WCA. The highest adhesion of *Streptococcus mutans* was observed in Printed, followed by Bis-acrylate, A-PMMA, and Milled-PMMA (p=.001). The highest adhesion of *Candida albicans* was noted on A-PMMA, followed by Printed, Bis-acrylate, and Milled-PMMA (r=.001). The adhesion of *Streptococcus mutans* (r=.660) and *Candida albicans* (r=.413) showed a positive correlation with Ra. A negative correlation was found between WCA of the materials and *Streptococcus mutans* adhesion (r= −.373).

Conclusions: Surface roughness plays an important role in the adherence of microorganisms. CAD/CAM PMMA-based polymers may be a better choice to reduce microbial adhesion in long-term use.

Key words: Interim material, microbial adhesion, surface properties.

Konvansiyonel, CAD/CAM Frezeleme ve 3D Baskı Yöntemleriyle Üretilmiş Geçici Materyallerin Yüzey Özelliklerinin *Streptococcus Mutans* ve *Candida Albicans* Tutunumuna Etkileri

Süreç

Geliş: 04/01/2023
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ÖZ

Amaç: Bu çalışmanın amacı konvansiyonel, CAD/CAM frezeleme ve 3D baskı yöntemleriyle üretilmiş geçici materyallerinin *Streptococcus mutans* ve *Candida albicans* tutunumu duyarlılıklarına göre karşılaştırılması ve yüzey pürüzlülüğü ve hidrofobikliğin buna etkisinin incelenmesidir.

Gereç ve Yöntemler: Otopolimerize polimetil metakrilat (A-PMMA), bis-akril kompozit (Bis-acrylate), CAD/CAM PMMA-bazlı polimer (Milled-PMMA) ve 3D baskı (Printed) rezinlerinden seksen adet disk şeklinde örnek üretildi. Örneklere 10,000 termal siklus (5-55°C) uygulandı ve kullanılan mikrobiyal süspansiyonlara göre örnekler iki gruba (n=10) ayrıldı: *Streptococcus mutans* ve *Candida albicans*. Örneklerin yüzey pürüzlülüğü (Ra) ve hidrofobikliği (WCA) ölçüldü. Örnekler Streptococcus mutans ve Candida albicans süspansiyonlarında 37°C'de 24 saat inkübe edilerek tutunum testi yapıldı ve yapışık hücreler koloni oluşturan birimler (CFU/ml) sayılarak değerlendirildi. Yüzeyleri analiz etmek için taramalı elektron mikroskobu (SEM) görüntüleri alındı (n=2). Veriler Kruskal-Wallis ve Mann-Whitney U testi ile analiz edildi. Ölçümler arasındaki korelasyonu belirlemek için Spearman korelasyon analizi kullanıldı (α=.05).

Bulgular: Restoratif materyalin türü, Ra ve WCA'yı önemli ölçüde etkiledi. En yüksek *Streptococcus mutans* adezyonu Printed'de gözlemlenirken, ardından Bis-akrilat, A-PMMA ve Milled-PMMA'da gözlemlenmiştir (p=.001). En yüksek *Candida albicans* adezyonu A-PMMA'da kaydedilirken, ardından Printed, Bis-acrylate ve Milled-PMMA'da (r=.001) kaydedilmiştir. *Streptococcus mutans* (r=.660) ve *Candida albicans* (r=.413) tutunumu ve Ra arasında pozitif korelasyon gözlemlenmiştir. Örneklerin WCA'sı ile *Streptococcus mutans* tutunumu arasında negatif bir korelasyon bulunmuştur (r=-.373).

Sonuçlar: Yüzey pürüzlülüğü mikroorganizmaların tutunumunda önemli rol oynar. CAD/CAM PMMA bazlı polimerler, uzun süreli kullanımda mikrobiyal yapışmayı azaltmak için daha iyi bir seçim olabilir.

Anahtar Kelimeler: Geçici diş restorasyonu, mikroorganizma tutunumu, yüzey özellikleri.

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Introduction

Interim fixed prostheses have an essential role in maintaining the patient's normal masticatory functions, in protecting the prepared teeth and periodontal tissues.[1] The interim restorations may be used for a longer duration to assess the course of treatment, particularly in multidisciplinary reconstructions. Therefore, the interim restorative materials should have certain mechanical and esthetic properties and biological behaviors.[1,2]

Accumulation of microorganisms on the surface of restorative materials contributes to the occurrence of caries, gingival inflammation, and denture stomatitis which is an important aspect related to the longevity of restorations.[2] Previous studies have reported *Streptococcus mutans (S. mutans)* as the primary etiological bacteria in the pathogenesis of caries.[2,3] *Candida albicans (C. albicans)* is the most prevalent fungus in the oral cavity and has been considered the major pathogenic agent related to denture stomatitis.[4] The factors that influence the quantity of microbial adhesion and biofilm formation on restorative materials include the chemical composition of the material, oral hygiene, salivary components, and dietary habits. Surface characteristics of materials such as surface free energy, surface roughness (Ra), and hydrophobicity were also reported to significantly influence the adhesion of microorganisms.[5-10] Surface irregularities can promote initial accumulation and provide niches in which microorganisms are protected against hydrodynamic shear forces.[11,12] Furthermore, manufacturing methods may affect the surface characteristics and biological behaviors of interim restorations. However, the relationship between these factors and microbial adhesion and subsequent biofilm formation still remains controversial.

Conventionally manufactured resin materials such as polymethyl methacrylate (PMMA) and bis-acryl composite resins are routinely used for fabrication of interim fixed prostheses because of their easy processability and relatively low cost. However, these materials have some disadvantages such that their fabrication is time consuming and their quality is dependent on hand skills.[13] Computer-aided design and computer-aided manufacturing (CAD/CAM) processes are increasingly used in dentistry to overcome some of the drawbacks of the conventionally manufactured materials.[14] Because CAD/CAM PMMA-based resin blocks are polymerized with a high degree of conversion and have a highly cross-linked structure, the surface properties and biocompatibility of milled interim restorations are better than those obtained with the conventional methods.[15,16]

The three-dimensional (3D) printing method produces restorations by building up a solid object from powdered or liquid-based material in layers and is a relatively low-cost alternative among digitally fabricating interim restorations.[17] The technology of stereolithography is commonly used in 3D-printing methods for dental applications and manufactured interim materials are largely based on photosensitive resin material in this technique.[18] Although acceptable mechanical and optical properties have been reported, investigations on the microbial accumulation on 3D-printed interim materials are limited.[17,19-21] Therefore, the purpose of this study was to investigate the effects of surface roughness and hydrophobicity of conventionally manufactured, milled, and printed interim materials on microbial adhesion of *S. mutans* and *C. albicans* in the presence of saliva after thermocycling. The first null hypothesis was that no difference would be found among the investigated interim materials by means of the quantity of *S. mutans* and *C. albicans* adhesion. The second null hypothesis was that the quantity of *S. mutans* and *C. albicans* adhesion would not be influenced by the surface roughness and hydrophobicity values of investigated interim materials.

Material and Methods

Four different interim materials, autopolymerized polymethyl methacrylate resin (A-PMMA), bis-acryl composite resin (Bis-acrylate), CAD/CAM PMMA-based polymer (Milled-PMMA), and 3D printed resin (Printed) were prepared to be assessed for their surface roughness, surface hydrophobicity, and susceptibility to adherence of the microorganisms after 10,000 thermal cycles (Table 1). Determination of sample size was performed by using a statistical power analysis program (G*Power 3.1.9.3; Heinrich-Heine-Universität Düsseldorf) which determined that 10 specimens per group provided a power of 0.8 at the significance level of 0.05.

Table 1. Materials used

Abbreviation	Product name	Manufacturer	Type	Interim material fabrication technique	Lot number
A-PMMA	Imident	Imicryl Dental	Conventional PMMA	Conventional; self-curing	20B976
Bis-acrylate	PreVISION® Temp	Kulzer GmbH	Bis-acrylate composite resin	Conventional; self-curing	72007838
Milled-PMMA	PMMA Disc Multi	Sagemax Bioceramics Inc	Polymethyl methacrylate-based polymer	CAD/CAM	YB357C
Printed	Temporis	DWS	Light curable nanocomposite	3D-Printing; SLA	1921741

A-PMMA: autopolymerized polymethyl methacrylate resin; CAD/CAM: computer-aided design and computer-aided manufacturing; 3D-Printing: Three Dimensional-Printing; SLA: stereolithography.

A-PMMA (Imident, Imicryl Dental, Konya, Turkiye) and Bis-acrylate (PreVISION® Temp, Kulzer GmbH, Hanau, Germany) Ø10×2-mm specimens were prepared by using stainless-steel mold. PMMA was mixed manually with a spatula and packed into the mold (n=20). Bis-acryl composite resin was mixed automatically and injected into the mold by using a cartridge system (n=20). Specimens then were polymerized according to the manufacturer's recommendations and removed from the mold after 15 minutes. Milled-PMMA specimens were designed as standard tessellation language files in the system software program (Nauta XFAB Edition, DWS, Thiene, Italy), 10 mm in diameter, and milled by using a CAD/CAM milling machine (Yenadent CAM 5.1, Yenadent Ltd, Istanbul, Turkiye) from CAD/CAM PMMA blocks (PMMA Disc Multi, Sagemax Bioceramics Inc, WA, USA). The specimens then were cut under water cooling to procure disk-shaped specimens 2 ±0.02 mm in thickness (n=20).

A virtual disk plate design (Ø10×2 mm) was performed by using the same software program and saved as a standard tessellation language file. The Printed specimens were fabricated by using an SLA-based 3D-printer machine (XFAB 2500PD, DWS, Thiene, Italy) with a galvanometer laser scanning technique from a composite resin material (Temporis, DWS, Thiene, Italy) according to the saved design (n=20). The thickness of the layer was 0.06 mm and the laser scanning speed was 5000 mm/sec. All printed specimens were cleaned with isopropyl alcohol for 1 minute and post polymerization procedures were completed in an ultraviolet curing unit (S2, DWS, Thiene, Italy) for 30 minutes.

After storage in 37 ºC distilled water for 24 hours, both sides of all specimens were trimmed and smoothed in an automatic polisher (LaboPol-20, Struers, Cleveland, OH, USA) with 500-, 800- 1000-, and 1200-grain sandpaper (SiC Foils, Struers, Cleveland, OH, USA), under water-cooling for 15 seconds for each. All specimens underwent additional polishing with a diamond solution on a felt disc (Struers, Cleveland, OH, USA) and were ultrasonically cleaned in distilled water for 5 minutes. Then all specimens were subjected to 10,000 thermocycles between 5 °C and 55 °C with a transport time for 15 seconds and a dwell time for 30 seconds by using a thermal cycler (MTE-101, Mod dental, Ankara, Turkiye) in distilled water to simulate 1-year clinical aging process.[22]

After thermocycling, the Ra values for each specimen were measured with a profilometer (MarSurf PS10, Mahr GmbH, Göttingen, Germany). The diamond stylus of profilometer (10 µm diameter) was moved across the surface under constant pressure and performed three measurements (cut-off: 0.8 mm; speed: 0.5 mm/sec) at different surface locations of each specimen, after which mean Ra values were calculated to obtain the general roughness profile of each specimen.

The surface hydrophobicity of all specimens was evaluated by using the static contact angle method with an automated contact angle measurement device (Attension Theta Flex, Biolin Scientific, Sweden) equipped with a digital camera and image analysis software (One Attension; 2.6 version). Specimens were cleaned with acetone and air dried. The water contact angle (WCA) values of all materials were obtained by dispensing a droplet (2 µL) of distilled water onto the specimens with 2 measurements for each droplet (right and left contact angle) at 20°C room temperature. Three WCA measurements were made from different areas of the surface for each specimen and then the average values were calculated.

To simulate the formation of an acquired salivary pellicle, unstimulated whole saliva was collected from two healthy volunteers after receiving their informed consent, as required by the Local Ethical Committee for Research (protocol 96/2021).[24] Volunteers refrained from oral hygiene for 24 hours, had no active dental disease, and did not have antibiotic therapy for at least three months before the experiments. All specimens were horizontally placed in presterilized 24-well plates, then were covered with 2 mL sterile human saliva prepared according to Baffone et al.[23] and incubated by shaking at 37 °C for 1 hour. Thereafter, the specimens were washed with 5 ml of saline and placed into the sterilized petri dishes.

Before biofilm adhesion test, all specimens were cleaned with an ultrasonic cleaner for 15 minutes and then disinfected with 70% alcohol, and ultrasonically cleaned with distilled sterile water for 15 minutes to remove any contaminants and residues from the surface, and then each material group was further divided into two subgroups based on the microbial suspensions used, with ten samples in each subgroup within the respective material group (n=10): *S. mutans* NCTC 10449 and *C. albicans* ATCC 10231 that were provided from a local laboratory (Near East University, Faculty of Medicine, Department of Medical Microbiology).

S. mutans obtained from stock were plated onto blood agar and incubated at 37°C in a 10% CO_2 atmosphere for 24 hours. Then transferred into tubes containing 5 ml of BHI and incubated at 37°C in a 10% CO_2 atmosphere for 18 hours. The tube contents were mixed using a centrifuge for 5 minutes. *S. mutans* suspensions were concentrated as $1.5x10^8$ bacteria/ml spectrophotometrically. The mixture of the *S. mutans* suspensions was applied to each specimen surface, and the *S. mutans* adhesion was provided for 15 minutes to the pellicle layer. BHI with 5% sucrose was added to each petri dish to cover all specimens, and dishes were placed into an incubator at 37°C in a 5% CO_2 atmosphere for 24 hours. The specimens were then placed into tubes containing 2 ml of PBS and mixed with a centrifuge for 30 seconds to separate the free *S. mutans*. After the incubation period, colony-forming units (CFU/ml) were determined and recorded for *S. mutans* for all groups.

C. albicans obtained from stock were plated onto SDA agar and incubated at 37°C in a 10% CO_2 atmosphere for 24-48 hours. Then transferred into tubes containing 5 ml of BHI and incubated at 37°C in a 10% CO_2 atmosphere for 24 hours. The tube contents were mixed using a centrifuge for 5 minutes. *C. albicans* suspensions were concentrated as $1.5x10^8$ yeast/ml spectrophotometrically. The mixture of the *C. albicans* suspensions was applied to each specimen surface, and the *C. albicans* adhesion was provided for 15 minutes to the pellicle layer. BHI with 5% sucrose was added to each petri dish to cover all specimens, and dishes were

placed into an incubator at 37°C in a 5% CO_2 atmosphere for 48 hours. The specimens were then placed into tubes containing 2 ml of PBS and mixed with a centrifuge for 30 seconds to separate the free *C. albicans*. After the incubation period, colony-forming units (CFU/ml) were determined and recorded for *C. albicans* for all groups.

For additional SEM evaluation, two specimens were randomly selected from each tested group (n=2), fixed for 1 h in 2.5% glutaraldehyde, dehydrated in increasing series of ethyl alcohol baths (10%, 25%, 50%, 75%, and 90% for 20 min and 100% for 1-h), and then dried overnight in a bacteriological incubator at 37°C. Then, specimens were coated with gold and evaluated under an SEM (Zeiss EVO 40, Carl Zeiss SMT, Cambridge, UK) at 20 kV, with magnifications of 1000× and 5000×. All images were examined by one observer.

Statistical analyses were performed by using a software program (Number Cruncher Statistical System 2007; NCSS Statistical Software). Descriptive statistical methods (mean, standard deviation [SD], median, minimum, maximum) and distribution of data were evaluated by the Shapiro-Wilk test. Because the data were non-normally distributed, the Kruskal-Wallis test was used to investigate the statistical difference between groups. The Mann-Whitney U test was then used to compare group pairs. Spearman's correlation analysis was used to determine correlation among the measurements (α=.05).

Results

The median, minimum, and maximum values of Ra and WCA for all tested interim materials are listed in Table 2. The Kruskal-Wallis test showed significant differences in the Ra and WCA results among groups (p<.05). The Ra values (0.21 to 0.83 µm) for all groups were higher than the plaque accumulation threshold (0.20 µm). The Mann-Whitney U test revealed the highest median Ra values for, Printed (0.61, p=.001) followed by A-PMMA (0.53, p=.001), Bis-acrylate (0.50, p=.001), and milled-PMMA (0.42, p=.001). The WCA values of all materials ranged between 71.15 and 101.65 degrees, so all had hydrophobic surfaces. The Mann-Whitney U test revealed the highest median WCA values for Milled-PMMA (93.38, p=.001), followed by A-PMMA (88.32, p=.001), Printed (84.94, p=.001), and Bis-acrylate (78.43, p=.001).

The median CFU/ml values for each group are presented in Figure 1. The Kruskal-Wallis test showed significant differences in the *S. mutans* adhesion results among groups (p<.05). The Mann-Whitney U test revealed the highest median CFU/ml values for Printed (220×10^6, p=.001), followed by Bis-acrylate (175×10^6, p=.001), A-PMMA (153×10^6, p=.001), and milled-PMMA (95×10^6, p=.001). The Kruskal-Wallis test showed significant differences in the *C. albicans* adhesion results among groups (p<.05). The Mann-Whitney U test revealed the highest median CFU/ml values for A-PMMA (275×10^6, p=.001), followed by Printed (200×10^6, p=.001), Bis-acrylate (150×10^6, p=.001), and Milled-PMMA (125×10^6, p=.001). As per the Mann-Whitney U test *S. mutans* adhesion was significantly higher than *C. albicans* adhesion in groups Bis-acrylate and Printed, whereas it was significantly less in groups A-PMMA and Milled-PMMA (p=.001). The representative SEM images of the S. mutans and C. albicans biofilm formation on the surface of the specimens are shown in Figure 2-3.

As per Spearman's correlation analysis, a statistically significant positive relationship was found between *S. mutans* adhesion and Ra values (r=.660, p=.000), and between *C. albicans* adhesion and Ra values (r=.413, p=.008). However, *S. mutans* adhesion showed a negative relationship with WCA values (r= −.373, p=.018), whereas the *C. albicans* adhesion did not show any significant relationship with the WCA values (r= −.133, p=.412).

Table 2. Median, minimum, and maximum surface roughness and water contact angle values for tested materials

	Surface roughness (µm)		Water contact angle (°)	
Groups	Median	Min/Max	Median	Min/Max
A-PMMA	0.53[a]	0.49/0.82	88.32[a]	79.6/99.69
Bis-acrylate	0.50[b]	0.43/0.58	78.43[b]	71.15/94.18
Milled-PMMA	0.42[c]	0.21/0.52	93.38[c]	79.31/101.65
Printed	0.61[d]	0.53/0.83	84.94[d]	75.35/94.08

A-PMMA: autopolymerized polymethyl methacrylate; Bis-acrylate: bis-acryl composite resin; Milled-PMMA: CAD-CAM PMMA resin; Printed: 3D-printed resin. Mean difference significant at p<.05. Means with same letters not statistically different.

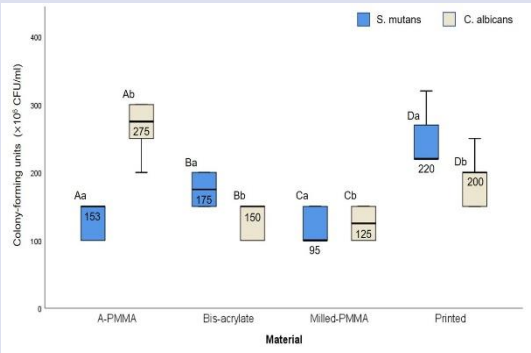

Figure 1. Box plot for colony-forming unit values of tested groups. Group codes as shown in

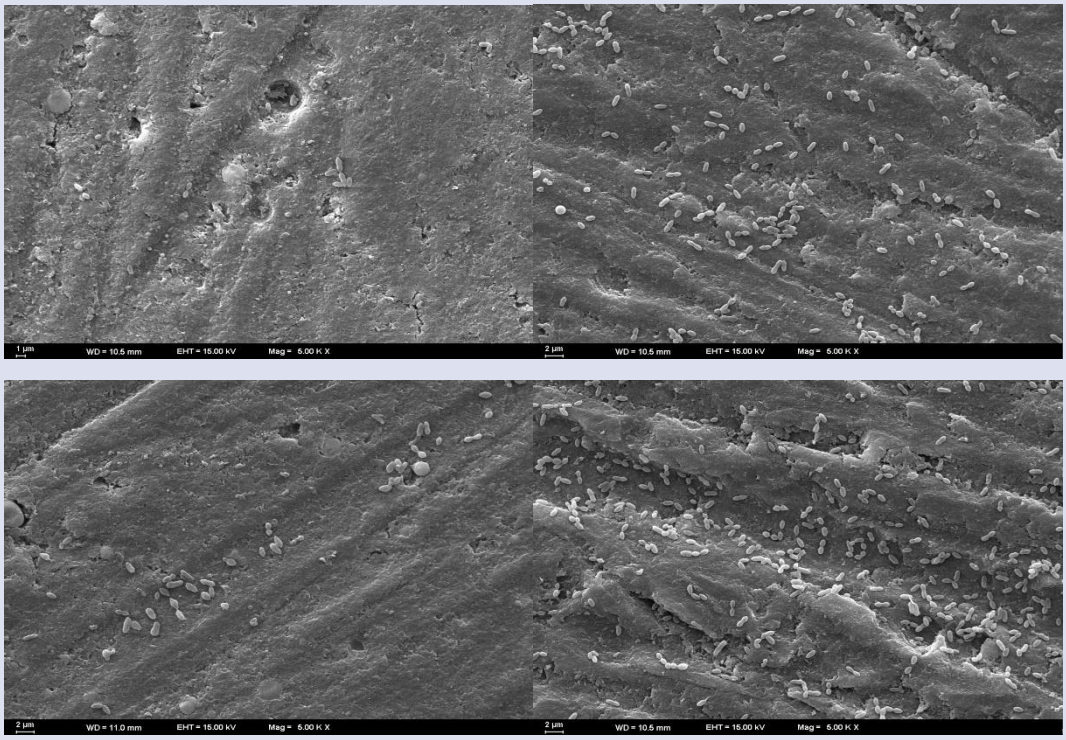

Figure 2. Representative scanning electron microscopy (SEM) images (5000×) of *S. mutans* adhesion on the specimens. A, A-PMMA. B, Bis-acrylate. C, Milled-PMMA. D, Printed

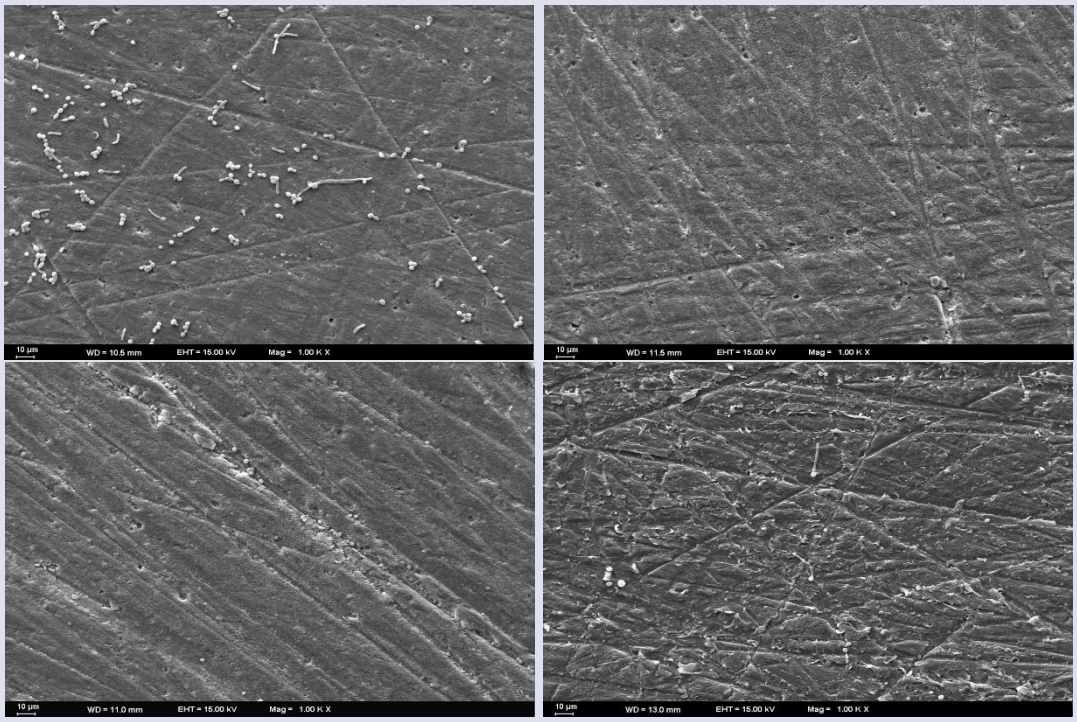

Figure 3. Representative scanning electron microscopy (SEM) images (1000×) of *C. albicans* adhesion on the specimens. A, A-PMMA. B, Bis-acrylate. C, Milled-PMMA. D, Printed.

Discussion

Based on the results of the current study, the first null hypothesis was rejected, as conventionally manufactured, CAD/CAM milled, and 3D-printed interim materials exhibited different susceptibility to adhere to *S. mutans* and *C. albicans*; the second null hypothesis was partially rejected, as correlations were observed among some of the tested parameters.

For restoration conditions in the oral environment to be simulated, all interim materials were submitted to thermocycling which mainly consists of water immersion and temperature change under standardized laboratory conditions, and after measurement of roughness, the hydrophobicity of all interim materials was evaluated.[22] The static contact angle method is an established method for obtaining hydrophobicity values of certain substrates.[6] In principle, the hydrophobic materials exhibit a higher water contact angle. Therefore, the sessile drop method, which involves measuring WCA, was used in the present study to evaluate the hydrophobicity of materials.[25] SEM observation is especially suited for the microscopic characterization of the surface topography and biofilm adhesion.[26] In the current study, scanning electron micrographs were used for supplementary verification of the results obtained from roughness and biofilm adhesion measurements.

Several researchers suggested that microbial adhesion is related to the components and compositions of materials.[11,27,28] Consistent with previous reports, [11,27,28] in present study, the quantity of adherent *S. mutans* and *C. albicans* cells varied depending on tested materials. Furthermore, higher amount of adherent *S. mutans* cells were observed in Bis-acrylate and Printed, which are composite-based materials, whereas higher amount of adherent *C. albicans* cells were observed in groups A-PMMA and Milled-PMMA, which are PMMA-based materials. In line with our study result, Ozel et al.[29] reported different susceptibilities of resin-based interim materials to the adherence of *S. mutans* and *C. albicans*. This may be related to the species-specific characteristics of different microorganisms, such as the cell surface protein antigen SpaP of *S. mutans* or the adhesion protein Hyphal wall protein 1 of *C. albicans*, which are associated with the initial adhesion of microorganisms to the surface, possibly resulting in the different sensitivities to materials.[30-33] However, a precise comparison concerning the interaction between bacterial adhesion and composition of the materials is not possible as manufacturers do not provide adequate information about the exact compositions of the materials.[17]

Surface roughness is generally regarded as an important surface property that affects microbial adhesion to restorative materials in the oral environment.[24,27,28,34,35] The current study found a significant, positive correlation between the Ra values and the quantity of adhesion *S. mutans* and *C. albicans* cells on the materials consistent with previous reports.[6,7,24,27,34,35] However, results of present study are in disagreement with other studies, where no correlation was observed between surface roughness of the resins and microbial adhesion.[36-38] In the present study, the lowest Ra values were observed on Milled-PMMA (0.42 µm). Additionally, the adhesion of *S. mutans* and *C. albicans* was found to be the least on Milled-PMMA, independent of the chemical composition and hydrophobicity of the materials. These findings supported that when Ra was above the threshold (Ra=0.2 µm), different susceptibilities to microbial adherence are primarily caused by variable Ra values. Corroborating the results of the current study, microscopic investigations reveal that the pits and fissures in substrata are responsible for initial adhesion of microorganisms.[12]

In the present study, Printed materials showed higher Ra than Bis-acrylate and Milled-CAD/CAM. These results are inconsistent with those of the previous study, which demonstrated that the Ra of bis-acryl and printed resin materials were similar and the Ra of 3D-printed resins were lower than that of conventionally fabricated and CAD/CAM milled interim materials.[39] These differences can also be related to the measurement of Ra at different aging periods. The layered nature of 3D-printing technology may initiate crack propagation between the layers due to the residual stress from temperature changes and result in increased Ra of the printed material.[17]

Hydrophobicity is another factor that has an influence on microbial adhesion and biofilm formation on material surfaces.[7] In the present study, Milled-PMMA showed a significantly higher WCA, and consequently higher hydrophobicity when compared to A-PMMA. This can be attributed to higher rates of residual components in A-PMMA which enhance its hydrophilic properties and to the reduced hydrolytic degradation processes in Milled-PMMA.[11,40] The lower WCA of Bis-acrylate and Printed groups in the present study confirmed that the composite-based resins are more hydrophilic and polar than PMMA-based resins. However, surface roughness may also affect the contact angle measurements, especially when Ra > 0.1 mm.[5,25] Therefore, it should be noted that dominant roughness values of the present *in vitro* study may obscure the effects of hydrophobicity on microbial adhesion.

The present findings showed a weak negative correlation between the hydrophobicity and *S. mutans* adhesion, which were in agreement with previous studies suggesting that the adhesion strength of *S. mutans* to hydrophobic surfaces was weaker than that to hydrophilic surfaces.[6,29] The published literature revealed different effects of the hydrophobicity on the adhesion of *C. albicans* to material surfaces. Several previous investigations[7-10] have indicated that increased hydrophobicity can lead to both higher and lower adhesion of *C albicans*; however, in the present study, surface hydrophobicity seemed to have no direct influence on the adhesion of *C albicans*, which is in agreement with other studies that found no correlation between hydrophobicity and Candida colonization.[38,41] Therefore, it is possible that the presence of an acquired

salivary pellicle has a homogenizing effect and masks originally distinct differences in material surface properties, which may explain the contradictory results in the literature.[41,42]

Limitations of the present study lie in its *in vitro* design, which did not fully imitate the complex and multifactorial process of the microbial adhesion mechanism. The biofilm formation is affected by the presence of other microorganisms in the dynamic environment of the oral cavity. It should also be noted that the design of the present *in vitro* study included the formation of monospecies *S. mutans* and *C. albicans* biofilm. Additionally, although saliva-coated specimens were evaluated in the present study, individual differences in saliva affect surface properties and microbial adhesion of the materials. Further clinical studies by using multispecies biofilm models on different brand materials in different incubation periods are needed.

Conclusions

Within the limitations of this *in vitro* study, the following conclusions were drawn:

1. The type of restoration material significantly influenced the surface roughness, hydrophobicity, and susceptibility of the tested interim materials to adhere to *Streptococcus mutans* and *Candida albicans*.

2. A significant positive correlation was established between the surface roughness of tested materials and the quantity of adhesion of *Streptococcus mutans* and *Candida albicans* cells on the materials.

3. A negative correlation was found between water contact angle values of the tested materials and the quantity of adhesion of *Streptococcus mutans* cells, whereas no correlation was found between hydrophobicity of the tested materials and the quantity of adhesion of *Candida albicans.*

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Conflicts of Interest Statement

The authors do not have any financial interest in the companies whose materials are included in this article.

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Clinical Findings Related to Musculoskeletal Disorders (MSDs) in a Group of Orthodontists

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ABSTRACT

Aim: Orthodontists may be exposed to a variety of occupational hazards and may be affected by conditions such as musculoskeletal disorders (MSDs). Prevention strategies are required to identify risk factors at different levels. Through an objective clinical evaluation, this study aimed to identify the factors related to the presence of MSDs in a group of orthodontists from Medellín (Colombia).

Methods: A cross-sectional study was conducted. A clinical evaluation, focusing on the occupational aspects related to orthodontics, was carried out by an occupational health physician, which was standardized for research purposes. Sociodemographic and clinical variables were collected. Descriptive and bivariate analyses were conducted on the prevalence of MSDs according to the study variables and Chi-square tests were carried out to observe statistically significant differences. Ethical approval was obtained.

Results: The prevalence of MSDs was 58.7% (95%CI 44.3- 71.7). The frequency of upper body MSDs was 45.7% (women: 51.4%), and in the Spine area was 23.9% (men: 45.5%). The prevalence of MSDs was higher in men those people ≤ 44 years, single/separate, from middle socioeconomic status, and with other medical previous conditions. Labor conditions were related to the presence of MSDs. The agreed percentage for self-perceived symptoms of MSDs and those found for clinical evaluation was for upper body MSDs and clinical Spine area MSDs considering positive cases was 50% and 29.2% respectively.

Conclusions: The orthodontists participating in the study manifested various MSDs and other pathologies related to their professional practice. Sociodemographic and clinical factors were related to the presence of MSDs. Epidemiological surveillance systems in occupational health and strategies in health and safety at work are requested.

Key words: Occupational Health, Musculoskeletal Diseases, Risk Factors; Orthodontists.

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Introduction

Dentists face numerous risk factors, including ergonomics.[1] When these risks are not controlled, musculoskeletal disorders (MSDs) can appear in dental professionals, including orthodontists.[1] MSDs are defined by the United States Center for Disease Control and Prevention as "*injuries or disorders of the muscles, nerves, tendons, joints, cartilage, and spinal discs and are considered a public health problem because it is one of the main causes of absenteeism*".[2,3] MSDs prevalence varies between 30-80%,[4-7] with clinical manifestations in the lumbar region, shoulders, and neck.[4,5]

MSDs are associated with risk factors like individual, physical, and workplace-related conditions such as non-ergonomic positions, long working hours, absence of active breaks, inadequate workplace design, and psychosocial characteristics.[8] We can mention other aspects such as general health conditions, body mass index (BMI), and previous history of pain or genetic predisposition.[5,6,9] Regarding the association of MSDs and individual factors, the evidence is not conclusive yet. While some authors have found that the presence of chronic diseases (such as cardiovascular disease, diabetes, varicose veins), obesity, and increased BMI, are correlated with a higher prevalence of MSDs[4,9], others have reported minimal evidence.[8,10,11] Therefore, it is necessary to elucidate which health factors are specifically related to MSDs, through clinical evaluations that allow an objective assessment and find possible associations.

There exists a large number of studies that have been conducted worldwide reporting the high prevalence of MSDs in general and specialist dentists. These results have been based on self-reported surveys.[4-7,12,13] This situation in many cases, can generate the feeling that people who have suffered some symptomatology are more willing and interested to participate in this type of study and, in addition, only the complaints and individual perceptions of the participants are collected, but not supported in medical diagnoses.[6] This could indicate that there is a difference between self-perception and objective findings after a medical evaluation and diagnosis.

Accordingly, this study aimed to identify the factors related to the presence of MSDs in a group of orthodontists from Medellín (Colombia), through a clinical medical evaluation.

Material and Methods

This manuscript is part of a multi-step project that uses different methodological approaches. In a first part, a cross-sectional survey was applied in 100 orthodontists who graduated between 1993 and 2018 from the Faculty of Dentistry at the University of Antioquia (Medellín, Colombia). Data were provided by the Association of Orthodontists of the University of Antioquia, Asociación de Ortodoncistas de la Universidad de Antioquia in Spanish (https://ortodoncistasudea.com). For more details of the study, please check the publication referenced here.[4] Subsequently, a clinical examination was provided in volunteers that participated in the survey.

Design and setting

A cross-sectional study was conducted on orthodontists that voluntarily agreed to participate in a clinical examination. A clinical record was carried out in a specialized clinic and was in charge by a medical practitioner focused on occupational medicine. The final sample was 46 (response rate: 46%), considering secondary participation in this step of the research. According to the study design, our work hypothesis is that there exist several factors that influence the presence or the absence of MSDs and there are differences between the prevalence of MSDs when the subjective perception and the clinical diagnosis are considered.

Clinical examination

The participants were called to the clinical evaluation by personal invitation, and they make the appointment according to their time of preference. At the appointment, an expert medical practitioner in occupational health, previously calibrated theoretically with one of the researchers (DMRO), proceeded to make the medical evaluation that contained: Patient identification data, history, evaluation by systems and physical evaluation focused on the musculoskeletal system. The participant was given a summary result of their evaluation (occupational certificate), while the research team was provided with the result of the complete examination.

Variables

According to the clinical records supplied, the following variables were considered: 1) sex (male/female); 2) Body mass index (BMI): defined as a person's weight in kilograms divided by the square of his/her height in meters (kg/m2), and with this information and according to the parameters of the WHO,[14] the following characteristics were determined: a) underweight: BMI ≤ 18.50; b) normal weight: BMI between 18.50 and 24.99; c) overweight obesity: BMI ≥ 25.00; 3) practice of sports; 4) Medical previous conditions.

The research team reviewed all the clinical records and according to the findings, classified the information that proceed from the diagnosis and grouped in several categories: 1) Upper body MSD; 2) Spine Area MSD; 3) Lower body MSD; 4) Cephalea/Migraine; 5) Visual disturbances; 6) Other pathologies; 7) We grouped the variables 1, 2 and 3 in a new variable named presence of MSDs.

Additional variables were collected from the database obtained from the previous study:[4] 1) Experience as an orthodontist (years); 2) Labor activity; 3) Written contract; 4) Working hours per week; 5) Monthly income (Colombian peso and US dollars); 6) Labor satisfaction; 7) Stressful job; 8) Affiliation to the System of Labor Risks in Colombia. We used other health variables such as: Self-perceived Upper body MSDs and Self-Perceived Spine Area MSDs (previously collected).

Data analyses

First, the prevalence of clinical diagnosis was calculated by sex. Secondly, bivariate analyses were conducted for the prevalence of presence of MSDs (and the 95% confidence interval 95%CI) according to the study variables, and Chi squared test were calculated to observe statistically significant differences. Finally, we measure the agreed percentage (and the 95% confidence interval 95%CI) between the MSDs (clinical examination) and the self-perceived MSDs (self-administrated questionnaire) and we also calculated Chi square tests. SPSS version 22.0 was used to conduct these analyses.

Ethics

The Ethical Committee of the Faculty of Dentistry at the University of Antioquia approved the study (Act 07/2019). All the participants gave informed consent to participate, and confidentiality was guaranteed following Colombian regulations (Resolution number 008430/1993 by the Ministry of Health and Social Protection).

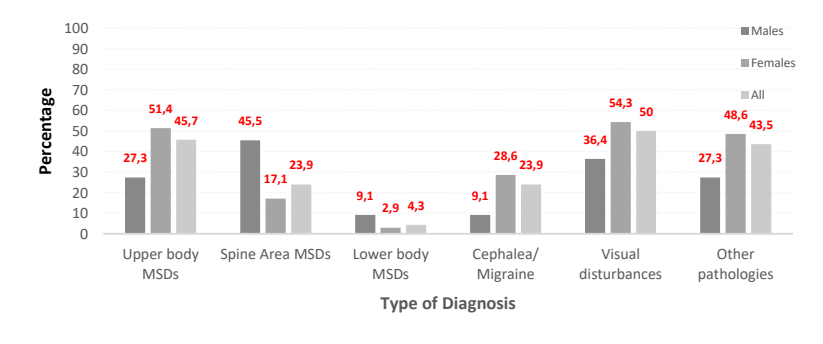

Figure 1. Prevalence of clinical pathologies in orthodontists according to sex (n= 46)

Table 1. Intragroup comparison of plaque index from baseline to 90 days.

Variables	Sample		Prevalence MSDs	
	n	%	%	95% CI
Sociodemographic				
Sex				
Men	11	23.9	63.6	35.4- 84.8
Women	35	76.1	57.1	40.9- 72.0
Age				
≤ 44	25	54.3	64.0	44.5- 79.8
≥ 45	21	45.7	52.4	32.4- 71.7
Marital status				
Single/Separate	19	41.3	73.7	51.1- 88.2
Married /Cohabitated	27	58.7	48.2	30.7- 66.1
Socioeconomic status*				
Middle	15	33.3	73.3	48.1- 89.1
High	30	66.7	53.3	36.1- 69.8
Body Mass Index (BMI)				
Normal	35	76.1	62.9	46.3- 76.8
Overweight/Obesity	11	23.9	45.5	21.3- 72.0
Practice of Sports				
Yes	29	63.0	51.7	34.4- 68.6
No	17	37.0	70.6	46.9- 86.7
Medical previous conditions				
Musculoskeletal history				
Yes	30	65.2	76.7**	59.7- 88.2
No	16	34.8	25.0**	10.2- 49.5
Other pathologies history				
Yes	37	80.4	62.2	46.1- 75.9
No	9	19.6	44.4	18.9- 73.3
Labor conditions				
Experience as an orthodontist(years)				
≤ 10	22	47.8	63.6	43.0- 80.3
≥ 11	24	52.2	54.2	35.1- 72.1
Labor activity (Yes)				
Teaching/research	13	28.3	46.2	23.2- 70.9
Clinical assistance	46	100.0	58.7	44.3- 71.7
Administrative	7	15.2	71.4	35.9- 91.8
Written contract				
Yes	34	73.9	58.8	42.2- 73.6
No	12	26.1	58.3	32.0- 80.7
Presence of several contracts				
Yes	24	52.2	50.0	31.4- 68.6
No	22	47.8	68.2	47.3- 83.6
Working hours per week				
≤ 40	34	72.3	52.9	36.7- 68.6
≥ 41	12	26.1	75.0	46.8- 91.1
Monthly income (Colombian peso)				
≤6,999,999 (U$1,843)	15	32.6	53.3	30.1- 75.2
≥7.000.000 (U$ 1,844)	31	67.4	61.3	43.8- 76.3
Labor satisfaction				
Satisfied	39	84.8	56.4	41.0- 70.7
Unsatisfied	7	15.2	71.4	35.9- 91.8
Stressful job				
Yes	35	76.1	60.0	43.6- 74.5
No	11	23.9	54.6	28.0- 78.7
Affiliation to the System of Labor Risks				
Yes	31	67.4	64.5	47.0- 78.9
No	15	32.6	46.7	24.8- 69.9
All	**46**	**100.0**	**58.7**	**44.3- 71.7**

* Missing values for socioeconomic status (n=1) **p-value <0.01

Table 2. Intragroup comparison of Gingival index from baseline to 90 days.

Variables		Clinical upper body MSDs	
		Yes	No
Self-perceived upper body MSDs	Yes	**18**	18
	No	3	**7**
Agreement percentage (positive & negative cases): 54.3 (95%CI 40.2- 67.9)			
Agreement percentage (positive cases): 50.0% (95%CI 34.7- 65.5)			
Agreement percentage (negative cases): 70.0% (95%CI 39.7- 89.2)			
p-value= 0.2613			
Variables		**Clinical spine Area MSDs**	
		Yes	**No**
Self-perceived spine Area MSDs	**Yes**	**7**	17
	No	4	**18**
Agreement percentage (positive & negative cases): 54.3 (95%CI 40.2- 67.9)			
Agreement percentage (positive cases): 29.2% (95%CI 14.9- 49.2)			
Agreement percentage (negative cases): 81.2% (95%CI 61.5- 92.7)			
p-value= 0.3829			

Results

Figure 1 shows the main diagnosis as provided for the medical records according to sex. When the MSDs are considered, a higher frequency of upper body MSDs was observed (especially in women). 45% of men suffered of MSD in the spine area. In the case of other pathologies, half of the participants were diagnosed with visual disturbances.

Table 1 indicates the prevalence of MSDs in a general way according to different variables. The general prevalence of MSDs (considering all areas) is 58.7% (95%CI 44.3%- 71.7%). The higher frequencies of MSDs were found for men, orthodontists ≤ 44 years old, those single or separated, with middle socioeconomic status. Regarding medical previous conditions, participants, with a musculoskeletal history had a higher prevalence of MSDs ($p<0.01$), the same situation was observed for a medical history in other pathologies. Orthodontists that do not practice sports had a higher prevalence of MSDs. Considering the labor conditions, higher frequencies of MSDs were observed in orthodontists with working experience ≤ 10 years, working in administrative tasks, with monthly incomes ≥7.000.000 (U$ 1,844), unsatisfied and stressed at work. In addition, participants with Affiliation to the System of Labor Risks reported higher frequencies of MSDs.

Table 2 refers to the agreed percentage between the self-perceived symptoms of MSDs according to a previous survey and the diagnosis as provided for the medical records. Considering positive cases (participants suffering MSD symptoms), 50% agreement was observed for upper body MSDs. In case of spine area MSDs, 81.2% agreement was found in negative cases (participants are not reporting MSD symptoms).

Discussion

This study aimed to identify different factors related to MSD in orthodontists and diagnose the presence of MDSs, beyond the symptoms perceived by the participants. A prevalence of MSDs of 58.7% was found. As the first relevant aspect, since a decrease in prevalence is observed compared to other studies;[4, 15] where the evaluation was made through self-completed surveys, this research showed that the diagnosis of MSDs can vary when the evaluation by a specialized clinician is considered.

Regarding sociodemographic factors such as sex, no significant differences were found between men and women. This situation agrees with a previous study by Ramírez *et al.*[4], where no positive correlation was found between sex and the presence of MSDs, and similar findings were observed in other studies.[8,16] However, in the present study, despite no significant differences, men had a higher prevalence of MSDs. This result is comparable to that found by Sankar *et al.*[17] where men had ten times higher prevalence than women, with statistically significant differences. This could be explained because men may have higher workloads than women. On the other hand, these results differ from those found by Meisha *et al.* in general dentists in Saudi Arabia[2] and by Kerouso *et al.*[15] in orthodontists from Canada, where it was observed that women experienced more symptoms of MSDs than men and a possible explanation was given by less muscle mass and tone, hormonal changes and osteoporosis. In this sense, more studies with more significant samples are necessary, which allow greater reliability in the results, given the heterogeneity found in the literature.

Relation to age, another factor that has been related to MDSs in orthodontists, some authors have found a significant positive correlation, thus, older orthodontists are more likely to develop musculoskeletal symptoms.[18] In the same way, Sankar et al. associated the greater older work experience and higher prevalence of MSDs.[17] However, other authors have not found any relationship.[5,8,15] In the present study, on the contrary, a higher prevalence was found in the youngest orthodontists (<44 years). This can be explained by the little work experience, where there is no good postural hygiene, in addition to working more frequently, due to longer periods of time and in more work sites.[4]

General health conditions have been considered a risk factor related to the presence of MSDs.[8] Maintaining good health conditions is correlated with a lower report of musculoskeletal symptoms[18] and many authors, within their recommendations, suggest the activity physics as a mechanism to prevent MSDs.[6,16] In this study, previous medical conditions were evaluated and it was found that those with a greater history of musculoskeletal and other pathologies had a higher prevalence of MDSs. This may be because these pathologies accumulated over time can increase the presence of musculoskeletal symptoms.

Two factors closely related to general health conditions were physical activity and BMI. The practice of physical activity has proven to be a protective factor for the development of MSDs since dentists who practice sports and take active breaks have less musculoskeletal pain.[5,6,8,19] This situation was found in the Colombian study, where, despite not having significant differences, the percentage of orthodontists with musculoskeletal diagnoses and who did not practice physical activity was much higher. In this regard, it is important to call for attention, since studies show that the percentage of general dentists and orthodontists who perform physical activity on a regular basis is very low.[5,17] Regarding BMI, the literature is still not conclusive, authors such as Rafie *et al.*[10], found no relationship between BMI and high frequencies of MSDs, Sakzewski *et al.*[8] reported that there was minimal evidence that this factor was related to MDSs and Ramírez *et al.*[4] found a positive correlation between an increased BMI and high frequencies of MSDs. The present study did not find a positive correlation. However, those participants with a normal BMI had a higher prevalence of MSD. In this regard, more studies are needed to clarify how this factor is related to musculoskeletal symptoms.

Marital and socioeconomic status were also evaluated, it was found that those single or separated orthodontists had a higher prevalence of MSDs, possibly associated with a higher workload due to a lower possibility of distribution of expenses and household tasks. On the other hand, in relation to the socioeconomic aspect, those with a medium status had a higher prevalence, it can be related to a greater workload and economic commitments. No studies were found evaluating these factors, therefore, no comparisons were possible.

Regarding working conditions, it was found that orthodontists who have over less than ten years of experience reported a higher prevalence of MSDs. This agrees with a review carried out by Leggat *et al.*[20] who indicates that musculoskeletal symptoms decrease with age and duration of practice, as did an investigation carried out in dentists in Australia[21] which also revealed that younger and less experienced dentists reported greater discomfort at the musculoskeletal system. This is probably due to the fact that younger orthodontists they work in more places and see more patients per day. However, it is also likely that more experienced orthodontists have already adapted to work positions that avoid the presence of MSDs.[4] One study carried out in Germany[22] found that the years of work showed to increase the percentage of MSDs. This situation could be related with a greater workload because patients seek to be treated by a more experienced professional.

The number of working hours per week and the workload are highly predictive factors of the appearance of disorders of the musculoskeletal system in orthodontists,[18] and in this study a high correlation was found between the presence of MSDs and a higher monthly salary (or equal to U$ 1,844) and more than 40 working hours per week. In studies conducted in general dentists, Pope-Ford and Pope-Ozimba also indicated an increased risk of MSDs related to long hours of work.[23] Al-Gunaid *et al.*[24] found positive correlations between the working hours, the number of patients per day and the presence of MSDs in different parts of the body. Kumar *et al.*[5] refers that with more than 5 patients the risk is increased.

Isolation due to the SARS-CoV-2 pandemic forced people to change their habits and orthodontists were not the exception. To a great extent, the use of computers to perform administrative tasks increased. In this study a relationship was found between the presence of MSDs and deskwork. However, Kumar *et al.*[5] reported that even though people used the computers for 2 or 4 hours a day there was no significant increase in the development of MSDs.

Psychosocial factors within the work environment have been linked to the appearance of MSDs. Sakzewski *et al.*[8] have divided these factors into three categories, those associated with the internal work environment, those associated with the external environment, and the individual characteristics of the employee; and their interaction can generate a stress process that can also affect individual health and job performance. Considering the above, the results of this study coincide with this hypothesis since the participants who classified their work as stressful have a higher prevalence of MSDs. This situation was found in a German study where the stress level was a very important factor related with the presence of MSDs.[22]

In this study, other variables that showed a positive correlation with the presence of MSDs were considered, such as the absence of several contracts and dissatisfaction with the job. However, due to the little literature and the type of study that was carried out, it was not possible to make accurate comparisons. It seems important to reflect on aspects related to the quality of life, and its relationship with health indicators, employment and work conditions, work-life balance and job satisfaction, as has been expressed in studies on the subject.[25-27]

To the best of our knowledge, this is the first study carried out in Latin America that identifies the health factors related to MSDs in orthodontists by using clinical examinations and complements the information obtained from a previous study.[4] Caution should be considered with the findings since these data were collected during the SARS-CoV-2 pandemic, which limited the response of the study population for fear of contagion at the site where the medical examinations were performed. It is suggested to carry out a job evaluation to verify the influence of its design on the presence and development of MSDs.

Conclusions

The orthodontists participating in the study manifested various MSDs and other pathologies that were related to their professional practice and that were diagnosed through a clinical examination performed by an occupational health professional. Some sociodemographic and clinical factors were related to the presence of MSDs, and some discrepancies were found between what orthodontists perceive as symptoms of MSDs and the actual diagnosis of MSDs. Studies of greater scope and depth are required that lead to epidemiological surveillance systems in occupational health and strategies in health and safety at work. The entities whose purpose is to promote the health of the working population are fundamental in these strategies.

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Conflicts of Interest Statement

The authors have no actual or potential conflicts of interest.

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Ethical Approval

11-2019, Ethics Committee, Faculty of Dentistry, University of Antioquia, Medellin, Colombia.

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Effect of Montmorillonite Incorporation on Shear Bond Strength and Surface Hardness of Acrylic Base Soft Lining Material (An in Vitro Study)

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Research Article

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ABSTRACT

Objective: One problem with the soft lining material is that it detaches from the acrylic base of dentures after a particular time period. So, the aim of this work was to evaluate how adding montmorillonite nano clay would affect the soft lining material's shear bond strength and surface hardness.

Materials and Methods: The heat-cured acrylic denture material for the control group, polymer reinforced with (0.25%, 0.5% by wt.) of montmorillonite nanoparticles(MMNTPs) for experimental groups was used in this study. For the shear bond strength test 60 blocks of acrylic-based material were constructed, each pair connected with soft lining material, the other 30 specimens for the surface hardness tests were carried out to be tested with a shore A durometer. The data were statistically examined using the One-Way ANOVA test and Bonferroni multiple comparisons test.

Results: 0.5% by weight MMTNPS should significantly increase in both shear bonding strength and surface hardness of soft lining material.

Conclusions: MMTNPs were remarkably successful in improving the strength of the shear connection between acrylic and soft liners.

Key words: Montmorillonite, Nanoparticles, Soft Lining Material, Shear Bond Strength, Surface Hardness.

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Introduction

Soft liners are extremely useful in dental procedures due to their rheological properties, the function of which is to absorb impact stress, lessen and spread out the stress on supporting tissues from dentures, and improve the intaglio denture's appearance. For patients being treated for the atrophic ridge, bruxism, xerostomia, or bone undercuts, these liners are clinically useful.[1]

One of the most common requirements for denture liners is that they must bind well enough to polymethylmethacrylate to prevent early breakage during use. However, using soft lining materials has a number of drawbacks, the main one being the lack of a strong attachment to the denture foundation.[2,3]

Denture base and liner materials debonding provide a platform for microbial growth resulting in the development of plaque and calculi. So, soft liners often need to be checked and replaced on a regular basis.[4] These issues have since been resolved with a number of improvements and techniques that enhance bonding of soft liners. These techniques consist of adding nanoparticles like glass fiber and zirconia silver nanoparticles with alumina. One of the nanomaterials used in polymers is MMTNPs, which has great biocompatibility and aesthetics.[5,6]

The 2:1 phyllosilicate mineral montmorillonite (MMT) is the most popular layered silicate mineral nano clay utilized in nanocomposite manufacturing. MMTNPs is an off-white powder that shouldn't have a negative impact on the denture base's aesthetics. According to reports, the material is biocompatible and has good physical and mechanical qualities.[6,7]

The chemistry of the nano clay, its dispersal, polymer reactivity, and process conditions all influence the efficiency of nano clay in improving the properties of nanocomposite materials. Clay exfoliation at less than 5% by weight improves nanocomposite properties significantly.[8]

Most nanoparticles have distinct features that make t hem useful in a wide range of biomedical applications, le ading to the production of amazingly effective diagnostic and therapeutic tools.

Furthermore, nanoparticles might be used in pharma ceutical delivery systems to lessen the negative effects th at are usually associated with standard drug use.[9]

Because of its multiple applications, nanotechnology has had an influence on all aspects of life in recent years and is interconnected with many other scientific areas.[10]

In this study, (MMTNPs) had been incorporated to acrylic soft lining material in amounts of 0.25%wt and 0.5%wt, along with control specimens, to enhance the strength of shear bonds.

The null hypothesis H_0 was that the incorporation of montmorillonite has no effect on soft lining material.

Table 1. The results of shear bonding strength test in pilot study.

Sample	Control	0.25%	0.5 %	0.75%	1%	1.5%
1	12	17	17	18	22	22
2	14	15	16	21	19	24
3	12	14	18	19	21	20
Mean	12.6	15.3	17	19.3	20.6	22

Table 2: The results of surface hardness test in pilot study.

Sample	Control	0.25%	0.5%	0.75%	1%	1.5%
1	66.2	68.2	70	71.4	78.3	76.8
2	67.4	68	68.8	72	77	77.5
3	65.4	67.4	69.2	71.8	76.3	78.5
Mean	66.3	67.8	69.3	71.7	77.2	77.6

Material and Methods

Ethical approval

The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional regional), the study protocol was reviewed and approved by an ethical committee of the College of Dentistry, University of Baghdad according to the document number (741 in 1-12-2022).

Pilot study: A pilot study to assess the effect of various MMTNPs percentages on the soft lining material's shear bond strength and surface hardness was conducted. MMTNPs were used in three samples for each percentage of 0%, 0.25%, 0.5%, 0.75%, 1%, and 1.5% by weight. According to the result, the MMTNPs percentages of 0.25 and 0.5 wt.% were chosen because they had a considerable improvement in shear bond strength as presented in table 1 and 2.

The research evaluated the shear bonding strength and surface hardness of a heat-curable acrylic-based soft liner (Moon Star, Turkey) before and after the incorporation of MMTNPs (SIGMA-ALDRICH, USA), (Figure 3) showed some of material used in the study.

Shear bond strength test

I. Specimens preparation: Sixty specific acrylic blocks with dimentions which is not ISO but its suitable for testing device (length, width, and thickness: 75mm, 25mm, and 5mm respectively) with a stopper of approximately 3 mm were created to assess the shear bond strength test.[11]

The above-given dimensions were used to build plastic blocks (made in Iraq), (Figure 4/A) that would later be replicated in acrylic. To facilitate duplicating, laboratory silicone putty (Labosil, Spain) was employed (Figure 4/C). As instructed by the producers, heat-cured acrylic (SpofaDental, Czech) was combined at (2.2 gm to 1ml). To ensure fair distribution of the material, the silicone molds with acrylic was packed and cured.[12] After cooling the acrylic samples were removed and refined as presented in (Figure 4/D, E). An acrylic sample is layered on top of another, with space between for soft lining material to be applied (Figure 4/F, G).[13]

II. Soft liner application: The soft liner (Moon Star, Turkey) was used and as directed by the producer, 10g of powder and 7.8 ml of monomer were blended in a dry, clean glass beaker. The beaker was then tightly sealed it took around fifteen minutes to get to the dough stage., the material had been gradually added into the spaces between each pair of blocks. Once the spaces were overloaded, it was packed and cured. After cooling the specimens were retrieved as presented in (Figure 4/H, I) and placed in a container with distilled water for 48h.[13]

III. Incorporation of MMTNPs: For test specimens, MMTNPs powder at two distinct concentrations (0.25% and 0.5% by weight) was included in the liner monomer. By using a probe sonication instrument (Soniprep-150, England) (120W, 60 kHz) for three minutes, the nanoparticles are broken up into individual nanoparticles and spread out evenly throughout the monomer. to lessen the likelihood of aggregation of MMTNPs it was immediately mixed with soft liner powder.[14]

A universal testing equipment (WDW-20, Laree Technology Co., Ltd., China) 0.50 mm/min cross-head speed with a maximum 100 kg load capacity for the load cell was used for testing. The maximal force of failure is represented by readings taken from the machine. strength was estimated by dividing the maximal force at which a bond would break by the area of its cross-section of each sample. Bonding strength (N/mm2) = maximal load/cross-sectional area = F/A.[15]

The specimens of soft liner for surface hardness tests were fabricated with parameters of (65mm,10 mm, and 2.5mm) in length, width, and thickness respectively.[16] All the samples were submerged in distilled water and stored for 48 hours at 37°C Before evaluation to remove excess monomer. A shore A durometer (Time groupTH200, China) hardness test was performed on the lining materials. Each sample has undergone five separate analyses. After each penetration, there was a 5-second contact period, and the mean of these times was used for the result of the test, as presented in (Figure 5).[17]

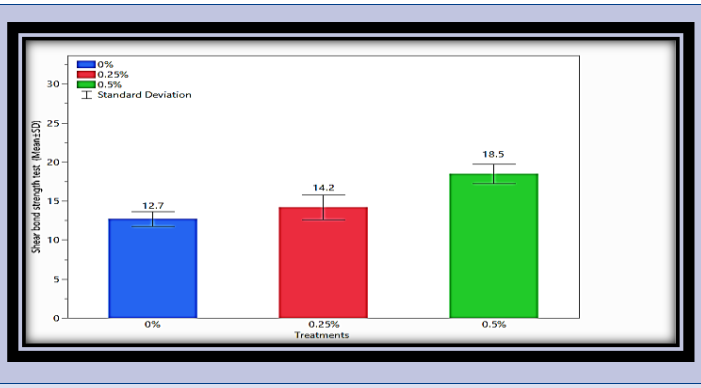


Figure 1. Bar chart representation the mean values and standard deviation of shear bond strength test (MPa) result among the studied groups. MPa: Megapascal

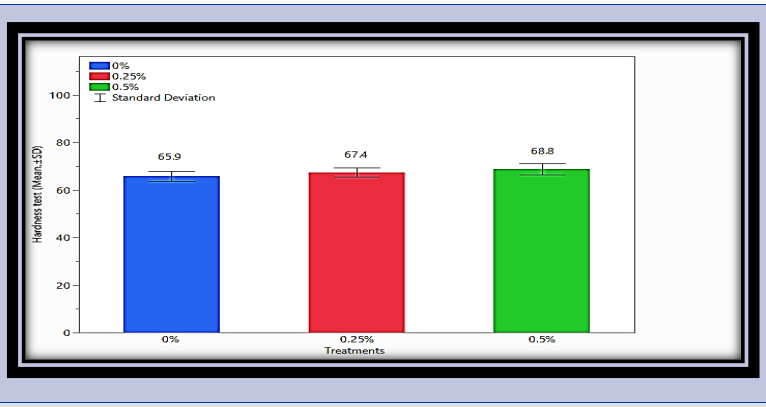
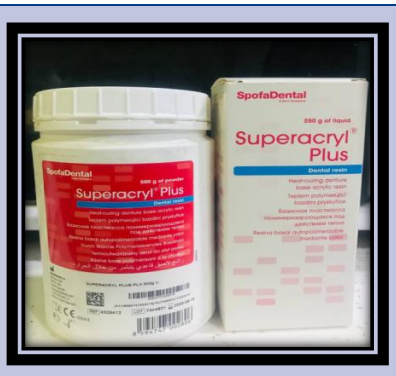


Figure 2. Bar chart representation the mean values of shore A hardness test result

A



B

C

Figure 3. A, Heat cure acrylic based material. B, Heat cure acrylic based soft liner. C, : Montmorillonite K10 nanaparticles.

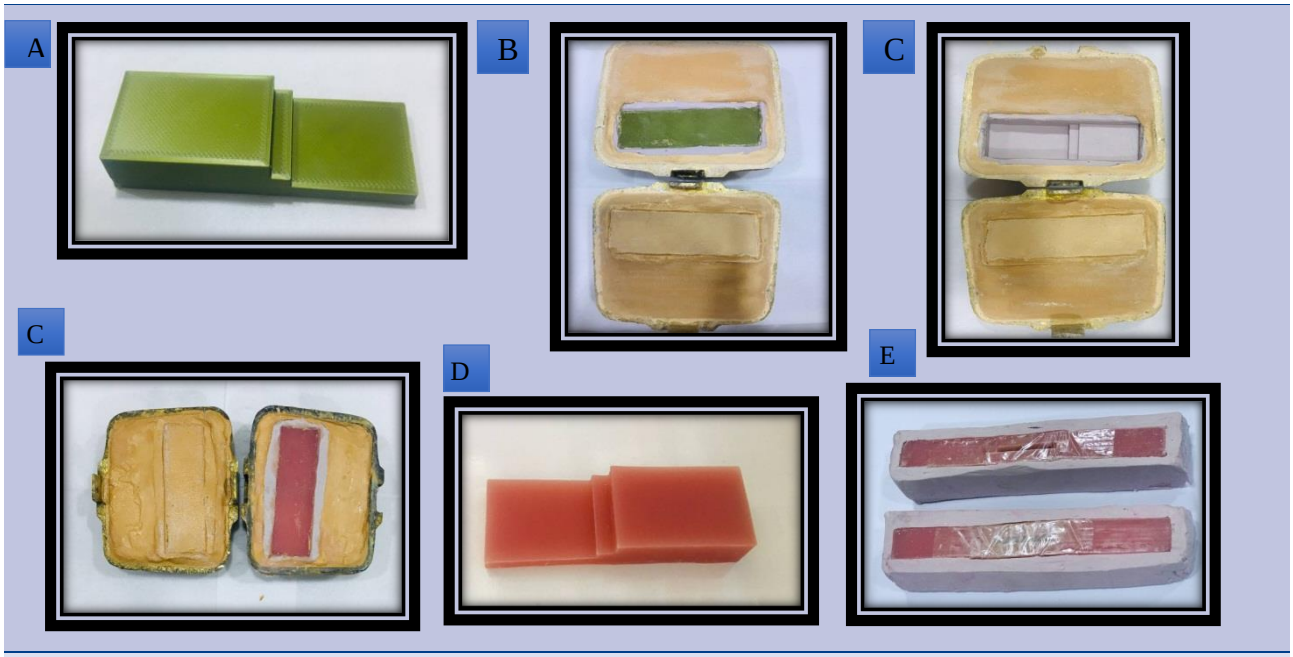


Figure 4: Shear bond strength specimens preparation. A, plastic mold. B, plastic mold placed in silicone and in stone. C, block removed. D, flask after curing. E, acrylic specimen after finishing. F, acrylic blocks invested in silicone. G, specimens invested in stone. H, specimens after curing. I, final specimens.

Figure 5: Surface hardness specimens ready for testing.

Table 3. One-way ANOVA test result of shear bond strength.

Analysis of Variance						
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F	Significant
Between Groups	2	181.26	90.63			
Within Groups	27	44.20	1.63	55.36	<.0001*	HS
C. Total	29	225.46				

DF: Degree of freedom, HS; Highly significant

Table 4. One-way ANOVA Table for hardness test results.

Analysis of Variance						
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F	Significant
Between Groups	2	42.71267	21.3563			
Within Groups	27	130.72100	4.8415	4.4111	0.0220*	S
C. Total	29	173.43367				

S:Significant

Results

Shear bond strength

The maximum value was obtained in a group containing (0.5%) wt. MMTNPs with (18.5) MPa, followed by a group containing (0.25%)wt. MMT with (14.2) MPa, while the lowest value of (12.7) MPa was obtained in the control group (Figure 1).

Statistical analysis using one way ANOVA test for shear bond strength showed a statistically highly significant difference among all studied groups (P. <0.01) as shown in (Table 3).

In dental treatment, soft lining materials are frequently adapted to reshape surfaces of dentures that are with an interface with the oral mucosa. They offer shock absorption that helps to distribute and reduce the functional forces. However, the primary drawback of soft liner materials is their tendency to separate from the denture base over time.[18]

This research was done to assess the soft liner's shear bond strength following the addition of MMTNPs in various concentrations and how that affected surface hardness.

The findings of this investigation revealed a significant rise in the average shear bond strength in 0.5% MMTNPS addition into soft liner compared to the two other groups. It is anticipated that the hydrogen bonds and the van der Waals interaction that form between the nanofiller and polymer will enhance the rigidity and shear strength of the material, in addition, the MMTNPs function as fillers by occluding voids between soft liner particles and improving the surface area for denture base material adherence, more importantly; the size of the sample used (25 mm long by 25 mm wide) is considered to be large, this might be used in the improvement of bonding.[19]

Hardness is among the most crucial characteristics of materials for soft denture liner since it reduces the effect of absorption.[20]

In this investigation, the distribution of MMTNPs throughout the matrix may be responsible for the rise in hardness. The particles cluster inside the voids of the soft lining matrix also as interparticle distance decreases and the bonding strength between particles increases, which in turn causes an increase in hardness.[21]

Hardness is directly related with nanoparticle concentration, as the concentration of MMTNPs increased the hardness increase and vice versa. with a low concentration (0.25% MMTNPs) resulting in a minimal effect related to a low number of networks, even so, there is no boundary to the spectrum of Shore a hardness values that can be used for therapeutic purposes.[22]

The study is limited by in vitro environment so we cannot generalized the result to the clinical situations.

Conclusions

Within the limit of this study, the following findings were obtained: after addition of 0.25% and 0.5% of

Surface hardness test

Maximum value (68.8) was obtained in 0.5% weight followed by the group containing 0.25% weight with (67.4), while lowest value of (65.9) was obtained in control group (Figure 2).

One-way ANOVA table for hardness test results showed significant difference between all tested groups (Table 4).

Discussion

MMTNPs into acrylic based heat cured soft denture lining material:

1-Incorporation of 0.5% MMTNPs into heat-cured acrylic-based soft denture liner material. showed a significant improvement in shear bonding strength in both concentration (0.25% and 0.5%) which a highly important improvement.

2-There was a significant increase in surface hardness of soft lining material in 0.5% MMTNPs but with 0.25% there was an acceptable value of hardness.

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Non.

Conflicts of Interest Statement

Non.

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Effects of T-PRF and A-PRF on the Osteogenic Biomarkers in Intrabony Defects of Periodontitis Patients

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Research Article

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ABSTRACT

Objectives: Different derivatives of platelet-rich fibrin (PRF) have been developed but the efficacy of these derivatives in tissue healing and regeneration is still unclear. The aim of this study was to evaluate the effects of titanium-prepared PRF (T-PRF) and advanced PRF (A-PRF) on osteogenic biomarkers in gingival crevicular fluid (GCF) and clinical parameters.

Materials and Methods*:* Seventeen systemically healthy participants with 30 bilateral intrabony defects were recruited. Following phase I periodontal therapy, intrabony defects were treated either with A-PRF+open flap debridement (OFD) or T-PRF+OFD. Plaque index (PI), gingival index (GI), pocket depth (PD), clinical attachment loss (CAL) was recorded at the baseline and 6th month after treatment. GCF samples were collected at the baseline and 3rd, 6th months after surgery. Nuclear factor receptor activator (RANK), receptor activator nuclear kappa-B ligand (RANKL), osteoprotegerin (OPG) and tumor necrosis factor alpha converting enzyme (TACE) in GCF samples were analyzed by human enzyme-linked immunosorbent assay (ELISA).

Results: In both groups, statistically significant changes were observed in clinical parameters, however, there was no difference between the groups. In terms of osteogenic biomarkers in GCF, there were no statistically significant differences between and within the groups.

Conclusions*:* Different derivatives of PRF can be used to enhance the clinical outcomes of intrabony defects in periodontitis.

Key words*:* Intrabony defects, A-PRF, T-PRF, periodontal surgery, osteogenic biomarkers.

Süreç

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ÖZ

Amaç: Trombositten zengin fibrinin (TZF) farklı türevleri geliştirilmiştir, ancak bu türevlerin doku iyileşmesi ve yenilenmesindeki etkinliği hala belirsizdir. Bu çalışmanın amacı, titanyum ile hazırlanmış TZF (T-TZF) ve geliştirilmiş TZF'nin (G-TZF) dişeti oluğu sıvısındaki (DOS) osteojenik biyobelirteçler ve klinik parametreler üzerindeki etkilerini değerlendirmektir.

Gereç ve Yöntemler: 30 çift taraflı kemik içi defekti olan 17 sistemik olarak sağlıklı katılımcı çalışmaya alındı. Faz I periodontal tedavinin ardından, kemik içi defektler ya G-TZF+açık flep debridmanı (AFD) ya da T-TZF+AFD ile tedavi edildi. Plak indeksi (PI), gingival indeks (GI), cep derinliği (CD), klinik ataçman kaybı (KAK) başlangıçta ve tedaviden 6 ay sonra kaydedildi. DOS örnekleri başlangıçta ve ameliyattan sonraki 3., 6. aylarda toplandı. DOS numunelerindeki nükleer faktör reseptör aktivatörü (RANK), reseptör aktivatörü nükleer kappa-B ligandı (RANKL), osteoprotegerin (OPG) ve tümör nekroz faktörü alfa dönüştürücü enzim (TACE), insan enzim bağlantılı immünosorbent testi (ELISA) ile analiz edildi.

Bulgular: Her iki grupta da klinik parametrelerde istatistiksel olarak anlamlı değişiklikler gözlendi ancak gruplar arasında fark yoktu. DOS'taki osteojenik biyobelirteçler açısından, gruplar arasında ve gruplar içinde istatistiksel olarak anlamlı bir fark yoktu.

Sonuçlar: Periodontitiste kemik içi defektlerin klinik sonuçlarını iyileştirmek için TZF'nin farklı türevleri kullanılabilir.

Anahtar Kelimeler: Kemik içi defektler, G-TZF, T-TZF, periodontal cerrahi, osteojenik biyobelirteçler.

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Introduction

Periodontitis is a progressive chronic inflammation characterized by irreversible loss of connective tissue attachment and alveolar bone. The aim of periodontal therapy is to control active inflammation, halt the progression of the disease and, where appropriate, reconstruction of structures that are lost as a consequence of inflammatory response.[1]

Non-surgical or surgical therapy can be employed to treat periodontitis. Within the context of surgical therapy, open flap debridement (OFD) has been defined as a standard approach in the treatment of residual pockets. A systematic review mentioned that treatment of deep persisting periodontal pockets with OFD resulted remarkable improvement both in PD and CAL compared to non-surgical therapy.[2] As a major challenging condition in clinical approaches, intrabony defects (IBDs) cannot be thoroughly treated by only OFD. In these cases, application of biological agents and bone substitutes produced marked enhancements in PD and CAL compared to OFD alone.[3]

Development of biologically active compounds has been the focus of clinical research for a long while. Surgical adjuvants has been commenced with fibrin glue applications to wound edges[4] which is followed by development of blood derived platelet concentrates such as platelet rich plasma (PRP)[5] and platelet-rich fibrin (PRF).[6] In particular, second generation platelet concentrate, PRF has a strong fibrin matrix enriched with growth factors (GFs) such as Fibroblast Growth Factor (FGF), Vascular Endothelial Growth Factor (VEGF), Platelet-derived Growth Factor (PDGF) and Transforming Growth Factor (TGF).[7] In vivo and in vitro studies have shown the vital role of these GFs on periodontal ligament cells during tissue regeneration.[8,9] These growth factors, when applied exogenously, change the response of periodontal hard and soft tissues during healing phase.[10]

Today, PRF has been used in a broad range of medical applications including plastic,[11] maxillofacial surgeries[12] and sports medicine.[13] Furthermore, it has been employed in dental practice embracing the treatment of gingival recession,[14] intraosseous defects,[15] furcation areas.[16] Various studies have shown the effect of PRF on periodontal healing and regeneration following surgical applications. For instance, Chang *et al*.[17] reported exceptional results in clinical parameters in addition to radiographic filling of the intraosseous defects 6 months following the therapy.

The progress in platelet concentrates was not limited to PRF. Monocytes involve in vascularization, bone growth and synthesis of VEGF. Choukron *et al*.[18] integrated this cells into PRF and obtain advanced platelet-rich fibrin (A-PRF) which accelerate soft tissue healing, possess more BMPs and cytokines than PRF. The other advancement arouse in response to a health hazard concern about unavoidable contact with silica in glass-tubes during the preparation of PRF. Tunali *et al*.[19] introduced titanium-prepared platelet-rich fibrin (T-PRF) which has higher platelet activation and wider fibrin network than PRF.

Osteoclastogenesis is regulated by osteoprotegerin (OPG), nuclear factor kappa B ligand (RANKL), and nuclear factor receptor activator (RANK). These three proteins play key roles in bone metabolism and osteoclast biology. RANKL binds to osteoclast precursors and RANK receptors in dendritic cells, causing bone resorption by affecting the differentiation, proliferation and activation of osteoclasts. OPG competes with RANKL for RANK.[20] In periodontal disease, RANKL and OPG regulate tissue destruction. Higher levels of RANKL and lower levels of OPG have been detected in GCF of periodontitis patients.[21] Chang *et al*.[17] reported elevated protein kinase phosphorylation, osteoprotegerin and alkaline phosphatase activity in periodontal ligament fibroblasts upon treatment with PRF under in vitro conditions.

Tumor necrosis factor alpha converting enzyme (TACE) is a type I transmembrane protein belongs to extracellular zinc-linked protease family and released from T lymphocytes and monocytes. RANKL is a substrate of TACE activity and plays a key role in stimulating bone resorption. The release of TACE increases simultaneously with RANKL release.[22] RANKL levels are higher in GCF samples of individuals with periodontitis, and TACE provides the release of RANKL from the cell membrane more effectively than other enzymes.[23] Therefore, TACE enzyme activity is the target of treatment approaches to prevent bone resorption.

We hypothesized that delayed resorption period and larger fibrin network features of T-PRF provide a long term support during the healing phase and stimulate periodontal enhancement more effectively than A-PRF. Therefore, the aim of this study was to evaluate the effect of T-PRF and A-PRF on clinical parameters and osteogenic biomarkers in bilateral intrabony defects in chronic periodontitis patients.

Material and Methods

Patient Population

In this randomized, split-mouth design, double-blinded, controlled clinical trial, seventeen systemically healthy, non-smoker individual (7 females and 10 males; age range 30-60 years; mean ± SD: 42.7 ± 8.91 years) who had two interproximal intrabony defects were included. The study was completed in the Department of Periodontology, Faculty of Dentistry at Kirikkale University from 2016 to 2018. The study design was approved by the ethics committee of Kirikkale University (Number: 13/03-Date: May 25, 2015) and guided in accordance with the Declaration of Helsinki and written informed consent from all participants was obtained.

Following non-surgical periodontal treatment, two- or three-wall intrabony defects ≥ 3 mm deep along with an interproximal probing depth ≥ 5 mm were included into the study. The exclusion criteria were determined as presence of systemic conditions and any medication, pregnancy and lactation and plaque index > 1.

Presurgical therapy

Phase I periodontal therapy was completed at first visit. 6 weeks after this initial periodontal treatment [scaling and root planning with curets (Hu-Friedy, Chicago, Illinois, USA) and polishing], a re-evaluation visit was performed to confirm the eligibility of sites to periodontal surgery. In the re-evaluation, individuals who were decided to have an OFD procedure were given an appointment 1 week later. Immediately before the periodontal surgery, clinical parameters (including PI, GI, PD and CAL) were recorded and GCF samples were collected (Figure 1).

Collection and preparation of GCF Samples

After isolation of sample sites, GCF samples were collected with the standardized strips (Periopaper; Ora Flow Inc., Amityville, New York, USA) at baseline and the 3rd and the 6th months after surgery (Figure 1) and GCF volume was measured on a precalibrated device (Periotron 8000; Oraflow Inc., Plainview, New York, USA). All samples were stored at -80 C until analysis. Total amounts of RANKL, RANK, OPG and TACE were measured by ELISA using commercial kits according to the manufacturer's instructions.

PRF preparation

Two tubes of 10 ml venous blood samples were collected from each participant to make T-PRF and A-PRF. To obtain T-PRF, blood sample was transferred to the titanium tube. After centrifugation (2700 rpm, 12 minutes for T-PRF; 1300 rpm, 8 minutes for A-PRF) at room temperature, PRF clots were removed from the tubes with sterile tweezers and were placed on sterile woven gauze and kept humidified until application to the intrabony defect.

Surgical procedures

All surgical procedures were performed by the same operator (S.S.S.). Before surgery, 0.12% chlorhexidine solution and an iodine solution were used for intraoral and extraoral antisepsis. Following local anesthesia, buccal and lingual/palatinal sulcular incisions were made and a full-thickness flap was reflected. The defects were debrided and root planed with area-specific curettes. The selected sites were randomly (by coin-toss method) assigned to the T-PRF and A-PRF group. Each surgery site were treated with only assigned PRF. 4-0 non-absorbable silk suture (Ruschmed, 4-0 Silk Black, Istanbul, Turkey) was used for the closure of the flaps.

Postoperative care

After surgery, an analgesic (Sanovel, Istanbul, Turkey) (100 mg flurbiprofen, two times per day, for 5 days) and chlorhexidine digluconate rinses (Drogsan, Istanbul, Turkey) (0.12%, twice daily for 10 days) were prescribed. The sutures were removed 10th day postoperatively. Gentle brushing with a soft toothbrush for 2 weeks and appropriate interdental brush devices after 4 week were recommended. If necessary, professional plaque control and reinforcement of oral hygiene were reinformed.

Statistical analysis

To achieve 90% power and detect differences among groups, 24 defects were essential for each group. The Shapiro-Wilk test was used for the normality of the data distribution. Non-normally distributed data were expressed as median (interquartile range).

The differences between groups and to determine the groups leading to differences were examined by Friedman nonparametric repeated measurements analysis of variance test and Bonferroni correction, respectively. The SPSS program (SPSS Inc., Chicago, Illinois, USA) was used for statistical analyses and $p<0.05$ was accepted for statistical significance level.

Results

Demographics

Ten male and seven female, totally 17 individual with chronic periodontitis met the inclusion criteria of the study. 30 bilateral intraosseous defects were treated according to study protocol and out of this number, on 22 defects all clinical measurements completed at all study period. Participants age and gender distribution were shown in Table 1. Postoperative wound healing was uneventfully in all participants. No side effects were observed in patients related to the use of anti-inflammatory medication prescribed following surgical procedure. Only antimicrobial mouthwash discoloration was recorded on teeth and the tongue of the patients.

Clinical Outcomes

Periodontal clinical parameters including PI, GI, PD and CAL were recorded at baseline and 6th month following periodontal surgery. PI was not statistically different between and within the groups at baseline and 6th month (Table 2). In both A-PRF and T-PRF groups, GI was markedly reduced at 6th month compared to baseline. However, there was no significant difference between two groups in terms of GI. In line with GI records, we observed significant improvement in PD and CAL. In both treatment groups, PD and CAL were significantly decreased at 6th month compared to baseline measurement. There was no significant change between the PRF groups.

Osteogenic Biomarkers in GCF

Figure 2 (A-E) showed the change of osteogenic biomarkers in GCF samples collected at baseline, 3rd and 6th months following open flap surgery. At baseline, in both A-PRF and T-PRF groups, RANK, OPG and TACE levels were not statistically different. Similarly, following OFD with PRF application at 3rd and 6th months, RANK, OPG and TACE expressions were not statistically different between two treatment groups. When we compared the osteogenic markers within the group during the time frame of the study we did not observe any remarkable change.

In both treatment groups, at baseline total RANKL levels were not statistically different but at the 6th month following surgery in T-PRF group total RANKL level decreased while it increased in A-PRF group. At the 6th month following surgery, total RANKL expression was markedly different between two treatment groups. But we didn't observe any remarkable alteration in terms of RANKL expression at baseline, 3rd and 6th months following surgery within both T-PRF and A-PRF group.

RANKL and OPG has a role in bone turn over and were reported in periodontal destruction. Increased RANKL levels decreased OPG were detected in GCF samples of chronic periodontitis patients. In this study, we evaluated the RANKL/OPG ratio and we observed statistically different RANKL/OPG ratio between A-PRF and T-PRF group at all time points. This ratio was significantly less in T-PRF group than A-PRF group at baseline, 3rd and 6th months following surgical therapy.

Table 1: Descriptive statistics of study population

Age (years); Mean±SD	42.7±8.91
Gender, N (%)	7 female (41.2); 10 male (59)

SD, Standart deviation

Table 2: Comparison of clinical parameters of treatment groups at baseline and 6th month following periodontal surgery

	GROUPS		
	A-PRF	**T-PRF**	**p**
PI			
Mean (SD)			
Baseline	0.413 ± 0.54	0.478 ± 0.51	0.328
6th month	0.478 ± 0.57	0.456 ± 0.52	0.803
p	0.705	0.883	
GI			
Median (IQR)			
Baseline	1 (0)	1 (0)	1.000
6th month	0.5 (1)	0 (1)	0.266
p	0.005*	0.001*	
PD			
Mean (SD)			
Baseline	6.13 ± 1.28	5.54 ± 1.11	0.091
6th month	4.06 ± 1.31	3.86 ± 0.66	0.506
p	0.000*	0.000*	
CAL			
Mean (SD)			
Baseline	6.47 ± 1.70	5.86 ± 1.15	0.132
6th month	4.82 ± 2.00	4.47 ± 1.01	0.281
p	0.000*	0.000*	

*PI: Plaque index, GI: Gingival index, PD: Probing depth, CAL: Clinical attachment loss, IQR, interquartile range; SD, standard deviation. * Significant difference within groups*

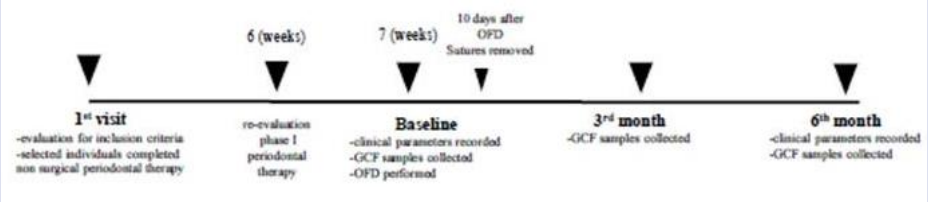

Figure 1: Study flowchart showing the each visit.

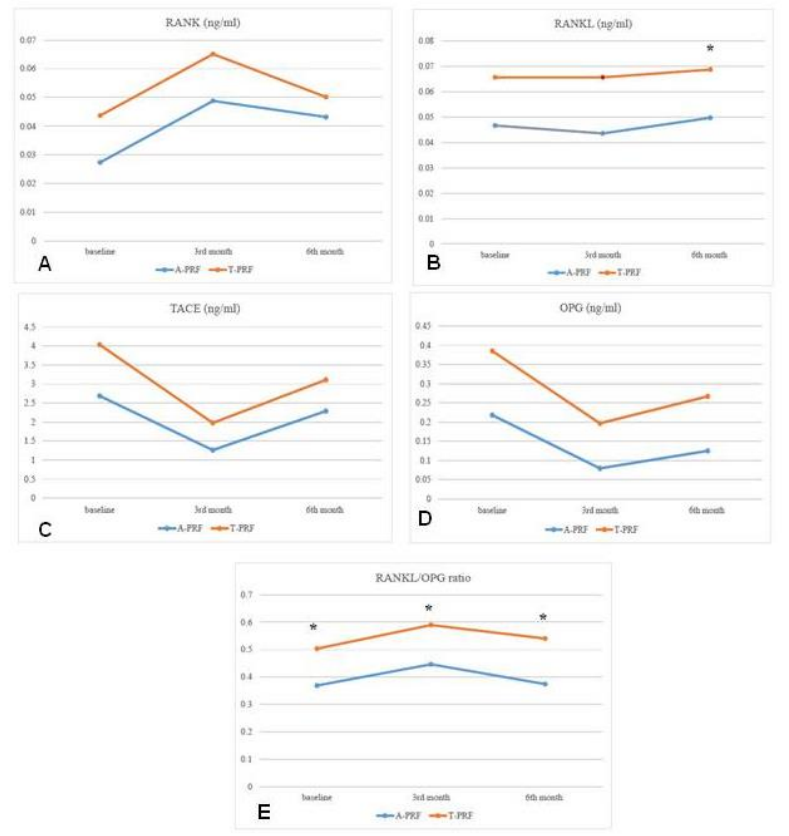

Figure 2: Comparison of osteogenic biomarkers in GCF of treatment groups at baseline, 3rd and 6th months following periodontal surgery.
*GCF concentrations of A)RANK, B)RANKL, C)TACE, D)OPG, and E)RANKL/OPG ratio in the time course of study in T-PRF and A-PRF groups. *indicates significant differences between the groups; p<0.05.*

Discussion

The aim of the periodontal therapy is to control the inflammation which leads to destruction of periodontal tissues and to regenerate lost periodontal structures. Successful periodontal regeneration is based on the formation of new cementum, new periodontal ligament and alveolar bone with the regeneration of the junctional epithelium.[24]

PRF is the second generation platelet concentrate widely used to accelerate soft and hard tissue healing.[25] Previous findings showed that PRF improves early wound closure, maturation of bone grafts, peri-implant and periodontal soft tissue aesthetic outcomes. Its ease of preparation, application and affordable rates can be counted as an advantages of this autogenous material.[14]

The fibrin network in the PRF structure provides the migration of endothelial cells during angiogenesis and the wound healing process is accelerated by the release of growth factors such as PDGF, TGF-β, IGF-1. Besides its positive effects on soft tissue healing, PRF has a function as a supportive matrix for bone morphogenetic proteins. These properties supports PRF use in periodontal and maxillofacial surgery.[12] Application of PRF in addition to flap debridement in the periodontal intra-osseous defects without graft material was evaluated in a systematic review and they concluded that the level of clinical attachment increased and the depth of the intra-osseous defect decreased.[26] This finding suggests that intraosseous defects can be treated by PRF without an exogenous graft material.

Besides aforementioned benefits, PRF has a disadvantage due to silica particles in glass tubes. This health hazard concern is eliminated by Tunali *et al*.[19] by developing T-PRF in which the activation of the clot is performed in titanium tubes. Moreover, authors reported higher and a wider fibrin network in T-PRF. In their rabbit model, T-PRF stimulated the formation of new bone and connective tissue in 30 days following application. Histomorphometric analysis in that study showed thicker fibrin network in T-PRF. Therefore, in relation to thick fibrin structure, growth factors may be released longer duration in T-PRF compared to PRF. Chatterjee *et al*.[26] compared the use of PRF and T-PRF without graft material in intra-osseous defects during open flap debridement and obtained significant results in clinical and radiographic parameters in both groups compared to open flap surgery alone. Any significant difference was observed between PRF and T-PRF in terms of clinical parameters and bone filling percentages. These findings obtained at the 9th month after surgical therapy and showed that bone filling can be achieved by using different platelet concentrates in intra-osseous defects

without any graft material.[27] In addition to T-PRF, A-PRF developed by Choukran *et al*.[18] and has a loose structure between fibrous spaces and contains more granulocytes compared to standard platelet-rich fibrin.

Evaluation of molecules involved in osteoclastogenesis to prevent bone damage is important in developing therapeutic approaches. Osteoclastogenesis is regulated by members of the tumor necrosis factor superfamily such as OPG, RANKL and RANK. RANKL binds to osteoclast precursors and RANK receptors on dendritic cells, causing bone damage by affecting the differentiation, proliferation and activation of osteoclasts. OPG competes with RANKL to bind to the same receptor.[20] Increased RANKL/OPG levels in GCF samples of periodontitis patients have been observed.[21] TACE is a protein released from T lymphocytes and monocytes which plays a role in stimulating bone resorption. It effectively ensures the release of RANKL from the cell membrane.[23]

To our knowledge, this is the first study to compare the expression of osteogenic biomarkers and to evaluate periodontal clinical parameters in intrabony defects treated either with T-PRF+OFD or A-PRF+OFD. Similar to other studies reported[3,10], statistically significant results were obtained at the 6th month following periodontal surgery in clinical parameters including GI, PD and CAL in both PRF groups. When T-PRF and A-PRF groups were compared, no difference was observed between the groups in terms of these clinical parameters.

In addition to clinical parameters we focused on osteogenic biomarkers and the total amount of these molecules in GCF was determined by ELISA at baseline, 3rd and 6th months following OFD and PRF application. There was no significant difference in RANK, OPG and TACE levels within and between the groups. We observed remarkable difference in RANKL levels between the T-PRF and A-PRF groups at 6th month. However, there was no difference within the group compared to the baseline. This change observed at the 6th month between the two groups may be due to the difference in baseline levels.

The increase in RANKL/OPG ratio in GCF is an indicator of the destruction in periodontal tissues. This ratio was evaluated in this study and we found no difference within the group. A significant difference was observed between the groups at the baseline, 3rd and 6th months following surgical therapy. At the 3rd and 6th months, RANKL / OPG ratio was higher in the T-PRF group than the A-PRF group. This state may be due to the difference between the groups at the baseline values. Arabaci *et al*.[27] evaluated the ratio of RANKL / OPG in GCF samples at 2, 4, and 6 weeks following the application of PRF and T-PRF in intrabony defects, and they reported significant decrease of RANKL/OPG ratio in T-PRF group at the 4th and 6th weeks after the surgery. Therefore, the lack of significant changes in the levels of osteogenic markers evaluated in this study may be due to the time points selected. Third and 6th months measurements might not include the early changes observed in osteogenic biomarkers. Considering the resorption times of PRF derivatives in the defect area, significant changes in these markers might be observed in an early period. Increased RANKL/OPG ratio is a valuable indicator of active periodontal disease. However, it is controversial that periodontal therapy reduce this ratio and provide an acceptable value for the treatment of periodontitis. For instance, in a study, gingival tissue samples were collected 4-6 weeks after initial periodontal therapy and RANKL, OPG gene expressions were determined by PCR. Authors reported no significant change in RANKL/OPG ratio.[28] Furthermore, Buduneli *et al*.[29] evaluated the effect of periodontal therapy on RANKL/OPG ratio in GCF. They observed significant increase in this ratio 4 weeks after the treatment. Another group reported no significant difference in RANKL/OPG ratio at the 4th month after initial periodontal therapy.[30] These findings suggest that RANKL/OPG ratio is a good marker for periodontal disease activity but it might not reflect the periodontal treatment outcomes.

Conclusions

This study showed that different derivatives of PRF might be a preferable option in the treatment of intraosseous defects in individuals with chronic periodontitis, without utilizing allografts. In addition to improved clinical outcomes observed in this work, further studies needs to elucidate the effect of different types of PRF on bone regeneration and bone filling.

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Conflicts of Interest Statement

The authors declare no conflict(s) of interest.

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Evaluation of the Effect of Brushing on Vickers Microhardness of Acrylic Denture Base Resins Polymerized by Different Techniques

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ABSTRACT

Objectives: To prevent the negative effects of brushing on the microhardness of the acrylic resin, different polymerization techniques may be taken into consideration while choosing the denture base material. This study's objective was to assess how brushing affected the Vickers microhardness of acrylic denture base resins polymerized using various methods.

Materials and Methods: From each acrylic resin (Integra and FuturaJet), 100 disk-shaped specimens (15 mm in diameter and 2 mm thick) were created. A total of five distinct polymerization processes—the traditional water-bath method, short and long autoclave polymerization, injection-molding polymerization, and auto-polymerization—were examined (n=20). An automatic brushing machine was used to imitate brushing on half of the specimens, applying 54 000 brush strokes each specimen. All specimens were then subjected to a Vickers hardness test with a 300-g force for 15 s. Data analysis was done using the Mann-Whitney U test, Kruskal-Wallis test, and Dunn's post-hoc test; statistical significance was set at $p<0.05$.

Results: In all polymerization methods, a statistically significant difference was seen between the control and brushing groups. The autopolymerized acrylic resin group substantially had lower microhardness values than the control and brushing groups' short, long autoclave, and water bath-polymerized resins.

Conclusions: The microhardness of acrylic denture base resins should be taken into consideration when considering polymerization procedures because the autopolymerization method may have certain drawbacks in terms of preventing negative effects of brushing on the microhardness.

Key words: Denture Bases, Polymerization, Hardness Tests.

Farklı Tekniklerle Polimerize Edilen Akrilik Protez Kaide Rezinlerinin Vickers Mikrosertliğine Fırçalamanın Etkisinin Değerlendirilmesi

Süreç

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ÖZ

Amaç: Protez kaide materyali seçiminde, fırçalamanın akrilik rezinin mikrosertliği üzerindeki olumsuz etkilerinden kaçınmak için farklı polimerizasyon teknikleri dikkate alınabilir. Bu çalışmanın amacı, farklı tekniklerle polimerize edilen akrilik protez kaide rezinlerinin Vickers mikrosertliği üzerindeki fırçalama etkisinin değerlendirilmesidir.

Gereç ve Yöntemler: Her bir akrilik rezinden (Integra ve FuturaJet) 100 adet disk şeklinde örnek (15 mm çap ve 2 mm kalınlık) üretildi. Geleneksel su banyosu polimerizasyonu, kısa ve uzun otoklav polimerizasyonu, enjeksiyon kalıplama polimerizasyonu ve oto-polimerizasyon olmak üzere 5 farklı polimerizasyon tekniği test edildi (n=20). Örneklerin yarısı, her numune için 54 000 fırça darbesi kullanılarak otomatik bir fırçalama makinesinde simüle edilmiş fırçalamaya tabi tutuldu. Tüm örnekler daha sonra 15 saniye boyunca 300 g yük ile Vickers sertlik testine tabi tutuldu. Sertlik verilerinin analizi için Mann-Whitney U testi ve Kruskal-Wallis testi ve ardından Dunn's post-hoc testi uygulandı, sonuçlar $p<0,05$ için istatistiksel olarak anlamlıydı.

Bulgular: Tüm polimerizasyon tekniklerinde kontrol ve fırçalama grupları arasında istatistiksel olarak anlamlı bir fark bulundu. Otopolimerize akrilik rezin grubu, kontrol ve fırçalama gruplarında su banyosu, kısa ve uzun otoklav polimerize rezin gruplarına göre anlamlı derecede daha düşük mikrosertlik değerleri gösterdi.

Sonuçlar: Otopolimerizasyon tekniği, fırçalamanın mikrosertlik üzerindeki olumsuz etkilerinden kaçınmak için bazı dezavantajlara sahip olabilir, bu nedenle polimerizasyon teknikleri belirlenirken, akrilik protez kaide rezinlerinin mikrosertliği açısından düşünülmelidir.

Anahtar Kelimeler: Protez Kaideleri, Polimerizasyon, Sağlamlık Testleri.

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Introduction

Denture wearers must maintain adequate denture hygiene because it helps control oral and systemic infections, especially in elderly and immune-compromised patients with decreased salivary flow rates, and it inhibits biofilm collection on the inner surfaces of complete dentures.[1,2] It was discovered that biofilm colonization and denture stomatitis are related.[3,4] In order to prevent oral and systemic disorders in edentulous individuals, thorough denture cleaning is crucial.

Complete dentures can be cleaned by mechanical, chemical, and combined methods. Chemical cleaning with hypochlorite, peroxides, enzymes and acids corrodes the metal components of the dentures, spoils the acrylic resin components, causes color changes and increased surface roughness.[5] In one study, it was reported that brushing and denture cleansers were more effective than placebo in reducing the amount of plaque and the microbial load on the plaque on the complete denture base.[6] The mechanical removal of organic debris and stains with brushing with the use of a toothbrush, dentifrice and water is a simple, inexpensive and effective technique commonly used by denture wearers.[3,7] However, it may cause the wear of the denture base or denture lining materials.[8] Many factors, including the abrasiveness of the dentifrice, the hardness of the bristles, brushing method, frequency, and strength, as well as the microhardness of the acrylic resins used in the denture foundation, might affect surface alterations that may happen from brushing.[9] In tests to ascertain how the brushes interact with the substrates, brushing with water may be advised to control for these factors.[8]

The most used acrylic material for denture bases is polymethyl methacrylate (PMMA). To overcome its poor mechanical qualities, numerous approaches have been tried.[10] To reduce residual monomer production, conventional denture base acrylic resins are polymerized in a lengthy hot water bath. The entire dentures, however, have undergone a few alterations as a result of this process, including internal stress development, dimensional changes brought on by polymerization shrinkage, and internal porosities brought on by monomer dissolution.[10] The fracture resistance of the denture base materials may be reduced by these chemical changes. Different polymerization or molding methods, including autoclave polymerization, dry polymerization, and injection molding, have been tested to reduce the problems associated with polymerization methods.[14]

By permitting the creation of denture bases that withstand the stresses from occlusion, mechanical denture cleaning, and abrasion, the hardness of acrylic resins is a critical component that extends the longevity of complete dentures.[15] Different hardness tests have been utilized to forecast in vitro wear behavior[16] and the elastic modulus[17] of dental materials. Rigid polymer hardness can be accurately assessed using the Vickers microhardness test. This test is based on a material's surface resistance to point penetration under a specified load.[18] The measurement of hardness has been used to forecast dental material deterioration.[16]

There are still few studies examining the impact of various polymerization processes on the microhardness characteristics of PMMA denture base resin and the change in of hardness values dependent on daily care.[8,9,11,12,19] It would appear crucial to assess both the abrasion resistance of various denture base resins polymerized using various processes as well as the impact of brushing acrylic resins with water on their microhardness. Therefore, the purpose of this study was to determine how brushing affected the Vickers microhardness of denture base resins polymerized using various polymerization methods. The first null hypothesis stated that brushing would not have an impact on the microhardness of the acrylic resin materials used. The second null hypothesis was that the microhardness of the acrylic resin materials would not be impacted by the polymerization processes.

Material and Methods

The Clinical Research Ethics Committee of Afyonkarahisar Health Sciences University granted its approval for this study (Date/ID Number: 04.03.2022/122). Table 1 lists the denture base resins used for this study. Working molds were created from stainless steel master dies with dimensions of 15 mm in diameter and 2 mm in thickness, from which 100 disk-shaped specimens were produced. The samples (n=20) were put through the paces using standard water baths, short and long autoclave polymerization, injection molding, and autopolimerization methods. According to references from previous studies, the sample size was determined.[19,20]

The specimens were fabricated with following polymerization techniques. For standard water-bath polymerization, conventional PMMA resin was prepared in accordance with the manufacturer's recommended powder to liquid ratio, and the specimens were then polymerized in a water bath at 70°C for 90 minutes before being heated to 100°C for 30 minutes.

In order to polymerize materials in an autoclave (Ar-El Group SAN, Greece), samples were either put through a short cycle at 60°C for 30 minutes, followed by 10 minutes at 130°C, or a long cycle at 60°C for 30 minutes, followed by 20 minutes at 130°C.

Models were maintained under constant pressure during the injection-molding polymerization process using specialized cap and pressure equipment. Vibration was used to combine pre-dosed acrylic capsules for five minutes. The cap assembly was submerged in tap water for 20 minutes after the mixture had been poured into the cap under 6 bar pressure for 35 minutes of polymerization. The pressure device was then taken off, and the cap was left to soak in tap water for an additional ten minutes.

For autopolymerization, the mixed material was pressed directly into the mold. Then, it was placed in a pressure chamber containing water at 40°C at 2 bar pressure for 15 minutes.

Since it is known that porosity will adversely affect the hardness values, it was ensured that there was no porosity in any of the specimens.[21] After the polymerization processes, all specimens were removed from the molds. A

skilled dental technician hand-polished all specimens using a laboratory polishing lathe machine (Reno, Roberson Machine Company) at 1500 rpm for 2 minutes each. Then, all specimens were kept in distilled water at 37°C for 48 hours.

Half of the specimens (n=10) had a linear brushing abrasion movement that involved a total of 54 000 strokes (forward and back), which is equivalent to three years of brushing.[8] According to ISO/DTS 145691, the brushing operation was carried out on a mechanical cross brushing equipment (Esetron MF-100, MOD Dental, Turkey).[22] The machine covered 3.8 cm at a speed of 356 rpm while brushing six specimens at once at a weight of 200 g. Only distilled water that was 23±3°C was used for the brushing. The type of toothbrush (Colgate professional soft, Colgate-Palmolive, Brazil) used had flexibility, uniform length, rounded ends, and smooth bristles. Brushes were replaced with new ones at each interval of 18 000 strokes. The specimens that were not subjected to brushing were immersed in distilled water at 23 ± 3°C. After being dried by air and cleaned with distilled water, each sample was put into a microhardness tester.

With a 300-g load applied for 15 seconds, the microhardness of all specimens was measured using a Vicker's Hardness Tester (Shimadzu HMV-M3, Japan). Each specimen was subjected to three measurements, each taken at a fixed distance from the center, with the third measurement serving as the arithmetic mean. At x40 magnification, Vickers indenter marks on the specimens were examined. The formula below was used to compute the Vickers hardness values (HV):

$$HV = 1.854\ (\frac{F}{D^2})$$

with *F* is the applied load (measured in kgf) and D^2 is the area of the indentation (measured in mm^2).

Using SPSS 20.0 software (IBM, IL), the microhardness data were analyzed using the Mann-Whitney U test, Kruskal-Wallis test, and Dunn's post-hoc test ($p<0.05$).

Table 1. Composition of acrylic resins tested in this study

Commercial Brand	Manufacturer	Polymerization technique	Composition
Integra	BG Dental, Ankara, Turkiye	Water bath heat polymerization	Powder: polymethyl methacrylate, catalyst, pigments Liquid: methyl methacrylate, dimethacrylate
Integra	BG Dental, Ankara, Turkiye	Autoclave polymerization (short cycle)	Powder: polymethyl methacrylate, catalyst, pigments Liquid: methyl methacrylate, dimethacrylate
Integra	BG Dental, Ankara, Turkiye	Autoclave polymerization (long cycle)	Powder: polymethyl methacrylate, catalyst, pigments Liquid: methyl methacrylate, dimethacrylate
FuturaJet	Schütz- Dental GmbH Rosbach, Germany	Injection molding polymerization	Powder: polymethyl methacrylate, copolymerand catalyst Liquid: mixture of MMA stab, dimethacrylate and copolymer
Integra	BG Dental, Ankara, Turkiye	Autopolymerization	Powder: Polymethyl methacrylate Liquid: methyl methacrylate, N,N dimethyl p-toluidine

Table 2. Vickers microhardness – mean and standard deviation (SD) and median values

Polymerization technique	Control group Mean ± SD Median	Brushing group Mean ± SD Median
Water bath	47.83±2.39 A a 48.89	39.32 ± 5.90 B a 38.11
Short autoclave	45.49±3.63 B a 47.05	38.55 ± 4.25A a 38.56
Long autoclave	53.25±3.38 A a 50.36	47.40 ± 2.69 B a 47.26
Injection molding	37.92 ± 2.52 B a b 36.51	34.80 ± 2.24 A a b 34.74
Autopolymerized	21.89 ± 1.10 A b 21.23	19.15 ± 2.34 B b 20.10

*Means followed by a distinct capital letter in the line and lower-case letter in the column differ statistically according to Dunn's post-hoc test ($p < 0.05$).

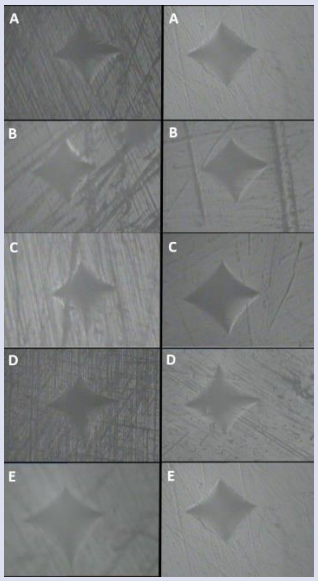

Figure 1: Traces formed in the Vickers microhardness test of the control (left) and brushing (right) groups. A. Conventional water-bath polymerization, B. Short autoclave polymerization, C. Long autoclave polymerization, D. Injection-molding polymerization, E. Auto-polymerization.

Results

The mean values and standard deviation of the Vickers microhardness of the examined acrylic resins are displayed in Table 2.

Auto-polymerized acrylic resin displayed significantly lower microhardness values than water bath, short and long autoclave polymerized resins in the control and brushing groups ($p < 0.05$). There is no significant difference between autopolymerization and injection molding ($p>0.05$). Regardless of brushing, long autoclave polymerized resin displayed higher microhardness values than those produced by injection molding and auto-polymerization ($p < 0.05$).

When comparing the control and brushing groups, it was possible to see that brushing had a negative impact on the Vickers microhardness of the acrylic resins (Figure 1). In all polymerization methods, a statistically significant difference was seen between the control and brushing groups ($p < 0.05$).

Discussion

In this study, the microhardness of denture base resins polymerized using various polymerization processes was assessed in relation to the impact of brushing with water. Acrylic resin specimens' microhardness was impacted by the brushing process. In all groups, microhardness values were reduced with brushing; in the water bath group, this reduction rate was around 18%, and in the autopolymerized group, it was approximately 12.5%. These findings demonstrated the rejection of the first null hypothesis of this study.

There was a 40% difference between the highest and lowest microhardness values for both the control and brushing groups when polymerization procedures were investigated. According to the results of the current study, which showed that the auto-polymerized acrylic resin had the lowest microhardness values, it was determined that specimens made using the auto-polymerization approach might wear out more quickly than those made using the other techniques. The high concentration of residual monomer left over after autopolymerization operations, which acts as a plasticizer, may be the cause of the current study's findings.[23,24] The evaluated acrylic materials' microhardness was strongly influenced by the polymerization processes used. The second null hypothesis was therefore disproved. Another study produced results that were similar to this one.[10] Anusavice and Phillips[18] claimed that autopolymerization results in a lower degree of polymerization than heat polymerization.

Acrylic resin used in injection molding had lower microhardness values than acrylic resin used in water baths. In a study, two varieties of acrylics polymerized using water-bath and injection-molding processes were examined for hardness and surface roughness. They came to the conclusion that both had similar levels of hardness and surface roughness, which was at odds with the findings of the present study.[25] The varied types of acrylic used in the water baths may be the cause of the variations in results. Another finding from this study showed that the microhardness of the two autoclave polymerization cycles (long and short) and water bath polymerization procedures did not significantly differ from one another. These findings were consistent with those of Abdulwahhab.[26] In a recent study, it was found that extended autoclave polymerization produced materials with higher hardness values than short autoclave polymerization and water-bath polymerization.[27] In the aforementioned study, autoclave polymerization under pressure sped up the initial polymerization by increasing the steam's temperature and the monomer's boiling point. This discrepancy in the result with the current study

may be explained by a decrease in the residual monomer content.

In order for patients who wear dentures to be able to wear them for an extended period of time, it is important to properly clean and care for the prosthesis at home to get rid of food particles, salivary mucus layer, and plaque deposits. Cleaning techniques are typically utilized for this purpose by washing with soap or brushing with dentifrice.[28] In earlier studies on the subject, it has been claimed that the denture base is harmed by the number of abrasives in toothpaste and/or different soaps or cleansers used at home.[28,29] These findings led to the goal of eradicating the damaging effects of chemical solutions on acrylic surfaces and demonstrating the relationship between mechanical cleaning and abrasion using simply water brushing.

The clinical life of the denture base material and the oral health of the tissues in contact with the prosthesis and the ability to perform adequate mechanical cleaning have a parallel effect.[30] In previous studies evaluating wear caused by brushing, different types of brushes and dentifrices were used as abrasives.[8,19,31] There are significant differences in the results of these studies due to the use of dentifrices containing different concentrations of different abrasive particles, making it difficult to compare the available data with each other. In the current study, a soft-bristled toothbrush was used because it is inexpensive, accessible to most patients and of high quality. Brushing with distilled water has been shown to cause minimal wear and minimal mass loss to the brushed substrate.[31] This study aimed to isolate the effect of brushes and dentifrices by analyzing the effect of brushing method with distilled water on the wear resistance of acrylic resins polymerized by different techniques.

In order to prevent loss of smoothness, to lessen aesthetic issues like plaque retention and discoloration, and to provide dentures with a longer serviceable life that will be more resistant to damage, the hardness of the acrylic resin used to make dentures for the elderly has been increased.[10] Clinicians should be aware that they can handle this circumstance using various polymerization techniques when creating dentures to address these issues. Regardless of the polymerization methods used, the microhardness values of the studied denture base materials declined during brushing. These findings suggest that complete denture wearers should be made aware that the fracture resistance of the denture may diminish even if they regularly clean it with a brush and water. In order to achieve long-term success, clinicians may be recommended to steer clear of autopolymerization and injection molding polymerization techniques, according to the study's therapeutic implications.

The limitations of this study include the use of in vitro tests rather than clinical trials and the evaluation of just two of the numerous denture base resins on the market. Additional research may focus on chemical and combination cleaning techniques, specialized denture brushes and dentifrices, or denture cleaning solutions in addition to mechanical cleaning. To continue the search for the best polymerization method for creating denture prosthetics, additional study using various mechanical testing and extended brushing times is required. Clinicians will learn more as a result of additional research on methods to improve the polymethyl methacrylate denture base material's wear resistance.

Conclusions

This study's findings could lead to the following conclusion:

1. In all groups, the microhardness values declined as the brushing process progressed.
2. The microhardness of acrylic resin specimens was influenced by the polymerization process.
3. The specimens produced from autopolimerized denture base acrylic resin had the lowest microhardness values.

Conflicts of Interest Statement

The authors declared that there is no conflict of interest.

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Evaluation the Marginal Adaptation for the Bio C Repair and Other Root end Filling Material by Using Scanning Electron Microscope (A Comparative In Vitro Study)

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ABSTRACT

Background: The ability of the retrograde filling material to adhere to dentin has a substantial impact on the success of endodontic procedures. The marginal adaptation of the root end filling materials is considered an important factor, since it demonstrates adaptation of the material to the dentinal walls.
Objective: Assess the marginal adaptation of Bio-C Repair material in contrast to MTA (Angelus) and Amalgam.
Material and method: A total of thirty human maxillary central incisors teeth that each had a single root were chosen. To keep the root canal length at 15mm, the crown was resected. The teeth underwent endodontic treatment, along with the resection of their root ends and preparation of root-end cavities. Based on the type of filling, the teeth were categorized into one of three groups, as follows: (A) Bio-C repair (Bioceramic reparative material), (B) MTA Angelus, (C) Amalgam. Teeth were sectioned longitudinally, and marginal gaps were measured by the use of a scanning electron microscope. Data was statically analyzed using ANOVA and Tukey Honestly Significant Difference (Tukey's HSD).
Results: Bio-C Repair material and MTA show better marginal adaptation with significant difference between them and amalgam, and no significant difference between the Bio-C repair group and MTA group.
Conclusions: Marginal adaptation is better for Bio-C Repair in comparison to MTA Angelus and Amalgam.

Key words: Bioceramic, Marginal Adaptation, MTA, Scanning Electron Microscope.

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Introduction

The main objective of endodontic treatment is to get a seal that prevents any fluid from leaking between the periodontium that surrounds the root canal system.[1] The routine endodontic treatment was done in orthograde approach, and if that is not possible, a retrograde approach, root-end preparation and filling with a surgical approach is recommended. Retrograde root-end filling, which comes following apicoectomy, is crucial. A sufficient amount of retrograde filling material must be used to fill the retrograde cavity to ensure that the root canal system is hermetically sealed, and for preventing the entrance of microorganisms and byproducts to the periradicular tissue.[2] numerous studies had examined the surgical endodontic treatment's clinical results, and most of these studies show that, The clinical outcomes of endodontic surgery are significantly influenced by the selection of retrograde materials.[3] Root end filling material should be antimicrobial, nontoxic, and biocompatible, radiopaque, non-resorbable, dimensionally stable, resist dislocating forces, withstand breakdown or dissolution by tissue fluids, be easily handled, and be able to adapt to the dentinal walls of root canal system .[4] Numerous materials had been used as root end filling material, Amalgam is one of the most used root-end filling materials its remain the standard to which other materials are evaluated .It is durable, less technically sensitive, easy to handle, has minimal technical time compared to other materials and its corrosion products seal the apex surface and prevent leakage of bacteria. Some of the disadvantages are local allergic reactions, mercury toxicity and lack of chemical bonding with dentin.[5]

Mineral trioxide aggregate (MTA) is one of the materials developed as retrograde filling material that has a noncytotoxic effect and promotes cementogenesis its presently utilized as a material for root-end filling.[6] However, MTA has certain drawbacks, including difficult handling, a prolonged setting time, an expensive cost, poor antibacterial properties, and discoloration.[7] MTA's composition was changed, and MTA Angelus was released in 2001, with calcium sulphate removed from its composition to improve utilization and decrease the setting time.[8] To overcome the problems of MTA the Bio-C Repair material have been created, its new material for root-end filling and because they become bioactive when they come into contact with vital tissues, they help with tissue repair and biomineralization.[9] Bio-C Repair have

better properties such as decreased moisture sensitivity, insolubility, and tissue inductive properties, They are the ideal substances for endodontic treatment.[10] It is simple to use , not provided in powder-and-liquid form; instead, it is offered as a single product stored in a syringe, thereby eliminating the need to manipulate the material and time saving.[11] CAMPI *et al.*[12] was the first investigate the physicochemical characteristics, bioactivity and cytotoxicity of the Bio-C Repair material, in comparison with MTA Angelus .The materials utilized as root end filling in this study are described in [Table1]. The success of surgical endodontic treatment is mostly dependent upon on how well a root end filling material adheres to the dentinal walls.[13] Given the importance of adequate marginal adaptation for the success of regenerative endodontics, studies should be performed to provide well-grounded information on the mechanical properties of Bioceramic cements, especially those recently released to the market were the Bio-C Repair material still in experimental phase. Therefore, the aim of the present study was to evaluate the marginal adaptation of Bio-C Repair material when used as root end filling material and compare it with MTA angelus, and Amalgam.

The null hypothesis stated that there is no significant difference in marginal gaps that found between dentin and root end filling material (Bio-C Repair, MTA, Amalgam).

Material and Methods

Ethical approval from the research ethics committee of the College of Dentistry-University of Baghdad (Ref. number: 56, Date: April 20, 2022). was gained for the use of extracted human teeth. The sample size calculation was performed by using G power 3.0.10 (Program written by Franz-Faul, Universitatit Kiel, Germany) with power of study=95%, alpha error of probability=0.05, doing pilot study on three groups find that the effect size of F is 1.11 (Large effect size), thus requiring 6 teeth for each group, 10 teeth were assigned for each group thus more calculated than G power.

The main steps in the methodology of this study summarizing in [Figure 1]. Thirty human maxillary central incisor, all the teeth extracted for patient treatment, that have the following criteria: single roots with completed root formation, without any anatomic variation, crack, or any resorption and without any previous endodontic treatment. The sample was cleaned by using scalar and pumice, and disinfected with NAOCL solution (Promida, Odunpazarı, Turkiye) in a concentration 2.5% for 30 minutes after disinfection the sample was stored in normal saline until used .[14]

The crown was sectioned by a diamond disc to standardize the root length (15mm), and a K-file of size 10 (DENTSPLY Maillefer, Ballaigues, Switzerland) was inserted in each root, the tip of the file was visible from the apical foramen at a distance of 1 mm. ProTaper rotary files (DENTSPLY, Maillefer, and Ballaigues, Switzerland) were utilized to prepare the root canals to the desired shape, the preparation began with the SX file and continued with the S1, S2, F1, F2, and F3 files. Between each file, the canal was irrigated with 2mL of 5.25% sodium hypochlorite canals was rinsed with 2ml of distill water then irrigated with 2 mL of EDTA at a concentration of 17% (Dental Produits Dentaires SA, Switzerland) canal was dried by using a paper point (DENTSPLY, Maillefer, and Ballaigues, Switzerland). Gutta-percha points (DENTSPLY, Maillefer, and Ballaigues, Switzerland) and MTA-Fillapex sealer (Angelus Odonto, Londrina, Brazil) were used to fill the canals using lateral condensation technique. The roots were kept at 100% humidity and 37°C for two days to ensure that the filling materials were fully set. The roots were precisely sectioned (3mm from the apex) Under continuous water cooling using a diamond disk. a root-end cavity measuring 3mm in depth and 1.5mm in diameter was prepared by using a fissure bur (FKG Dentaire, La Chaux-de-Fonds, Switzerland) with copious water. The root end cavities were irrigated by (EDTA) 17% (Dental Produits Dentaires SA, Vevey, Switzerland) for smear layer removal and rinsed with normal saline after that the cavities were filled with the root end filling material according to the manufacture's instruction. The samples were randomly classified (using www.random.org) into three groups according to the type of filling material that was utilized (n=10):

I. Bio-C Repair group: the retrograde cavities were filled with Bio-C Repair (Angelus Odonto, Londrina, Brazil).

II. MTA Angelus group: the retrograde cavities were filled with MTA (Angelus Odonto, Londrina, Brazil).

III. Amalgam group: the retrograde cavities were filled with Amalgam (NAIS, Sofia, Bulgaria).

Radiographs were taken to confirm the proper filling of the material. All teeth will be stored at 37º and 100% humidity for 7days.

The roots were then sectioned longitudinally using a slow-speed diamond saw and under continual water-cooling. The specimens have a gold sputter coating and analyzed by scanning electron microscope (Axia Chemisem, Thermo Scientific Fisher, USA,2021) without dehydration of the specimens.at six points on both sides of the specimen under magnifications 250X,500X,1000X,2000X,4000X.

The points are chosen so that points 1a, 1b and points 3a,3b represent the upper and lower edges of the preparation (0,5mm from the margin), points 2a,2b represent the mid distance between points 1 and 3 [Figure 2]. The full width of the marginal gaps was assessed in micrometers at magnification 2000X, gaps measured in micrometer (um) by using Image J software application (National Institutes of Health, Bethesda, MD).

Statistical analysis

The Statistical Package for Social Science (SPSS version -22, Chicago, Illinois, USA) was used for data analysis, Statistical tests included: Shapiro–Wilk test, ANOVA test, Tukey post hoc test**.**

Table 1. Materials' composition, manufacturers, and Technique of use.

Materials	Composition	Manufacturer	Technique of use
Bio-C Repair	Calcium silicate, calcium oxide, zirconium oxide, iron oxide, silicon dioxide, dispersing agent	Angelus, Londrina, PR, Brazil	Ready for use.
MTA-Angelus	Powder: Tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium oxide, calcium tungstate istead of bismuth oxide. Liquid: Distilled water	Angelus, Londrina, PR, Brazil	For 30 s, mix the content of 1 sachet of MTA-Angelus (or 1 spoon of MTA-Angelus with 1 drop of distilled water. The mixture should be homogeneous and with a consistency similar to wet sand.
Amalgam	Ratio of Alloy/ Mercury 1:1 Alloy: Silver 43%, tin 32%, copper25%.	NAIS, Sofia, Bulgaria	12-15 s, mix the Amalgam capsule in the amalgamator.

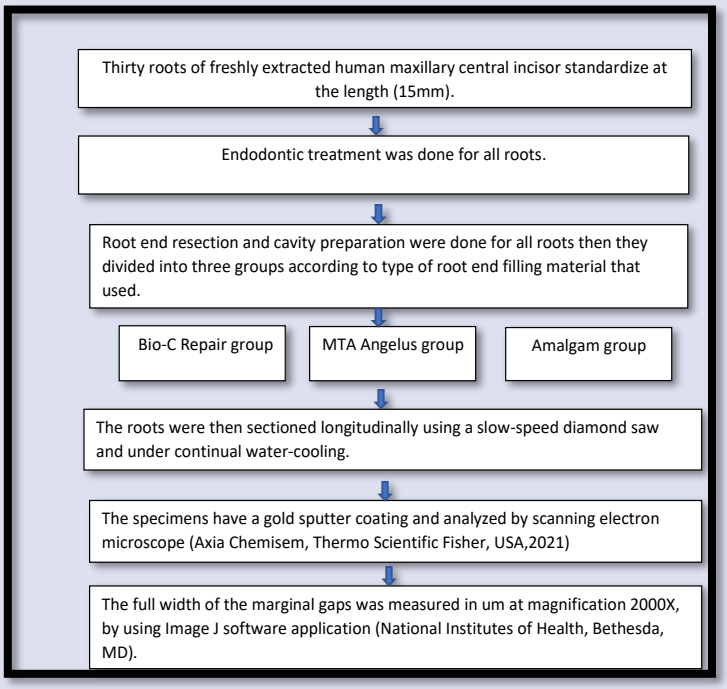

Figure 1. Flow chart summarizing the main steps in the methodology of this study.

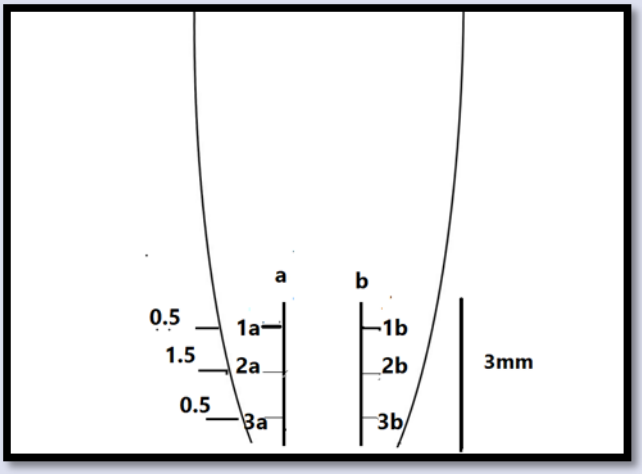

Figure 2. Flow chart summarizing the point of gaps measurement in SEM photomicrographs.

Results

The gaps at filling-dentin among three different groups tested for normality of disruption using Shapiro Wilk test at p >0.05 The results were revealed that the data were normally distributed.

The finding of each group including Minimum, Maximum, Mean, Standard Deviation, and Standard Error of gaps at filling-dentin among groups was shown in [Table 2] [Figure 3]. SEM photomicrographs of all groups are presented in [Figure 4].

The difference in mean value of marginal gaps between groups was evaluated using One way Analysis of Variance (ANOVA) [Table 3] The results showed that there was significant difference among groups (p<0.05). Further comparison using Tukey's HSD test after ANOVA revealed that the difference between Bio-C repair group and MTA angelus group is not significant(P>0.05), and there's significant difference between them and Amalgam group (p<0.05) as shown in [Table 4].

Table 2. Descriptive statistics of gaps among groups in um.

Groups	Mean	Std. Deviation	Std. Error	Minimum	Maximum
Bio-C Repair	2.962	0.481	0.152	1.830	3.652
MTA ANGELUS	3.810	1.451	0.459	2.896	7.791
Amalgam	6.602	0.908	0.287	5.031	8.240

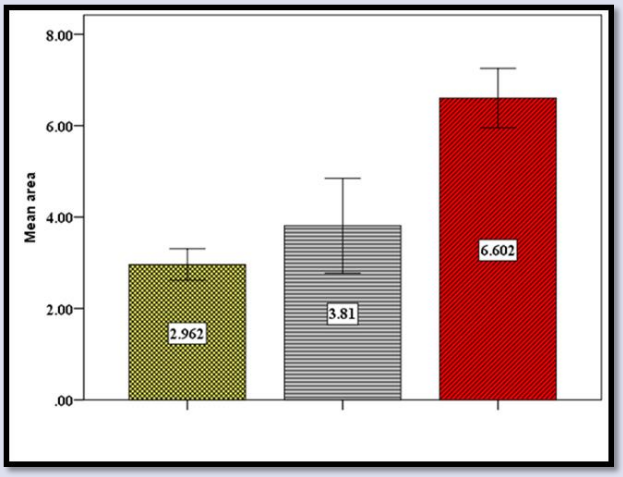

Figure 3. Means of gaps which measured in the groups in um.

Table 3. Statistical test of gaps among groups using One way Analysis of Variance.

	Sum of Squares	df	Mean Square	F	P value
Between Groups	72.553	2	36.277	34.440	0.000*
Within Groups	28.440	27	1.053		
Total	100.993	29			

*=significant difference
p=0.000

Table 4. Multiple pairwise comparison of SEM among groups suing Tukey HSD.

(I) Groups	(J) Groups	Mean Difference (I-J)	P value
Bio-C Repair	MTA ANGELUS	-0.848	0.174^
	Amalgam	-3.640	0.000*
MTA ANGELUS	Amalgam	-2.792	0.000*

*==^ non-significant difference
*=significant difference

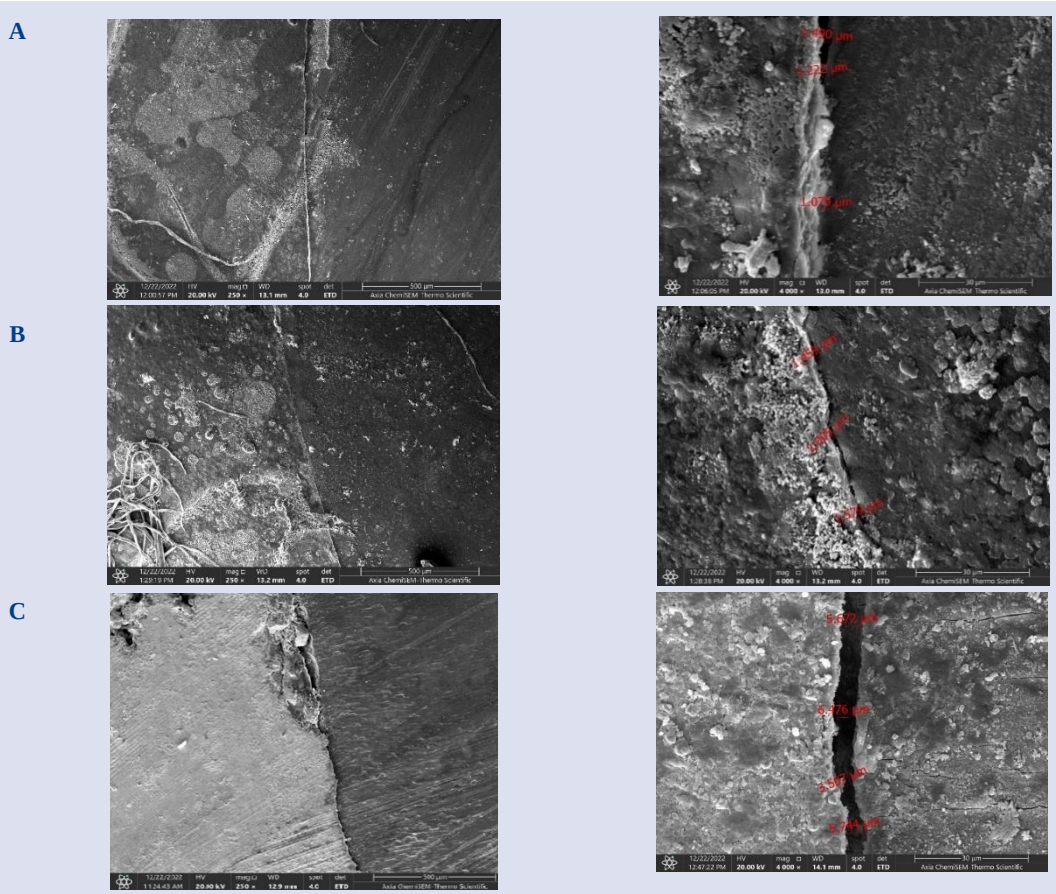

Figure 4. Scanning electron micrograph show marginal gaps between retrograde filling materials and dentin. (A) Bio-C Repair at magnification (a) 250x (b) 4000x. (B) MTA ANGELUS at magnification (a) 250x (b) 4000x. (C) Amalgam at magnification (a) 250x (b) 4000x.

Discussion

Marginal adaptation of the retrograde filling material considered the most significant factor for successful long-term treatment of surgical endodontics.[13] An optimal root-end filling material, as stated by Torabinejad *et al.*, should have the ability to adhere to the dentinal walls for complete seal of the root canal system. It must not be toxic, it cannot corrode, must not irritate the periradicular tissues, accelerate healing. It should be easy to manipulate and should be radiopaque, among other requirements, addition to these properties, it must be dimensionally stable, non-resorbable, and impervious to moisture.[4] Bioceramic root end filling material has shown to have success rate 86.4-95.6 in the last years and its showed high success rate in comparison to amalgam [2]. MTA was tested extensively in many vivo and vitro studies over the last years. These studies show better properties for the MTA, if compared with traditional retrograde filling materials.[15] Bio-C Repair material recently added in the marketplace, used as root-end fillings, because of their superior qualities such as insolubility, decreased moisture sensitivity, and tissue inductive properties, recently they are the materials of choice in the surgical endodontic treatment.[16] The addition of silicate-based materials leads to decrease setting times, this overcoming on the drawbacks of MTA. Bio-C Repair (Angelus)is not supplied in powder and liquid form; rather, it is sold as a single product that is packed in a syringe; as a result, there is no requirement for the material to be manipulated .[17] Bio-C Repair is not only biocompatible, but it also has the ability to biomineralize when it comes in contact with live tissues.[18,11] In this present in vitro study marginal adaptation of Bioceramic reparative material was evaluated and compared with MTA and amalgam which considered standard filling in this study. amalgam was used as root end filling material in the past, therefor it considers control when new root end filling material tested. In this study, 3mm from the apical part of the root was eliminated to reduce the apical ramifications and lateral canal. the retrograde cavity was prepared by bur to avoid crack formation in the root.[19] The marginal adaption test is an indirect method that is used to examine the sealing ability of different root-end filling materials. Studies of color penetration, bacterial leakage, fluid filtration, and radioisotope methods, along with confocal microscopy, micro-computed tomography (CT), and scanning electron microscopy were used in order to assess the quality of the material's marginal adaption. In many studies, Through the use of longitudinal sections of the tooth, scanning electron microscopy (SEM) has been utilized to examine the marginal adaptation of a variety of

root-end filling materials to the surrounding tooth structure. [20,21]

SEM show highest means value of gaps recorded in the Amalgam group the poor adaptation of amalgam may be due to contraction during setting, lowest mean of gaps in the Bio-C Repair group, gaps in MTA smallest than this which found in Amalgam that's confirm with results in other previous studies.[13]

The good marginal adaptation of MTA because its composition consists of tricalcium silicate, tricalcium aluminate, tricalcium oxide, silicate oxide, and various mineral oxides , all of which combine to produce a hydrophilic powder that solidifies in the presence of water.[22] It demonstrates the precipitation of calcium-phosphate at the interface of the two substances This interface layer lessens the likelihood of marginal percolation and paves the way for potentially long-term clinical success.[23,24] In the Bio-C Repair The capacity of these materials to seal is improved by the presence of mineral precipitate at the interface between calcium silicate-based cement and dentin.[25]

In this study Bio-C Repair show better marginal adaptation than MTA this may be due to volumetric change that occur in MTA is higher than this in Bio-C Repair as mentioned in other previous studies.[26,10]

Conclusions

The result of this study shows improved marginal adaptability for the Bio C repair; better handling this material make its better to use as retrograde material in comparison to other.

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Conflicts of Interest Statement

There is no potential for bias or conflict.

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Analyzing Content and Information Quality of Instagram® Posts About #teethwhitening

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ABSTRACT

Objectives: Getting information on health issues from social media applications provides autonomy to patients; however, different types of content can lead to the development of inappropriate norms. This study therefore aimed to evaluate the content and information quality of Instagram® posts about teeth whitening.

Materials and Methods: The first 100 posts were included in the three search periods, which started in October 2022 and were carried out at two-week intervals. (n=300) The #teethwhitening hashtag, which was determined as a trend topic in each search period, was used. The content of the posts was analyzed over eight questions, whereas their general information quality was analyzed according to the modified DISCERN analysis on seven questions. The demographic data of the posts were evaluated in two parts: a) account and b) data of the post. The distribution of the user questions and other hashtags (#) mentioned in the posts were also analyzed. Data normality checked via the Shapiro Wilk test, then applied Mann Whitney U, Kruskal Wallis, Bonferroni tests, and Spearman correlation as needed for non-normal data.

Results: 191 posts were excluded from the research. 42% (n=46) of the posts had "low" information content. In addition, the mean value of the content was 2.77 (min 0, max 8/n=109). The mean score in the modified DISCERN score was 11.83 (min 7, max 35/n=109). 53% (n= 57) of the posts were related either to prosthetic treatments alone or to combined treatments that included prosthetic procedures. Most of the posts mentioned the hashtag "#veneer/s". Questions such as cost, duration of treatment, and appointment procedures were also asked as comments under the posts.

Conclusions: It can be stated that the content and information quality of Instagram® posts about #teethwhitening are insufficient and may direct patients to more interventional invasive treatment options.

Key words: Misinformation, Social Media, Teeth Whitening, Internet, Dental Esthetics.

#Diş Beyazlatma Hakkında Instagram® Gönderilerinin İçerik ve Bilgi Kalitesinin Analizi

Süreç

Geliş: 08/03/2023
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ÖZ

Amaç: Sosyal medya uygulamalarından sağlıkla ilgili konularda bilgi alabilmek hastalara özerklik sağlamaktadır. Öte yandan içerik bolluğu, zararlı görüşlerin gelişmesine yol açabilmektedir. Çalışmamızda diş beyazlatma ile ilgili Instagram® görsellerinin içerik bilgisi ve genel bilgilendirme kalitelerinin değerlendirilmesi amaçlanmaktadır.

Gereç ve Yöntemler: İlk 100 gönderi, Ekim 2022'de başlayan ve iki haftalık aralıklarla gerçekleştirilen üç arama döneminde çalışmaya dahil edildi. (n=300) Her arama döneminde trend konu olarak belirlenen #teethwhitening etiketi kullanıldı. Gönderilerin içeriği sekiz soru üzerinden, genel bilgi kalitesi ise yedi soru üzerinden modifiye DISCERN analizine göre analiz edildi. Gönderilerin demografik verileri, a) hesap ve b) gönderiye ait veriler olmak üzere iki bölümde değerlendirildi. Gönderilerde bahsedilen kullanıcı sorularının ve diğer (#) etiketlerin dağılımı da analiz edildi. Verinin normal dağılımı Shapiro Wilk testi ile kontrol edildi, daha sonra normal dağılım göstermeyen veriler için gerektiği gibi Mann Whitney U, Kruskal Wallis, Bonferroni testleri ve Spearman korelasyonu uygulandı.

Bulgular: 191 gönderi araştırma dışı bırakıldı. Paylaşımların %42'si (n=46) "düşük" bilgi içeriğine sahiptir. Ayrıca bilgilendirme puan ortalaması 2.77'dir (min 0, max 8/n=109). Modifiye DISCERN değerlendirmesinde ortalama puan 11,83'tür (min 7, max 35/n=109). Gönderilerin %53'ü (n= 57) ya sadece Protetik ya da kombine tedavilerin protetik işlemleridir. Paylaşımlara en çok "#veneer/s" etiketi eklenmiştir. Sorularda ise daha çok maliyet, tedavi süresi, randevu oluşturma işlemleri sorulmuştur.

Sonuçlar: Diş beyazlatma ile ilgili Instagram® paylaşımlarının bilgi içerik ve kalitelerinin yetersiz olduğu ve hastaları daha çok girişimsel invaziv tedavi seçeneklerine yönlendirebileceği söylenebilir.

Anahtar Kelimeler: Yanlış Bilgilendirme, Sosyal Medya, Diş Beyazlatma, İnternet, Dental Estetik.

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Introduction

Today, social media applications play an important role in reaching the information that patients are curious about.[1-4] Because of patients' refrain from asking face-to-face questions, long waiting times in clinics, and the danger of COVID-19 virus transmission, it becomes more preferable to get medical information over the internet, and this encourages people to research through social media applications.[5] In many developed countries, most patients do internet research before being examined.[6] Dentistry has also been affected by this situation. Many patients do internet research about the problems they experience before their dental treatments.[7] Social media is frequently used to promote dental treatments and share their results.[8] In addition, patients frequently use social media to ask questions and express their concerns, doubts, and advice, especially about oral and dental health.[9,10]

Patients would like to have a beautiful smile and also demand to have whiter teeth. They state that they are not satisfied with the normal color of their teeth as well as color changes due to external staining.[11,12] In studies containing data from many different countries, it was reported that the rate of those who are not satisfied with their tooth color varied between 32.3% and 64.1%.[13-15] Teeth whitening is one of the most popular cosmetic procedures for patients and can be conducted in the office and/or at home with different materials.[11]

Instagram®, one of the popular social media applications where visual sharing is dominant, is widely used by patients and physicians.[16] The app has an estimated 1.44 billion monthly users as of December 2022.[17] Users can share different types of posts (photos, videos, etc.) by adding their comments and hashtags (#). They can add up to 30 hashtags (#) to these posts and increase the visibility of their posts by gathering these posts under the same title.

Being able to get information about possible treatments from social media applications, make decisions and receive education on health-related issues provides patients with autonomy.[18,19] However, the abundance of content in social networking ecosystems causes patients to have difficulty in filtering posts on the subject, which leads to the consumption and production of misinformation and ultimately to the development and dissemination of harmful health information.[20-22] In the literature, there are studies evaluating the media content on the YouTube™ platform, especially in the field of eliminating aesthetic concerns and teeth whitening.[23,24] However, there is no study evaluating Instagram® posts under the topic of teeth whitening. This study, in this context, aimed to evaluate the information and general quality of Instagram® images shared with the teeth whitening hashtag (#teethwhitening) for patients.

Material and Methods

A new Instagram® account was created on 10.10.2022 so that the shares to be included in this study would not be affected by any search algorithms. The first search was made on 14.10.2022. The autocomplete feature of the Instagram® search engine that users frequently search for trending hashtags (#) was utilized. By typing #teeth in the search section, trending tags (#) related to teeth whitening were determined according to the popularity level and in line with the purpose of this study. This procedure was performed 3 times in total, with an interval of two weeks. In each search period, the most popular hashtag (#) was identified as “#teethwhitening” (over 3.5 million), and the study data was completed through the consideration of this hashtag (#).

The first 100 posts shared with the hashtag “#teethwhitening” in each search period were collected in different folders using the Instagram® save feature. In addition, the negative effects of data variability were avoided by taking the screenshots of the shares and the demographic data of the account that shared the information, and the information was fixed. Only posts in English that were open to all users were included in the study. 191 out of 300 posts were not suitable for the research topic; and irrelevant posts (59), posts containing treatments from other areas of dentistry (40), non-English posts (20), identical posts (10), posts with funny content (4), posts with clinical promotion (48), and posts with product promotions (10) were excluded from the study.

The demographic data of the posts included for the study were collected under 2 main headings as account and sharing data. The number of followers of the accounts, their professional information, if any, their contact information and location information were recorded. The method of sharing the post, the treatment method applied, the number of likes, the time (hours) after sharing, the number of comments, and the other top 10 hashtags (#) shared with the post were recorded. The last period information was accepted in the demographic data (likes number, number of comments, number of followers, etc.) of the posts belonging to the same accounts included in the study by entering the first 100 shares in different search periods. In addition, the comments made on the posts were evaluated in terms of the questions that users wondered one month after the last data collection, and the first question in the comment sections of the study posts was recorded.

The posts were evaluated according to the information they contained and their overall quality rating. In addition, the Interaction Coefficient (IC) was determined for each post included in the study: [(Number of Likes + Number of Comments) / Time of posting (hour)]. The content analysis of the posts was performed using the parameters in Table 1, and the scores were determined by two restorative dentistry specialists, M.B. and T.M., using a double-blind evaluation method. In the posts where their scores were different, the evaluators made their decisions by evaluating together. The posts received 1 point for each review item they included, and scores between 0-2 points were considered to be low, scores between 3-5 points were considered to be moderate, and scores between 6-8 points were considered to have a high information level.

In the quality analysis of the shares, the DISCERN guide was used. The DISCERN Guide is used to evaluate the quality and reliability of the material created for the purpose of presenting information in the field of health. In the DISCERN guide, which consists of 3 sections and 16 questions in total, 8 questions in the first part are used to measure the reliability of the information presented, 7 questions in the second part are used to measure the quality of the information given about treatment and care options, and the last question is used to measure the overall evaluation of the sharing. In this research, the questions in the 2nd and 3rd sections of the DISCERN Guide were used (Table 1). Each question was scored between 1 and 5 (1: not suitable, 5: appropriate). A low score in the evaluation indicates low quality, and a high score indicates high quality.[25,26] Before the DISCERN evaluation, it was determined that there was a positive correlation between the observers by looking at the coefficient of agreement between the physicians. The posts were evaluated by three Restorative Dentistry specialists (M.B., T.M. and G.A.). Since the study was conducted on publicly available data, ethics committee approval was not required.

Statistical Analysis

Test-retests were applied to investigate whether the values re-measured by different people for the same variables were similar or not. Concordance between categorical variables was checked with Kappa statistics, and concordance between continuous variables was checked with Pearson correlations. The interobserver correlation coefficients for the modified DISCERN total score were above the minimum value of 0.70. (W:0.911, *p<0.05*), (W:0.938, *p<0.05*), (W:0.963, *p<0.05*). Statistically significant, positive and very high-level relationships were obtained. In the study, the descriptive statistics (number, percentage, mean, standard deviation, minimum and maximum) of the data were given. As the first step of the statistical analysis, the assumption of normality was checked with the Shapiro Wilk test. In the cases where the normal distribution assumption was not met, the Mann Whitney U test was applied. The Kruskal Wallis test was used to compare the means of three or more groups that did not have a normal distribution. The Post Hoc Bonferroni test was applied to reveal the group or groups that made the difference. The Spearman correlation was used to examine the relationships between the non-normally distributed continuous variables. The analyzes were performed with the IBM SPSS 25 program.

Results

The descriptive statistics of the Instagram® data used in the study are given in Table 2. When the distribution ratios of the posts are examined, "Dentist" ranked first in occupational groups, "Nano Influencer" in the number of followers, and "Prosthetic Treatment" groups in treatment methods. It was determined that only 1 (one) post received a 'High' score in the information level scale. The most informative topics in the posts were "Explanation" and "Before-After" information. (Figure 1) The number and order of the other hashtags (#) in the posts are as in Figure 2. The number of posts with questions asked in the comment section was determined as 55, and all of the questions were listed in the “Questions” section of the article.

The difference between the IC mean values of the groups formed according to the number of followers was statistically significant *(p<0.05).* Statistically significant differences were obtained between the Nano influencer and Micro and Mid-tier influencer groups. *(p=0.000 and p=0.000)*. The mean values of the Micro and Mid-tier influencer groups were higher than the mean value of the Nano influencer group. There was no statistically significant difference between the mean of informing content and Modified DISCERN Scores *(p>0.05)*, (Table 3).

The difference between the mean IC values in the groups determined according to the treatment methods was statistically significant *(p<0.05*), (Table 3). Statistically significant differences were determined between the Bleaching, Prosthetic and (Orthodontic+Prosthetic) treatment groups *(p=0.003 and p=0.015)*. The mean scores of the “Prosthetic” and “Orthodontic+Prosthetic” treatment groups were higher than those of the “Bleaching” treatment. A statistically significant difference was found between the mean scores of information *(p<0.05).* Statistically significant differences were determined between the "No info" group and the "Prosthetic", "Combined Treatment" and "Orthodontic+Prosthetic" treatment groups *(p=0.003, p=0.011 and p=0.007)*. The mean values of the “Prosthetic”, “Combined Treatment” and “Orthodontic+Prosthetic” treatment groups were higher than those of the “no info” group. A statistically significant difference was obtained between the modified DISCERN Score mean values *(p<0.05)*. Statistically significant differences were found between the "no info" group and the "Prosthetics" and "Combined treatment" groups (*p=0.001 and p=0.003*). The mean values of the “Prosthetics” and “Combined treatment” groups were higher than the mean values of the no info group (Table 3).

As a result of the Spearman correlation analysis, a statistically significant, positive and high-level relationship was obtained with a correlation coefficient of 0.735 calculated between the Information score and the Modified DISCERN score (*p<0.05*), (Table 4). The same relationship did not exist between the IC and Modified DISCERN Score and the Content Score.

Table 1. Evaluation parameters of Instagram® Posts' Content and Modified Discern Evaluation Questions.

Content Parameters	Modified Discern Questions
Definition	Does it describe how each treatment works?
Indications	Does it describe the benefits of each treatment?
Contraindications	Does it describe the risks of each treatment?
Advantages	Does it describe what would happen if no treatment were used?
Procedures involved	Does it describe how treatment choices affect overall quality of life?
Complications	Is it clear that there may be more than one possible treatment choice?
Prognosis and survival	Based on answers to the above questions, rate the overall quality of the publication as a source of information about treatment choices
Cost	

Questions

1. "How did his teeth end up like it was before??"
2. "We also want to work and study in the USA. How can we go there?"
3. "Does it take a long time to fit that many veneers?"
4. "Are these porcelain or emax?"
5. "What did she do?"
6. "Great work here bro - all milled same day?"
7. "How much did this cost!?"
8. "Great work but - Why? Not necessary at all!!! Great natural teeth before."
9. "How long does the procedure take?"
10. "Is this dangerous?"
11. "If one of my teeth is crooked, do I need to fix it first before getting veneers? I'm assuming I need Invisalign. I had braces for 6 years so they aren't so bad just a little crook on the bottom set."
12. "What does no prep mean?"
13. "What's the aftercare like?"
14. "Expensive?"
15. "Hi, are there any issues if I'm on blood thinners?"
16. "How long does this process take from start to finish?"
17. "How much are your veneers?"
18. "If it's okay with you, could I design you a logo with a cartoon illustration or a new logo?"
19. "How much is this?"
20. "What was done to her teeth?"
21. "What is the cost for just the upper?"
22. "Which lab do you use, doc?"
23. "How far in advance do you need to book an appt?"
24. "How did his teeth end up like it was before??"
25. "How much is something like this?"
26. "How much does this procedures cost?"
27. "I've always wondered why people usually get veneers on their lower teeth?"
28. "What did they do?"
29. "How much does veneers cost?"
30. "How do I organize a consultation?"
31. "Hello I am a graphic designer, do you need any of my services? note - I'm not a bot, kindly check my pages."
32. "We also want to work and study in the USA. How can we go there?"
33. "How to order?"
34. "How much does it cost?"
35. "What's the difference between bonding and veneers?"
36. "Was it too expensive?"
37. "Do you offer non-prep veneers without grinding down your own teeth?"
38. "How long does the procedure take?"
39. "How many visits did this take?"
40. "Looks great! Which color shade are these?"
41. "How many visits did this take?"
42. "Did she get a deep clean on her teeth first??"
43. "Is zoom whitening expensive on its own?"
44. "That sounds really promising, and who wouldn't want whiter teeth?"
45. "Can you do minimal prep veneers that you've seen so many dentists talk about lately? It's always been more appealing to me to get veneers if I don't have to completely ruin my natural teeth?"
46. "How much does veneers cost?"
47. "What color are these? Om3?"
48. "How long does it take to make this?"
49. "Is it painful having them put on?"
50. "What did he do?"
51. "You are so so talented and beautiful! Are you only on tik tok or do you have a YouTube channel? I just found your page, and damn girl!"
52. "I've always wondered why people usually get veneers on their lower teeth?"
53. "That first slide was hideous. Was it infected?"
54. "Do your teeth need to be straightened before you have veneers?"
55. "How much is it per veneers?

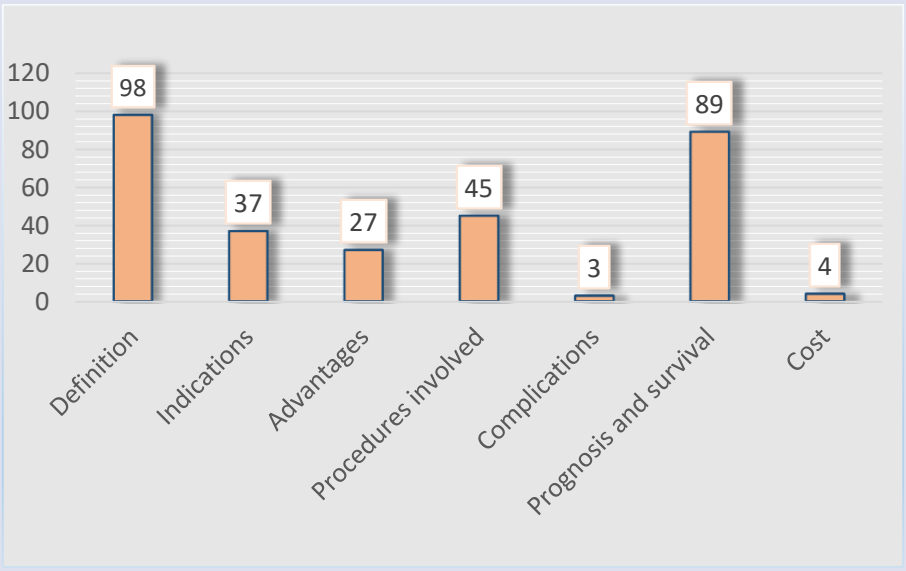

Figure 1: Evaluated Content parameters. (n=109) The numbers indicate the counts of visuals containing the relevant evaluation parameters.

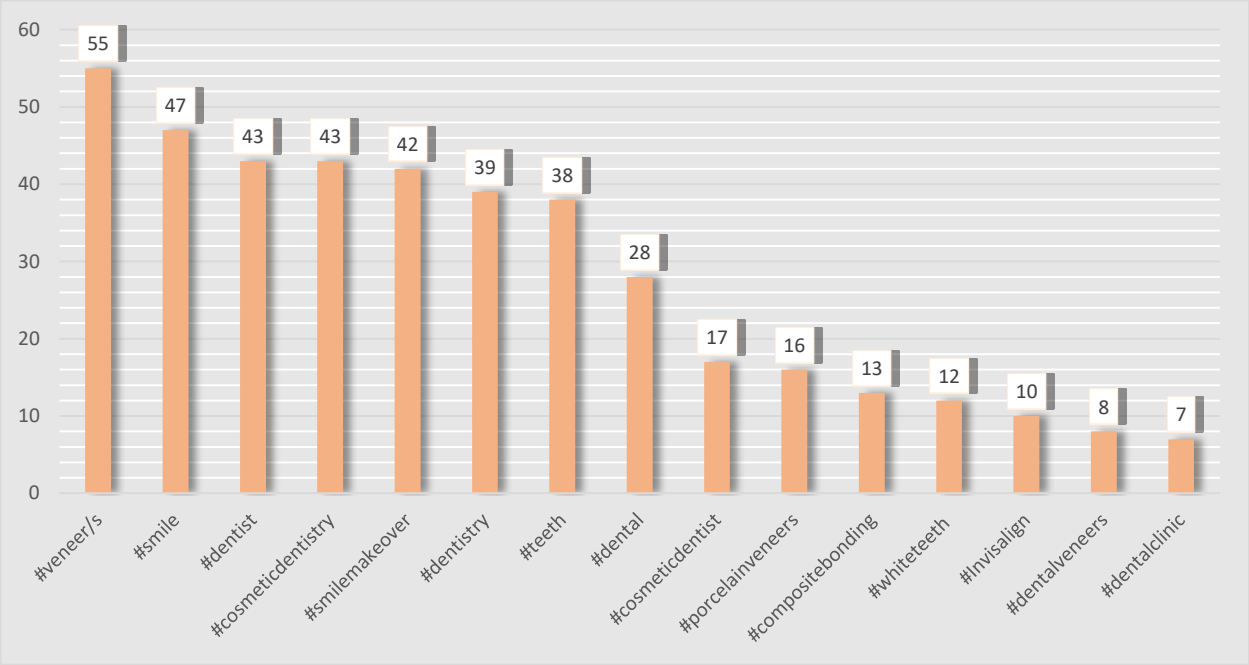

Figure 2: Other mentioned hashtags with #teethwhitening.

Table 2. Demographic distributions by Instagram® account and sharing data.

		n	%
Source of upload	Dentist	52	47.7
	Clinic	39	35.8
	Influencer	5	4.6
	Cosmetics Industry	13	11.9
Number of followers	Nano influencer (1.000-10.000)	56	51.4
	Micro influencer (10.001-50.000)	22	20.2
	Mid-tier influencer (50.001-500.000)	30	27.5
	Macro influencer (500.001-1.000.000)	1	0.9
Treatment method	Prosthetic	40	36.7
	Whitening	30	27.5
	Orthodontics and Prosthetics	6	5.5
	Combined Treatment	6	5.5
	No information	21	19.3
	Other	6	5.5
Content level	0 Point	4	3.7
	1 Point	13	11.9
	2 Point	29	26.6
	3 Point	29	26.6
	4 Point	26	23.9
	5 Point	7	6.4
	6 Point	1	0.9
Content level-group	Low	46	42.2
	Moderate	62	56.9
	High	1	0.9

	n	Minimum	Maximum	Mean	Standard Deviation
Number of followers	109	75.0	552100.0	37709.11	77067.68
Number of likes	109	0.0	8290.0	932.85	1488.39
Number of comments	109	0.0	232.0	19.35	33.41
Time (hour)	109	1.0	720.0	72.15	120.06
Interaction Coefficient	109	0.0	304.5	27.64	61.13
Modified DISCERN	109	7.0	22.0	11.83	3.77

Table 3. Comparison of Interaction Coefficient, and Content and Modified DISCERN scores according to follower numbers and treatment methods.

	Number of followers	n	Mean	Standard Deviation	Rank Mean	Test Statistic	p
Interaction Coefficient	Nano inf.	56	2.41	2.71	39.20	28.932	0.000*
	Micro inf.	22	40.41	69.73	69.77		
	Mid-tier inf.	31	64.17	85.73	73.06		
DISCERN Score	Nano inf.	56	11.39	3.94	50.44	3.273	0.195
	Micro inf.	22	13.27	4.63	64.50		
	Mid-tier inf.	31	11.61	2.38	56.50		
Content Score	Nano inf.	56	2.66	1.42	52.18	1.294	0.524
	Micro inf.	22	3.00	1.20	60.84		
	Mid-tier inf.	31	2.84	1.04	55.95		
							Kruskal Wallis; *p<0.05
							Bonferroni; (p=0.00 and p=0.00)
	Treatment Method	**n**	**Mean**	**Standard Deviation**	**Rank Mean**	**Test Statistic**	***p***
Interaction Coefficient	Prosthetic	40	42.84	74.93	64.80	21.271	0.001*
	Whitening	30	5.52	14.82	36.58		
	Ort.+ Pro.	6	40.46	61.52	83.17		
	Combined Tr.	6	2.29	2.52	41.33		
	No Info	21	40.88	78.48	61.21		
	Other	6	3.17	3.13	45.50		
DISCERN Score	Prosthetic	40	13.50	4.11	68.14	24.713	0.000*
	Whitening	30	10.87	3.03	47.75		
	Ort.+ Pro.	6	12.83	3.37	64.50		
	Combined Tr.	6	15.00	3.29	83.08		
	No Info	21	9.48	2.27	34.74		
	Other	6	9.67	2.88	37.00		
Content Score	Prosthetic	40	3.20	1.02	65.86	29.130	0.000*
	Whitening	30	2.50	1.20	47.02		
	Ort.+ Pro.	6	4.00	0.89	84.83		
	Combined Tr.	6	4.00	1.10	83.00		
	No Info	21	1.90	1.22	35.07		
	Other	6	2.00	1.10	34.42		
							Kruskal Wallis; *(p<0.05)
							Bonferroni; (p=0.003 and p=0.015)
	Content Score	**n**	**Mean**	**Standard Deviation**	**Rank Mean**	**Test Statistic**	***p***
Interaction Coefficient	Low	46	26.41	70.63	23.50	0.000	0.000*
	Medium	62	29.01	54.04	77.50		
							Mann Whitney U; *(p<0.05)

Table 4. Relationships between Interaction Coefficient, and Content score and Modified DISCERN scores.

		Content Score	DISCERN Score
Interaction Coefficient	Rho	0.135	0.139
	p	0.161	0.148
Content Score	Rho		0.735
	p		0.000*

*Spearman correlations; *(p<0.05)*

Discussion

Nowadays, the number of patients who would like to have a good smile and shining white teeth is quite high. Patients think that a good smile can make them appear healthier, happier and more attractive. This situation can sometimes give them an advantage even in finding a job.[24,27] While acknowledging the personal, sociological and psychological benefits of a good smile and shining white teeth, planning treatment procedures completely according to the patient's wishes is also a very important and controversial issue. The aesthetic treatment applications that patients would like may not always be necessary and the right choice. Although the treatment plan is usually made by dentists after the examinations of the patient, the patient's expectations are met within the ethical limits. However, there are multiple treatment options/methods for the desired smile and bright white teeth. It has therefore become important that treatment options and planned procedures are carried out by adhering to ethical principles (Primum non nocere).

In a survey conducted among dentists, it was stated that there was an increase in the demands of patients for

aesthetic dental treatments and that the reason for this was social media. It was reported that patients wanted to have "teeth whitening", "Hollywood smile", "dental veneers", and "Invisalign". This is in line with the data of this study. It was stated that patients requested such procedures because it was a "trend" on social media platforms.[27,28]

In this study, the accounts in which the images were shared belonged to dentists or clinics at a high rate. In addition, it was determined that laypersons also shared such images. (n=18, % 16,5) In the study of Şimşek *et al.*, in which they examined teeth whitening videos uploaded to the YouTube™ platform, the rate of those sharing from non-professional accounts was stated as 60%.[23] Matheus Lotto *et al.* reported that more than half of the participants were regular people in their Instagram® study on fluoride.[29] This rate was lower in this study.

The number of followers of user accounts is a very important factor in reaching the information to different users. The IC used in this study was used to measure the comments and likes received by the posts on an hourly scale. According to the results of the study, the ICs of the accounts with a high number of followers were also high, as expected. However, in quality and content evaluations, the information and content values were low in all of them, without making a comparison between the sub-groups of the number of followers. In the study of Şimşek *et al.*, it was observed that the interaction coefficients of the videos with a low level of information were high, while in this study, the group with a medium level of information score had a higher interaction score than a low level of information.

The purpose of the Instagram® application is not to be an intermediary in presenting right treatment options and methods in health, and not to take responsibility for the correct and high quality of the information and quality of posts. If there are no complaints, the company does not interfere with the posts of users. However, it was also reported by other studies that it could be a direct source of incorrect practices and incomplete information, given the changing conditions of use.[27,29-32] A similar result was found in this study. According to the information scale, the majority of the shares had insufficient information content. Only 3 posts were informed about possible complications. The situations where the procedures are contraindicated were not mentioned in any of the posts. (Figure 1) In the evaluation made according to the modified DISCERN scoring, the mean value of the posts was only 4 points higher than the lowest score. In the Modified DISCERN evaluation, where the lowest 7 and the highest 35 points can be obtained, only 6 images scored 20 and above, while 45 images scored 10 and below. In the last question of the Modified DISCERN Guide, where the general evaluations of the shares are made, the score of 79 images was below 3.

That almost half of the treatment applications in the posts shared under the #teethwhitening hashtag were either only prosthetic or prosthetic procedures as a step of combined treatments (Periodontology, Orthodontics, etc.) is the most important issue that can cause misdirections. Prosthetic approaches, one of the most invasive and operational treatment plans required to have white teeth, show that patients demand a general change not only in tooth color but also in tooth morphology and smile design. In a survey with 502 participants, the total rate of patients who were dissatisfied with the color, shape, position of their teeth and their smile in general was 65%.[24] This general desire for change may lead both dentists and patients to comprehensive general aesthetic prosthetic procedures involving too many teeth.[27] This is also evident from the distribution of other hashtags shared under the study posts, which is another step of the research. The hashtags, which are connected to each other in the interaction network, also refers patients to prosthetic applications or aesthetic and cosmetic areas and to shares that include a comprehensive general change and smile design planning.

In the first questions written under the posts, the users generally wondered about the cost and duration of the process, and how to make an appointment. The most curious issue was the details of the process steps. This was also the case with the low number of posts containing this information in the study. There were also questions such as the durability of the material used, which of the treatment options is good, and which color teeth are made. Only one user emphasized that the operation was unnecessary for a patient who already had natural and beautiful teeth, in order to question the necessity of the operation.

Limitations of the Research

Since this study is a cross-sectional research, evaluations were made within the specified time interval and on a limited number of data. Since the Instagram® platform is a very variable and dynamic platform, information about data may change over time, shares can be deleted by users and new shares can be added. The keyword researched was the most trending teeth whitening hashtag (#teethwhitening) during the study period. The research did not include information about the posts with other hashtags that may be related to the subject. Since only posts in English were taken into account, information about teeth whitening in other languages was not available.

Conclusions

Within the limitations of the study, it can be stated that the information content and quality of the Instagram® posts about teeth whitening were insufficient and may direct patients to more interventional invasive treatment options. Since the normalization of aesthetic perceptions through advanced interventional dental treatments may reveal different health problems in the short, medium and long term, the subject should be supervised by experts in the field. In different dental treatments and other social media applications, patient and dentist communication,

the role of social media in treatment procedures, and demands should be analyzed in detail with further studies.

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Conflicts of Interest Statement

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Determination of the Effect of Periimplantitis-Induced Bone Defects on Implant Stability by Resonance Frequency Analysis Method: An Ex-Vivo Study

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ABSTRACT

Objectives: Periimplantitis is an infectious disease that causes the resorption of the alveolar bone around the implant. This resorption compromises osseointegration by affecting bone-implant contact. This study aimed to determine the effects of experimentally created 3-walled periimplantal defect models at different depths on osseointegration.

Materials and Methods: This study was designed as an ex-vivo study. Fresh bovine ribs were used in this study. A total of 14 dental implants of 3.5x10 mm size were placed on the fresh beef rib, and then periimplantal bone defects of different depths were experimentally created. There are a total of 4 groups in the study, they are respectively; healthy group, 1.5 mm deep defect, 2.5 mm deep defect, and 5 mm deep defect group. For all of these groups, osseointegration was evaluated with the Osstell penguin device using the resonance frequency analysis method from four regions of each implant, mesial-distal buccal palatinal, to determine the osseointegration level according to the amount of bone-implant contact.

Results: While the highest ISQ values were observed in the healthy group, the difference between the other groups and the healthy group was not statistically significant, except for the 5 mm defect group. The results of the 5 mm defect group were significantly lower than those of the other three groups.

Conclusions: It has been observed that there will be a significant decrease in osseointegration according to osstell scores in periimplantal defects with a defect depth of 5 mm.

Key words: Osseointegration, Peri-Implantitis, Resonance Frequency Analysis.

Peri-İmplantitise Bağlı Kemik Defektlerinin İmplant Stabilitesi Üzerine Etkisinin Rezonans Analiz Yöntemiyle Belirlenmesi: Ex-Vivo Çalışma

Süreç

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ÖZ

Amaç: Peri-implantitis, implant çevresindeki alveolar kemiğin rezorbsiyonuna neden olan enfeksiyöz bir hastalıktır. Bu rezorpsiyon, kemik-implant temasını etkileyerek osseointegrasyonu azaltır. Bu çalışma, deneysel olarak oluşturulan 3 duvarlı periimplantal defekt modellerinin farklı derinliklerde osseointegrasyon üzerindeki etkilerini belirlemeyi amaçlamaktadır.

Gereç ve Yöntemler: Çalışma ex-vivo olarak tasarlanmıştır. Çalışmada taze sığır kaburgası kullanıldı. Sığır kaburgası üzerine 3.5x10 mm boyutlarında toplam 14 adet dental implant yerleştirildi ve ardından deneysel olarak farklı derinliklerde periimplantal kemik defektleri oluşturuldu. Çalışmada toplam 4 grup vardır, bunlar sırasıyla; sağlıklı grup, 1,5 mm derinlikte defekt, 2,5 mm derinlikte defekt ve 5 mm derinlikte defekt grubu. Tüm bu gruplar için, kemik-implant temas miktarına göre osseointegrasyon düzeyini belirlemek için ostell cihazı ile her bir implantın mezial-distal bukkal palatinal olmak üzere 4 bölgesinden rezonans frekans analizi yöntemi kullanılarak osseointegrasyon değerlendirildi.

Bulgular: En yüksek ISQ değerleri sağlıklı grupta iken, 1,5 mm derinlikte defekt, 2,5 mm derinlikte defekt grupları ile sağlıklı grup arasındaki fark istatistiksel olarak anlamlı değildi. 5 mm defekt grubunun sonuçları diğer 3 grubun sonuçlarına göre anlamlı derecede düşük bulundu.

Sonuçlar: Defekt derinliği 5 mm olan periimplantal defektlerde osstell skorlarına göre osseointegrasyonda anlamlı bir azalma olduğu saptandı.

Anahtar Kelimeler: Osseointegrasyon, Peri-Implantitis, Rezonans Frekans Analizi.

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Introduction

Periimplantitis, defined as an inflammatory disease of the tissues around an implant, is one of the most important inflammatory diseases that affect the long-term success of dental implant treatment. Periimplantitis is characterized by bleeding on probing and marginal bone loss with a pocket depth of more than 4 mm around the implant.[1]

The diagnosis of periimplant bone loss is made using periapical radiographs, but these radiographs do not always provide clear results, especially in imaging initial bone loss.[2] The early detection of periimplantitis in clinical examinations is key to bone loss prevention and periimplant health. any studies that the radiological determination of periimplant bone loss is difficult, especially in the early period; to overcome this difficulty, a search was made for diagnostic devices that can make more precise measurements, which are easy to measure and repeatable.[3,4]

Approximately 20 years ago, Meredith *et al.* developed a resonance frequency analysis (RFA) method to determine osseointegration. The OsstellTM is a manufactured tool for measuring implant stability using RFA. (SmartpegTM; Osstell, Gothenburg, Sweden). Recently, a mobile device called PenguinTM (MultipegTM; Penguin Integration Diagnostics, Gothenburg, Sweden) was developed for this purpose. Using this method, both instruments can measure the implant stability quotient (ISQ) of implants.[5]

In studies on the RFA method, it has been stated that the amount of bone-implant contact is the main determinant method.[6-9] However, studies on how much the ISQ value will change in bone loss, especially in the periimplantal region, are limited.

The purpose of this study was to investigate whether ISQ values can predict periimplantal defects with different morphologies.

Material and Methods

Ethical approval of this study was approved by Nuh Naci Yazgan University Scientific Research and Ethics Committee (2022/001-006). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and the Helsinki Declaration of 1975, as revised in 2008.

The current study was a planned ex-vivo study. Fresh, young beef ribs were used in this study. The Bovine Tissues were not frozen and were used fresh directly for the study. The ribs were stabilized during drilling. The soft tissues and periosteum were carefully dissected using with blunt dissection and the bone surface was exposed. The implant sockets were then prepared by a single operator using the drilling protocols of the manufacturer's instructions. The bovine rib was morphologically similar to the type III–IV bones. While the coronal (2–3 mm) portion was cortical bone, the bone in the apical region of the implant consisted mostly of spongiosa.[10]

When ex vivo studies using bovine ribs related to periimplantitis were examined in the literature. Miotk *et al.* In a study of periimplantitis diagnostic accuracy in CBCT, Minsua *et al.* in which they measured buccal bone thickness, and experimental periimplantitis studies by Yao *et al.* showed that the model we used in the current study concept is a frequently preferred study model.[11-13]

Periimplant defects were created at different depths with trephine burs after osteotomy *(Figure 1(a-c))*. 4 groups in total are used in the study; they are as follows: Group 1: Control group, group 2:3-walled defect with a 1.5 mm defect depth, group 3:3-walled defect 2.5 mm defect depth and group 4:3-walled defect 5.0-mm defect depth. In 2,3 and 4. group defects were created in the vestibule walls. In determining the morphology of periimplant defects reference studies based on Monje *et al.* were taken into account in *Table 1.*[14]

At the each of groups, after placement of the implants (n=14) (SLA surface, conical geometry, V-shaped, 3.5 x10 mm; Nucleoss T6, İzmir/ Türkiye) from the mesial, distal, palatal vestibule surfaces of each implant, Penguin TM device (MultipegTM; Penguin Integration Diagnostics, Gothenburg, Sweden) made 4 consecutive ISQ measurements.

The mean of the four values was calculated as the final ISQ of each implant. All data were analyzed using descriptive methods. A two-sample t-test was used to compare the mean differences between the two groups. The Wilcoxon rank sum test and Kruskal–Wallis test were used to analyze non-parametric data. To compensate for multiple testing situations, the Mann–Whitney U-test was applied, and p-values were corrected using the Bonferroni adjustment procedure and compared with an alpha level of 0.05. All statistical analyses were conducted by using SPSS version 21 software (SPSS, Chicago, IL, USA)

Table 1. Distribution of study groups

Defect Depth Type of defect	Group 1: Control	Group 2: Depth :1.5 mm (≤%25 of implant length)	Group 3: Depth:2.5 mm (≤%25-50 of implant length)	Group 4: Depth :5 mm (≥50 of implant length)
No Defect	n= 14	-	-	-
There walls	-	n=14	n=14	n=14

Figure 1: a: Preparation of implant bed, b: Implant placement, c: ISQ measurement with Ostell Penguin TM (MultipegTM; Penguin Integration Diagnostics, Gothenburg, Sweden), d: Experimentally created periimplantal defect with 3 wall depth 2. 5 mm, e: Experimentally created periimplantal defect with 3 wall depth 3. 5 mm, f: Experimentally created periimplantal defect with 3 wall depth 5 mm.

Table 2. Statistical Comparison of the control and bone defects score of ISQ Among the Four Groups

	Total (n=56)	Group 1 (n=14)	Group 2 (n=14)	Group 3 (n=14)	Group 4 (n=14)	p value
Ostell	72.28±4.53	74.68±1.9[a]	74.29±1.87[ac]	74.75±1.64[ace]	65.39±2.97[bde]	0.0001

Results are expressed as mean – standard deviation.
There are no significant differences between the averages shown with the same letter in the same row (Tukey's HSD).

Results

The ISQ value measurement methodology was developed using the average of four ISQ measurements taken from various surfaces of each sample (mesial-distal, palatinal, and lingual). The results of the ISQ measurements are displayed in Table 2, according to statistical assessments.

There were no statistically significant differences between Groups 1, 2, and 3. There was a statistically significant difference between group 4 and all other groups ($p<0.001$).

Discussion

Implant stability is primarily determined by bone-implant contact, which has been noted in numerous studies. Although a connection exists between the degree of bone-implant contact (BIC) and ISQ scores, this connection is not well understood.[8,15] Some researchers suggest that there is no direct relationship between BIC and ISQ scores because the viscoelastic structure of the alveolar bone makes it difficult to predict how the bone will react to mechanical stimulation. Additionally, it should be remembered that the BIC may also be impacted by the bone's mineral density and histological structure. Studies have revealed, for instance, that cortical bone makes a greater contribution to ISQ values than spongiosum bone.[16,17]

Ito *et al.* evaluated RFA scores in defects at different depths, which represent different amounts of bone loss. The findings of this study indicated that the most substantial reduction in RFA occurred when the screws in the implants' most coronal regions were loosened, whereas there was no discernible difference when the screws in the implants' more apical regions were loosened.[8]

Shin *et al.* examined the impact of the defect type and depth on implant stability. The cortical bone thickness in the current investigation ranged from 2.7 to 3.18 mm, and circumferential defects between 2.5 and 5 mm were produced around the implant. The ISQ values in the 5 mm defects were found to be substantially lower than those in the control and 2.5 mm defects. These results led the author to the conclusion that implant stability and ISQ values are decreased by cortical bone loss.[18]

In a cadaveric study, Turkyilmaz *et al.* found a linear association between the development of vertical defects and the loss of bone around implants.[19]

In the current study, 3 walled bone defects of four different sizes were created to represent different amounts of bone loss (bone destruction was experimentally created from the vestibule). The study's findings showed that for every 1 mm change in the ISQ score, there was an average decline of 2.7 mm. Loss in the first 2 mm of the coronal bone led to a significant decrease.

The morphological features of periimplantal defects have been extensively investigated in previous studies.[20-22] One of the first studies on this subject was by Schwarz *et al.* They described the periimplantitis defect configuration[23] Then Monje et al modified it. In this study, we used the classification proposed by Monje *et al.* The classification is as follows:

Class I: Infraosseous defect Class Ia: Buccal dehiscence,

Class Ib: 2-3 walls defect, Class Ic: Circumferential defect,

Class II: Supracrestal/horizontal defect,

Class III: Combined defect; 2-walled, 3-walled, peripheral bone defects have been observed. In addition, Monje *et al.* determined that the defects originating from periimplantitis were mostly in the 3-walled Clas1b type.[14]

The minimum ISQ score was used for osseointegration. According to studies by Sennerby and Meredith, loading implants requires a minimum ISQ value of 65, and ISQ values lower than 45 are highly likely to result in loss of osseointegration.[24]

Three limitations of the current study were previously planned ex vivo. These results included one type of bone: a cow-bone-like type 3 bone. If we study different types of bones, the results of the study could be different from those of the current study. Second, we only investigated the defects of the three walls. Within the scope of this study, other periimplantal defects weren't included in the study. Implant stability was determined using only one parameter. If we had one more parameter for implant stability measurement, the results of this study would be more powerful.

Conclusions

If the depth of the periimplant defect is equal to or higher than ½ of the total implant length, the RFA scores significantly decreased, and the risk of losing the implant increase.

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Conflicts of Interest Statement

The authors declare that they have no conflicts of interest.

Author Contributions

Protocol development: TEK.
Data collection and analysis: TEK and OK.
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Comparison of the Effect of the Same Polishing Method on the Surface Roughness of Conventional, CAD/CAM Milling and 3D Printing Denture Base Materials

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ABSTRACT

Objectives: The aim of this study was to evaluate the effect of both the same polishing method and those with and without thermal aging on the surface roughness of conventional, CAD/CAM milling and 3D printing denture base materials.

Materials and Methods: A total of 30 round shaped specimens were obtained by 3 different methods: Conventional, CAD/CAM milling and 3D printing. After applying the same polishing technique to all groups, surface roughness values were measured. Profilometer device was used for surface roughness measurement. Then, after the thermal aging of all samples, surface roughness values were measured and the roughness values between no-thermocycling and thermocycling were compared. Tukey, Mann Whitney U and Kruskal Wallis tests were used statistically. P values of ≤ 0.05 were considered significant.

Results: As a result of the same polishing process, there was a difference in surface roughness in all groups. While the highest surface roughness values were seen in 3D printing, the lowest roughness value was seen in the CAD/CAM milling and was statistically significant. Thermocycling did not show a statistically significant difference in surface roughness.

Conclusions: The same polishing process caused different surface roughness values in the denture base materials obtained with different methods, and the lowest surface roughness value was seen in the CAD/CAM milling.

Key words: Denture base material, CAD/CAM Milling, 3D Printing, Polishing, Surface Roughness.

Konvansiyonel, CAD/CAM Kazıma ve 3D Baskılı Protez Kaide Materyalleri Üzerine Uygulanan Aynı Polisaj Yönteminin Yüzey Pürüzlülüğüne Etkisinin Karşılaştırılması

Süreç

Geliş: 21/06/2023
Kabul: 21/08/2023

ÖZ

Amaç: Bu çalışmanın amacı, hem aynı cilalama yönteminin ve hem de termal yaşlandırma işlemi yapılan ve yapılmayanların geleneksel, CAD/CAM kazıma ve 3D baskılı protez kaide materyallerinde yüzey pürüzlülüğüne olan etkisini değerlendirmektir.

Gereç ve Yöntemler: Konvansiyonel, CAD/CAM kazıma ve 3D baskılı olmak üzere 3 farklı yöntemle toplam 30 adet yuvarlak numune elde edildi. Tüm gruplara aynı şekilde polisaj tekniği uygulandıktan sonra yüzey pürüzlülük değerleri ölçüldü. Yüzey pürüzlülük ölçümü için profilometre cihazı kullanıldı. Daha sonra tüm örneklere temal yaşlandırma yapıldıktan sonra yüzey pürüzlülük değerlerinin ölçümü yapıldı ve termal işlem yapılmamış ve termal işlem yapılmış örnekler arasındaki pürüzlülük değerleri karşılaştırıldı. İstatistiksel olarak Tukey, Mann Whitney U ve Kruskal Wallis testleri kullanıldı. ≤ 0,05 olan p değerleri anlamlı kabul edildi.

Bulgular: Aynı polisaj işlemi sonucunda tüm gruplarda yüzey pürüzlülüklerinde farklılık görüldü. En yüksek yüzey pürüzlülük değerleri 3D baskıda görülürken, en düşük pürüzlülük değeri CAD/CAM kazımada görüldü ve istatistiksel olarak anlamlı bulundu. Termal siklus, yüzey pürüzlülüğünde istatistiksel olarak anlamlı bir fark göstermedi.

Sonuçlar: Aynı polisaj işlemi farklı yöntemlerle elde edilen protez kaide malzemelerinde farklı yüzey pürüzlülük değerlerine neden olmuş ve en düşük yüzey pürüzlülük değeri CAD/CAM kazımada görülmüştür.

Anahtar Kelimeler: Protez Kaide Malzemesi, CAD/CAM Kazıma, 3D Baskı, Parlatma, Yüzey Pürüzlülüğü.

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Introduction

Polymethyl methacrylate (PMMA) is the most widely used denture base material (DBM). It is still the main material used because of acceptable aesthetic results, ease of use and manipulation, non-toxicity, ease of repair and polishing. Despite its many advantages, PMMA resin has low flexural strength and low flexibility, so different methods and materials suitable for these methods have been developed.[1,2] With the advancement of technology, computer-aided design and computer-aided manufacturing (CAD/CAM) systems have started to take their place in the field of dentistry. Prostheses produced with CAD/CAM have many clinical advantages such as eliminating laboratory steps, saving time, eliminating traditional impression methods, minimizing the possibility of cross contamination, and making prosthesis in a single session compared to conventionally produced prostheses.[3,4] CAD software is used to design the prosthesis. The design is transferred to the CAM program and produced. There are two types of CAD/CAM production. One of them is subtractive (milling) and the other is additive (printing).[5] The base resin produced with both CAD/CAM and 3D printing produces a more accurate denture base than the base resin produced with the conventional technique.[6]

Surface roughness is defined as small irregularities that affect surface wetting, adhesion quality and shine quality. The surface roughness of the DBM is an important factor that directly or indirectly affects the microbial plaque formation and bacterial adhesion on the tissue surfaces of the prosthesis. Rough surfaces cause bad breath and are more vulnerable to discoloration than smooth surfaces. The roughness of the DBM is affected by the structural feature of the material, the polishing method and oral hygiene.[3,6,7]

According to ISO 4287 standards, surface roughness symbol 'Ra' unit is determined as 'µm'. Studies have indicated that a roughness of 0.2 µm can be obtained with finishing and polishing processes in laboratories. For this reason, it is imperative to finish and polish the prosthesis in order to minimize the surface roughness of the prosthesis surfaces.[7,8]

Evaluation of physical and mechanical properties is essential in determining the durability and success of these materials. For this reason, thermal cycling is used in laboratory studies to imitate the oral environment by changing the temperature.[6,9,10]

The purpose of this study was to evaluate the effect of the same polishing method on the surface roughness of DBMs manufactured by conventional, CAD/CAM milling and 3D printing before and after thermocycling. The null hypotheses were that the same polishing technique would not effect on surface roughness of the conventional, CAD/CAM milled and 3D-printing DBM, that thermocycling would affect the surface roughness.

Material and Methods

In this study, the surface roughness of the DBMs manufactured by conventional, CAD/CAM milling and 3D printing was evaluated with no-thermocycling (NT) and thermocycling (T). In addition, the effect of the same polishing method on the surface roughness was also evaluated.

In the study, DBMs manufactured by 3 different methods were used: Heat cured acrylic resin (Meliodent, Kulzer, Germany) in the conventional method, prepolymerized pink acrylic block (Yamahachi, Yamahachi Dental Mfg, Japan) in the CAD/CAM milling method and fluid resin (MACK4D, Dentona, Germany) in the 3D Printed method. In order to evaluate the surface roughness measurement in the study, a total of 30 samples were prepared (n=10), in the form of discs with a diameter of 20 mm and a thickness of 2 mm (Figure1).

Metal molds were used to obtain the samples with the conventional method. First, samples were obtained by dripping wax into these molds. After the samples were muffled, negative spaces were formed by melting them. Then, heat polymerized acrylic resin (Meliodent, Kulzer, Germany) was prepared and polymerized according to the manufacturer's instructions. The muffle was placed in boiling water for the polymerization process. After the heat source was turned off, the muffle was kept in hot water for 15 minutes. Then the heat source was turned on again and the water was boiled for 20 minutes. Then It was allowed to cool slowly.

For the samples to be obtained by the CAD/CAM method, at first the design was made in the form of a disc (20mm diameter, 2mm thickness) in the CAD program (Solid Works 2022, Dassault Systemes S.A, service pack 5.0, France). After the design file was transferred to the CAM device (Redon Hybrid Full, İstanbul, Turkey), the samples were obtained by milling.

For the samples to be obtained with a 3D-Printer, at first a disc-shaped sample was designed with AutoCAD software (Autodesk, USA). The samples was created using a 3D printer (Free Shape 120 Printer, Ackuretta, China) and MACK4D resin (Dentona, Germany). After the samples were obtained, they were cleaned by keeping them in isopropyl alcohol for 5 minutes in an ultrasonic cleaner (Ackuretta Cleaning Kit, Taiwan). Then, final curing was performed in a UV light curing device (Ackuretta UV Oven, Taiwan) with a wavelength of 405 nm for 3 minutes.

After all samples were obtained, the excess was trimmed with a tungsten carbid bur. Then, one surface of the samples was sanded by one operator with 100, 120, 400, 600 grit abrasive paper (Atlas, England) respectively for 30 seconds under water. The specimens were then ultrasonically cleaned for 5 minutes to remove debris. Polishing paste (Universal Polishing; Ivoclar Vivadent) was applied for 90 seconds with a felt attached to the polishing motor (Schütz Dental, Germany) on one surface of the prepared samples. After each polishing, the sample was washed ultrasonically for 5 minutes and the residual polishing pastes were removed. After all samples were kept in distilled water at 37 ± 1°C for 48 ± 2 hours, surface roughness measurements of the polished surfaces were made. Profilometer device (Mitutoyo/Kawasaki, Japan) was used to obtain the measurement values (Figure 2). While making the measurements, measurements were made from three different points of the sample surfaces and the surface roughness value (Ra) was obtained by taking the average. All samples for which initial measurements were made were thermocycled for 5000 cycles with a dwell time of 30 seconds in water at 5°C and 55°C (Gökçeler Machinery, Turkey). After the thermal aging process, measurements were made from three different points by using a profilometer device for the final measurement values of all samples and Ra values were obtained by taking the average. The surfaces of the NT and T

samples were examined under a scanning electron microscope (SEM) (Tescan MIRA3 XMU, Brno-Kohoutovice, Czech Republic) (x5000 magnification).

The obtained data were analyzed with statistical software (IBM SPSS Statistics, v22.0; IBM Corp). Analysis of variance was performed when parametric test assumptions were fulfilled in the evaluation of the data. As a result, Tukey test was used to find the groups that made a difference when the significance decision was made, and the Mann Whitney U test was used to find the groups that made a difference as a result of the Kruskal Wallis test when the parametric test assumptions could not be fulfilled (p=0.05).

Results

The mean surface roughness and standard deviation values of the samples in the study are shown in Table 1. The roughness values of same polishing on the CAD/CAM milling were significantly lower than 3D printing and Conventional in NT (p=0.024) and T (p=0.044). While the lowest surface roughness values were found in CAD/CAM milling after same polishing, the highest value was found in 3D-Printing. There is no statistically significant difference in surface roughness each groups between NT and T. However, when the values are examined, a decrease in surface roughness was observed after the thermocycling in other groups except for CAD/CAM milling. It was observed that the surface roughness values in all groups had a statistically significant affected by same polishing, while it was not affected by the thermocycling. The SEM images from all groups with no-thermocycling and thermocycling after same polishing are showed in Figures 3,4,5.

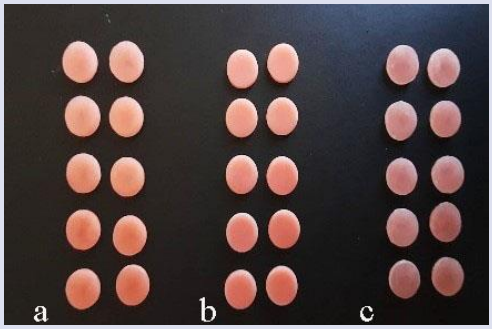

Figure 1: Samples obtained by different methods; a: CAD/CAM milling, b: 3D Printing, c: Conventional.

Figure 2: Profilometer device and surface roughness measurement.

Table 1. Surface roughness values (µm) of all groups no-thermocycling and thermocycling

Groups	No-thermocycling X±SS(µm)	Thermocycling X±SS(µm)	
Conventional	0.32±0.10[a]	0.30±0.05[c]	t=1.13 p=0.282
CAD/CAM Milled	0.24±0.6[a.b]	0.25±0.04[c.d]	t=1.16 p=0.276
3D Printing	0.35±0.12[b]	0.32±0.09[d]	t=0.51 p=0.617
	F=4.22 p=0.024*	F=3.79 p=0.044*	

*The difference between the means shown with the same lowercase letter in the vertical direction is statistically significant (p<0.05)

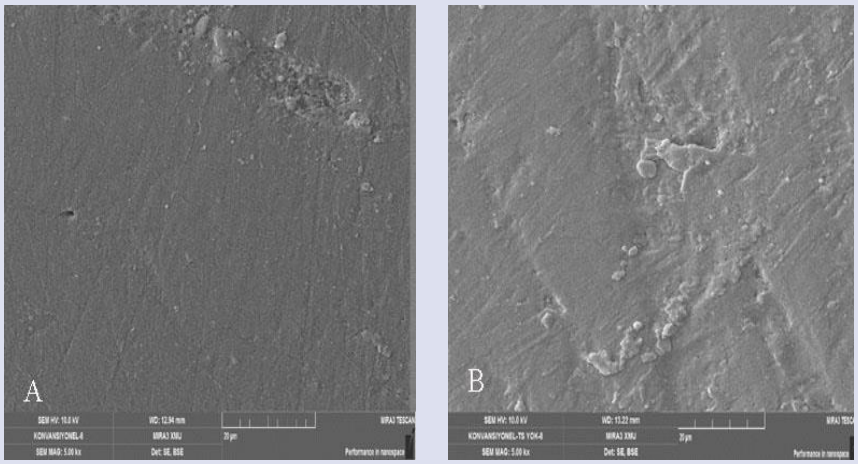

Figure 3: Image of surface properties of samples obtained by Conventional at x5000 magnification under SEM. A: No-thermocycling, B: Thermocycling.

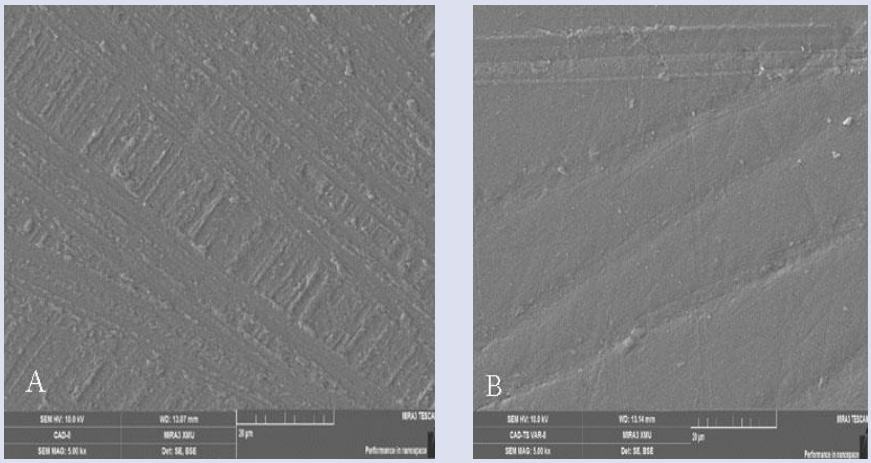

Figure 4: Image of surface properties of samples obtained by CAD/CAM milling at x5000 magnification under SEM. A: No-thermocycling, B: Thermocycling.

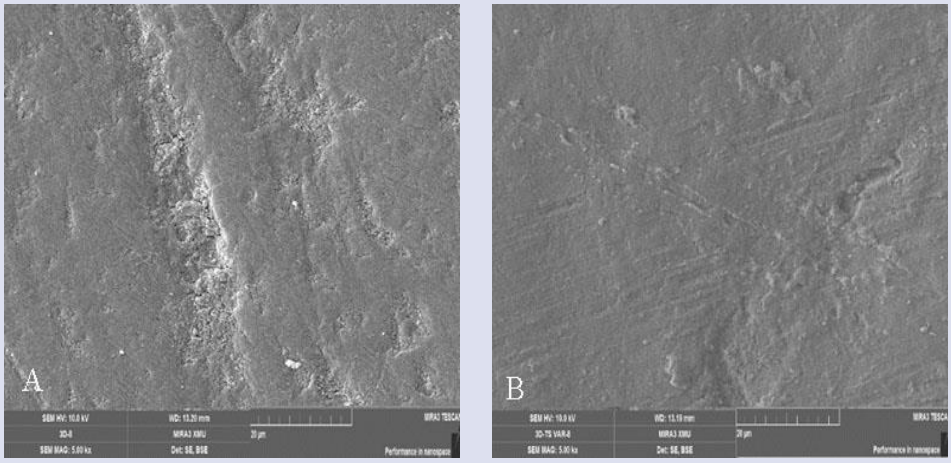

Figure 5: Image of surface properties of samples obtained by 3D printing at x5000 magnification under SEM. A: No-thermocycling, B: Thermocycling.

Discussion

The effect of both the same polishing method on the denture materials produced by conventional, CAD/CAM milling and 3D printing and the thermocycling on the surface roughness were evaluated. The null hypotheses, that no difference would be found in the surface rougness of the Conventional, CAD/CAM milled and 3D printing resins, that thermocycling would affect the surface roughness, and that same polishing would not affect the surface roughness, were rejected.

The surface roughness of the materials is very important since the base materials are related to the oral tissues.[11] The surface roughness of DBMs is an inherent physical property that varies depending on the person's dexterity, polishing method and the structure of the material.[3,12] Polishing increases the smoothness of acrylic resins. Polishing protocols must be chosen correctly to avoid high roughness protocols. If the current protocol does not affect the Ra values after polishing, this indicates that the polishing protocol is not suitable for each type of denture base.[13] For this reason, in study, we aimed to see how the same polishing technique would affect the base materials obtained by different methods.

In the study, while 3D printing showed a high Ra value, smoother surface in CAD/CAM milling was similar to Helal *et al.*[14] and Gad *et al.*[15] studies. The increase in roughness in 3D printing may be due to decreased polymerization degree and monomer leakage and increased porosity.[16] 3D printing produces progressive edges between layers in addition to layered printing.[16-18]

In the study of Murat *et al.*[19] comparing the surface roughness values of the base materials obtained by the CAD/CAM milling method and the heat polymerized method, the Ra values were measured after applying the thermal aging process to the samples. In the results obtained, it was seen that the CAD/CAM milling method showed lower values than heat-polymerized acrylic resins. In the study of Al-Fouzan *et al.*[20], they found that the samples produced by the CAD/CAM milling method showed less surface roughness than conventional heat-polymerized acrylics. It is similar to the results of the above studies.

Freitas *et al.*[21] In the study in which the surface roughness values of the base materials produced by heat polymerized, CAD/CAM milling and 3D printing methods were examined, they concluded that the samples produced by the 3D printing method had the highest surface roughness value. In the study by Fiore *et al.*[13], heat cured acrylic resin, CAD/CAM milling method and 3D printing method were compared and stated that CAD/CAM had the lowest roughness. Meirowitz *et al.*[22] also stated in their study that CAD/CAM milling had the lowest roughness value. The above studies show parallelism with our study. In our study, CAD/CAM samples showing lower Ra values compared to other groups were confirmed in the images at x5000 magnification under SEM (Figure 3,4,5).

In the study, it was determined that the thermal cycle increased the surface roughness in CAD/CAM milling, but no significant result was obtained, and although it decreased in conventional and 3D printing, there was no significant difference. Gad *et al.*[15] ınterlayer thicknesses may vary in 3D printing due to temperature changes and changes in water absorption during the thermal cycle. The thickness of each layer indicates that it has a great influence on the surface roughness. however, reducing the layer thickness reduces the surface roughness but increases the machining time.[23,24] We can also say that the polishing method used can make a difference in roughness.

Limitations of the study include that the in vitro situation does not fully reflect the clinical situation. In addition, polishing by the operator may affect the roughness. The denture base materials obtained by different methods have different properties and a single polishing method should not be seen as a definitive result. Different polishing methods should also be tried and compared.

Conclusions

Based on the findings of this in vitro study, the following conclusions were drawn:

1. CAD-CAM milled showed lower surface roughness than conventional and 3D-printing denture base specimens both before and after thermocycling.
2. Thermocycling did not cause a statistically significant effect on surface roughness.

After the same polishing applied, the lowest surface roughness was seen in the CAD/CAM milled, while the highest value was seen in 3D Printing.

Acknowledgments

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Conflicts of Interest Statement

The authors declared that there is no conflict of interest.

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Effect of Brushing with Whitening Toothpaste on Color Stability and Surface Roughness of Color-Adjustment Resin-Based Composites

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Research Article

History

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ABSTRACT

Objectives: Aim of this study is to compare the color stability and surface roughness of four different color-adjustment composites produced using smart chromatic technology and a highly esthetic nano-hybrid composite after brushing with whitening toothpaste**.**

Materials and Methods*:* 4 different color adjustment composites and 1 nano hybrid composite are divided into 5 main groups according to their contents. A total of 120 disc-shaped specimens (8 x 2 mm) were prepared, 24 in each group. The prepared samples were sequentially kept in solutions (tea, coffee and cherry juice) for 12 days and randomly divided into 3 subgroups (n=8). Subgroup 1, brushing with distilled water; Subgroup 2, brushing with whitening toothpaste; Subgroup 3, home bleaching agent were applied (14 days). Color measurements were made with a spectrophotometer, ΔE values were calculated using the CIELAB formula. Surface roughness values were made using a profilometer device. Samples from each group were selected for SEM surface analysis.

Results: Among the composite groups, the highest coloration was observed in the Omnichroma, while the lowest ΔE values were observed in the Vittra composite group. Although the ΔE values on the 14th day were higher than the 7th day in all groups, the differences between them were statistically insignificant. In all composite groups (except Omnichroma), the ΔE values of brushing with whitening toothpaste at the end of the 14th day were higher than brushing with distilled water.

Conclusions*:* Although the application of home bleaching agent was found to be more effective in a short time in whitening color-adjustment composites; brushing with whitening toothpaste at the end of the 14th day was found to be as effective as the application of home bleaching agent. Coloring and bleaching procedures applied to color-adjustment restorative materials did not have a significant negative effect on surface roughness values.

Key words*:* Color Adjustment, Roughness, Color Stability, One Shade Composites.

Beyazlatıcı Diş Macunu ile Fırçalamanın Renk Uyumlu Rezin Bazlı Kompozitlerin Yüzey Pürüzlülüğüne ve Renk Stabilitesine Etkisi

Süreç

Geliş: 21/06/2023
Kabul: 04/08/2023

ÖZ

Amaç: Bu çalışmanın amacı yüksek estetiğe sahip bir nanofil kompozit ve akıllı kromatik teknoloji kullanılarak üretilen dört farklı renk uyumlu kompozitlerin beyazlatıcı diş macunu ile fırçalama sonrası renk stabilitesinin ve yüzey pürüzlülüğünün karşılaştırmalı olarak değerlendirilmesidir.

Yöntemler: Çalışmada 4 farklı renk uyumlu kompozitler ile bir supra nanofil (kontrol) kompozit içeriklerine göre 5 gruba ayrıldı. Her grupta 24 adet olmak üzere toplam 120 disk şeklinde örnek (8 x 2 mm) hazırlandı. Hazırlanan örnekler 12 gün boyunca sırayla solüsyonlarda (çay, kahve ve vişne suyu) bekletildi ve rastgele 3 alt gruba ayrıldı (n=8). 14 gün boyunca 1. alt gruba distile su ile fırçalama, 2. alt gruba diş macunu ile fırçalama, 3. alt gruba ise ev tipi beyazlatıcı ajan uygulaması yapıldı. Renk ölçümleri spektrofotometre ile yapıldı, ΔE değerleri CIELAB formülü kullanarak hesaplandı. Yüzey pürüzlülük değerleri profilometre cihazı kullanılarak yapıldı. SEM Analizi Yüzey analizinin yapılması için her gruptan seçilen örnekler incelendi.

Bulgular: Kompozit grupları arasında en fazla renklenme Omnichroma kompozit grubunda görülürken, en düşük ΔE değerleri Vittra kompozit grubunda görülmüştür. Tüm gruplarda 14. gün ΔE değerleri 7. güne göre yüksek çıkmasına rağmen, aralarındaki farklar istatistiksel olarak önemsiz bulunmuştur. Tüm kompozit gruplarda (Omnichroma hariç) 14. Gün sonunda beyazlatıcı diş macunu ile fırçalama ΔE değerleri, distile su ile fırçalamaya göre daha yüksek değerler elde edilmiştir.

Sonuçlar: Renk uyumlu kompozitlerin beyazlatılmasında, ev tipi beyazlatma ajanı uygulanması kısa sürede daha etkili bulunmasına karşın, 14. gün sonunda beyazlatıcı diş macunu ile fırçalama ev tipi beyazlatma ajanı uygulanması kadar etkili bulunmuştur. Renk uyumlu restoratif materyallere uygulanan renklendirme ve beyazlatma prosedürleri yüzey pürüzlülük değerlerinde kayda değer negatif bir etki yapmamıştır.

Anahtar Kelimeler: Renk Uyumlu, Pürüzlülük, Renk Stabilitesi, Tek Renk Kompozit.

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Introduction

Composite resins, which have a wide range of applications in Restorative Dentistry, are continuously being developed and updated according to the needs and demands of patients. Advances in composite resins have increased patients' interest in aesthetic dentistry by enabling the creation of aesthetically pleasing restorations that resemble natural teeth.[1] In recent years, color-adjustment resin-based composites, which can match all shades with a single shade, have been produced to facilitate the color selection procedure, reduce the time spent on the patient's chair, and minimize the variables that depend on the dentists.[1-4] Different methods are used to obtain various colors during the production of composites. In the chemical color matching method, color is obtained by adding dyes and pigments. In the structural color matching method, color is obtained using inorganic fillers of the same size without pigments or dyes. Besides in the blending method, there is an optical interaction of the dental materials used.[3,5,6] In restorative dentistry, it is based on the principle of imitating the colors of the surrounding structures of the material known as the "chameleon effect mixing effect" in the production of color-matched resin-based composites. Resin composites should be translucent character that transmits light in be able to have a blending effect with the surrounding tooth structure.[1,7,8]

In the development process of composite resins, the different properties they acquire can cause changes in the surface structure of the material and respond differently to aging factors. Discoloration may be due to intrinsic and extrinsic reasons causes as one of the most common aging factors in composite resins.[9-11] Factors causing intrinsic discoloration are related to the more chemical properties of the composite, such as the structure of the resin matrix, matrix-particle interface, size and volume of filler particles. External discoloration is associated with physical factors such as oral hygiene, occupational factors, smoking, and contact with food and drink.[12,13] Various studies examining the color stability of composite resins have reported that different beverages (such as coffee, tea, wine, cola, fruit juice, etc.) and mouthwash solutions have varying degrees of staining effects.[14,15] According to these studies, the highest discoloration was observed in samples immersed in coffee, followed by tea, red wine and cola.[16] A wide variety of methods are used to detect color changes, including visual methods and measurement methods using different instruments.

Currently, color measurement devices used in dentistry include colorimeters, spectroradiometers, spectrophotometers, and digital cameras.[17,18] Spectrophotometers or colorimeters, which provide numerical results with low error rates, are mainly used for color evaluation.[19] Colorimeters are low cost and easy to use color measurement devices, but they are not suitable for use in dental practice as they are designed to measure flat surfaces.[20] On the other hand, spectrophotometers are mainly used in more professional fields, such as scientific research, color identification, and quality control. In addition, spectrophotometers can discriminate and diagnose metamerism, distinct to colorimeters.[17] The two most commonly used systems for calculating color changes are reported to be the CIE L*a*b* and CIEDE 2000 systems. When examining studies related to dental materials, it is stated that the CIE L*a*b* system is preferred and is the most commonly used color measurement system in the literature for investigating the color differences of materials.[21]

Many whitening techniques have been proposed to remove discoloration and prevent aesthetic problems in teeth and composite restorations. In addition to applying whitening agents with different chemical compositions, mechanical methods such as tooth brushing can also be used to remove discoloration.[22, 23] Whitening toothpastes contain agents such as abrasives, surfactants, calcium chelators, enzymes, and polymers to clean teeth and remove discoloration. Depending on the mechanisms of action of the agents they contain, toothpastes can provide a whitening effect on teeth through physical, chemical, or optical means. Physical agents such as abrasives and chemical agents such as peroxides can be used together to achieve a synergistic effect.[24]

The surface properties of composite resin materials may be altered or compromised when brushed with toothpaste. Abrasive particle sizes in toothpaste are important factors in the deterioration of the type of toothbrush used and the composite resin used.[25] Studies have reported that brushing restorative materials with toothpaste can affect the surface roughness values of the materials.[26] In addition to quantitative methods such as mechanical (two-dimensional) and optical (three-dimensional) profilometry techniques that can measure surface roughness, qualitative methods such as AFM and SEM are also used to evaluate surface structures of composite resins. Many researchers recommend using multiple techniques to examine the surface structures of composite resins.[26]

The aim of this study is to investigate the effect of brushing with whitening toothpaste on the color stability and surface roughness of color-adjustment resin-based composites after staining due to beverage consumption. The null hypothesis of this study was that there would be no significant difference in color stability and surface roughness between brushing color-adjustment resin composites with whitening toothpaste and applying home bleaching agent.

Material and Methods

Preparation of Samples

Ethics Committee approval dated 17.11.2021 and numbered 2021-11/24 was obtained by Sivas Cumhuriyet University Non-Interventional Clinical Research Ethics Committee to start the study. A total of 120 disc-shaped samples with the dimensions of 8 mm × 2 mm were prepared by using teflon-molds. The tested composite resins increment by compressing between two glass slides and mylar strip types. Specimens were polymerized with a light-curing unit (Valo Cordless light device (Ultradent, USA) with a

wavelength of 395-480 and a power of 1000 mW/cm$_2$) for 40 s from both, upper and bottom surfaces. After removing the excess residues on the upper surfaces of the samples with arkansas stone, the Soflex Spiral Diamond (3M ESPE, St. Paul, USA) diamond polishing system in 2 stages; Beige spiral rubber for pre-polishing and pink spiral rubber for high gloss was applied by using a slow-speed handpiece (15.000-20.000 rpm) under wet condition for 15-20 seconds. The prepared samples were kept in distilled water at 37°C for 24 hours.

Experimental Groups

Four different color-adjustment resin based composites and one supra nano spherical filler content multishade composite were divided into 5 main groups according to their contents. The tested composite resins and their compositions are given in Table 1.

Omnichroma Group: Tokuyama Omnichroma Single-shade composite (Tokuyama Dental Tokyo, JAPAN) specimens were prepared using teflon-molds and subjected to processes as described above (n=24).

Vittra Group: Vittra Unique APS Single-shade composite (FGM Dental, BRAZIL) specimens were prepared using teflon-molds and subjected to processes as described above (n:24).

Charisma Group: Charisma Diamond Topaz One Single-shade composite (Kulzer, Tokyo, JAPAN) specimens were prepared using teflon-molds and subjected to processes as described above (n=24).

Zenchroma Group: Zenchroma Single-shade composite (President Dental, GERMANY) specimens were prepared using teflon-molds and subjected to processes as described above (n=24).

Asteria Group: Tokuyama Estelite Asteria multi-shade composite -A2 color preferred- (Tokuyama Dental, JAPAN) specimens were prepared using teflon-molds and subjected to processes as described above (n=24).

The main groups were randomly divided into 3 subgroups according to their bleaching procedures (n=8).

Sub-group 1: Brushing with Distilled Water: Oral-B Smart 4 (Braun Oral B Procter & Gamble / USA) electronic electric toothbrushes with pressure sensors were used for brushing the samples. The brushing simulator (HİYELLAB Makine Arge ve Inovasyon San.ve Tic.) we designed was prepared to ensure standardization in the use of electric toothbrushes and to eliminate the variables depending on the practitioner. In order to ensure that the brushes contact the samples and apply an equal amount of force, 200 N weights were attached to the samples and their positions were fixed. The prepared samples were subjected to a brushing procedure with the help of a brushing simulator for 10 seconds twice a day with distilled water for 14 days.

Sub-group 2: Brushing with Whitening Toothpaste: The prepared samples were subjected to a brushing procedure with the help of a brushing simulator for 10 seconds twice a day with whitening toothpaste (Colgate Optic White Expert, Palmolive, USA) for 14 days. The tested whitening toothpaste and its composition is given in Table 2. Toothpaste was mixed with distilled water at a ratio of 2:1 and applied on the samples by using an applicator. Distilled water was applied to the samples with the help of an applicator every 5 seconds during brushing.

Sub-group 3: Home Bleaching Agent: The home bleaching agent (Opalascence, Utradent, USA) was applied to the polished upper surface of the samples by using an applicator, without applying pressure, in accordance with the manufacturer's instructions. The tested home bleaching agents and its composition is given in Table 2. The samples were kept in a closed container with moist cotton pellets at room temperature for 8 hours. After the bleaching agent application, the samples were washed under running tap for 10 seconds and kept in distilled water. The whitening process was continued in the same way for 14 days.

Coloring Procedure

After the initial color and roughness measurements of the samples were made, they were kept in each solution for 4 days respectively tea (Lipton Yellow Label, Unilever, Turkey), coffee (Nescafe Classic, Nestle, Turkey) and cherry juice (DimesFruit Juice, Dimes, Turkey) for simulating 1 year coloring procedure. At the end of the coloring processes, the samples were placed in opaque containers after washing under running tap for 10 seconds. During the entire staining procedure, solutions were refreshed daily and samples were kept at room temperature.

Measuring Color Change Values (ΔE)

Color measurements were made with a Vita Easyshade Advanced 4 (Vita Zahnfabrik, Bad Sackingen, Germany) spectrophotometer. Measurements were repeated three times for each sample, their averages were recorded as L0*, a0* and b0* values, and the device was calibrated after all three measurements. Color measurements were made in 4 stages: beginning, post-colouring, 7th day end of bleaching procedure and 14th day end of bleaching procedure. The following formula was used to calculate the ΔE values according to the CIELAB color system:

$$\Delta E^* = [(L1^*-L0^*)^2+(a0^*-a1^*)^2+(b0^*-b1^*)^2]^{½}$$

Measuring Surface Roughness Values

In the measurement of the surface roughness values of the samples; a profilometer device (Mitutoyo, surftest SJ-301, JAPAN) was used with a scanning length of 4 mm and a surface cutting length of 0.25 mm. The arithmetic average of the values obtained by measuring the roughness from three different regions of each sample was taken and calculated as the Ra value; It was recorded in 3 stages: at the beginning, after the coloring procedure and after the bleaching procedures.

SEM Analysis

After the roughness measurements of the samples were made after the bleaching procedures, 1 sample from each of the 15 subgroups was coated with 90 A° gold-palladium in an airless environment using a coating device (Quorum Q150R ES, UK) and then SEM images were examined under 20.000x and 50.000x magnification.

Statistical Analysis

Variation data of were analyzed using the SPSS statistical software program (22.0 version, SPSS Inc., Chicago, USA). The data were subjected to statistical analysis with using two-way analysis of variance and Tukey's test to examine pairwise differences at a significance level of 0.05.

Results

Color changes in composite specimens after coloring procedures are given in Table 3. The highest level of color change among composite groups was observed in the Zenchroma group following the Omnichroma group, while the lowest color change was observed in the Vittra group (Table 3). The differences between the vittra, charisma and asteria groups were insignificant ($p>0.005$), on the contrary differences between the other groups were statistically significant ($p<0.005$).The color changes in the composite specimens at the end of the 7th and 14th day after the bleaching procedures are given in Table 4. Color change values after the bleaching procedures were found to be higher in all groups on the 7th day compared to the groups brushed with distilled water while only Charisma Groups color change values were significant ($p<0.05$). On the 14th day, the differences in ΔE values between groups brushed with whitening toothpaste and distilled water were statistically significant in all composites except for Omnichroma ($p<0.05$). As a result of brushing with whitening toothpaste; while the highest whitening effect was seen in Charisma group, the least whitening effect was seen in Omnichroma group.

In all groups in which home bleaching agents were applied, a greater whitening effect was observed at the end of the day compared to the groups brushed with distilled water. But only Asteria and Charisma groups' color change values were observed significant changes ($p<0.05$). At the end of the 14th day, color change values in all groups applied with home bleaching agents were statistically significant (except for Vittra Unique). The maximum ΔE value was observed in the Asteria group, while the minimum ΔE value was observed in the Zenchroma group, and the differences between them were statistically insignificant ($p>0.05$). Although coloration and bleaching procedures applied to composite resins caused some changes in surface roughness values, the differences between them were statistically insignificant ($p>0.05$). When the composite groups were compared among themselves, at the end of the 14th day, the least roughness was observed in the groups that applied home bleaching agent, in the Asteria and Zenchroma groups, and the difference between the Charisma group was significant ($p<0.05$). Home bleaching agent application provided greater whitening effects in 7th day follow-ups compared to brushing with a whitening toothpaste, but similar whitening effects were observed as the end of 14th day(except for Omnichroma). SEM images of all the groups after bleaching procedures are given in figure 2-6.

Table 1. The tested composite resins and their compositions

Groups	Type	Filler Contents
Estelite Asteria	Supra- nano sferical	Bisphenol A di(2-hydroxy propoxy) dimethacrylate (Bis-GMA), Bisphenol A polyethoxy methacrylate (Bis-MPEPP), 1,6-bis(methacrylethyloxycarbonylamine) timethyl hexane (UDMA), triethylene glycol dimethacrylate (TEGDMA), Mequinol, Dibutyl hydroxyltoluene, UV absorbers, 82% by weight (71% by volume) silica zirconia fillers
Omnichroma	Supra- nano sferical	79% by weight (68% by volume) spherical silica-zirconia fillers (average particle size: 0.3 µm, particle size range: 0.2 to 0.4 µm) and composite fillers, 1.6-bis (methacryl-ethyloxy) carbonyl amine), trimethyl hexane (UDMA), Triethylene glycol dimethacrylate (TEGDMA), Contains Mequinol, Dibutyl hydroxyl toluene and UV absorber.
Vittra Unique	Zirconium oxide glass particle	Methacrylate monomer mixture UDMA, TEGDMA, photoinitiator composition (APS), initiators, stabilizers, silane, boron-aluminum-silicate glasses. (72-80% by weight, 52-60% by volume)
CharismaDiamond Topaz One	TCD matrix	Urethane dimethacrylate (UDMA), TCD- DI-HEA, Triethylene glycol dimethacrylate (TEGDMA), barium, Aluminum, boron, fluorine, silicon glass, PPF, silicon oxide 75% by weight (59% by volume) inorganic filler
Zenchroma	Radio-opaque glass with filler microhybrid	Glass powder, diurethane dimethacrylate, silicon dioxide, Bisphenol A dimethacrylate (Bis-GMA), tetramethylene dimethacrylate. 75% by weight (by volume 53% inorganic filler (0.005-3.0μm)

Table 2. The tested whitening agents and their compositions

Trade Name	Type	Contents	Company
Colgate Optic White Expert	Whitening Toothpaste	Glycerin, propylene glycol, sodium monofluorophosphate, calcium pyrophosphate, PEG-12, PVP, PEG/PPG-116/66 Copolymer, disodium pyrophosphate, pentasodium triphosphate, sodium laurly sulfate, silica, flavor, sodium saccharin, hydrogen peroxide, lemon	Colgate/Palmolive Company, New York, NY, USA
Opalescence	Home Bleaching Agent	16% Carbamide Peroxide, Deionized Water, 0.5% Potassium Nitrate, 0.11% Sodium Fluoride, Carbopol, Glycerin	Ultradent Products Inc, South, Jordan, Utah, USA

Table 3. Color change values after staining procedure

Groups	ΔE (mean)	(SD)
Estelite Asteria	11.39[a]	2.92
Omnichroma	16.08	2.11
Vittra Unique	7.97[b]	2.81
Charisma Diamond One	9.75[a.b]	2.75
Zenchroma	13.39	1.80

F=37.561, P=0.000, p<0.05, [a,b] there is a statistical no difference between the groups shown with the same lower case letters.

Table 4. Color change values after bleaching procedures, Mean Values (Standard Deviasion)

Whitening Procedures	Time	Estelite Asteria	Omnichroma	Vittra Unique	Charisma DiamondOne	Zenchroma
Brushing with Distilled Water	7th day	2.20 (0.66)[a]	2.61 (1.67)	3.15 (2.11)	1.99(0.48)[f.g]	2.23 (0.88)
	14th day	2.99 (0.53)[b.c]	3.34 (1.45)[d]	3.46 (1.52)[e]	3.00(0.59)[h.i]	2.84 (0.66)[k.l]
Brushing with Whitening Toothpaste	7th day	4.20 (1.27)	3.19 (0.56)	4.13 (2.46)	4.65 (0.91)[f]	3.09 (0.46)
	14th day	5.75 (0.88)[b]	4.30 (0.96)	5.94 (1.11)[e]	5.95 (1.00)[h]	5.65 (1.57)[k]
Application of Home Bleaching agent	7thday	5.18 (1.83)[a]	3.84 (0.92)	4.32 (1.12)	4.63 (0.89)[g]	3.61 (0.53)
	14th day	6.79 (1.17)[c]	5.93 (0.64)[d]	5.37 (1.05)	6.04 (1.49)[i]	5.31 (1.06)[l]

F=10.248, P=0.000, p<0.05, [a,b,c,d,e,f,g,h,i,k,l] there is a statistical difference between the groups shown with the same lower case letters.

Figure 1: Brushing simulator used for brushing the samples.

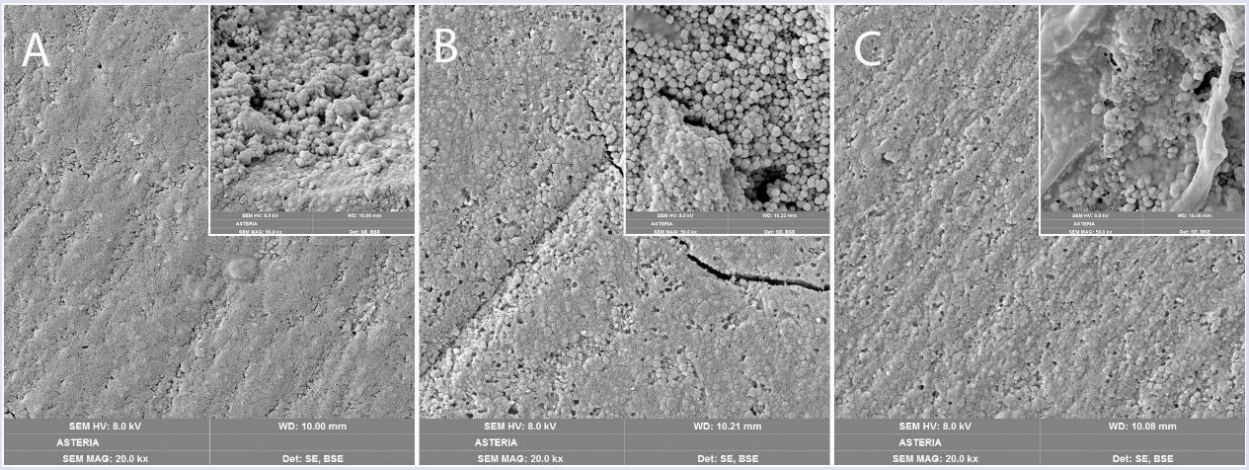

Figure 2: After bleaching procedures SEM images x20.000 x50.000 magnification respectively, Asteria Group, (A-Brushing Distiled Water, B- Brushing Whitening Toothpaste, C- Home Bleaching Agent Application).

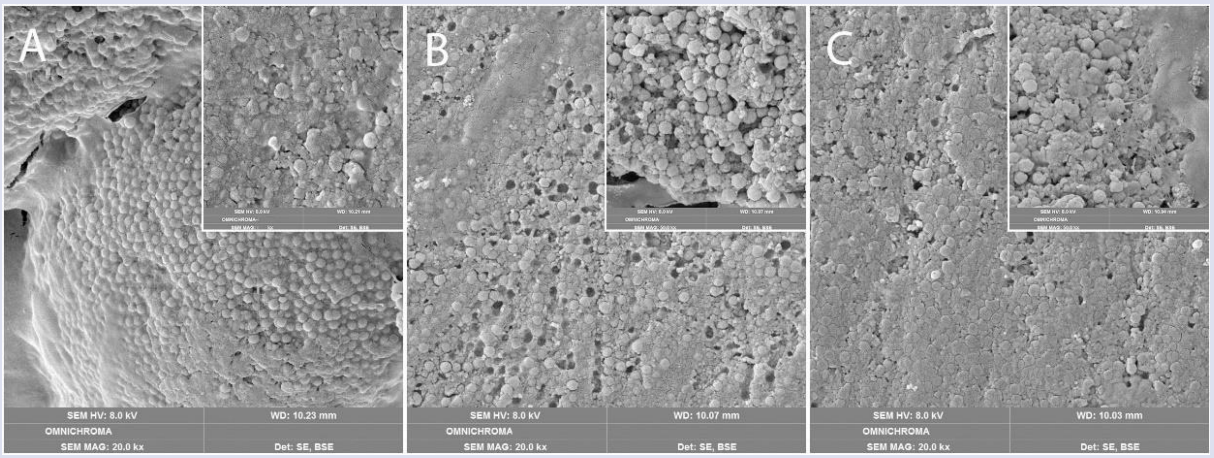

Figure 3: After bleaching procedures SEM images x20.000 x50.000 magnification respectively, Omnichroma Group, (A-Brushing Distiled Water, B- Brushing Whitening Toothpaste, C- Home Bleaching Agent Application).

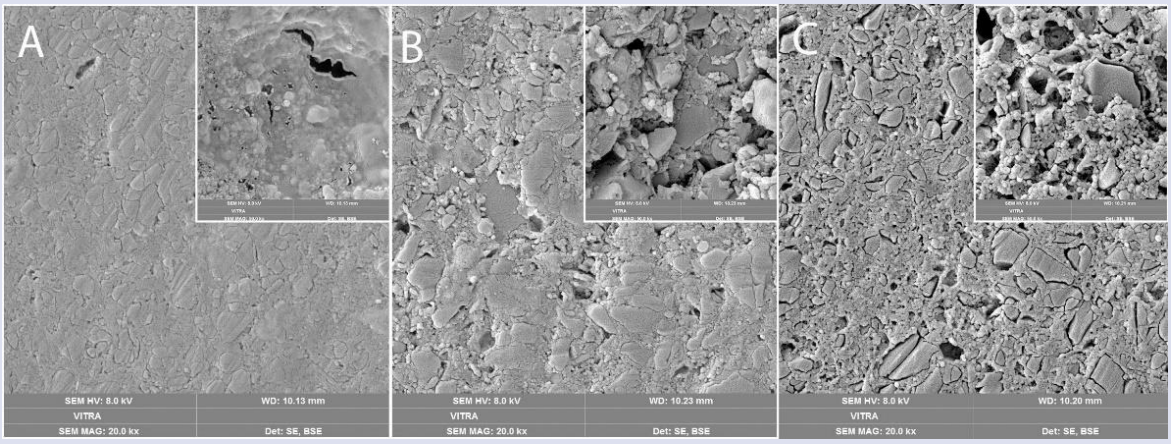

Figure 4: After bleaching procedures SEM images x20.000 x50.000 magnification respectively, Vittra Group, (A-Brushing Distiled Water, B- Brushing Whitening Toothpaste, C- Home Bleaching Agent Application).

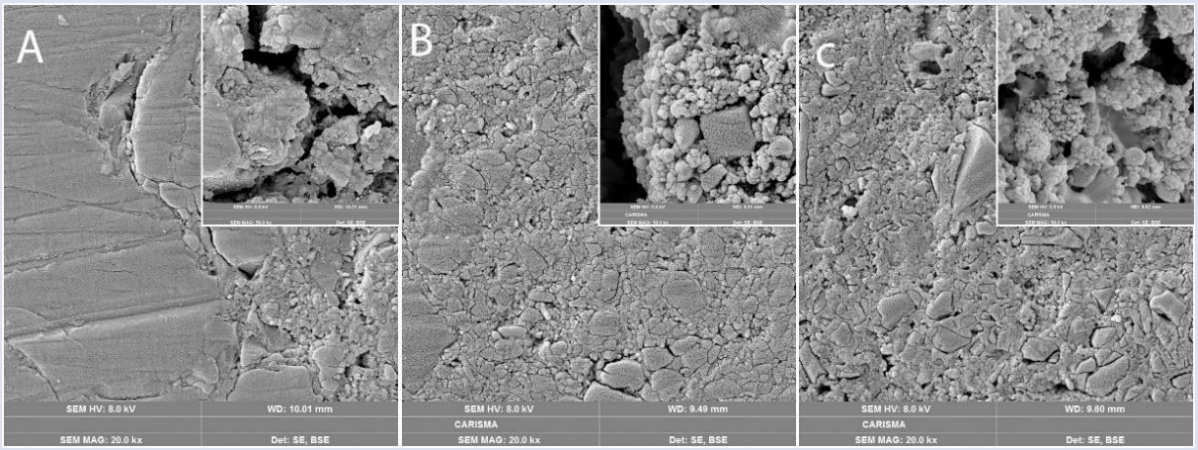

Figure 5: After bleaching procedures SEM images x20.000 x50.000 magnification respectively, Charisma Group, (A-Brushing Distiled Water, B- Brushing Whitening Toothpaste, C- Home Bleaching Agent Application).

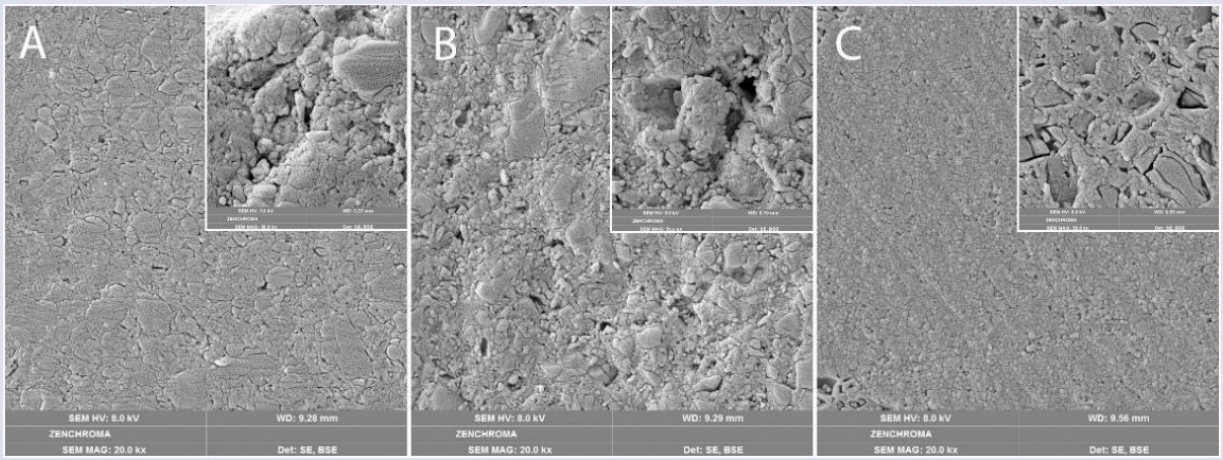

Figure 6: After bleaching procedures SEM images x20.000 x50.000 magnification respectively, Zenchroma Group, (A-Brushing Distiled Water, B- Brushing Whitening Toothpaste, C- Home Bleaching Agent Application).

Discussion

In clinical practice, multiple composites with different properties should be layered to create restorations compatible with the natural tooth structure, which entails a complex color selection process. To simplify this process, color adjustment resin-based composites that can match all shades with a single composite have been developed.[1,4] In De Abreu *et al.*[1]'s study examining restorations performed on teeth with different color properties, there was no significant difference in color harmony between single shade and multi shade composite resins.

Color is an important factor for optimum aesthetics in dental restorations. In addition to the initial color harmony, color stability is also one of the criteria that should be considered for the continuity of the optimum aesthetics of restorations. One of the main reasons for replacing composite resin restorations is the failure to achieve sufficient color stability.[25] Many factors such as the structure of the resin matrix, the amount of polymerization, oral hygiene, food and beverages, water absorption and surface roughness can affect the color change process of composite resin materials.[26]

In the literature review, although there are many studies on the color adjusting abilities of single shade composites, there is limited research on their color stability and mechanical properties. Therefore, in this study, we investigated the effect of brushing with whitening toothpaste on the color stability and surface roughness of four different single-shade resin-based composites after simulating 1-year coloring procedure.

Akgül *et al.*[29] investigated the color changes and surface roughness of different resin composites after immersing them in various solutions (distilled water, cola, coffee, and orange juice) for 12 days. All samples immersed in coffee showed significant color change. The least color change was observed in Filtek Universal composite while the highest color change was observed in Omnichroma composite. Fidan *et al.*[30] investigated the effect of coffee staining on color change and translucency

of different composite resins for 12 days in coffee. It was reported that both the highest color chance and highest translucency value were observed in the Omnichroma group. Aydin *et al.* [31] examined the effect of staining with coffee solution on surface roughness and color changes of a single shade composite (Omnichroma) and multiple shade composites. They reported that the highest coloration was observed in the Omnichroma group at all measurement times (1st, 7th, and 30th day) and this result may be related to the hydrophilic nature of TEGDMA monomer present in Omnichroma's organic structure.

In various studies[28-30], it has been reported that TEGDMA and Bis-GMA in the composite resin matrix structure exhibit more hydrophilic properties than UDMA and Bis-EMA, and the addition of TEGDMA to the matrix structure to dilute Bis-GMA and UDMA increases hydrophilicity, leading to more coloration in composite resins as hydrophilic character increases.

Ilie[32] investigated the aging behavior, quasi-static and viscoelastic behavior analyses of universal chromatic resin-based composites. As a result, they stated that the mechanical properties of the Omnichroma composite are lower than the Venüs Pearl One (≅ Charisma Diamond One) composite. The reason for the low stability of the Omnichroma against aging procedures; It has been reported that the production of inorganic fillers by clustering around an organic-inorganic core using by sol-gel method may be due to the observation of a more porous and weak structure in the interphase (Figure 3-A). In addition, the better mechanical properties of Venüs Pearl One composite might releated that the urethane groups in the TCD matrix structure facilitate the polymerization reaction, enabling the formation of multiple polymer chains and contributing to the color stability by reducing the amount of residual monomer.

In this study, the highest color change was observed in the Omnichroma composite group following the coloring procedure. The result of this study, Akgul et al.[29], Fidan et al.[30], Aydın et al.[31] and Ilie[32] consistent with the results of their study. Omnichroma's low color stability possibly be related to the effect of TEGDMA hydrophilic character however TEGDMA is also present in the structures of the other composites (except Zenchroma) in this study. Therefore color stability could be not only releated composite resins's hidrophilic characters but also differences in inorganic filler types and sizes, differences in photo initiators and the presence of pre-polymerized monomers.

Graf and Ilie[33] examined the long-term mechanical properties and translucency characteristics of color-matching resin-based composites. They found that the TCD matrix structure of the organic matrix in Charisma Diamond One improves the mechanical properties of the material and creates a structure that is more resistant to aging processes. Additionally, they reported that the presence of additional pre-polymerized monomer in the organic matrix structure of Charisma Diamond One may contribute to the long-term mechanical stability of the composite (Figure 4-B).

Pedrosa *et al*. [34] compared the color stability of different composite resins after polymerization and water storage (30 days). While Vittra APS showed the least color change after polymerization, the highest color change was observed after water storage. The least color change after water storage was observed in the Z350XT and Charisma Diamond One. They suggested that the minimal color change of Vittra APS after polymerization may be due to the different photoinitiator system.

Özara *et al.*[35] investigated the color stability of different resin materials after being stored in different beverages (coffee, tea, cola, and saliva) for 14 days. While Charisma Diamond One showed the highest color change in cola, it showed less color change in other beverages. They stated that the higher color stability of Charisma Diamond One may be due to its hydrophobic structure.

In literature review, we could not come across a study specifically focused on the color stability of Zenchroma composite. Atalı *et al.*[36] compared the physical and mechanical properties of different shade-matched resin-based composites. They stated that the composites with the highest conversion degree were Vittra APS Unique and Charisma Diamond One, followed by Zenchroma and Omnichroma. As the degree of conversion increased, the mechanical properties of the material improved and the color stability increased.

In this study, the least color change after the coloring process was in Vittra APS and Charisma, followed by Asteria and Zenchroma. The reason why the highest color stability was seen in the Vittra APS in the studies of Pedrosa *et al.* [34] was releated might be due to the composition, which is free of Bis-GMA and smaller quantities of campheroquinone. Researchers stated that the campheroquinone/amine and BisGMA/TEGDMA ratios play an important role in the coloring of the composites and that the a* and b* values from the color coordinates vary depending on these ratios. In this study, while a significant increase was observed in b* parameters in Charisma, Omnichroma and Zenchroma groups depending on the tea and coffee we used in the coloring procedure, while a very little change was observed in the b* parameter in the Vittra Unique.

In the literature, there is no direct study on brushing with whitening toothpaste and the application of home bleaching agent to color-adjustment resin-based composites. In their study, Mehrgan *et al.*[38] investigated the effects of brushing with different whitening toothpastes on coffee-stained Spectrum TPH composite samples over a period of two weeks. According to their results, the whitening toothpaste containing 2% hydrogen peroxide (Colgate Optic White) was more effective than abrasive-containing and charcoal-containing whitening toothpastes.

Similarly, Al-Shalan *et al.*[39] investigated the effect of brushing with different whitening toothpastes on the color stability of cylindrical samples prepared using different restorative materials. The samples were subjected to brushing with the whitening toothpastes for 1 hour, twice daily for 15 days to simulate brushing habits. They reported that the glass ionomer cement group brushed with Colgate Optic White Expert toothpaste showed important color change.

In various studies, the perception of the human eye was determined as a reference for the color change to be clinically

acceptable. It has been reported that the critical threshold values for visual perception should be above ΔE ≥3.3.[38,39] As a result of this study, all color-adjustment resin-based composites had an effective whitening efficacy above clinically acceptable threshold value (ΔE ≥3.3) after brushing with whitening toothpaste and home bleaching procedures. The highest whitening effect was observed in Charisma (ΔE=5.95) and Vittra APS (ΔE=5.94), and the least color change was observed in Omnichroma composite (ΔE=4.30). The reason for this may the micro pits formed as a result of the very intense separation of relatively large spherical fillers (260nm) from the surface during brushing when the Sem images of Omnichroma were examined in our study (Figure 3-B).

Villata *et al.*[39] investigated the effects of 2 staining solutions (coffee and red wine) and 3 bleaching systems on the color stability of two dental composites. They reported that agents containing carbamide peroxide with different concentrations were effective in removing coloration regardless of concentration ratios. Asdagh *et al.*[40] applied composite restorations (Gradia Direct, GC/Japan) to Class V cavities prepared on extracted central teeth, followed by 14 days of tea staining. They reported that the application of a whitening agent containing 10% carbamide peroxide for 14 days (8 hours a day) after staining was effective on both teeth and composite resins. Similarly, in this study, the highest color change was observed in the Asteria composite (ΔE=6,79), while the least color change was observed in the Zenchroma composite (ΔE=5,31) after home bleaching agent applying. These differences between all composites were not statistically significant (p>0.05).

Amaral *et al.* [42] compared the effects of brushing with 5 different toothpastes using a simulator on the surface roughness of the resin materials. They stated that the experimental paste containing 1.5% hydrogen peroxide and Rembrant Plus Whitening containing 3.6% hydrogen peroxide had the lowest roughness values and also reported that low concentrations of hydrogen peroxide in the toothpastes did not increase surface roughness.

Demir et al[43.] investigated the effect of manuel brushing (electric toothbrush) using different toothpastes on the color stability and surface roughness of colored composite disc samples (6 x 2 mm). As a result of the study, they stated that while the color change was observed the most in the colgate optic white, the surface roughness of the samples in this group were increased.

Yüzügüllü *et al.*[44] examined the effect of home bleaching agent containing %20 carbamide peroxide on the surface roughness of composite resins for 8 days (6 hours per day). They reported that there was no significant difference between samples applied bleaching agent and stored in distilled water in terms of surface roughness.

In another study, Sharafeddin *et al.*[45], investigating the effect of high concentration bleaching agent (35% carbamide peroxide) application on the surface hardness and roughness of composite resins, and they reported that carbamide peroxide application did not have a significant effect on the surface roughness of composites.

Similarly in this study, although a slight increase in surface roughness values were observed in all composite groups after bleaching procedures, these differences were found to be statistically insignificant (p>0.05) (Table 4). Our study results are parallel with the findings of Amaral *et al.*[42], Yüzügüllü *et al.*[44] and Sharafeddin *et al.*[45] in terms of not observing a significant change in surface roughness. On the other hand, results of this study are in conflict with Demir *et al's* [43] study results. The reason of difference may be related that the composition of composites with different contents, variations in brushing time and polishing systems. In addition, the use of a brushing simulator in this study may have contributed to achieving more realistic results by minimizing the variables depending on the practitioner.

Conclusions

All color- adjustment resin based composites showed coloration above the clinically acceptable threshold in this study. The least color change was observed in Vittra Unique composite while the highest color change was observed in Omnichroma composite, Home bleaching agent application provided greater whitening effects in short-term follow-ups compared to brushing with a whitening toothpaste, but similar whitening effects were observed as the duration extended (except for Omnichroma). Further, whitening procedures did not negatively affect the surface roughness of all color-adjustment resin-based composite resins.

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Conflicts of Interest Statement

None.

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The Effect of Pulp Treatments on the Survival of Zirconia Crowns in Primary Teeth

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Research Article

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ABSTRACT

Objectives: This study evaluated the effect of pulp treatment on the survival rate of zirconia crowns (ZCs) placed in primary anterior teeth of children under general anesthesia.
Materials and Methods: A total of 80 anterior teeth of 32 children aged 18-60 months who underwent ZCs under general anesthesia were followed for 18 months. Failure cases of were categorized. Statistical analysis included independent sample t-test, chi-square test, and Kaplan-Meier survival method.
Results: There was no difference in survival of ZCs between pulp treated and untreated teeth (p>0.05). Considering the survival time of ZCs according to the presence or absence of pulpal symptoms, it was observed that 4 out of 80 teeth were symptomatic (with or without crown loss) at the end of 18 months, and the success rate was 95%.
Conclusions: Pedodontic ZCs had a high survival rate in anterior primary teeth in children treated under anesthesia.

Key words: General Anesthesia, Primary Teeth, Zirconia Crown.

Süt Dişlerinde Zirkonyum Kuronların Sağ Kalımı Üzerine Pulpa Tedavilerinin Etkisi

Süreç

Geliş: 06/07/2023
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ÖZ

Amaç: Bu çalışma, genel anestezi altındaki çocukların süt ön dişlerine yerleştirilen zirkonya kruonların sağ kalım oranına pulpa tedavisinin etkisini değerlendirdi.
Gereç ve Yöntemler: Genel anestezi altında zirkonyum kuron uygulanan 18-60 aylık 32 çocuğun toplam 80 ön dişi 18 ay takip edildi. Zirkonyum kuronların başarısızlık vakaları kategorize edildi. İstatistiksel analiz bağımsız örneklem t-testi, ki-kare testi ve Kaplan-Meier sağ kalım yöntemini içermiştir.
Bulgular: Zirkonyum kuronların sağ kalımında pulpal tedavisi olan veya olmayan dişler arasında önemli derecede fark yoktu. Semptomların varlığına veya yokluğuna göre zirkonyum kuronların hayatta kalma süresine bakıldığında, 18 ay sonunda 80 krondan sadece 4'ünün semptomatik olduğu (kron kaybı olsun veya olmasın) gözlendi ve başarı oranı %95 idi.
Sonuçlar: Pedodontik zirkonyum kuronlar, anestezi altında tedavi edilen çocuklarda anterior süt dişlerinde yüksek sağ kalım oranına sahipti.

Anahtar Kelimeler: Genel Anestezi, Süt Dişleri, Zirkonyum Kuron.

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Introduction

Dental caries, which is an important public health problem, is one of the most common chronic diseases in childhood.[1] In a study conducted in Turkey in 2004, the prevalence of caries in 5-year-old children was 69.8%; It has been reported as 61.1% in children aged 12 years.[2] Since the enamel in deciduous teeth is structurally different from that of permanent teeth, caries in deciduous teeth progress faster than in permanent teeth. In particular, primary incisors may need treatment due to dental caries as well as trauma or developmental defects. There are different treatment options and different materials that can be used for the restoration of these teeth. The choice of the treatment applied and the material used; It is affected by many factors such as the amount of remaining tooth structure, isolation conditions, aesthetic expectations and the level of compliance of the patient.[3]

There are many materials used in full coronal restorations of primary incisors from past to present. Resin strip crowns, preveneer stainless steel crowns, open-face stainless steel crowns and prefabricated zirconia crowns (ZCs) are among the recommended treatment approaches.[4] Previous researches have determined that the failure rate of composite resins placed under general anesthesia is up to 45%.[5,6] Today, with the demands of parents and children, the need for aesthetic restorations is increasing day by day.[7] In a previous study on the preferences of children and parents in the selection of restorative materials, it was determined that tooth-colored materials were preferred the most.[8]

In recent years, ZCs have attracted more attention than other treatment options in primary anterior teeth with excessive material loss, due to both meeting aesthetic expectations and high mechanical strength.[9] Various studies have been conducted on ZCs used in anterior primary teeth with excessive material loss. In a study by Holsinger et al.[10] in which they evaluated the success of ZCs made under general anesthesia and sedation, retention values were found to be 96% at the end of 20 months. Likewise, Seminario et al.[11] in their study evaluating the survival of ZCs performed under general anesthesia, they determined the survival rates at the end of 12 and 36 months as 93% and 76%, respectively. Although there are several other studies showing the survival rates of ZCs, data from clinical studies are insufficient.

It is not possible to treat children with complicated and multi-interventional teeth in the clinic conditions. Children who have high anxiety and anxiety, especially children before the age of 6, and children with systemic diseases and/or disabilities are difficult patients in terms of performing dental procedures in a clinical setting in a harmonious manner.[12] Pediatric dentists can perform their treatments under general anesthesia in such cases. General anesthesia application in pediatric dentistry; It is frequently used in patients with early childhood caries and severe early childhood caries.[13-15] It is noteworthy that the application of dental general anesthesia has increased in the last 10 years, and this increase is especially in the 3-6 age group.[16]

This study evaluated the effects of factors such as age, gender and pulp treatments on the survival of ZCs applied to the anterior teeth of children under general anesthesia. The null hypothesis of the study is that pulp treatments, which are among the factors, have no effect on success and survival in teeth with ZCs.

Material and Methods

Ethical approval for the study was obtained from Nevşehir Hacı Bektaş Veli University Ethics Committee (no:2023/189).

Sample Selection

Between October 2020 and October 2021, severe early childhood caries were diagnosed and dental treatments were performed under general anesthesia in a private hospital in Kayseri; The files of patients younger than 60 months who used one or more ZCs in their treatment were scanned, and information about follow-up appointments and visit information of the children who met the inclusion criteria were recorded. During the 18-month follow-up, the data on the success or failure of the restorations of the patients who came to the control appointment at 6-month intervals were recorded. (Figure 1)

The condition of the crown was classified using the following criteria; accordingly, crown survival has been noted to be unsuccessful if one of the following conditions is observed.

a) No clinical failure
b) Debonding without complication: debonding of crown but no pulp-related pain or abscess in the patient
c) Debonding with complication: debonding of the crown and the patient's complaints such as pulp-related pain, abscess and tooth mobility
d) Failure without debonding: the crown not debonded, but the patient has clinical symptoms such as toothache, food impaction, abscess, tooth mobility

The survival of crowns was recorded at the end of the one-and-a-half year follow-up (18 months), during the follow-up period when debonding and pulpal failure were first noticed (6, 12, or 18 months).

Independent t-test and chi-square test were used to compare differences in parametric variables (age) and non-parametric variables, respectively. Kaplan-Meier survival curves were obtained for the effect of pulp treatment on the success of ZCs. Statistical analyzes were performed in SPSS 21.0 (IBM Corp., Armonk NY, USA) statistical program at 5% significance level.

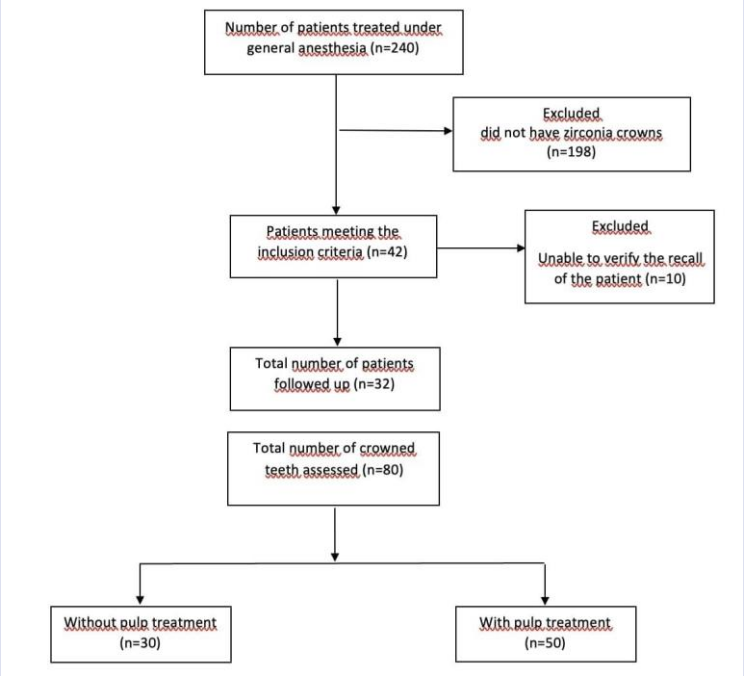

Figure 1: Study flowchart

Table 1. Types of failure according to the presence of pulp treatment

		Pulp Treatment		
		No	Yes	Total
Failure	***No clinical failure***	27	41	68
	Debonding without complication	3	5	8
	Debonding with complication	0	3	3
	No debonding	0	1	1
		30	**50**	**80**

Table 2. Types of failure observed.

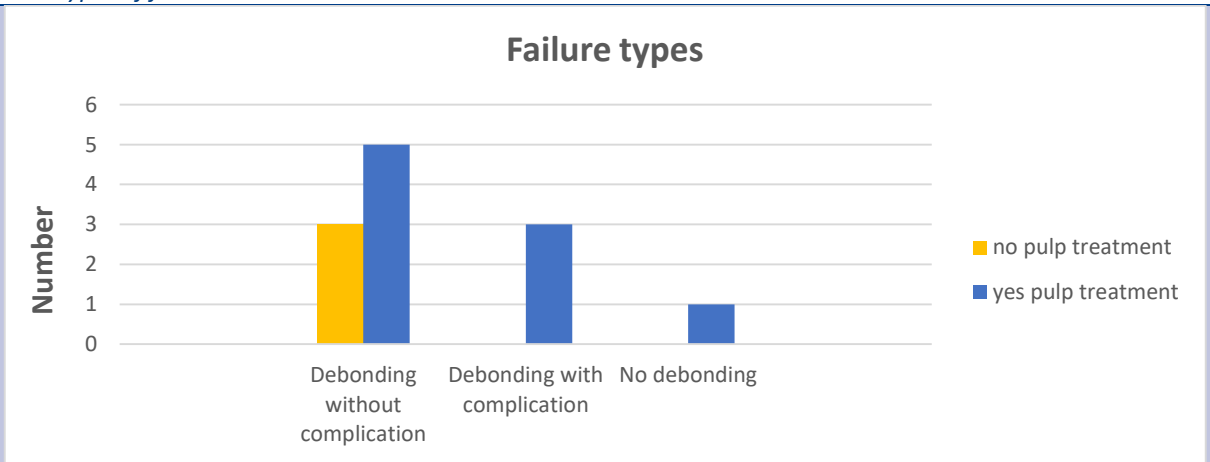

Figure 2: Kaplan–Meier survival curve for mean survival time of zirconia crowns.

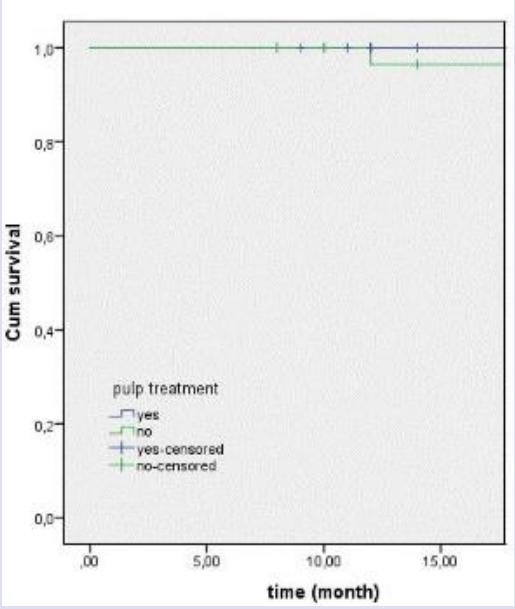

Figure 2: Kaplan–Meier survival curve for mean survival time of zirconia crowns.

Results

The sample consisted of 32 patients (15 girls, 17 boys) aged between 18-60 months (mean 38 months). There was no significant difference in the success of ZCs in terms of gender and age ($p>0.05$). Pulp treatment (50 patients) was applied to a significant majority of the 80 teeth examined before crown application (Table 1). Most of them were treated with pulpectomy (38 patients), while pulpotomy (12 patients) was applied to the others. Debonding was the most common cause of failure, while pulpal failures were recorded only in teeth that underwent pulp treatment.

Of the 80 crowns observed over a one-and-a-half year period, 12 were recorded as clinically unsuccessful, however, there was an overall survival rate of 85%. Debonding failure was the dominant failure type (n =11) (Table 2). Failure without debonding of the crown occurred in only 1 patient, which also occurred in the pulpal treated group and as a result of dental trauma. It was observed that the mild pain and mobility observed in the first control of the involved tooth disappeared in the ongoing follow-up appointments. Likewise, failure in 3 patients with pulpal complications and debonding was also the result of dental trauma. And these 3 patients were from the pulpal treated group, and 2 of the debonded crowns were re-cemented in the clinical setting. However, crown cementation could not be performed in 1 patient due to very young age and insufficient cooperation.

Looking at the survival time of ZCs according to the presence or absence of symptoms, only 4 out of 80 ZCs were observed to have pulpal symptoms (with or without crown loss) after 18 months. Survival functions for pulp-treated and non-pulp-treated crowns were plotted using Kaplan-Meier survival charts (Figure 2). The survival rate for ZCs with pulp treatment was 82%, while the survival rate for ZCs without pulp treatment was 90%. There was no statistically significant difference between the survival of ZCs with and without pulp treatment ($p>0.05$).

Discussion

With the increasing public awareness of aesthetics, the demands of parents for tooth-colored restorations have increased.[4] For primary incisors, tooth-colored restorations that completely cover the tooth have their own advantages and disadvantages.[17] Composite strip crowns, which have been used for a long time in aesthetic restorations of primary incisors, offer advantages such as diversity in color options, allowing multiple dental treatments and ease of repair; Difficulties in providing saliva and blood isolation during application and contamination sensitivity of the technique are among the disadvantages.[18,19] On the other hand, when the veneered stainless steel crowns are compared with the strip crowns; It can be listed as an advantage that they are less affected by saliva and blood contamination, which may affect the retention, color and resistance of the crown, and that they require less time for treatment.[20] However, it requires too much tooth cutting, deterioration of adaptability in multiple dental treatments, and fractures that occur in the inflexible veneer structure are among its disadvantages.[21]

Prefabricated pediatric ZCs, on the other hand, were developed to be an aesthetic alternative to stainless steel crowns and veneered stainless steel crowns, which are considered the gold standard in full coronal restorations of primary teeth. There is no cooperative tendency for restorative treatments due to insufficient cognitive skills in young children with high early childhood caries. It is possible to prevent failures that may occur in dental treatments at this age, in treatment conditions such as sedation or general anesthesia where stabilization can be achieved definitively. The children included in our study had early childhood caries and were in the younger age group. Therefore, their treatment was completed under general anesthesia.

When the literature is reviewed, there are studies that evaluate the clinical performance of prefabricated pedodontic ZCs and compare them with other restorative materials.[22,23] However, there is limited information regarding the effect of pulpal treatments on the survival of pedodontic ZCs. Therefore, in our study, the effects of pulpal treatments on the survival of ZCs, as well as factors such as age and gender, were evaluated.

Yanover et al.[24], in their study in which they followed the success of pedodontic ZCs for more than 30 months; They stated that it is a satisfactory treatment option for decayed primary maxillary incisors in terms of marginal integrity, gingival health and aesthetics. Gill et al.[25], reported that ZCs were clinically acceptable in a 12-month follow-up study conducted in children aged 2 to 4 years, showing that they showed high survival and marginal integrity.

Although there are studies suggesting pulpal treatment of teeth to be crowned, there is no need to perform pulpal treatment before placing ZCs on teeth where dental caries does not reach the pulp. In primary teeth, clinical examination is the only way to accurately determine the pulp status of the tooth.[26] Therefore, in our study, it was decided whether pulp treatment would be performed or not, whether pulp exposure was observed after caries removal.

Our findings showed that there was no difference in the survival of ZCs applied to pulp treated and untreated teeth. It can be thought that crown retention may be less than the group without pulp treatment, since teeth requiring pulp treatment have more material loss due to deep caries. As a matter of fact, in a previous study[27], they revealed that crown loss was higher in teeth that had pulpal treatment. However, there was no difference in our study; It can be attributed to increasing the retention of the crowns by compensating the tissue losses in pulpal treated teeth with filling materials with high adhesion strength before the ZCs is placed.

In our study, the survival rate of ZCs was 85%. In a previous study[28], clinical performance of ZCs and composite strip crowns was followed for 18 months. According to the study findings, ZCs gave more satisfactory results in terms of retention and restoration success than composite strip crowns. Retention success of ZCs was 100%, whereas that of strip crowns was 77.8%.

Some limitations should be considered when evaluating the results of our study. This study was conducted retrospectively and therefore results may not be as strong as those from split mouth clinical studies. However, the Kaplan-Meier survival curve has been shown to be a valid tool for predicting the success of restorations used in children. The role of oral hygiene on the survival of crowns was a factor not evaluated in our study. In addition, the limited number of patients included in the study can be considered as one of the limitations of the study. Despite these limitations, this study provides clinicians with an insight into the impact of pulpal treatments on the survival of ZCs.

Conclusions

ZCs applied under general anesthesia in uncooperative children have a high survival rate in teeth with or without pulpal treatment. In the future, clinical studies with longer follow-up periods are needed to evaluate the effects of different variables on the survival of ZCs in primary teeth.

Conflicts of Interest Statement

The authors deny any conflicts of interest related to this study.

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Ultrasonographic Evaluation of Intracranial Pressure during Rapid Maxillary Expansion

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ABSTRACT

Objectives: The objective of this research was to assess the impact of rapid maxillary expansion on intracranial pressure in individuals with maxillary transverse deficiency. This was achieved by measuring the optic nerve sheath diameter using ultrasonography (US).

Materials and Methods: This prospective observational study included 25 young patients (mean age 13.10 ± 1.20) with bilateral posterior cross bite. Acrylic cap splint hyrax appliances were given to all patients for rapid maxillary expansion (RME). Prior to the initial screw activation (T0), the patient underwent monitoring, collection of vital signs, and measurement of optic nerve sheath diameter (ONSD) using US. Subsequent measurements were taken at 1 minute (T1), 10 minutes (T2), and 60 minutes (T3) following the first activation. During the final session of the rapid maxillary expansion therapy, the same measurement procedure was repeated (T4, T5, T6, and T7) as in the initial activation session. The patients' perception of pain during screw activation (T1, T5) was also assessed using a four-category verbal rating scale (VRS-4). A p-value of less than 0.05 was considered statistically significant for the conducted analysis.

Results: The ONSD values, at T1 and T5, showed a significant increase within 1 minute following screw activation. However, there was no significant difference observed between the initial (T0) and final (T7) ONSD values throughout the active RME therapy.

Conclusions: Intracranial pressure rises immediately after screw activation, but it auto regulates at the end of the active RME therapy.

Key words: Optic Nerve Sheath Diameter, Rapid Maxillary Expansion, Intracranial Pressure, Ultrasonography.

Hızlı Üst Çene Genişletme sürecinde Kafa içi Basıncının Ultrasonografik Değerlendirilmesi

Süreç

Geliş: 17/07/2023
Kabul: 25/08/2023

ÖZ

Amaç: Bu çalışmanın amacı; üst çene transversal yetersizliği bulunan hastalarda uygulanan hızlı genişletme protokolünün kafa içi basıncı üzerine etkilerini optik sinir kılıf çapını ultrason ile ölçerek değerlendirmektir.

Yöntemler: Bu prospektif gözlemsel çalışma posterior çapraz kapanışı olan 25 genç hastadan (ortalama yaş 13.10 ± 1.20) oluşmaktadır. Hastaların tamamına hızlı üst çene genişletme için akrilik kaplı hyrax apareyi uygulandı. İlk vida aktivasyonundan hemen önce (T0) hastalar monitörize edildi, vital bulgular kaydedildi ve optik sinir kılıfı çapı ultrason ile ölçüldü. Bu ölçümler 1 (T1), 10 (T2) ve 60 dakika (T3) sonra tekrarlandı. Aktif genişletme tedavisinin son seansında aynı ölçümler ilk aktivasyon esnasındaki gibi uygulandı. (T4, T5, T6 ve T7). Hastaların vida aktivasyonları (T1, T5) sırasındaki ağrı değerleri de not edildi. (VRS-4). Sonuçlar istatiksel olarak $p<0.05$ düzeyinde anlamlı olarak kabul edilmiştir.

Bulgular: Optik sinir kılıf çapının vida aktivasyonundan sonraki ilk 1 dakika içinde anlamlı derecede arttığı görüldü. Aktif genişletme tedavi sürecinde başlangıç (T0) ve bitiş (T1) optik sinir kılıf çaplarında ise anlamlı değişim görülmedi.

Sonuçlar: Kafa içi basıncı vida aktivasyonunu takiben artmakta anca aktif genişletme tedavisinin sonunda otoregule olmaktadır.

Anahtar Kelimeler: hızlı üst çene genişletme, intrakraniyal basınç, optik sinir kılıfı çapı, ultrasonografi.

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Introduction

In RME therapy, high level orthopedic forces lead to a splitting of mid-palatal suture, causing maxillary halves to separate from one another.[1] These heavy forces affect not only adjacent structures in the face but also the structures in the cranium caused by unpredictable transmission of forces.[2] Research studies have indicated that utilization of RME has been associated with notable alterations in cranial and circummaxillary sutures, as well as mid-palatal sutures, in developing individuals.[3,4] Therefore, several researchers have focused on potential effects associated with RME therapy upon visceral structures and the neurocranium.[5,6] According to a study, the application of significant forces during expansion exerts stress on the structures of the cranial base, which results in the widening of various areas such as the superior orbital fissure, spinous foramen, oval foramen, optical foramen, round foramen, and carotid sulcus. Consequently, there is a possibility of micro-fractures and potential damage to nerves and blood vessels in these regions.[5] *Li et al.*[6] determined that intensive anatomical stresses on craniofacial structures during the expansion process can induce cerebral hemodynamic changes, including increased cerebral blood volume (CBV) and cerebral blood flow (CBF). *Romeo et al.*[7] observed that the use of RME may increase intracranial pressure, impairing venous drainage by affecting cerebral venous circulation on magnetic resonance imaging (MRI).

The total volume of the brain, which consists of cerebrospinal fluid (CSF) and blood, influences the intracranial pressure (ICP).[8] Within the central nervous system, the optic nerve is surrounded by the infraorbital subarachnoid space that exhibits pressure closely resembling and correlating with the ICP.[9]

Optic nerve sheath diameter (ONSD) has been suggested as a dependable method for detecting elevated ICP.[10] Research studies have demonstrated that changes in ONSD occur almost simultaneously with rapid fluctuations in ICP, reflecting an immediate response.[9,10] Today, invasive and radiological methods have been introduced to measure raised ICP such as direct ICP monitoring via an intracranial catheter and a computerized brain tomography (CBT).[10] Nevertheless, more recent researches have also documented an alternative approach, which is non-invasive, for assessing ICP by means of US to measure the diameter of the ONSD. The measurement of ONSD for determining ICP is also easy-to-apply, reliable, and non-invasive, in comparison to other methods.[9]

There are many clinical and simulative works that provide clinicians with valuable information about skeletal and dental effects of RME therapy as a part of orthodontic treatment.[3] While earlier investigations have predominantly concentrated on examining the impact of forces on craniofacial structures in patients undergoing RME treatment, the potential secondary consequences on the brain hemodynamics, intracranial vascular compartment and intracranial pressure remain unexplored and undefined.[5,11] Thus, the aim of this research was to assess the potential impact of RME therapy on intracranial pressure through the measurement of the ONSD.

Material and Methods

This research received approval from the Scientific Research Ethics Committee at XXX University Faculty of Medicine (2020/xxx, 06/11/2020), and parental consent was obtained for all patients. The sample size was established using existing data as a reference.[6] By considering an alpha level of 0.05, beta of 0.20, and effect size of 0.65, it was calculated that a total of 22 participants would be needed. To eliminate potential data loss, final sample size was designed with at least 25 patients (15 females and 10 males). The average age of the participants was 13.10 ± 1.20 years, falling within a range of 10 years and 11 months of age to 14 years and 11 months of age. Every patient exhibited both bilateral posterior cross bite and maxillary constriction, indicating suitability to undergo RME treatment. Their skeletal maturity varied between the CS1 and CS3 stages according to the Cervical Vertebral Maturation (CVM) index. The exclusion criteria were pathologic periodontal conditions, major skeletal asymmetries, previous or ongoing orthodontic treatment, and acute or chronic ophthalmic diseases.

RME treatment was administered using a bonded acrylic splint and a hyrax screw appliance following the method outlined by McNamara and Brudon (Figure 1). The appliance was activated by turning the screw a quarter of a rotation (0.25 mm) every 12 hours, which was initiated immediately after it was placed.[12] On the days when intracranial pressure (ICP) measurements were conducted, the researcher activated the screws, while the patients or their parents performed daily activations throughout the expansion process. The average duration of the treatment period was 14 days.

ICP measurements were conducted by a single operator using an US device from General Electric (GE, Vivid-e, Wauwatosa, USA) equipped with a 7.5 MHz linear probe. During the US imaging, patients were positioned supine with their head elevated at ~30°. To protect the closed upper right eyelid, a transparent film dressing was applied followed by a layer of water-soluble US gel. The operator carefully moved the probe to obtain the optimal image of the optic nerve entering the eyeball (Figure 2). The operator measured the ONSD behind the globe, approximately 3 mm, using the ultrasound images (Figure 3). Each bulb was examined two times by the operator and the mean values of the ONSD were used for data.

The measurement of ONSD was carried out immediately prior to the initial screw activation (T0). Subsequently, measurements were repeated at intervals of 1 minute (T1), 10 minutes (T2), and 60 minutes (T3) after the first activation. On the day when the expansion process concluded, the same measurement procedure was repeated, mirroring the protocol employed during

the initial activation appointment (T4, T5, T6, and T7). The study design and measurement times are provided in Figure 4.

Physiological parameters including heart rate, mean arterial pressure (MAP), and peripheral oxygen saturation (SpO2) were documented both before and after the screw activation intervals (T0, T1, T4, and T5) using conventional monitoring techniques (Spacelabs Healthcare, USA).

The patients' experience of pain during screw activation was assessed using a verbal rating scale (VRS-4) at T1 and T5. Each patient was requested to rate their pain level on the VRS-4, which consisted of self-explanatory categories ranging from no pain to mild, moderate, or severe pain.

Statistics

The statistical analysis was conducted utilizing the NCSS (Number Cruncher Statistical System) Statistical Software, developed in Utah, USA. The study data were assessed using descriptive statistical techniques, including calculation of mean and standard deviation. Additionally, the Shapiro-Wilk test was employed to evaluate the normality of the data. Repeated one-way analyses of variance and binary evaluations with a Bonferroni correction were used for comparisons among the time points of more than two normally distributed quantitative variables. A paired sample t-test was used to evaluate the differences between the each time point during the study period. A significance level of less than 0.05 was deemed significant for the conducted analysis.

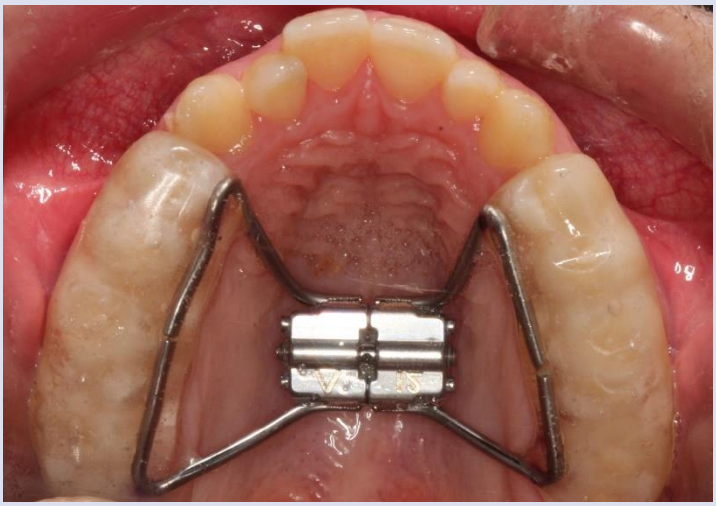

Figure 1: Bonded rapid maxillary appliance (Hyrax screw, Dentaurum, Germany).

Figure 2: Measurement of optic nerve sheath diameter ultrasonographically.

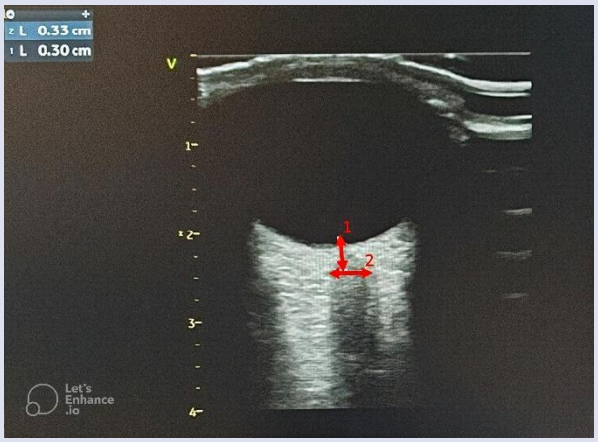

Figure 3: Ultrasonographic image of optic nerve sheath diameter measurement (1.Distance behind the optic disc where the optic nerve sheath diameter (ONSD) is measured in its width, 2. ONSD measurement).

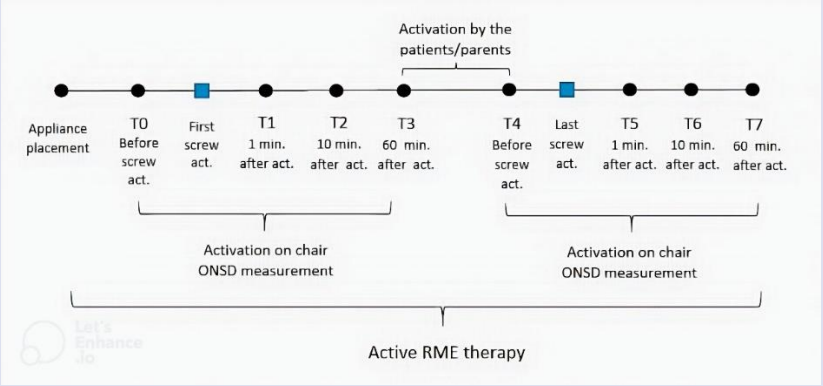

Figure 4: Study design and measurement times.

Results

A cohort of 25 individuals participated in the study. The average age was 13.10 ± 1.20 years. Detailed demographic information of the patients can be found in Table 1.

Mean ONSD measurements were 0.35 ± 0.03 at T0, 0.37 ± 0.05 at T1, 0.36 ± 0.05 at T2, 0.35 ± 0.04 at T3, 0.33 ± 0.031 at T4, 0.36 ± 0.04 at T5, 0.34 ± 0.03 at T6, and 0.34 ± 0.03 at T7. The results of the pairwise comparisons showed that the ONSD values, 1 minute after screw activations (T1, T5), were significantly higher compared to the ONSD values before screw activations (T0, T4) ($p = 0.001$). Nonetheless, a notable and statistically significant decline was observed in the ONSD measurements taken 60 minutes after screw activation (T3, T7) in comparison to the ONSD measurements taken 1 minute after activation (T1, T5). In contrast, changes in ONSD values at both 10 minute and 60 minutes following screw activations (T0-T2, T0-T3, T4-T6, T4-T7) were not statistically significant ($p>0.05$). Additionally, no significant alteration was observed between the initial (T0) and final (T7) ONSD measurements ($p>0.05$). Detailed comparisons of the mean ONSD values and corresponding descriptive statistics at different time points can be found in Figure 5 and Table 2.

After comparing the heart rate, SpO2, and MAP values of the patients before the initial activation (T0) with those after the first activation (T1), no statistically significant differences were observed ($p>0.05$). Similarly, there were no significant variations in these vital parameters between T4 and T5. A comprehensive depiction of the potential influence of hemodynamic parameters on ICP can be observed in Figure 6.

A total of 3 patients had severe pain score during screw activation at both the initial and final appointments of active RME treatment. 22 patients did not have mild or moderate pain score during the activations. Pain scores of the patients were insignificant at T0 and T1, and T4 and T5 ($p>0.05$).

Table 1. Demographic data of the patients

	Min - Max	Mean ± SD
Age	11 - 15	13.10 ± 1.20
Gender	Number	%
Female	15	60.0
Male	10	40.0

Table 2. Comparison of optic nerve sheath diameter (ONSD) values at each time point

	Mean ± SD	Median (Min/Max)	P
T0-T1	0.03 ± 0.03	0.03 (-0.03/ 0.10)	0.001
T4-T5	0.02 ± 0.02	0.02 (0/ 0.07)	0.001
T1-T3	-0.03 ± 0.02	-0.02 (-0.08/ 0.02)	0.001
T5-T7	-0.02 ± 0.03	-0.02 (-0.06/ 0.03)	0.007
T0-T2	0.02 ± 0.03	0.01 (-0.03/ 0.09)	0.113
T0-T3	0.01 ± 0.03	0.01 (-0.05/ 0.07)	1.000
T4-T6	0.01 ± 0.01	0.01 (-0.01/ 0.04)	0.110
T4-T7	0 ± 0.02	0 (-0.03/ 0.05)	1.000
T0-T7	-0.01 ± 0.03	0 (-0.08/ 0.03)	1.000

Repeated Measure Test & Post-Hoc Bonferroni Test *p < 0.05

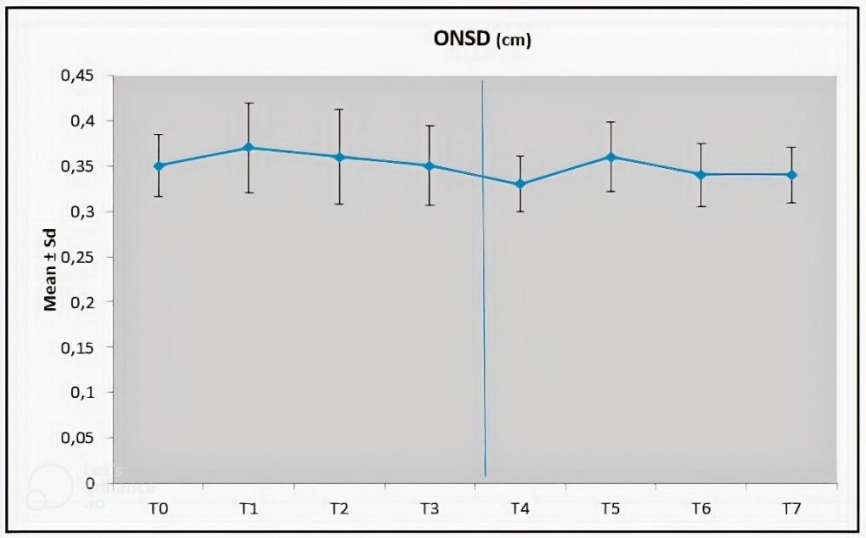

Figure 5: Changes in optic nerve sheath diameter (ONSD) values at each time point.

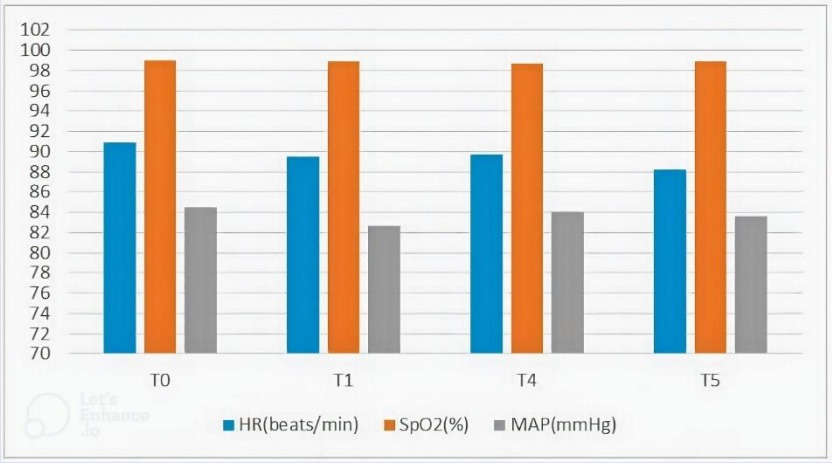

Figure 6: The hemodynamic parameters (HR, Spo2, MAP) before and after screw activation periods.

Discussion

In this current prospective observational study, we discovered that the activation of the screw during active RME therapy leads to a noteworthy enlargement of the optic nerve sheath diameter and a subsequent compensation in ICP over the course of one hour. In our extensive literature search, no previous study assessing intracranial pressure through ultrasonography-guided ONSD measurements in patients undergoing RME therapy was identified.

RME therapy has been initially applied during circumpubertal age, and the closure of median palatal suture usually occurs at ages 11 to 16 years.[13,14] *Narula and colleagues*[15] reported a substantial correlation between the CVM index and the maturation of sutures. The participants in the current study had an average age of 13.10 ± 1.20, and their skeletal maturity ranged from CS 1 to CS 3 stages, as determined by the CVM index. Considering the disruptive factors of midpalatal suture fusion and possible high stress accumulation on craniofacial structures, patients going through the CS 4 stage and above were excluded from the study.

Enlarged ONSD is a robust predictor of raised ICP.[10] Several studies have documented the rapid changes in intracranial pressure (ICP) associated with immediate variations in ONSD.[10] Several methods have been introduced in prior research to measure raised ICP.[16,17] Invasive methods such as intraventricular catheter are the gold standard for ICP monitorization.[2] However, these methods can lead to complications such as bleeding and infection.[18] In our investigation, we utilized ultrasound imaging to measure optic nerve sheath diameter (ONSD) and assess the impact of active RME therapy on intracranial pressure (ICP). Notably, no complications were detected throughout the active treatment procedure.

Several researches have hypothesized that the forces generated by palatal expansion have the potential to extend to deeper anatomical structures. Previous studies have highlighted the proximity of these structures to critical and delicate vessels that are crucial for cerebral blood supply.[6,11,19] Importantly, the strain exerted around various cranial base foramina can lead to the enlargement of adjacent blood vessels. [7]

In a study on rabbits by *Li et al*[6], it was reported that anatomical stresses on craniofacial structures during RME therapy cause cerebral hemodynamic changes, including increases in CBF and CBV. In a case report, it was reported that RME treatment may cause raised intracranial pressure in patients with impaired venous drainage and pseudotumor cerebri syndrome (PTCS) by affecting cerebral venous circulation on MRI.[7]

In our current investigation, there was a notable increase in ONSD one minute after screw activations (T1, T5) in comparison to the ONSD values prior to screw activations (T0, T4) ($p=0.001$). While the precise mechanisms underlying the elevation of ICP are not yet fully understood, we speculate that this phenomenon might be attributed to varying levels of stress and cerebral hemodynamic alterations within the intracranial region.

Our study revealed a significant decrease in ONSD values 60 minutes after screw activation (T3, T7) when compared to the ONSD values 1 minute after activation (T1, T5) ($p<0.05$). This observation suggests the involvement of the brain's autoregulatory capacity and mechanisms. A prior investigation utilizing perfusion CT reported an initial increase in CBF during the initial stages of RME therapy followed by a return to normal levels.[6]

When comparing the ONSD values immediately before activations (T0 - T4) to those 60 minutes after activations (T3 - T7), no statistically significant differences were observed ($p>0.05$). Furthermore, the correlation between the initial (T0) and final (T7) ONSD values was also found to be non-significant ($p>0.05$). These findings suggest that the optic nerve sheath diameter, which serves as an indicator of ICP, increases during activation and subsequently returns to its baseline value within an hour. Moreover, repetitive activations do not appear to have an impact on this process.

Researchers have pointed out that pain raises ICP by increasing blood pressure and causing respiratory irregularity.[20,21] In our study, patients' perception of pain during screw activation was evaluated by a four-category verbal rating scale (VRS-4) at T1 and T5. In both initial and final appointment of active RME treatment only 3 patients had noticeable pain during screw activation, and the pain scores were insignificant at T0 - T1 and T4 - T5 ($p>0.05$). Therefore, we suspect that pain was not the reason for the raised ICP.

In the study, ICP measurements were performed only during a short period of the active RME treatment, and only among adolescents. There is therefore a need to investigate the long-term effects of total RME treatment on intracranial pressure, and to assess these factors in adults.

Conclusions

This study has contributed to the assessment of rising intracranial pressure in patients undergoing RME treatment. Raised ICP is likely normalized by autoregulatory mechanisms of the brain and body for healthy people. We propose that healthcare professionals should take into account the potential hazards associated with elevated ICP, and ask patients about previous conditions before RME treatment, especially for adolescent patients.

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Not applicabble.

Conflicts of Interest Statement

All authors declare that no conflict of interests.

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The Effect of Food-Simulating Liquids and Thermal Aging on Surface Roughness and Color Stability of Bulk-Fill and Conventional Composites

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History

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ABSTRACT

Objectives: The aim of this study was to evaluate the effect of food-simulating liquids (FSLs) and thermal aging on the surface roughness and color stability of bulk-fill and conventional composites.

Materials and Methods: A total of 320 disc-shaped samples were prepared, with 40 samples from each of 4 different bulk-fill composites (Filtek Bulk Fill, X-tra fil, Beautifil Bulk Restorative, and Estelite Bulk Fill Flow), and 4 conventional nano-filled composite resins (Filtek Z550, CeramX SphereTEC one, Admira, and Kalore). The prepared samples were randomly divided into subgroups for exposure to FSLs (ethanol, heptane, citric acid) and thermal cycling (TC) (n=10 per subgroup) for 28 days. Initial profilometric surface roughness measurements (Ra_0) of all samples and AFM and SEM analyses of selected samples were followed by exposure to FSLs and TC. After completion of aging protocols, measurements and analyses were repeated to obtain the Ra_1 (post-treatment surface roughness), and change in surface roughness (ΔRa_{1-0}) was then calculated. Subsequently, initial color measurement of the samples was conducted using a spectrophotometer, followed by immersion of the samples in a coffee solution for 24 hours. Color measurements were repeated, and color change (ΔE) was calculated. Two-way repeated measures ANOVA was used to compare Ra_0 and Ra_1 values and one-way ANOVA for comparing ΔRa and ΔE values. Post-hoc Tukey tests were employed for pairwise comparisons. The significance level was set at α=.05.

Results: While the surface roughness of bulk-fill composites was affected by the protocols applied ($p<0.05$), most of the conventional composites generally remained unaffected. Bulk-fill composites exhibited greater ΔRa and ΔE values. The highest ΔRa and ΔE values were observed in the Beautifil Bulk Restorative group, with the greatest discoloration seen after immersion in citric acid.

Conclusions: Thermal cycling and immersion in FSLs affect surface roughness and color stability of composite resins depending on the content and structure of the composites.

Key words: Bulk-Fill Composites, Food-Simulating Liquids, Thermal Cycling, Surface Roughness, Color Stability.

Gıdaları Taklit Eden Sıvıların Ve Termal Yaşlandırmanın Bulk-fill Ve Konvansiyonel Kompozitlerin Yüzey Pürüzlülüğü Ve Renk Stabilitesi Üzerine Etkisi

ÖZ

Amaç: Bu çalışmanın amacı gıdaları taklit eden sıvılar ve termal yaşlandırmanın bulk-fill ve konvansiyonel kompozitlerin yüzey pürüzlülüğü ve renk stabilitesi üzerine etkisini değerlendirmektir.

Gereç ve Yöntemler: 4 farklı bulk-fill kompozit (Filtek Bulk Fill, X-tra fil, Beautifil Bulk Restorative ve Estelite Bulk Fill Flow) ve dört konvansiyonel nano dolduruculu kompozit rezin (Filtek Z550, CeramX SphereTEC one, Admira ve Kalore) kullanılarak disk şeklinde her birinden 40 toplam 320 adet örnek hazırlandı. Hazırlanan örnekler gıdaları taklit eden sıvılarda (etanol, heptan, sitrik asit) bekletilmek ve termal siklus uygulaması için rastgele 4 alt gruba ayrıldı (n=10). Gruplardaki örneklerin ilk profilometrik yüzey pürüzlülük ölçümü (Ra_0) ve seçilen örneklerin AFM ve SEM işlemi sonrası gıdaları taklit eden sıvılarda 28 gün bekletme ve termal siklus uygulamasına geçildi. İlgili işlemler sonrası ölçüm ve analizler tekrarlanarak (Ra_1) değerleri elde edilerek pürüzlülük değişimi (ΔRa_{1-0}) hesaplandı. Daha sonra örneklerin ilk renk ölçümü spektrofotometre ile yapılarak 24 saat kahve solüsyonunda bekletildi ve sonrasında renk ölçümü tekrarlandı ve renk değişimi (ΔE) hesaplandı. Ra_0- Ra_1 değerlerinin karşılaştırılması için two-way repeated measures ANOVA, ΔRa ve ΔE değerlerinin karşılaştırılması için one-way ANOVA, ikili karşılaştırmalar için post-hoc Tukey testi kullanıldı (α=,05).

Bulgular: Bulk-fill kompozitlerin yüzey pürüzlülükleri uygulanan protokollerden etkilenirken ($p<0,05$) konvansiyonel kompozitlerde grupların çoğu etkilenmemiştir. Bulk-fill kompozitler daha yüksek ΔRa ve ΔE değerleri göstermiştir. En yüksek ΔRa ve ΔE Beautifil Bulk Restorative grubunda görülürken en fazla değişim ise sitrik asitte bekletme sonrası görülmüştür.

Sonuçlar: Gıdaları taklit eden sıvılarda bekletme ve termal siklus uygulaması, kompozit rezinlerin içeriği ve yapısına göre yüzey pürüzlülüğü ve renk stabilitesi üzerine etkilidir.

Anahtar Kelimeler: Bulk-Fill Kompozitler, Gıdaları Taklit Eden Sıvılar, Termal Siklus, Yüzey Pürüzlülüğü, Renk Stabilitesi.

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Introduction

In recent years, the use of resin-based composite (RBC) materials in dental restorations has increased due to their ability to meet aesthetic expectations and technological advances in matrix, filler structure, and content.[1] Nano-filled composite resins, which are among the most commonly preferred composites today, are routinely used in the restoration of both posterior and anterior teeth in the clinical setting. Bulk-fill composites that can be inserted into cavities in a single layer with a thickness of 4-5 mm have been introduced as an alternative to these conventional composite resin materials, placed in 2 mm increments using layering techniques.[2] This innovation has overcome shortcomings of layering techniques, such as interlayer contamination, air entrapment, and weak interlayer bonding, which require technical precision and extended time for the restoration of large and deep cavities.[3,4] Bulk-fill composites are available in two different types: flowable (low-viscosity) and sculptable (high-viscosity). Studies comparing the mechanical properties of bulk-fill and conventional composites have yielded contradictory results.[2,5,6]

Over time, RBC restorations in the oral cavity are exposed to various factors such as the forces generated during chewing and the moisture introduced by saliva, as well as chemical compounds present in food and beverages, and thermal effects due to the varying temperatures of the food ingested.[7] RBC materials are susceptible to changes caused by chemicals found in food and beverages, leading to alterations in surface structure and physical properties. Consequently, increased surface roughness can occur over time, resulting in the loss of material's aesthetic qualities and aging.[8,9] The increase in surface roughness resulting from chemical degradation can lead to issues such as discoloration of restorations, increased plaque accumulation, irritation of soft tissues, and the development of recurrent caries.[10,11]

In order to assess the impact of chemical compounds present in the oral environment on dental composites, food simulating liquids (FSLs) have been established by the Food and Drug Administration (FDA).[12] Thermal aging and aging using FSLs are commonly employed methods for the artificial aging of composite resins.[13,14] Thermal aging is intended to simulate temperature fluctuations within the oral cavity. For this purpose, ethanol and citric acid can be used to simulate certain alcoholic beverages, soft drinks, vegetables, fruits, mouthwashes, candies, and syrups, while heptane is used to simulate butter, fatty meats, and vegetable oils.[15,16] It has been observed that the chemical composition and physical-mechanical properties of the composites are affected by the use of these solutions, with the matrix being more affected than the filler component.[17] In various studies, color changes and color stability of composites after exposure to FSLs and thermal aging have been examined, showing differential results based on the materials and solutions used.[18,19]

Profilometers are among the most commonly used devices to evaluate the surface roughness of dental composites. Profilometers provide quantitative measurements of surface roughness, typically expressed as Ra, for the examined samples. In addition to profilometric measurements, scanning electron microscopy (SEM) and atomic force microscopy (AFM) can be used to analyze surface roughness and characteristics.[20] AFM has the ability to provide detailed three-dimensional topography of the examined surface at a nanometric level. Thus, AFM is regarded as a reliable method for investigating the surface properties of dental materials following various procedures.[21]

In the literature, there are studies reporting the effects of FSLs on the surface and physical properties of bulk-fill composites.[14,22,23] Unlike previous studies, the present study aimed to investigate the effects of both FSLs and thermal aging on surface properties and color stability using conventional and bulk-fill composites with different content and characteristics, as well as to compare these properties among composites. The null hypotheses tested in our study were that FSLs and thermal aging would not have an impact on the surface roughness and color stability of the composite materials used, and that the surface roughness and color stability of the composites would not change according to the aging protocol.

Material and Methods

In our study, a total of 320 samples were prepared using four different bulk-fill composite resins (Filtek Bulk Fill, X-tra fil, Beautifil Bulk Restorative, and Estelite Bulk Fill Flow) and four conventional nano-filled composite resins (Filtek Z550, CeramX SphereTEC one, Admira, and Kalore), with 40 samples from each composite. Detailed information about the composites used is provided in Table 1. Teflon discs with a width of 8 mm and thickness of 2 mm were used for sample preparation. After placing the composites onto the discs, they were polymerized for 20 seconds using a LED light-curing device (Elipar DeepCure-S, 3M ESPE, St. Paul, MN, USA) with an output of 1470 mw/cm^2. At this step, the light intensity of the device was periodically monitored using a radiometer (Radiometer, Peng Lim Enterprise Co., Ltd., Taiwan). Following polymerization, the sample surfaces were sanded using 1000-grit sandpaper to achieve standardization. Subsequently, polishing was carried out using fine and extra-fine steps of a polishing system (Optidisc, Kerr Corporation, Orange, CA, USA) in a standard manner. Next, the samples were rinsed with water-air spray to remove residues from the surface and then dried. Finally, the samples prepared from each composite were divided into four subgroups (n=10 per subgroup) based on the solution in which they will be immersed (aging method).

Surface Roughness, SEM and AFM Analyses

Surface roughness and color measurements were conducted for all samples, and one sample from each

group was selected for SEM and AFM analyses. A contact-type profilometer (Surftest SJ-301, Mitutoyo, Japan) was used to measure the surface roughness of the samples, setting the cut-off length (λc) at 0.8 mm, tracing length at 4 mm, and stylus speed at 0.25 mm/s. Calibration of the profilometer was checked before starting the measurements and between the samples. Three measurements were obtained from different points on the surface of the samples, and the average of the measurements was used to determine the initial surface roughness value (Ra_0) for each sample, expressed as Ra.

One sample from each group was used for SEM and AFM analyses in this study. As the analysis would be repeated after the aging procedure following the initial SEM analysis, the samples were not coated. To enhance conductivity, the samples were wrapped with carbon tape to ensure no direct contact with the surface. The samples were then placed on the platform of the SEM device (Jeol Jsm 6510, Jeol Ltd., Tokyo, Japan). Representative areas were chosen for obtaining baseline SEM images at 1000x magnification under 5 kV electric current. Subsequently, for AFM analysis, the samples were placed in the AFM device (XE-100, Park Systems, South Korea), and images were acquired from a 20x20 µm area at a scanning rate of 1.0 Hz and a resolution of 512x512 pixels. The images from the AFM analysis were examined using the XEI Data Analysis Program, 1.6 Version (PSIA Inc., USA), and 3D topographic images were obtained. For each sample, the root mean square roughness (Sq) and average roughness (Sa) values were noted.

Aging with FSLs and Thermal Cycling

After completing the initial surface roughness, SEM, and AFM analyses, the samples underwent aging with three different FSLs and thermal cycling. For the first group consisting of each composite resin, thermal cycling (TC) was applied to the samples for a total of 5000 cycles of increasing temperatures from 5°C to 55°C (±2°C), with transfer times of 5 seconds and dwell times of 30 seconds. The second group of samples was immersed in a n-heptane solution (Tekkim Chemical Ind., Istanbul, Turkey) at 37°C for 28 days. The third group of samples was immersed in a 10% (w/v) citric acid solution (ChemBio Laboratory Research, Istanbul, Turkey) at 37°C for 28 days. The fourth group of samples was stored in a solution of 75% ethanol and water (Teksoll, Tekkim Chemical Ind., Istanbul, Turkey) at 37°C for 28 days. After completion of the immersion and thermal cycling steps, the samples were removed, rinsed, and dried. Surface roughness measurements were conducted as previously mentioned to determine the final surface roughness values (Ra_1). The change in surface roughness ($\Delta Ra_{1-0} = Ra_1 - Ra_0$) was calculated by subtracting the initial surface roughness values (Ra0) from the final Ra values (Ra1). Additionally, SEM and AFM analyses were repeated for one sample from each group.

Table 1. Type, chemical composition and manufacturer details of the composite resins used in the study.

Composite resins (Abbreviation)	Type	Contents	Filler particle size and ratio (%Wt/Vol)	Manufacturer
Filtek Bulk Fill (FBF)	High-viscosity bulk-fill	Bis-GMA, Bis-EMA, UDMA, Zirconia, Silica, Ytterbium trifluoride	0.004- 0.1 µm 76.5 / 58.4	3M ESPE, St. Paul, MN, USA
X-tra fil (XF)	High-viscosity bulk-fill	Bis-GMA, UDMA, TEGDMA Inorganic fillers in a methacrylate matrix	2 – 3 µm 86 / 70.1	Voco, Cuxhaven, Germany
Beautifil-Bulk Restorative (BF)	High-viscosity giomer based bulk-fill	Bis-GMA, UDMA, Bis-MPEPP, TEGDMA, S-PRG based on F-Br-Al- Si glass	No Data 87 / 74.5	Shofu Inc. , Kyoto, Japan
Estelite Bulk Fill Flow (EB)	Flowable bulk-fill composite	Bis-GMA, Bis-MPEPP, TEGDMA, SiO2, and ZrO2 fillers	avg 0.2 µm 70 - 56	Tokuyama, Japan
Filtek Z550 (Z550)	Nanohybrid	Bis-GMA, UDMA, Bis-EMA, PEGDMA, TEGDMA, Zirconia, Silica	0.02 - 3 µm 81.8 - 67.8	3M ESPE, St. Paul, MN, USA
Ceram.X SphereTEC One (CX)	Nanoceramic	Methacrylate modified polysiloxane, dimethacrylate, Barium-aluminum borosilicate glass, functional prepolymerized silicon dioxide	0.1 - 1.5 µm 77 / 55	Dentsply, Milford, USA
Admira (AD)	Ormocer	Bis-GMA, UDMA, Organic modified ceramic, silica	0.04-0.7 µm 79 - 56	Voco, Cuxhaven, GERMANY
Kalore (KAL)	Nanohybrid	UDMA, DX-511 (UDMA), Bis-EMA lanthanide fluoride, strontium glass, barium glass, fluoroalumina silicate glass, silicon dioxide	0.4–0.7 µm 82 - 69	GC Corporation, Tokyo, JAPAN

* Bis-GMA: Bisphenol A-diglycidyl methacrylate; Bis-EMA: ethoxylated bisphenol A glycol dimethacrylate; UDMA: urethane dimethacrylate; TEGDMA: triethylene glycol dimethacrylate; Bis-MPEPP: 2,2-bis(4-methacryloxypolyethoxyphenyl) propane; S-PRG: Surface pre-reacted glass-ionomer; PEGDMA: polyethylene glycol dimethacrylate;

Evaluation of Color Stability

To assess the color stability of the samples after aging, a contact-type spectrophotometer (Vita Easyshade® V, VITA Zahnfabrik GmbH & Co. KG, Germany) was used for color measurements. Color measurements of the samples were conducted on a white background under standard conditions. For each sample, color measurements were obtained three times consecutively from the center of the samples using the CIEL*ab* system. The average of three measurements was noted as the initial color values of L_0*, a_0* and b_0*.

Subsequently, the samples from each group were immersed in a coffee solution [Nescafé Classic 2 g (Nestlé, Switzerland) - 200 ml boiling water] for 24 hours, which corresponds to one month of coffee consumption [24]. After 24 hours, the samples were removed from the solution, rinsed with distilled water, dried using absorbent paper, and then subjected to the second round of color measurement. The color measurements were repeated three times as previously mentioned, and the average values were recorded as the final color values as L_1*, a_1* and b_1*.

The initial (L_0*, a_0*, b_0*) and final (L_1*, a_1*, b_1*) color values were used to calculate the color change (ΔE_{1-0}) for the samples using the formula;

$$\Delta E_{1-0} = [(L_1^* - L_0^*)^2 + (a_1^* - a_0^*)^2 + (b_1^* - b_0^*)^2]^{1/2}.$$

Statistical Analysis

Statistical analysis of the study data was performed using IBM SPSS Ver. 22.0 (Statistical Package for Social Sciences; IBM Corp., Armonk, NY) software. The normality of data distribution was checked using Kolmogorov-Smirnov and Shapiro-Wilk tests, while the homogeneity of the data was assessed using the Levene test. The results of these tests showed that the data exhibited normal distribution and homogeneity. For the comparison of initial and post-aging surface roughness (Ra_0/ Ra_1) values, a two-way repeated measures ANOVA was used, and Tukey's test was employed for comparisons among the Ra_1 values. One-way ANOVA was used to compare the data for color change (ΔE) and change in surface roughness (ΔRa), and Tukey's test was used to compare the differences among the groups. The statistical analysis of all data was conducted at a significance level of $p < 0.05$ with a 95% confidence interval.

Results

Changes in Surface Roughness Following Aging with FSLs and Thermal Cycling

Pre-aging (initial) and post-aging surface roughness values with standard deviations are presented in Table 2. After thermal aging and immersion in FSLs, the average surface roughness values of bulk-fill composite resins were found to be significantly different compared to the initial surface roughness values in all groups. In conventional composites, significant differences in Ra values were observed only in the groups where the CX composite resin was immersed in heptane and the AD composite resin was immersed in citric acid, while no significant differences were found in Ra values in other groups after aging.

Following thermal aging and immersion in FSLs, the BF composite resin group aged with citric acid showed the highest Ra value, while the lowest Ra value was observed in the KAL group subjected to TC. Except for the BF composite resin group, the final surface roughness values (Ra_1) were statistically similar among all composite resins. In the BF group, similar Ra_1 values were obtained after TC and heptane application, while the Ra_1 values observed after citric acid and ethanol applications were different. For the purpose of comparing the effects of TC and FSLs applications on surface roughness more accurately, surface roughness change (ΔRa) values were used for statistical analysis and comparisons were made based on these values. The ΔRa values and standard deviation values after TC and FSLs applications are displayed in Table 3, and the distribution by groups is shown in Figure 1. Considering the ΔRa values of the composite resins subjected to aging protocols, the highest ΔRa value was observed in the BF composite resin group after exposure to citric acid, while the lowest ΔRa value was found in the KAL composite resin group aged in heptane solution. The ΔRa values of bulk-fill composite resins were higher than those of conventional composites. Overall, all applications resulted in greater surface roughness change (ΔRa) in the BF composite resin compared to other composite resins. Except for the BF composite resin, all other bulk-fill composites showed similar surface roughness changes depending on the protocol applied after aging with TC and FSLs, while BF had higher ΔRa values compared to those of other bulk-fill composites. All protocols resulted in statistically similar ΔRa values in the FBF composite resin ($p=0.395$). For conventional composites, similar ΔRa values were observed after TC and ethanol immersion, while the KAL composite resin showed lower ΔRa values than other conventional composites after exposure to citric acid and heptane. Among other bulk-fill composite resins, the highest ΔRa values were obtained after ethanol application, whereas in the BF composite resin group, citric acid resulted in the highest ΔRa values.

Effects on Color Stability

The average color change (ΔE) and standard deviation values of the samples stained in coffee solution after TC and FSLs applications are presented in Table 4, and the distribution by groups is shown in Figure 2. Following immersion in coffee solution after aging with TC or FSLs, the ΔE values were significantly different depending on the aging protocol in all groups except for the FBF and XF composite resin groups ($p<0.001$). For the FBF and XF composite resin groups, the ΔE values resulting from staining solution after FSLs and TC applications showed no significant difference, indicating that they had no effect on color change ($p=0.094$ and 0.092).

The highest ΔE value was observed in the BF composite resin group after citric acid application, while the KAL composite resin group aged with TC showed the least color

change. After all applications, the highest ΔE values were observed in the BF composite resin in all groups, and the lowest ΔE values were observed in the KAL composite resin in all groups. For the BF composite resin, the ΔE values of the samples subjected to TC, heptane, and ethanol after staining were similar, while the color change of the samples treated with citric acid was statistically different. For the EB composite resin, the ΔE values of the groups exposed to TC and heptane were similar to those of the groups subjected to ethanol and citric acid. For the FZ550 composite resin, the ΔE values of the groups subjected to heptane and ethanol were comparable. For the CX composite resin, staining after heptane and citric acid treatment resulted in a similar color change which was greater than the color change caused by other applications. Compared to other conventional composites, the AD composite resin exhibited the greatest color change after exposure to staining solution following all aging protocols.

For the KAL composite resin, the ΔE values of the groups subjected to TC and heptane were similar to those of the groups treated with ethanol and citric acid. EB, FZ550, CX and KAL showed similar color changes after immersion in the staining solution following TC. Although KAL and XF composites exhibited similar color changes compared to FBF, EB, FZ550, CX following staining after immersion in citric acid, their color changes were different from each other. After all applications, BF exhibited significantly different color changes compared to other composites.

Table 2. Average surface roughness values (Ra_0/ Ra_1) and standard deviations before and after aging protocols

Composite Resins	Aging Types	Ra_0	Ra_1
Filtek Bulk Fill	Thermal Cycling	0.199 ± 0.012 ^{A}	0.222 ± 0.006 ^{B,a}
	Heptane	0.202 ± 0.008 ^{A}	0.221 ± 0.010 ^{B,a}
	Citric Acid	0.203 ± 0.012 ^{A}	0.226 ± 0.007 ^{B,a}
	Ethanol	0.201 ± 0.012 ^{A}	0.228 ± 0.009 ^{B,a}
			p=0.755
X-tra fil	Thermal Cycling	0.332 ± 0.015 ^{A}	0.351 ± 0.017 ^{B,a}
	Heptane	0.340 ± 0.014 ^{A}	0.362 ± 0.015 ^{B,a}
	Citric Acid	0.339 ± 0.016 ^{A}	0.368 ± 0.017 ^{B,a}
	Ethanol	0.336 ± 0.018 ^{A}	0.366 ± 0.019 ^{B,a}
			p=0.372
Beautifil-Bulk Restorative	Thermal Cycling	0.252 ± 0.013 ^{A}	0.314 ± 0.013 ^{B,a}
	Heptane	0.254 ± 0.013 ^{A}	0.313 ± 0.009 ^{B,a}
	Citric Acid	0.252 ± 0.011 ^{A}	0.428 ± 0.016 ^{B,b}
	Ethanol	0.254 ± 0.011 ^{A}	0.345 ± 0.010 ^{B,c}
			p=0.001
Estelite Bulk Fill Flow	Thermal Cycling	0.170 ± 0.012 ^{A}	0.190 ± 0.013 ^{B,a}
	Heptane	0.176 ± 0.013 ^{A}	0.194 ± 0.012 ^{B,a}
	Citric Acid	0.171 ± 0.011 ^{A}	0.193 ± 0.010 ^{B,a}
	Ethanol	0.180 ± 0.012 ^{A}	0.205 ± 0.012 ^{B,a}
			p=0.101
Filtek Z550	Thermal Cycling	0.178 ± 0.022 ^{A}	0.186 ± 0.022 ^{A,a}
	Heptane	0.183 ± 0.027 ^{A}	0.196 ± 0.024 ^{A,a}
	Citric Acid	0.176 ± 0.024 ^{A}	0.192 ± 0.029 ^{A,a}
	Ethanol	0.173 ± 0.022 ^{A}	0.185 ± 0.020 ^{A,a}
			p=0.400
Ceram.X SphereTEC	Thermal Cycling	0.212 ± 0.008 ^{A}	0.223 ± 0.005 ^{A,a}
	Heptane	0.208 ± 0.012 ^{A}	0.232 ± 0.015 ^{B,a}
	Citric Acid	0.220 ± 0.008 ^{A}	0.237 ± 0.011 ^{A,a}
	Ethanol	0.211 ± 0.014 ^{A}	0.227 ± 0.014 ^{A,a}
			p=0.124
Admira	Thermal Cycling	0.220 ± 0.013 ^{A}	0.231 ± 0.013 ^{A,a}
	Heptane	0.218 ± 0.012 ^{A}	0.232 ± 0.013 ^{A,a}
	Citric Acid	0.222 ± 0.019 ^{A}	0.241 ± 0.018 ^{B,a}
	Ethanol	0.217 ± 0.023 ^{A}	0.230 ± 0.024 ^{A,a}
			p=0.745
Kalore	Thermal Cycling	0.128 ± 0.012 ^{A}	0.135 ± 0.013 ^{A,a}
	Heptane	0.134 ± 0.013 ^{A}	0.139 ± 0.012 ^{A,a}
	Citric Acid	0.133 ± 0.011 ^{A}	0.141 ± 0.010 ^{A,a}
	Ethanol	0.137 ± 0.012 ^{A}	0.149 ± 0.012 ^{A,a}
			p=0.193

* Superscript uppercase letters indicate differences between Ra_0 and Ra_1 values in the same row. Superscript lowercase letters and p values indicate differences among the aging protocols in the same composite group.

SEM and AFM Analyses

Representative SEM and AFM images acquired for the purposes of observing any changes on the sample surfaces are provided in Figures 3-6. AFM analyses showed similar Sa values compared to those obtained with the profilometer. The surface topography and Sa values varied depending on the aging method and the composites used. Among the composite resins, the most irregular surface topographies were observed in the groups where BF composite resin was used. The BF composite resin stored in citric acid showed greater Sa values.

The results of SEM analysis were consistent with those of the AFM analysis. The increased susceptibility of the BF composite resin to FSLs was evident in the SEM images, where greater surface irregularities were observed. There were no significant changes on the surfaces of conventional composites.

Table 3. Average change in surface roughness values (ΔRa) and standard deviations following aging protocols

Composite Resins	Thermal Cycling	Heptane	Citric Acid	Ethanol	p values
Filtek Bulk Fill	0.023 ± 0.009 ^{A,a}	0.020 ± 0.005 ^{A,a}	0.023 ± 0.007^{A,a}	0.026 ± 0.006^{A,a}	p=0.395
X-tra fil	0.019 ± 0.005 ^{A,a}	0.022 ± 0.005^{A,ab}	0.028 ± 0.003^{A,b}	0.030 ± 0.008^{A,b}	p<0.001
Beautifil-Bulk Restorative	0.062 ± 0.002 ^{B,a}	0.060 ± 0.008^{B,a}	0.176 ± 0.005^{B,b}	0.091 ± 0.002^{B,c}	p<0.001
Estelite Bulk Fill Flow	0.020 ± 0.002 ^{A,ab}	0.018 ± 0.003^{A,a}	0.022 ± 0.002^{A,bc}	0.025 ± 0.003^{A,c}	p<0.001
Filtek Z550	0.008 ± 0.003 ^{C,a}	0.013 ± 0.007^{C,a}	0.016 ± 0.006^{C,b}	0.013 ± 0.005^{C,ab}	p<0.001
Ceram.X SphereTEC	0.011 ± 0.006 ^{C,a}	0.024 ± 0.009^{A,b}	0.017 ± 0.006^{C,ab}	0.016 ± 0.005^{C,ab}	p<0.001
Admira	0.011 ± 0.003 ^{C,a}	0.014 ± 0.005^{C,b}	0.019 ± 0.003^{C,c}	0.012 ± 0.003^{C,ab}	p<0.001
Kalore	0.007 ± 0.002 ^{C,ab}	0.005 ± 0.002^{D,a}	0.007 ± 0.002^{D,b}	0.011 ± 0.002^{C,b}	p<0.001
	p<0.001	p<0.001	p<0.001	p<0.001	

* Superscript uppercase letters indicate differences between the rows in the same column (composite resins). Superscript lowercase letters indicate differences between the columns in the same row (aging protocols).

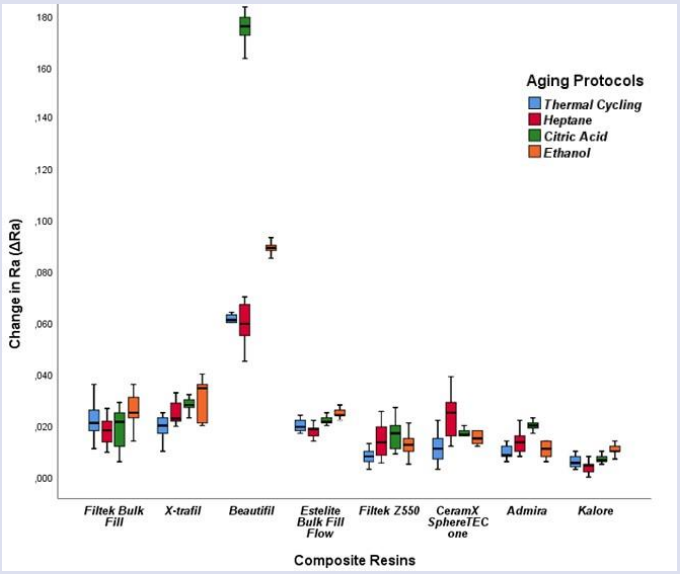

Figure 1: Comparison of change in surface roughness values (ΔRa) among composite resins after different aging protocols.

Table 4. Average color change (ΔE_{1-0}) values and standard deviations after immersion in staining solution, presented by aging protocols

Composite Resins	Thermal Cycling	Heptane	Citric Acid	Ethanol	p values
Filtek Bulk Fill	1.989 ± 0.318 ^{A,a}	2.072 ± 0.082 ^{ADE,a}	1.939 ± 0.167 ^{AB,a}	2.155 ± 0.147 ^{AB,a}	p=0.094
X-tra fil	2.328 ± 0.234 ^{B,a}	2.199 ± 0.084 ^{AF,a}	2.179 ± 0.183 ^{B,a}	2.333 ± 0.146 ^{A,a}	p=0.092
Beautifil-Bulk Restorative	3.643 ± 0.243 ^{C,a}	3.950 ± 0.273 ^{C,a}	4.548 ± 0.313 ^{C,b}	3.863 ± 0.206 ^{C,a}	p<0.001
Estelite Bulk Fill Flow	1.590 ± 0.216 ^{D,a}	1.729 ± 0.155 ^{DG,a}	2.141 ± 0.124 ^{AB,b}	1.971 ± 0.170 ^{BD,b}	p<0.001
Filtek Z550	1.625 ± 0.058 ^{D,a}	1.887 ± 0.199 ^{DE,b}	2.161 ± 0.097 ^{AB,c}	1.845 ± 0.097 ^{D,b}	p<0.001
Ceram.X SphereTEC	1.593 ± 0.067 ^{D,a}	2.074 ± 0.133 ^{E,b}	2.162 ± 0.099 ^{AB,b}	1.862 ± 0.094 ^{D,c}	p<0.001
Admira	2.838 ± 0.128 ^{E,a}	2.365 ± 0.155 ^{F,b}	2.509 ± 0.200 ^{C,b}	2.830 ± 0.213 ^{E,a}	p<0.001
Kalore	1.390 ± 0.216 ^{D,a}	1.529 ± 0.159 ^{G,a}	1.921 ± 0.125 ^{A,b}	1.771 ± 0.170 ^{D,b}	p<0.001
	p<0.001	p<0.001	p<0.001	p<0.001	

* Superscript uppercase letters refer to the differences between the rows in the same column (composite resins). Superscript lowercase letters refer to the differences between the columns in the same row (protocols).

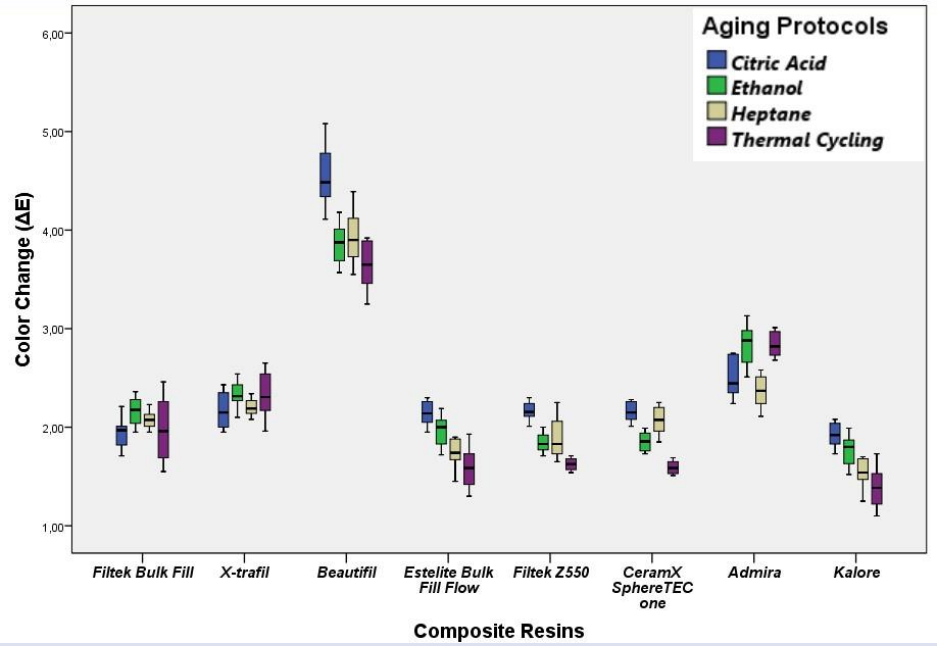

Figure 2: Comparison of color change (ΔE) among composite resins after different aging protocols

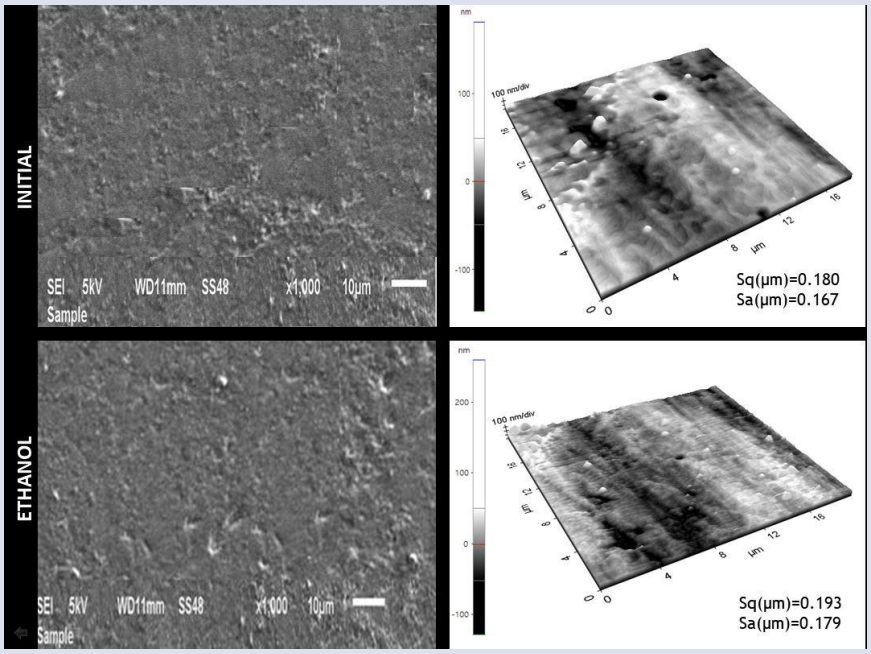

Figure 3: SEM and AFM images of FZ550 composite resin initial and after immersion in ethanol solution.

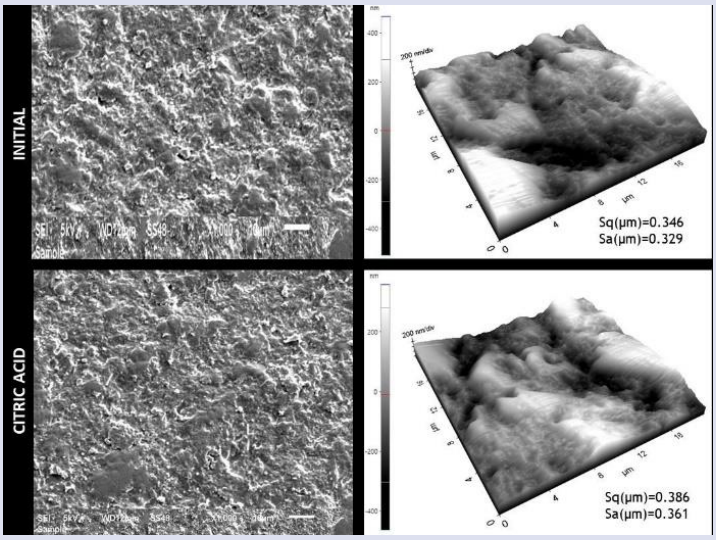

Figure 4: SEM and AFM images of XF composite resin initial and after immersion in citric acid.

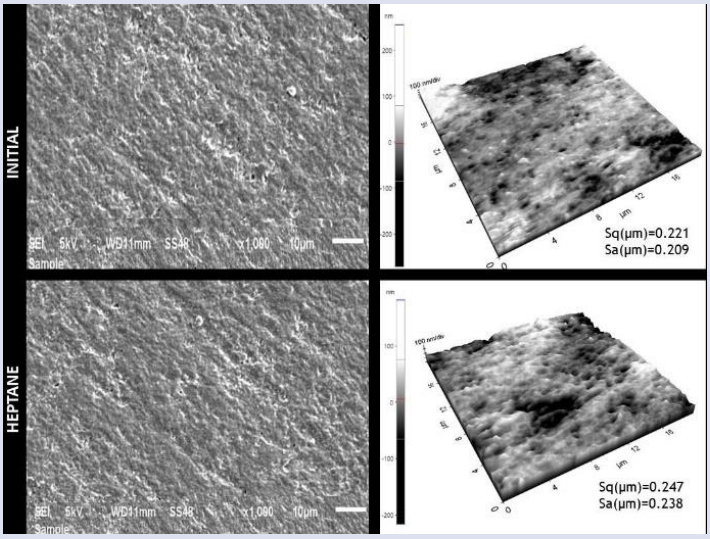

Figure 5: SEM and AFM images of AD composite resin initial and after immersion in heptane.

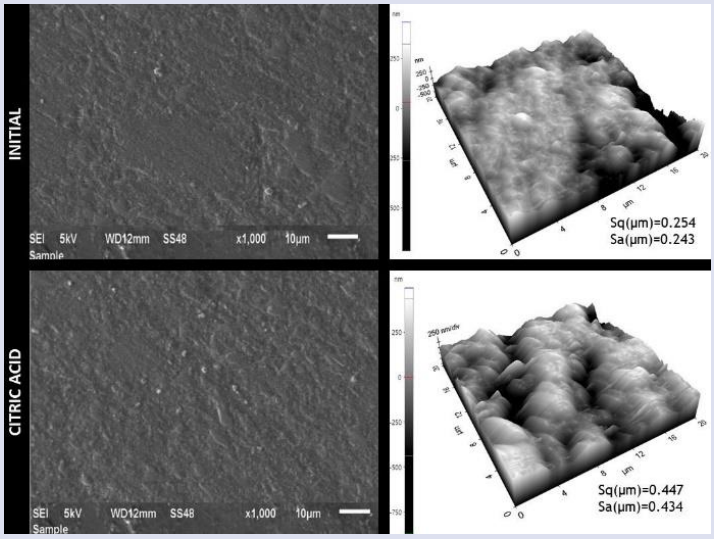

Figure 6: SEM and AFM images of BF composite resin initial and after immersion in citric acid.

Discussion

In this study comparatively evaluating the effects of thermal cycling and immersion in food-simulating liquids on the surface properties and color stability of bulk-fill and conventional composite resins, the protocols applied affected surface roughness and color stability of the bulk-fill composite resins, while for conventional composites, surface roughness was unchanged in all groups except for two groups. Moreover, changes in surface roughness varied depending on the protocol and composite used. The color stability of the stained composites also differed among the composite groups based on the protocol applied after thermal cycling and immersion in food-simulating liquids. Therefore, both null hypotheses were rejected.

Although improvements have been achieved in the materials used for dental restorations in modern dental practice, the longevity of dental composites in the oral environment remains a concern for clinicians. Dental composites can undergo changes over time in the oral cavity, such as discoloration, water absorption, dissolution, microleakage, increased roughness, and wear. Such changes are influenced by the type and ratio of fillers, as well as the content and monomers that make up the resin matrix.[25] Studies have reported that FSLs and TC which are commonly used to simulate the oral environment, lead to degradation, monomer release, dissolution, increased surface roughness, discoloration, reduced hardness, and accelerated aging of composite resins.[25, 26] These alterations have been attributed to the deterioration of the polymer matrix of the composite and the interface between resin and filler, as well as the loss of inorganic filler particles.[27, 28] In this study, the FSLs used, significantly increased surface roughness and discoloration particularly in bulk-fill composites, with variations observed among different composite resins. This discrepancy could stem from the structural differences among composite resins, as mentioned earlier.

The use of bulk-fill composites by clinicians is rapidly increasing due to the advantages they offer. Changes in the composition of bulk-fill composites have been designed to enhance polymerization depth compared to conventional composites. One of these changes includes increasing the size of filler particles. In bulk-fill composites, the aim of increasing the filler particle size is to enhance translucency to enable single-layer polymerization. The size, shape, and quantity of the filler particles and their relationship with the resin matrix are the key determinants of surface morphology.[29] The increase in filler particle size in bulk-fill composites leads to an increase in surface roughness, which also results in increased spaces between fillers, i.e., the resin matrix.[30, 31] Furthermore, irregularities resulting from the detachment of small particles from the surface are less pronounced. In our study, the greater increase in surface roughness and discoloration after FSLs and TC observed in the bulk-fill composites, especially those with larger particle sizes, compared to conventional composites might be attributed to the larger particle size and the greater ratio of resin matrix between the particles.

Giomers are a new generation of glass ionomer hybrid materials that release fluoride due to the surface pre-reacted glass ionomer (SPRG) particles embedded in their resin matrix. In our study, the highest ΔRa and ΔE values were observed in the BF group following aging protocols. Similar findings have been reported in other studies, where the greatest increase in surface roughness after FSL was found in giomer-containing materials, and particularly the high ΔRa and ΔE values observed in the groups exposed to citric acid were attributed to the potential degradation of SPRG particles in the presence of citric acid.[32,33] Looking at other composites, different aging protocols led to similar ΔRa values, and the variation in BF composite depending on the applied protocols supports the potential sensitivity of SPRG to citric acid. While there is no specific data on particle size for BF composite in the literature, it is believed that the void resulting from the degradation of SPRG particles might contribute to increased surface roughness and discoloration.[33] In a study by Cabadag and Gonulol, similar to our study findings, the surface roughness values of the BF increased compared to the initial values, but contrastingly, no significant difference was observed between the initial and final surface measurements of other bulk-fill composites; this discrepancy might be explained by variations in the duration of FSLs exposure in our study.[32]

Deterioration and discoloration of the composite restorations over time still represent their major disadvantages. Especially in today's world, where patients have higher aesthetic expectations, discoloration is one of the main factors affecting the replacement of composite restorations. The color stability of composite resins depends on various factors such as the resin matrix structure, water absorption, filler particle structure and size, and the matrix-filler relationship.[34] In our study, the higher ΔE values observed in bulk-fill composites indicating discoloration could be attributed to these factors. Additionally, the higher ΔE value of the AD composite compared to other conventional composite resins might be due to the presence of different types of fillers and their bonding to the resin matrix.[35] The better color stability of the KAL composite resin compared to other composites could be related to the hydrophilic properties of the UDMA and DX-511 monomers in its content, resulting in lower water absorption compared to Bis-GMA[36]. Degradation occurring at the filler particle-resin matrix interface and increased surface roughness due to mechanical and chemical deterioration can enhance discoloration.[33] It has been reported that the solutions used as FSLs, especially ethanol, reduce the surface hardness of composite resins and induce degradation at the filler particle-resin matrix interface, leading to the formation of microcracks.[7] In a study, the average ΔE value for the participants to notice color

changes was reported to be 1.8.[37] In our study, except for a few groups, color changes exceeding this value were observed in most groups.

As with any in vitro study, this study has a number of limitations. Restorations within the oral cavity are subjected to various conditions such as different temperatures of food and beverages, chewing forces, other mechanical factors, and the erosive effects of organic acids produced by bacteria. The inability to replicate combinations of these conditions in vitro is a limitation of this study. With advances in the characteristics and content of restorative materials, further research would be needed to fully assess the physical and chemical stabilization of these materials under various conditions.

Conclusions

Based on the data obtained from this study, it can be concluded that the food-simulating liquids and thermal aging have an impact on the surface roughness and color stability of the bulk-fill composites, while the surface roughness of conventional composites is less affected compared to bulk-fill composites. The findings of this study also suggest that the protocol applied and the composite structure affect the extent of surface roughness and color change. Moreover, the content of the products that people take with the diet is effective on the physical and chemical properties of the restorative materials used.

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Conflicts of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this article.

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Root Resorption in the Permanent Teeth. A Review

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Review

History

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ABSTRACT

Root resorption is losing dental hard tissue because of odontoclastic activity. It is undesirable and pathological in permanent teeth. Root resorption may happen within the root canal, called internal root resorption, or on the outer surface of the root, called external root resorption. Regardless of where it occurs, root resorption is irreversible, can cause pain for the patient, necessitates treatment, and in some circumstances, results in the early loss of the affected tooth. It might be challenging to diagnose and treat root resorption precisely. There is limited information within the literature on root resorption; therefore, this review aims to understand the clinical and radiographical characteristics of root resorption and evaluate their effect on the accurate diagnosis and management of root resorption in permanent teeth.

Key words: Root Resorption, External Resorption, Inflammatory Resorption, Internal Resorption, Replacement Resorption.

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Introduction

Root resorption is a destructive procedure, resulting in a gradual degradation and eventual loss of dentine and cement in the tooth root. It might be a normal or pathological occurrence. Root resorption in the primary teeth is normal except when it happens early.[1] However, the resorption of roots in primary dentition is a typical physiologic reaction. On the other hand, it has a pathologic origin in the permanent teeth, and the etiology necessitates two phases: injury and stimulus.[2] Injury can occur to the tissue covering the root's exterior surface (precementum) or inner surface (predentin). The injury might be mechanical due to trauma, surgery, or the compression of an impacted tooth.[3] It can also happen as a result of chemical irritation after using hydrogen peroxide to whiten teeth. Multinucleated cells inhabit denuded mineralized tissue and begin the resorption process.[4] However, under normal circumstances, the permanent tooth is surrounded by alveolar bone with very strong osteoblasts and osteoclasts without being affected by either of these cell types. According to many studies, this immunity is due to healthy cementoblast and odontoblast cell layers.[5] Consequently, after differentiation, an osteoclast's role is to resorb hard tissue (i.e., enamel, cementum, dentine). The process of hard tissue breakdown occurs by dissolving inorganic materials with acids, along with the degradation of organic components.[6]

Root resorption is self-limiting until the clastic cells are stimulated further. If the damage is minor, it can be repaired by cementum-like tissue in 2 to 3 weeks. The active resorption process will continue in the presence of a general stimulating factor of the osteoclastic cells, such as inflammation or pressure. Each form of root resorption has a different origin. As a result, the various forms of root resorption should be distinguished according to the factors that stimulate them. It will be feasible to stop the process by eliminating the etiological aspects once these stimulating factors have been discovered.[7]

This review aims to discuss the etiological factors as well as the radiological and clinical features of root resorption to summarize the current strategies of proper diagnosis and evaluate the effectiveness of any interventions to manage root resorption in permanent teeth.

Diagnosis of root resorption

Early detection is the most important and beneficial aspect of root resorption treatment because the faster treatment begins, the less extreme the resorption effects will be. Because the lack of pathognomonic symptoms makes an accurate diagnosis more complicated, a definitive diagnosis can be reached through a thorough clinical and radiological examination.[8] Because teeth with root resorption are usually asymptomatic, the diagnosis may be made by chance during a radiographic examination, and sensitivity testing may be effective for root resorption detection.[3] The most common method for diagnosis is intraoral radiography, and most clinical investigations utilize radiographs of an irregular root surface contour at various angles to establish which surface is involved.[9] However, using an intraoral radiograph with a panoramic radiograph has been suggested to improve assessment in

the anterior areas.[10] Because the limits of standard dental radiography are well known, numerous case studies have shown that cone-beam computed tomography (CBCT) can help diagnose resorptive lesions early in their progression. The precise visualization provided by CBCT leads to better definitive outcomes.[11,12]

Because several tiny resorptive channels stretch inside the dentine and are trapped beneath the root surface, the resorption cannot be adequately examined intraorally; however, a CBCT scan enables observation of these abnormalities, which is a good alternative for histopathological analysis.[12]

CBCT can provide the following information: the position, size, and form of the lesion, the existence of root perforations and apical bone pathology, the thickness of the root dentine, and the location of specific structures such as the inferior alveolar nerve canal, maxillary sinus, and mental foramen. These findings support the differential diagnosis and enable a prognosis evaluation of the tooth if the condition is treatable.[13] CBCT can also detect the root resorption complexity accurately and aid the clinician in determining the precise amount of the resorptive lesion.[14,15]

Classification of root resorption

1. Internal root resorption

It begins along the root canal surface and may cause the neighboring radicular dentine to deteriorate gradually. This entity occurs inside the canal system.[16]

Etiology

The exact etiological and pathogenic processes are yet unknown. Internal resorption may be linked to traumatized/replanted teeth, teeth with pulpotomy and crown preparations, partial pulp removal, caries, pulp capping with calcium hydroxide, intense heat, and a broken tooth. These agents activate the pulp tissue, triggering inflammatory reactions, which lead to the conversion of specific undifferentiated pulp cells into osteoclasts or macrophages, resulting in dentine resorption.[17,18]

Male patients have a higher prevalence than female individuals. The previous literature rarely discussed the prevalence of internal root resorption. Thoma (1935) showed only one case out of 1000 teeth of internal resorption studied.[19] Another study observed internal resorption in eight out of 28 teeth (28%) following coronal pulp amputation and calcium hydroxide capping.[20] 51.5% of this resorption in auto-transplanted maxillary canines was found in another study.[21] In these studies, the diagnosis of resorption was based on 2-dimensional radiography data, which would have underestimated the exact prevalence of internal root resorption.[22]

Internal root resorption has two sub-categories, as described by Sak et al. (2016):2

a. Internal inflammatory resorption

The pulp chamber or root canal enlarges in an ovoid or fusiform shape. The enlargement usually spreads in apical and lateral directions, as shown in Figure 1 (a). The pulp may be inflamed on a long-term basis. Although persistent inflammation is widespread in pulpal infections, it does not create the conditions for root canal inflammatory resorption to occur.[22]

Figure 1a: Internal inflammatory resorption.

b. Internal replacement root resorption

It is an uncommon type, and the canal space may increase unevenly. This root resorption shows metaplastic changes, and there are diffuse zones of heterogeneous radiopacities and radiolucencies, as shown in Figure 1 (b). It may result in obliterating the canal by cancellous-like bone. The exact cause for this occurrence is unknown. Dental pulp stem cells may produce the osteoid material in reaction to trauma, inflammation, or infection.[22]

Figure 1b: Internal replacement root resorption with metaplasia.[22]

Figure 2: Pink/red spot in the cervical area of a maxillary central incisor with internal resorption.[23]

Clinical features

Clinical features of internal inflammatory and internal replacement resorption are similar. Internal root resorption has different clinical features depending on its development and where it occurs. Most teeth with this resorption have no symptoms; however, when the resorption is actively proceeding, the tooth is at least partly vital and may present with pulpitis-like symptoms. In established internal resorption cases, the pulpal tissue may become necrotic and persistently infected, causing signs and symptoms of acute or chronic apical periodontitis. A pink or reddish discoloration visible through the crown of the concerned tooth may be produced by severe resorption of the coronal pulp, as shown in Figure 2.[23,24]

Treatment

Following the diagnosis of internal root resorption, the dentist must determine if the tooth is restorable. Root canal therapy is necessary if the tooth is judged restorable and has a good prognosis. Instrumentation and disinfecting the root canal with internal resorption presents a few unique problems not found in standard endodontic therapy. Because internal resorption lesions are inaccessible to chemomechanical debridement, activation of irrigants by ultrasonic after the preparation of the root canal system should be considered a necessary step in the cleaning and disinfection of the internal resorption defects to enhance the removal of necrotic tissue and biofilms from inaccessible parts of the root canal when used to agitate the irrigation solution (3% NaOCl, 17% EDTA,) and normal saline used as the final irrigation. An intracanal antimicrobial medicament should be employed to increase the disinfection of root resorption defects. Calcium hydroxide has been used as a dressing between appointments to eliminate microorganisms successfully that persist following chemomechanical instrumentation.[25,26]

Thermoplasticized techniques are recommended to enhance the seal of the internal resorption defect. If the resorption defect has perforated the root canal wall, bioactive hydraulic calcium silicate materials such as Biodentine or Mineral trioxide aggregate (MTA) must be used to repair the resorptive perforation defects.[27] Therefore, combining bioactive materials with thermoplasticized gutta-percha obturation will provide three-dimensional obturation and promote remineralization and healing.[28,29]

Recently, perforated internal root resorption has been treated using regenerative endodontic treatment. First, the entire root canal is debrided, followed by intracanal medicament such as calcium hydroxide for three months before MTA placement over the blood clot. This treatment method had shown encouraging results, as evidenced by the development of hard tissue in the perforation area and an increase in the thickness of the root canal wall after

2–3 years.[29,30] There is little information regarding the long-term result of this resorption and its management because of various treatment techniques that may result from the very few internal root resorption cases described in the literature.[31] Long-term clinical studies with good designs are necessary to evaluate the medium- to long-term results of internal resorption.

2. External root resorption

a. External inflammatory root resorption

Etiology

It is a resorptive defect that develops after a trauma, periodontal disease, or orthodontic therapy that causes an inflammatory reaction inside the periodontal ligament. This type of resorption is not a self-limited process and may result in massive damage.[32]

Clinical and radiographic features

The tooth is usually non-vital and may be either symptomatic or asymptomatic. It is characterized by abnormal mobility and the existence of a sinus tract. The tooth may be tender to percussion and palpation.[32] External inflammatory resorption is widespread in teeth with diseased necrotic root canal systems, and traumatic dental accidents do not necessarily cause it.[33] The usual radiographic appearance of these defects is scooping out radiolucency with an adjacent radiolucency in bone. In the resorption region, there is a total loss of the lamina dura, as shown in Figure 3 (a).[34,35]

Treatment

For teeth with extrinsic inflammatory resorption, the interceptive management strategy is to obtain access to the root canal if appropriate for treatment, explore the root canal, determine the working length, and do chemomechanical cleaning, then thoroughly irrigate and dry the canal. A Corticosteroid antibiotic paste, such as Ledermix paste, must be used as a root canal dressing and replace the Corticosteroid antibiotic paste intracanal dressing after six weeks. After that, obtain periapical radiography after another six weeks, and if the inflammatory resorption has not advanced, use a 50 - 50 combination of corticosteroid antibiotic paste and Ca (OH)2 to make a fresh root canal dressing. Then, obtain periapical radiography after three months and apply a Ca (OH)2 root canal dressing to stimulate hard tissue healing if the inflammatory resorption has not proceeded. The Ca (OH)2 root canal dressing must be replaced every three months till the resorptive lesion's hard tissue healing is visible in the periapical radiography. Once a hard tissue has healed, apply the gutta-percha and cement as root canal filling. Plan a six-month assessment, followed by a review for at least five years.[35]

If gutta-percha is utilized when this resorption occurs in the apex, extreme caution is necessary to ensure a proper cone fit because accidental over-extension of the root filling is likely. Root filling with calcium silicate bioactive materials may be advantageous due to their excellent biocompatibility, sealing ability, the potential to repair the periodontal ligament and cementum, the ability to improve the conditions for hard tissue repair, and the suppression of clastic activity.[16,36] However, Long-term evidence on this therapy approach is inadequate.

b. External replacement resorption or ankylosis external replacement resorption.

Etiology

The root substance is eventually replaced by bone, resulting in a direct connection between bone and root tissue. The most common reason is a traumatic injury, including severe luxation, such as lateral luxation, intrusion, and avulsed tooth replantation. In cases of the avulsion, replacement resorption was more frequently observed (87.2%), followed by intrusive luxation (57.1%), according to Soares et al. (2015) retrospective study.[37]

Clinical and radiographic features

On percussion, the tooth looks to be very hard in its socket, producing a high metallic sound. There is no evidence of tooth mobility, and the tooth is asymptomatic. The resorption area is filled with bone, and the periodontal ligament spaces disappear. So, there is no radiolucency, and the whole root may be substituted by bone, as shown in Figure 3 (b).[38,3]

Treatment

Replacement resorption, which tends to be persistent until the root is substituted by bone, currently has no therapy available. The resorptive process is delayed (but not prevented) in teeth when immersed in fluoride before replantation.[39]

A case series showed the use of regenerative endodontic treatment to manage three external replacement resorption cases. As a scaffold to encourage stem cell differentiation, they selected platelet-rich fibrin rather than an artificial blood clot, and then they added Biodentine on top of the scaffold. The resorption was arrested and, in some cases, reversed after three years of follow-up.[40]

c. Cervical root resorption

Etiology

The etiology is incompletely understood, the conjunction of damage to the periodontal ligament and the cementum, as well as other factors, has been suggested as a cause of cervical root resorption start. The most commonly reported risk factors were orthodontic treatments and a history of injury. The teeth most typically involved are the maxillary incisors, canine, first molar, and mandibular first molar.[41] It was found that when 35 percent hydrogen peroxide was utilized for whitening methods, these resorptions were detected in 6% - 8% of instances and 18% - 25% of patients whenever the hydrogen peroxide was thermally activated.[42] When 30 % hydrogen peroxide was placed in the pulp chamber of teeth that lost part of the cementum layer, 82 percent of the bleaching agent diffused to the periodontal tissues around the root.[43,44]

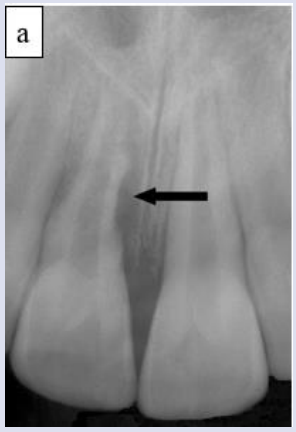

Figure 3a:External Inflammatory resorption.[35]

Figure 3b: Ankylosis external replacement resorption.[3]

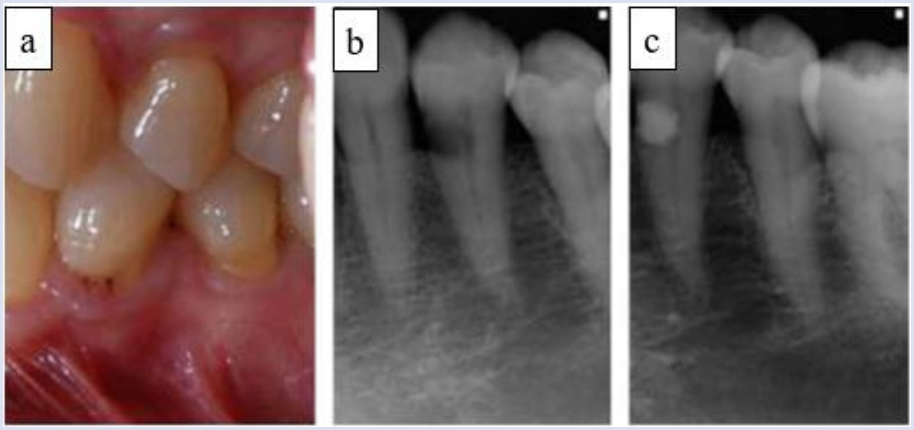

Figure 4: a) Cervical root resorption on the lower left first premolar. b) A radiolucent defect having ill-defined edges. c) After the cervical root resorption cavity was repaired using glass ionomer cement.[45]

Clinical and radiographic features

The symptoms of cervical root resorption vary widely and are influenced by many factors, including the position and the stage of advancement. Until there is a superimposed infection late in the resorptive procedure, the afflicted tooth will usually stay asymptomatic unless there are pulpal or periodontal complaints. Teeth without a pulp infection typically respond well to vitality tests. There is a lot of spontaneous and copious bleeding on probing, and around the resorptive cavity, there is the sharp, thinned-out margin. It has a wide range of radiographic appearances, which are determined by the size and type of the lesions. It usually shows an uneven, asymmetrical radiolucent area with a discernible root canal outline, as shown in Figure 4.[45,46]

Treatment

Treatment depends on the intensity of the lesion, its location and if it is perforated to the root canals, and the tooth's restorability. Many therapy regimens have been recommended depending on the form of the resorption defect. Therapy includes removing all resorptive tissues and filling the resultant defect with a tooth-colored plastic restoration. In situations when the cervical root resorption defect has perforated the root canal system, endodontic therapy may be necessary. Internal repair is suggested when the resorption is near or has already perforated the root canal, and a surgical approach is not feasible because of improper accessibility or if surgical access will result in the removal of an excessive amount of sound tooth structure. After endodontic therapy, the access cavity is restored. Under a dental microscope, long shank burs and ultrasonic tips help to eliminate the resorptive lesion. Then, Biodentine may be used to restore resorbed dentine, and its high pH may aid in stopping the osteoclastic action of any remaining osteoclast.[47,48]

Conclusions

Early discovery of root resorption and accurate documentation of the patient's history are essential steps for successful management, prognosis, outcome of root resorption, and treatment at the appropriate time, which will prevent tooth loss. With the current advancement of sophisticated imaging methods, such as cone-beam computed tomography, which is an effective screening method for confirming the existence of root resorption, and bioceramic-based endodontic materials, which allows for the extension of the limits for tooth conservation, the treatment of root resorption has become more predictable and successful.

Clinical studies are necessary to understand the etiology and pathogenesis of the various root resorption types. In addition, a deeper understanding of this area is essential since root resorption diagnosis and management can be difficult for clinicians and result in misdiagnosis.

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None

Conflicts of Interest

None

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Sclerostin - The Silent Bone Breaker

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Review

History

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ABSTRACT

A disparity between host defense and periodontopathogens leads to periodontitis, which is an inflammatory disease of the periodontium of high prevalence. The dysregulated host immune response brought on by the disease's ongoing progression may result in tissue and bone destruction, which ultimately leads to tooth loss. Interpretation of bone metabolism has enhanced as a result of the identification of sclerostin and its function as a bone mass regulator. Primarily, osteocytes express sclerostin, an SOST gene known to inhibit formation of bone. The canonical Wnt pathway involved in bone homeostasis, is significantly suppressed by Sclerostin. It is thought to result in resorption of bone by altering the ratio of OPG and RANKL. Characteristics, mode of action and significance of sclerostin in periodontal diseases are discussed in this review.

Key words: Sclerostin, RANKL, SOST, Periodontitis, Osteocytes, Osteoclasts, Alveolar bone.

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Introduction

Bone destruction is a hallmark of periodontal disease. Bone is a highly dynamic and active tissue that constantly renews itself in response to nutritional, mechanical and hormonal factors and a balance between the related processes of bone formation by osteoblasts and bone resorption by osteoclasts is necessary to maintain bone homeostasis.[1]

In general, GBR[2] which could be invasive, results in potential surgical concerns, and autologous or allogeneic bone transplants are frequently used in alveolar bone reconstructive therapy.[3] Periodontal regenerative medicine would benefit from treatments that can enhance bone formation, bone quality and increase bone volume with minimal invasion in order to satisfy this requirement.[4]

Developments in the field of research in medicine have identified a bone matrix glycoprotein known as sclerostin, which is produced primarily by mature osteocytes and is critical in the regulation of bone homeostasis.[1] It has a detrimental impact on bone formation and is known to be a potent antagonist of the Wnt signalling pathway.[5]

Sclerostin was discovered as a result of research on two rare bone diseases van Buchem disease and sclerosteosis, both which have higher bone mineral density and bone formation induced as a result of skeleton sclerosis.[6] It is produced by osteocytes, as OPG and RANKL and inhibits bone formation by competitively binding to LRP 5/6, reducing its ability to combine with Wnt proteins, which blocks the activation of the Wnt/β-catenin pathway. RANKL is a receptor of OPG which downregulates bone resorption and is a primary regulator of osteoclast differentiation and activation.[7] Sclerostin initially was considered to have anabolic properties but has shown to play a role in bone catabolism according to recent research.[8]

Structure of Sclerostin

The DAN/Cerebrus family of glycoproteins includes the 190-residue secreted glycoprotein sclerostin. It is a three-loop structure surrounding a cysteine knot with a long and highly flexible C- and N-terminal arms. Four extremely conserved cysteine residues make up the cystine-knot motif, which then forms two intra-chain disulfide bonds that generally have 8-14 residues. Additionally, sclerostin has a core that binds a semi-flexible loop and heparin, which blocks Wnt signalling.[1]

In order to give the protein a structured core, loops 1 and 3 have cysteine knots at their bases and additional disulfide bonds at their tips. Loops 1 and 3 have a substantial hydrophobic patch that could be a protein interaction site. Loop 2 is a binding site for antibodies also known as the "target site". The binding site for LRP 5 is located on loop 3. In monomeric form, sclerostin weighs between 27 - 28 kDa.[9,10]

Regulation and Expression of Sclerostin

Sclerostin has been discovered in chondrocytes and osteoclasts in addition to being primarily secreted by osteocytes. Sclerostin has been identified in bone, bone marrow, cartilage, aorta, pancreas, kidneys, and liver.[11,12,13]

Age, Mechanical stimulation, vitamin D, estrogen levels, PTH, PGE2, TGF-β, glucocorticoids, as well as other factors, affect the synthesis of sclerostin. According to numerous studies, Mechanical loading impacts SOST expression. Mechanical regulation of sclerostin under loading and unloading conditions was investigated by Robling et al. In both rats and mice, sclerostin synthesis was upregulated in a loading model and downregulated in an unloading model. Additional animal studies have shown that transgenic mice's osteocytes lose sclerostin when subjected to mechanical loading.[1]

Age is associated with higher serum sclerostin levels. Age-related impairments in bone formation is the cause of this increase. Circulating sclerostin levels are known to drop with estrogen, and its synthesis is enhanced by oestrogen deficiency. Sclerostin synthesis was found to be greater in men compared to women and serum levels were markedly lowered in postmenopausal women who received estrogen therapy.[14,15]

Sclerostin and vitamin D both inhibit the Wnt pathway. In patients with vitamin D deficiency, a reduction in serum levels of sclerostin was seen following vitamin D therapy. Dawson-Hughes *et al*. found that serum sclerostin levels were increased in healthy older men, in response to vitamin D and calcium treatment in comparison to women.[16,17]

Osteoprogenitor cell proliferation and differentiation are well-known to be regulated by prostaglandin E2. A significant reduction in SOST expression occurs through cyclic AMP, BMP signalling and EP2 receptor Ptger2. PGE2 thus inhibits sclerostin, stimulating the Wnt signalling pathway (18). Expression of sclerostin is downregulated by PTH. Runx2, which breaks down into prosteosomes in the presence of PTH, upregulates expression of SOST and PTH inhibits SOST expression by cyclic AMP/PKA pathway activation.[19,20,21]

Sclerostin production in osteocytes is increased by an increase in glucocorticoids. Prednisolone-treated mice exhibited increased SOST expression, indicating the involvement of glucocorticoids in inhibiting formation of bone mediated by the Wnt pathway. According to Thiele *et al*. serum levels of sclerostin were upregulated in mice after glucocorticoid administration but reduced in human mesenchymal stem cells and people on glucocorticoid therapy.[22,23]

Sclerostin's Biological Aspects

The Wnt signalling pathway is directly inhibited by sclerostin which prevents Wnt from attaching to LRP 5 and LRP 6. As a result, degradation of β-catenin is blocked, antagonising the Wnt/β-catenin pathway. Interaction between sclerostin and LRP 4, fosters sclerostin's antagonistic effects on Wnt/β-catenin signalling. Osteoblasts and bone formation are thought to be negatively regulated by sclerostin by affecting the differentiation and proliferation of osteoblast and inhibits mineralisation of osteoblasts. Additionally, there is suppression of osteoblastogenesis that causes the apoptosis of osteoblastic cells. It inhibits Wnt signalling, which promotes an unbalanced bone turnover. Along with inhibiting formation of bone, it also promotes resorption of bone. Sclerostin's ability to trigger bone resorption has thus been established.[1,24,25]

Sclerostin Distribution Within Oral Tissues

Recent research has demonstrated that expression of sclerostin in oral tissues along with alveolar bone osteocytes are found in odontoblasts, cementocytes, periodontal ligament cells (PDLCs), dental pulp stem cells (DPSCs) and in GCF as well. The diverse manner in which sclerostin is expressed in oral cells and tissues has revealed the ability of sclerostin to regulate dental homeostasis.[26,27,28]

Sclerostin has also shown to be expressed in mouse and human cementocytes. Deficiency of SOST gene seen in mice also causes thickening of the buccal and lingual cementum, reflecting the reduction in cementogenesis caused by sclerostin. Another study conducted on mice showed the lack of production of sclerostin during early stages of cementogenesis. At four weeks it was expressed in the apical cellular cementum, with an increased expression at eight weeks. This finding raises the possibility of the involvement of sclerostin in regeneration and maintenance of homeostasis of cementum.[29,30,31]

The Relationship Between Sclerostin and Periodontal Disease

Recent research shows that sclerostin regulates the alveolar bone catabolism and anabolism, which could lead to periodontitis. Inhibition of sclerostin could restore the morphology of the periodontal ligament and increase alveolar bone mass. According to a study, increased expression of sclerostin and RANKL was associated with increased formation of osteoclast and a decrease in the formation of osteoid in rats with ligature-induced periodontitis.

Sclerostin expression is decreased with an increase in osteoid formation, which emphasises the significance of sclerostin and RANKL in causing loss of bone.[32,33]

For formation of periodontal ligament, periostin is a crucial matrix protein. Periodontal ligament integrity is lost due to periostin deficiency, which also causes loss of alveolar bone, inflammation of periodontal tissue, formation of periodontal pocket, along with other periodontitis-like manifestations. [33]

Periostin and periodontal homeostasis are regulated by the regulatory role of sclerostin.[34] It is expressed more strongly in gingival tissues and GCF of periodontitis patient according to *in vivo* studies. Patients with periodontitis have been reported to have elevated GCF and salivary levels of RANKL. In addition, periodontitis patients' crevicular fluid contains higher levels of sclerostin, which may provide to be a more accurate indicator of the disease's diagnosis or prognosis than RANKL. Patients with chronic periodontitis showed an increase in sclerostin in gingival biopsies and peri-implantitis patients were also found to have increased levels of sclerostin in their PICF.[35,36]

Based on an *in vitro* study by Wijenayaka *et al*., exogenous administration of recombinant sclerostin

increased the production of RANKL. This suggests that sclerostin may promote osteoclastogenesis through RANKL. Additionally, sclerostin-induced osteocyte development has been linked to higher resorptive activity. Sclerostin has anti-anabolic properties, but it also causes pathogenic bone loss in periodontitis due to inflammation-induced sclerostin expression.

It was also evaluated how NSPT affected the production of sclerostin. Following NSPT, Balli *et al*. found reduction in the sclerostin levels in GCF, which showed an improvement in clinical parameters.[37] Patients with chronic periodontitis had 1.6 times higher levels of sclerostin expression than at baseline, but Beiler *et al*. found no significant difference between salivary sclerostin levels prior to and following NSPT.[38]

Role of Sclerostin in Dental Implantation

Dental implants are a credible procedure to replace missing teeth. For dental implants to successfully osseointegrate after placement, sufficient bone density at the edentulous ridge is essential. Accelerating regeneration of alveolar bone to reduce healing time of implant and uphold enduring stability is a challenge for stable osseointegration.[39,40] For clinicians, the treatment and prevention of peri-implant diseases are becoming more pivotal.

Sclerostin levels in patients with periimplantitis are higher in comparison to patients with perimucositis and healthy peri-implant tissues and the region around inflamed implants have significantly higher levels of sclerostin, according to results from *in vivo* studies. These finding raise a possibility that sclerostin could be a useful biomarker for peri-implantitis.[41,10]

Conclusions

Early discovery of root resorption and accurate documentation of the patient's history are essential steps for successful management, prognosis, outcome of root resorption, and treatment at the appropriate time, which will prevent tooth loss. With the current advancement of sophisticated imaging methods, such as cone-beam computed tomography, which is an effective screening method for confirming the existence of root resorption, and bioceramic-based endodontic materials, which allows for the extension of the limits for tooth conservation, the treatment of root resorption has become more predictable and successful.

Clinical studies are necessary to understand the etiology and pathogenesis of the various root resorption types. In addition, a deeper understanding of this area is essential since root resorption diagnosis and management can be difficult for clinicians and result in misdiagnosis.

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Evaluating the Predictability and Regenerative Capacity of Novel Platelet Concentrate (PC)-Titanium Platelet Rich Fibrin (T-PRF) in the field of Dentistry- A Narrative Review

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Review

History

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ABSTRACT

Periodontal disease treatment is always a challenging task. Various treatment modalities have been applied for treating this painless chronic condition. With the advancement in the field of research in dentistry, researchers shifted towards autologous products hence came the usage of platelet concentrates in various branches of dentistry. Initially, fibrin glue and platelet-rich plasma (PRP) have been tried, but because of their drawbacks, platelet-rich fibrin (PRF) came into play. Due to possible contamination of silica particles in silica tubes or silica-coated plastic tubes and shorter resorption time, titanium attracted the researchers. This led to the introduction of titanium platelet-rich fibrin (T-PRF), a second-generation platelet concentrate. This had a thicker fibrin meshwork, better cellular entrapment, and greater resorption rate, and titanium tubes are inert, better hemocompatible, and non-corrosive. It also eliminates the possible contamination of silica test tubes and silica-coated plastic tubes. The present article is a review of T-PRF and its usage in the field of dentistry.

Key words: Periodontitis, Platelet Rich Fibrin, Platelets.

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Introduction

Periodontitis is a dysbiotic multifactorial disease that results in the destruction of the periodontium.[1] Regeneration of lost periodontal tissues is always a challenging task for a periodontist, how experienced and skill full he is may be.[2] Several non-surgical and surgical treatment modalities have been tried over several decades in order to treat these periodontal bone defects to achieve a firm and healthy periodontal structures along with regaining of lost alveolar bone and other periodontal structures.[3] Gradually, there has been a trend of incorporating several biomaterials like bone grafts and collagen membranes as a part of regenerative treatment strategies besides open flap debridement (OFD) alone.[4] During this search for better biomaterials, scientists came across platelet concentrates, which were considered a boon to dentistry as they were autologous and prepared from patients' own blood.

Initially, first, generation platelet concentrates (PC) like fibrin glue and platelet-rich plasma were introduced. But because of the use of bovine anti-thrombin, there might be some hypersensitivity reactions. Robust release of growth factors within the first half an hour after the insertion at the surgical site. This rapid release was not sufficient for the activation of progenitor and wound-healing cells of periodontal regeneration. Hence standard or leukocyte platelet-rich fibrin (S/L-PRF) and advanced PRF (A-PRF) were introduced, which were totally autogenous without anticoagulants.[5,6]

Initially Fibrin glue, Platelet Rich Plasma (PRP) were introduced, but because of the usage of bovine anti-thrombin as a part of activating platelets which might cause hypersensitivity reactions and robust release of growth factors within first half an hour of insertion at surgical site not sufficient for activation of cells for regeneration led to further research where Standard or Leukocyte Platelet Rich Fibrin (S/L-PRF), Advanced PRF (A-PRF) were introduced that is totally autogenous without anticoagulants.[5, 6]

For easy identification of PCs, they were categorized into generations. First generation – PRP, second generation-Leucocyte –PRF, A-PRF, injectable –PRF (i-PRF), pure-PRF (P-PRF) and Titanium-PRF (T-PRF). Some additional PC's are A-PRF+, Concentrated Growth Factors (CGF), etc.[7] Recently, Kobayashi *et al.*[8], introduced Albumenized PRF (Alb-PRF), where PRF clot was dipped in albumin gel. Based on centrifugation type another advancement happened which is called Horizontal PRF (H-PRF) by Miron *et al.*[9], but these advancements are under protocols with in-vitro studies, and much research is not performed on humans. Some protocols of various PCs are shown in Table 1.

Table 1. Shows the protocols of some of the platelet concentrates used in the field of dentistry

Platelet Concentrates	Centrifugation Protocol
Platelet Rich Plasma[18]	1st Centrifugation 3000rpm for 3 minutes 2nd Centrifugation 3000rpm for 13 minutes
Leucocyte- PRF[11, 19]	2700 rpm for 12 minutes; 3000 rpm for 10 minutes
Advanced PRF[20]	1500 rpm for 14 minutes
Injectable PRF[21]	400-700 rpm for 7-8 minutes 3300 rpm for 2-3 minutes
Titanium PRF[13, 15, 17]	2700 rpm to 12 minutes 3000 rpm for 10 minutes 3500 rpm for 15 minutes
Concentrated growth factors (CGF)[22]	30 seconds acceleration phase; 2 minutes- 2700 rpm; 4 minutes- 2400 rpm; 4 minutes- 2700 rpm, 3 minutes- 3000 rpm; 36 seconds deceleration phase and stop
Albuminized PRF[8]	Initially, blood was centrifuged for 8 minutes with 700 grams of rotational force. Platelet-poor plasma was gathered and heated at 75°C for 10 minutes to create denatured albumin gel. Then buffy coat and denatured albumin gel to form Alb PRF.
Horizontal PRF[23]	700 grams rotational force in a horizontal centrifugation machine for 8 minutes.

PRF acts as a scaffold with entrapment of platelets, several white blood cells (WBCs), holds various growth factors like vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), insulin-like growth factor (IGF) and epidermal growth factor (EGF), it also holds several stem cells. Thus collectively acts at the surgical site and helps in rapid wound healing and regenerating the periodontal tissues[11]. But the resorption rate of this S/L-PRF & A-PRF was only 7-11 days, and O'Connell stated the possible silica contamination during their centrifugation preparations in silica or silica-coated test tubes.[11, 12] So there was again a search by researchers for a better biomaterial in order to eliminate these drawbacks, and this led to the introduction of a third generation of platelet concentrate Titanium Platelet Rich Fibrin (T-PRF).[13] Articles were searched in PubMed/ Medline, Google Scholar, Ebsco, Embase, etc., and gathered information so that a detailed report could be prepared. To the author's knowledge, we have included almost all recent studies, including systematic reviews and meta-analyses. The present review article briefly and exclusively discusses T-PRF regarding its history, histological analysis, centrifugation protocols, and its usage in various areas of dentistry as well as periodontology.

History of T-PRF

Due to the drawbacks of first-generation PCs, where silica particles within the test tube may get entrapped, sediment during the centrifugation, and contaminate the sample made, the researchers go on a hunt for better biomaterial. During this search, titanium metal has gained their attention and led to T-PRF preparation. As it is already established that titanium is regularly used in the preparation of dental implants, in 2013, Tunali M *et al.*,[13] introduced this T-PRF where sterile titanium tubes grade IV were used for centrifugation of blood immediately after drawing from patients. Titanium is a better biocompatible, haemocompatible, non-corrosive, and can passivate itself into the titanium dioxide (TiO_2) layer on the inner surface of the tube. This TiO_2 layer will activate platelets in a similar manner to that of silica particles and help in thicker fibrin meshwork and denser membranes with greater cellular entrapment.[14] This titanium also plays a role in the activation of osteoblast cells and progenitor cells of periodontium during the process of osseointegration after implant placement. It is stated that the resorption time of T-PRF was 21 days which was demonstrated on rabbits by Tunali M *et al.*, 2013.[13, 14]

Centrifugation Protocols:

Tunali M *et al.*,[13] stated a standard protocol of 2700 rotations per minute (rpm) up to 12 minutes where they have reported a properly polymerized PC clot. Chatterjee A *et al.*,[15] standardized their own protocol of 3000 rpm for 10 minutes. All recent studies followed the standard protocol of Tunali M *et al.* Study was done by Bhattacharya HS *et al.*,[16] and Gummaluri SS *et al.*,[17] used the modified Tunali M *et al.*,[13] protocol and obtained the T-PRF clots at 3500 rpm for 15 minutes. The basic method of preparation was that after the obtainment of fresh blood from the antecubital vein, it was directly transferred to sterile titanium tubes (Figures 1 and 2) with no delay then it was centrifuged with the appropriate protocols. Similar to normal PRF, three layers were reported as the top layer was plasma, the middle layer was a fibrin clot along with a buffy coat, and the bottom layer of RBC clot.

Figure 1: Shows the Image of T-PRF tubes

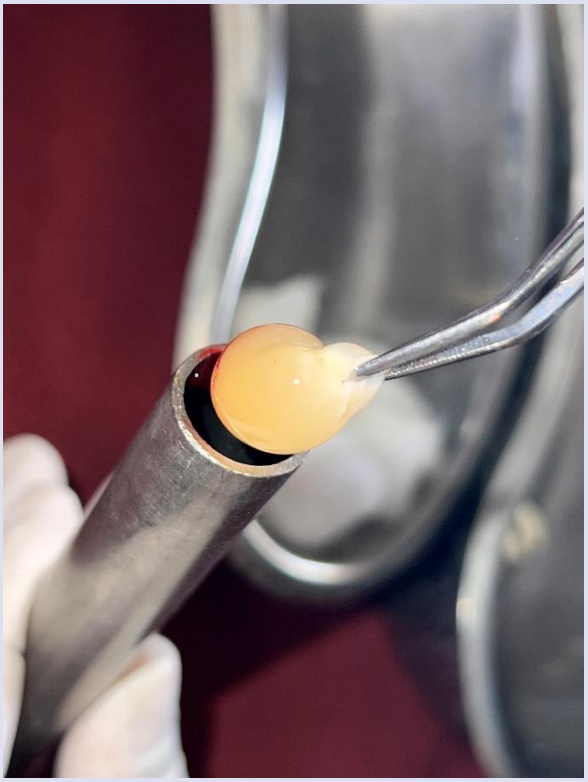

Figure 2: Shows the Image of the T-PRF clot retrieved from a titanium test tube.

Light Microscopic and Scanning Electron Microscopic Analysis

The structural and fibrin network pattern of T-PRF was evaluated by Tunali M *et al.*[13], where they concluded that T-PRF had a thicker fibrin meshwork with continuous integrity and covered a greater area of fibrin network than L-PRF. Further, in SEM analysis, a well-organized pattern with thicker fibrin meshwork and better entrapment of platelets were noted. Bhattacharya HS *et al.*[16], in their immune histochemical analysis, concluded that strong positive staining was reported for the distribution pattern of cells. For the labeling index also, positive staining was obtained for T & B- lymphocytes. Regarding localization of cells, positive staining was reported for platelets in T-PRF and for stem cells in L-PRF. A stronger significant positive staining regarding cell pattern was reported for neutrophils in L-PRF and B- lymphocytes in T-PRF. Further, Chatterjee A *et al.*[15], in their cell cytology study, stated that a thicker well-organized fibrin network pattern was recorded in T-PRF of healthy patients than in L-PRF. Hypertensive and smoker patients showed lesser fibrin border prominence in both the PC while adequate fibrin meshwork pattern was found in T-PRF clot when compared to L-PRF clot. These findings of thicker fibrin meshwork in T-PRF were also reported by Mitra DK *et al.*[24], in their histological evaluation.

Yajamanya SR *et al.*[25], in their cytology study, where the comparison between young and old age groups regarding fibrin network patterns of T and L-PRF stated that age plays a role in the quality and fibrin patterns of PRFs. Younger people have thicker fibrin meshwork and greater entrapment of red blood cells (RBC), platelets, and white blood cells (WBC) than older individuals. In older people, the pattern of PRFs was thin and loose, with less number of cells entrapped. Overall from the reports of the above studies, these histological evaluations are one mode of indirect evaluation for assuming the amount of healing and formation of new tissues after the placement of PCs at surgical sites. Thus T-PRF had better cellular entrapment with thicker fibrin meshwork and can hold an ample amount of growth factors which would stimulate the cells at the surgical site to recreate the lost tissues to as much extent as possible.

A recent study was done by Bhattacharya HS *et al.*[26], where histological sectioning of T-PRF and L-PRF was performed by collecting blood from 10 healthy volunteers. These T&L-PRF clots were subjected to processing according to protocols of Bancroft's manual and made into slides for LM analysis, and some clot samples were sent to SEM analysis. The results of their study concluded that there was no significant difference between T&L-PRF on histological analysis. SEM analysis also showed non-significance regarding fibrin thickness, cell entrapment, and structure, and significance was recorded in the body region of the clot. Thus. T-PRF can be a better alternative by eliminating the hazardous effects of silica.

Ravi S and Santhanakrishnan M[27], in their in-vitro study, compared T-PRF, L-PRF, and A-PRF, stated that T-PRF had high tensile strength, modulus of elasticity with greater time to degrade than L and A-PRF's. But the amount of release of GF, particularly PDGF AA, was rapid in T-PRF, while in A-PRF, it was a sustained release. Thus they concluded A-PRF as the most favorable PC for regenerative periodontal therapy

Uses of T-PRF in Dentistry

T-PRF applications in periodontal treatment

Various authors have utilized this T-PRF for the treatment of periodontal disease. Arabaci *et al.*,[28] used this T-PRF in the treatment of OFD, and the release of GF was observed. Results of the study stated that OFD+T-PRF helped in better soft tissue and surgical wound healing with lesser post-operative recession. Regarding GF's release, T-PRF had maintained a greater level of release of them up to 6 weeks post-operatively. Further, lesser levels of relative Receptor activator of nuclear factor kappa beta ligand/ Osteoprotegerin (RANKL/OPG) were reported, which indicated lesser post-operative bone loss and greater stabilization of surrounding periodontal tissues. Mitra DK *et al.*,[24] in their study concluded that both T and L-PRF groups had reduced probing depth (PD) and relative attachment levels (RAL) when compared from baseline to 9 months post-operative for intra-group comparisons, while on inter-group comparisons, both the groups showed no significant difference regarding the clinical and radiographic parameters of defect depth reduction.

A study was done by Paribas HG *et al.*,[29] where they utilized allograft as the control group, allograft+ T-PRF as the test group and assessed for PDGF-BB, VEGF-A, FGF-2, Anjiogenin (ANG), and Angiostatin (ANT) in gingival crevicular fluid samples at days 3, 7, 14 and 30th day. Where there was no significant difference regarding the release of these GF at all the time points, and T-PRF has no significant effects on angiogenic markers in the treatment of periodontal disease where bone grafts were treated with allograft+T-PRF. Chatterjee A *et al.*,[30] in their study treated the 90 intrabony defects in 38 patients by dividing into 3 groups, OFD alone, OFD+PRF and OFD+T-PRF and concluded that there was a significant improvement of decreased PPD, gain in CAL along with defect depth reduction 9 months post-operatively on comparison with OFD alone. While T-PRF+OFD and L-PRF+OFD showed no significant difference. Thus they concluded that both PC had shown improvements and could be used regularly for the treatment of periodontal disease.

In a study done by Gummaluri SS *et al.*,[17] treated 34 IBD with L & T-PRF and followed up to 9 months. They have concluded that both the groups have shown good improvements in clinical parameters indicated for soft tissue healing while T-PRF showed a more defect fill percentage indicating a greater hard tissue healing. They also stated that T-PRF can be a better alternative to L-PRF for treating IBDs.

A study was done by Ustaoglu G et al.,[31] on endo-period lesion-associated IBDs and stated that T-PRF had shown similar results with that of GTR membranes than OFD alone. Thus indicating that T-PRF can be used for the soft and hard tissue healing as an alternative to GTR membrane. A recent study done by Razi AM et al.,[32] used

PRF and T-PRF in 140 patients to manage endo-period lesions. They assessed the PPD and CAL at baseline 3 & 6 months and concluded that both the PC helped in good healing of the surgical site with no significant difference for inter-group comparisons, while intra-group statistical significance was reported from baseline to follow-up time periods.

Clinical pictures of T-PRF placement at surgical sites were depicted in Figure 3 and 4.

T-PRF application in treating gingival recession and assessment of palatal wound healing

Ustaoglu G *et al.*[33], also used T-PRF in palatal wound healing and histoconduction. In this, they used T-PRF clots and placed them at the donor sites where free gingival grafts were harvested and compared with the control group palatal site where no T-PRF clots were placed. In this palatal soft tissue thickness (PSTT) and wound healing of palatal mucosa, pain, bleeding, and post-operative consumption of painkillers were assessed. They followed up with the patients for up to 6 months and concluded that T-PRF helped in good palatal mucosal wound healing based on hydrogen peroxide bubble tests, significant color match scores at 1st and 2nd weeks, bleeding also reduced in the initial two days, and lesser intake of pain killers were noted in T-PRF group. There was no significant difference between baseline and 6-month follow-up of PSTT in the T-PRF group, while time-dependent thinning happened in the control group. They also concluded that T-PRF can be an alternative to sub-epithelial CTG (SCTG) because of its superior properties and equal functioning with that of SCTG.

Recent systematic review and meta-analysis done by Mahale SA *et al.*[34], stated that within limitations, T-PRF can be used for the treatment of IBD as it helped in reduced PPD and gain in CAL along with improvement in bone parameters. But the conclusions drawn were provisional because of a limited number of studies, shorter follow-ups, and histological evaluation was not performed that might indicate the actual amount of periodontal regeneration.

Figure 3: Shows the image of the T-PRF membrane placed in the intrabony defect

Figure 4: Shows the image of the T-PRF membrane placed on a gingival recession

Another systematic review done by Reshma AP *et al.*[35], also concluded that limited evidence was reported regarding the superiority of PRF over PRP and T-PRF over PRF for the treatment of osseous defects. There was no significant difference among the platelet concentrates. Hence the long number of randomized trials that would be published need to be considered and come to a proper conclusion.

At present, only a few randomized control trials have been performed regarding the usage of T-PRF as a biomaterial in the treatment of gingival recession. A study was done by Gummaluri SS *et al.*[36], and Ahmed S et al.,[37] where they treated Class I millers gingival recession with T-PRF as biomaterial using coronally advanced flap and modified coronally advanced tunneling technique (MCATT) and concluded that T-PRF is a good alternative biomaterial to connective tissue graft, eliminate the second surgical site and help in complete root coverage. Case report treated by Agarwal MC *et al.*[38], also stated that when T-PRF was used underneath the vestibular incision sub-periosteal tunnel access (VISTA) procedure in the anterior region helped in better healing and complete recession coverage of the surgical site. In a study done by Agarwal MC *et al.*[39], T-PRF was used as a biomaterial in pinhole surgical technique (PST) for the treatment of GR. During their follow-up periods, they concluded this PST was a conservative approach; it doesn't hamper blood supply, and patients experienced lesser post-surgical pain. Further, there was an increased post-surgical keratinized tissue width.

Koyuncuoglu CZ *et al.*[40], treated 62 miller class I/II gingival recessions using CTG and T-PRF with MCATT technique and followed up to 36 months where there was a root coverage with decreased recession depth, recession width and increased keratinized tissue width. They have concluded that T-PRF showed a similar amount of complete root coverage (80% and 56% for CTG at 6 months and 36 months, while it remained at 64% in T-PRF at 36 months) and acted as a good alternative treatment modality where the patient is not willing for a second surgical site.

Recent case series published by Bhattacharya HS et al.,[41] 2023 used T-PRF as a biomaterial underneath the coronally advanced flap for the treatment of Cairo type I recession defects. During their follow-ups, they concluded that T-PRF helped in the reduction of recession depth, recession width, and increased width of keratinized tissue with a mean root coverage percentage of 91%. They also stated that T-PRF eliminated the possible silica contamination and resulted in thicker fibrin membrane. Second surgical site preparation for SCT graft harvesting was also eliminated, as T-PRF recorded equal results with that of SCTG.[40]

T-PRF usage in Sinus Elevations and Dental Implants

Olgun E et al.,[42] compared the T-PRF with allograft in the treatment of sinus floor elevation, where they treated 18 posterior maxillary areas and randomly assigned 10 sites for T-PRF and 8 sites with allograft and followed up to 6 months. Radiographically allograft group showed better results, but histomorphometrically, T- PRF showed better bone formation, and it was accelerated to 4 months, and a greater time was reported with allografts. Thus they concluded that T-PRF alone usage in sinus operations had gained good histomorphometric and clinical results.

In implants, T-PRF was used by Ustaoglu et al.,[43] where it was compared with CTG. Soft tissue thickness (STT) and keratinized tissue width (KTW) were measured and compared at baseline and 3 months postoperatively. No significant difference was recorded for STT at the occlusal part of the alveolar crest measurement during inter-group comparison. During follow-up STT and KTW were increased, thus indicating T-PRF as an alternative to CTG in the treatment. Moreover, there was no crestal bone loss recorded in both T-PRF and CTG groups.

T-PRF as Drug Delivery System

This is one of the thrust areas where research will be performed in the near future. To the author's knowledge, only one study was published. Ercan E et al.,[44] compared T-PRF with Collagen Membrane loaded/injected with doxycycline gel. Further, anti-microbial efficacy and drug kinetics release for doxycycline. They have concluded that T-PRF holds the drug longer time and greater loading capacity than collagen. T-PRF loaded with doxycycline also had a thicker meshwork than collagen membrane on SEM images, it had long-term drug-carrying capacity, and the bibliophilic property of Doxy was mainly responsible for T-PRF's thicker fibrin meshwork.

T-PRF usage in Nerve conduction damage

Senturk F et al.,[45], in their study, utilized T-PRF in the treatment of facial nerve regeneration. It was an experimental study done on 27 male New Zealand rabbits. There were three groups. Where first group had a facial nerve dissected and left, while 2nd group had nerve dissection and suturing of that surgical site, and in 3rd group, apart from dissection & suturing, the T-PRF membrane was wrapped around the surgical area. Rabbits were followed up for 1, 3, 5, 7, and 10 weeks for whisker movements, motor reflexes, and lowering of ears. From their findings, they have concluded that T-PRF helped in partial nerve healing in both electrophysiology and functional levels.

Apart from this positivity, titanium tubes are costly; hence usage is still limited. On a brighter note, procuring them is like a one-time investment, and preparation of T-PRF at your clinical setup becomes much more regular as tubes are sterilizable and re-used.

Conclusions

Thus, within the limitations of this review, T-PRF can be a better alternative to L-PRF or A-PRF as it is autologous, eliminates the possible contamination of silica particles, Titanium metal is inert, non-corrosive, better haemocompatible, and activates platelets similar to silica particles. Thicker fibrin meshwork, longer duration of resorption, and greater holding of drug and sustained release made it an important biomaterial among PC and being regularly used in dentistry. It also eliminated the second surgical site and produced equal results with that of CTG. Further extensive research needs to be performed to establish ground-level firm evidence regarding its usage in the near future.

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Nil.

Competing interests

Authors declare Nil conflicts of interest

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Intramuscular Hemangioma of the Masseter Muscle Mimicking Parotid Sialolithiasis

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Case Report

History

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ABSTRACT

Intramuscular hemangioma occurs in less than 1% of soft tissue hemangiomas. It is equally prevalent in men and women. We examined a case of a masseter muscle hemangioma with evidence of a suspected parotid sialolithiasis. A 24-year-old woman with a 3-year history of swelling and mild pain in the cheek and ear area was referred to our Department. Due to the calcification in the anterior region of the masseter muscle observed in earlier CT images, sialography was scheduled for the differential diagnosis of sialolithiasis. Ultrasound imaging (Sonography) was performed as an initial examination that showed a complex tubular mass of 20 × 35 mm in the anterior region of the masseter muscle. The mass appeared hypervascular on color Doppler ultrasound, confirming its vascular nature. Due to its proximity to the facial nerve, a diode laser was adopted for treatment.

Key words: Hemangioma, Sialolithiasis, Masseter Muscle, Ultrasonography, Computed Tomography (CT).

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Introduction

A hemangioma is a neoplasm of endothelial origin that consists of vascular spaces composed of endothelial cells. It usually occurs in infants in their first year of life and disappears with age. [1]Hemangioma is more apparent on the skin and in the subcutaneous tissue. A rare type of hemangioma is intramuscular hemangioma (IMH), with an equal prevalence in men and women. It accounts for less than 1% of soft tissue hemangiomas[1], of which about 13.8% occur in the head and neck area, usually in the masseter, sternocleidomastoid, and trapezius muscles.[2]

IMH etiology is unknown. Among the factors that have been mentioned are Excessive muscle contraction, trauma, menstrual cycle, and pregnancy.[3] Magnetic resonance imaging (MRI), computer tomography (CT), and ultrasound imaging help diagnose IMH.[4] Unlike other hemangiomas, IMH does not regress spontaneously and is usually diagnosed in the second or third decades of life. In addition, IMH can be mistakenly diagnosed as parotid or other salivary neoplasms, emphasizing the importance of correctly diagnosing this.[5]

In this study, we examined a case of a masseter muscle hemangioma with evidence of a suspected parotid sialolithiasis in a 24-year-old woman.

Case report

A 24-year-old woman was referred to our radiology department with a 3-year history of swelling and pain in her right cheek that sometimes spread to the muscles of the ear and neck. Due to the swelling in the parotid region and the suspicion of parotid sialolithiasis, the patient was initially prescribed panoramic radiography, during which no specific findings were observed. A CT with contrast was then performed for further examination. On CT, an opacity in the anterior region of the masseter muscle indicated the presence of sialolithiasis (Figure 1). The patient was referred for a sialography examination for confirmation of sialolithiasis to the oral and maxillofacial radiology department.

On clinical examination, a smooth, round swelling was observed in the anterior region of the masseter muscle (Figure 2). It was well-circumscribed soft mobile swelling with pain and tenderness which was noticeable when chewing, manipulating, or palpation. However, no evidence of sialolithiasis-like sialagogue stimulation swelling was found. This swelling was well circumscribed soft mobile Due to swelling of the masseter muscle region, an ultrasound imaging before the sialography opted for the initial examination which was done with her consent. An ultrasound of the cheek and anatomical proximity was performed by grayscale sonography (E-CUBE7, Opinion, South Korea) with a multi-frequency linear probe (3-12 MHz) at frequencies of 8-10 MHz on the sonograms, a mixed echo tubular mass of 35 × 20 mm was observed in the anterior region of the soft tissue of the right cheek, which was

hypervascular on the color Doppler, suggesting a hemangioma and vascular malformations (Figure 3). Phlebolith echogenic foci of 3 mm diameter were also observed, which confirmed the diagnosis. A low-power diode laser was suggested for treatment because of its proximity to the facial nerve and the impossible surgery. The patient's consent was obtained to publish the case report.

Discussion

Studies show that IMH is more common in people under 30, although it might also occur in older ones, according to some case reports. IMH is equally common in both sexes. However, the involvement of masseter muscles is more prevalent in men.[6] The swelling is either painless [7, 8 9]or painful [6], which is the patient's chief complaint. The patient in our study was a 24-year-old woman with painful swelling in the cheek area. Jain[10], Chandrasekar[11], ElHariti[8], and Murugan[9] case reports were women too. while in contrast to our patient, Righini[3], Suraj[2], Lee[12], Kim[13], and Murugan[9] case reports, age patients were over 30 years old. The swelling was chief complaint in all of the patients (Table 1).

Although the etiology of IMH is not yet fully understood. Studies suggest trauma plays a role in the development of hemangioma and may be related to the etiology and growth of the lesion.[1] Our patient had a history of trauma in the past three years and swelling in the same area afterward.

The turkey wattle sign is an unusual pathognomonic manifestation of hemangioma within the masseter or the parotid gland. The lesion reshapes and enlarges when the jaw is clenched or the position of the head is changed. This symptom refers to swelling of the arteries in the lesion, preventing venous return from the head to the superior vena cava. Usually, no changes are observed on the skin's surface, while clenching the jaw causes the lesion to become firm and prominent. While manipulating the target area in our patient or clenching the jaw, a swelling was visible, a symptom of the turkey wattle sign. In rare cases, the area's skin may be bluish, with increased regional warming associated with hypervascularization.[8] However, there were no specific findings on the skin surface of our patient.

Only 8% of IMH cases are diagnosed before surgery.[8] The lack of symptoms is one of the factors that cause its misdiagnosis, which is confirmed by the long history of similar cases (Table 1). Ultrasound imaging, CT, and MRI are used to diagnose IMH along with clinical examination. Conventional radiological techniques help identify phleboliths and calcifications.[14] However, these are not specific, as our patient's panoramic radiography did not contain any findings. The hemangioma appears as an ill-defined mass on non-enhanced CT scans with a similar attenuation to the muscles. Phlebolith is too small to be detected on radiography. A significant enhancement is obtained after injecting the contrast agent.[13] In our patient, because of the presence of swelling and pain in the clinical examination, CT scan was requested. On contrasted CT scan, phlebolith be enhanced. it was similar to sialolithiasis which led to a misdiagnosis. ElHariti and Lee used of CT scan like our case and report high density[8] and calcification[12] on it (Table 1).

Because of the patient's history of swelling and pain, and since no signs of sialolithiasis were observed during the clinical examination, we used ultrasound imaging for further examinations; it is inexpensive, without radiation dose and initial imaging step in patients with soft tissue swellings .[14] On the sonograms, the presence of a well-defined hypoechoic mass along with heterogeneous echotextures in the head and neck might indicate the presence of a hemangioma. In IMH, color Doppler ultrasound is helpful to diagnose vascular structures within and around the muscle, assess pathological changes such as fibrosis, and identify calcifications. Vascular lesions are characterized by abundant vascular and high blood flow.[14]

The shape of phlebolith and calcification has also been described as a diagnostic feature of hemangiomas.[8] In our patient, the hemangioma was identified on the patient's sonograms as a mixed echo tubular mass in the anterior region of the soft tissue of the right cheek, which was hypervascular on the color Doppler and confirmed the diagnosis. Chandrasekar *et al.* and Lee *et al.* reported similar observations as a mixed echo mass with calcification in the right muscle of the masseter.[12, 14] Murugan *et al.*[9] found an isoechoic lesion in the masseter muscle, while Jain *et al.* and Makkad et al. observed a lobular and hypoactive mass in the muscle.[5, 10] In these case reports, the presence or absence of phlebolith resulted in a difference in the sonograms as mixed echo or hypoechoic. Most of the articles used the MRI T2 images for IMH diagnosis, which were seen as heterogeneous and mixed hyper and hypointense. But since ultrasound has better access and less cost than MRI, it was used in our case. In the differential diagnosis of IMH of the masseter muscle, conditions including hypertrophy, myofascial pain, sialolithiasis, parotid gland tumor, lymphangioma, lymphoma, rhabdomyosarcoma, and schwannoma are considered. For our case, due to evidence such as myofascial pain, cheek swelling on the differential diagnosis of the masseter muscle hypertrophy, and the CT with contrast, parotid sialolithiasis was present and suggested.

Various methods have been proposed for treating hemangiomas, including sclerosing agents, radiation, and surgery. However, the optimal accepted treatment is the excisional surgery of the lesion and the muscle around it. However, excisional surgery is associated with an increased risk of damage to the facial nerve. On the other hand, the diode laser is also effective in treating hemangiomas and vascular malformations. It reduces the lesion size and the disease symptoms and is better tolerated by the patient. Because of the reduced risk of facial nerve damage along with the benefits of using a diode laser [15], low-power laser treatment was suggested for our patient. For follow-up, the ultrasound was not performed on the patient, but after a few months, the patient expressed an improvement in pain and swelling in the area clinically.

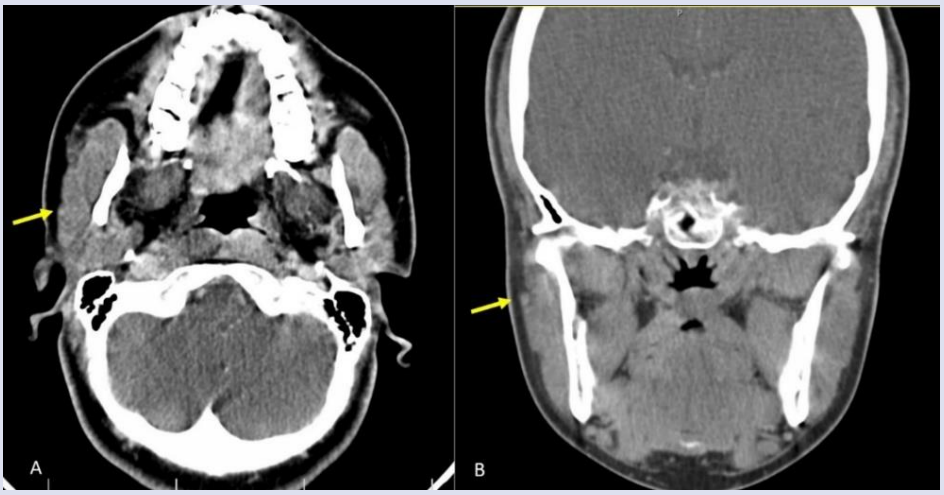

Figure 1. CT scans axial and frontal view: hypo to Iso attenuation mass in the right masseter muscle with phlebolith(arrow).

Figure 2. Slight swelling was observed in the anterior region of the right masseter muscle

Figure 3. Ultrasound image revealing a mixed echo lesion with a phlebolith in the right masseter

Table 1. Intramuscular Hemangioma case reports and radiographic findings

Study	Year	Age	Gender	Chief complain	History	Imaging modality	Imaging findings
Jain et al. (13)	2011	8	Female	Facial swelling	3 year	USG Color Doppler USG MRI	USG: Lobulated hypoechoic mass Color Doppler ultrasound: Evidence of color flow (internal vascularity) MRI: Isointense on T1 Nonhomogeneous hyperintense in T2 and PD
Righini et al. (3)	2014	70	Male	Firm painless well-contoured swelling	2 year	MRI	MRI: Well-Contoured mass, contrast-medium uptake
Chandrasekar et al. (10)	2014	23	Female	Painful swelling	6 months	USG Color Doppler USG MRI	USG: Mixed echoic lesion with a speck of calcification Color Doppler: Dilated vascular channels with good flow MRI: T2 mixed (hypo and hyperintense) mass signal, space-occupying lesion
Surej Kumar et al. (2)	2016	35	Male	Firm swelling	3 year	MRI Angiogram	Enhanced- well-circumscribed intramuscular mass
Lee et al. (12)	2016	42	Male	Muscular hypertrophy and Swelling during clenching Several months after treatment for hypertrophy: painful swelling	At childhood	USG CT	USG: Non-homogenous echo CT: swelling with calcification
ElHariti et al.(8)	2017	16	Female	Painless, well-circumscribed, firm swelling	2 year	CT scan MRI	CT: High density MRI: Voluminous encapsulated tissue mass in iso signal T1 and T2
Kim et al. (13)	2017	48	Male	Swelling	--	MRI External carotid angiography	MRI: strongly enhanced with heterogeneous T2 With multiple vascularity as signal voids. External carotid angiography: The blushed mass by contrast agent injection and gradually disappeared with time
Murugan et al. (9)	2018	37	Female	Painless dependent swelling	4 year	USG Color Doppler USG MRI	USG: Isoechoic lesion Color doppler USG: minimal color flow MRI: Intermediate signal on T2W1 and strong enhancement embedded in the anterior side of the masseter muscle
Makkad et al. (5)	2021	25	Male	Incidental finding of well-swelling	Gradual growth	USG Color Doppler USG	USG: lobulated, hypoechoic mass Color Doppler USG: Enhanced vascularity within the lesion
Present Case report	2023	24	Female	Dull pain and swelling	3 year	CT USG Color Doppler USG	CT: Opacity anterior of the masseter USG: Mixed echo tubular mass Color Doppler USG: Hyper vascular

Conclusions

Masseter muscle hemangioma can be associated with vague symptoms such as myofascial-sialolithiasis pain, parotid gland tumor, and masseter muscle hypertrophy. A careful ultrasound examination of the parotid gland and the anatomical proximity of the area, especially the masseter muscle, can help diagnose the condition promptly. It is suggested that in such cases, similar to the present case, before prescribing CT, which has a high amount of radiation,

ultrasound should be used as the initial image, and after that, CT should be prepared if necessary.

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Conflicts of Interest Statement

The authors declare that they have no conflict of interest.

Informed consent

Informed consent of the patient has been obtained for this case report.

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Comparing Facial Esthetic and Volumetric Changes of an Edentulous Patient After Either Conventional or Neutral Zone Technique

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Case Report

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ABSTRACT

The aim of the present case report was to compare the soft tissue measurements, and volumetric differences on the face of the patient whose complete dentures were fabricated either with conventional or neutral zone impression techniques. Two different complete dentures were fabricated according to conventional and neutral zone impression techniques. The measurement of soft tissue changes was evaluated on lateral cephalograms; conventional technique (L1), neutral zone technique (L2) and with no dentures (L3). Three images (I) were taken on the same day respectively with dentures completed by conventional technique (I1), dentures completed by neutral zone technique (I2), and with no dentures (I3). The superimposition of the images was evaluated. The cephalometric soft tissue measurements of the present case showed that upper and lower lips were positioned anteriorly in both conventional (L1) and neutral zone techniques (L2) on the sagittal plane. These results could be attributed to the fact that the placement of the prosthesis in the mouth helps direct lip position with big linear measurements. Volumetric difference between conventional (I1) and no denture (I3) images was more pronounced compared to neutral zone (I2) superposition with no denture (I3).

Key words: Facial Esthetic, Neutral Zone, Complete Denture, Lateral Cephalometric Measurement, 3dMD.

Dişsiz Bir Hastada Konvansiyonel veya Nötral Zon Tekniği Sonrası Fasiyal Estetik ve Hacimsel Değişikliklerin Karşılaştırılması

ÖZ

Bu vaka raporunun amacı konvansiyonel ve nötral zon teknikleriyle tam protezleri yapılan bir hastanın yüzdeki yumuşak doku ölçümleri ve volumetrik değişimlerinin kıyaslanmasıdır. Hastaya konvansiyonel ve nötral zon ölçü teknikleri ile 2 tam protez yapılmıştır. Yumuşak doku değişimleri lateral sefalogramda 3 ayrı şekilde değerlendirilmiştir: (L1) konvansiyonel teknik ile yapılan protez ile, (L2) nötral zon tekniği ile yapılan protez ile, (L3) protez kullanılmazken. Aynı gün, hastadan konvansiyonel teknik (I1) ile yapılan protez ile, nötral zon tekniği (I2) ile yapılan protez ile ve protezsiz iken (I3) yüz görüntü kayıtları alınmıştır. Görüntülerdeki superimpozisyonlar değerlendirilmiştir. Yapılan sefalometrik yumuşak doku ölçümlerine göre sagittal düzlemde konvansiyonel (L1) ve nötral zon tekniği (L2) ile yapılan protezleri taktığında, hastanın üst ve alt dudağının daha önde konumlandığı tespit edilmiştir. Bu durum protezlerin takıldıklarında dudağı yönlendirmesi ile açıklanabilir. Hacimsel farklılıklar, konvansiyonel (I1) ve protezsiz (I3) görüntü çakıştırmalarında, protezsiz (I3) ve nötral zon tekniği (I2) çakıştırmasına göre daha belirgin bulunmuştur.

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Introduction

The tongue, cheeks, and lips are the source of muscular forces that collectively specify the teeth positions and eventual dental arch form in the oral cavity. The effects of these muscular activities intensively influence the volume of the oral cavity space and continue throughout life even after tooth loss.[1] "Neutral zone" is defined as the potential space between the lips and cheeks on the buccal side, and the tongue on the lingual side. In this space, the forces between the tongue and cheeks or lips are equal.[2] Knowledge of the neutral zone concept provides advantages when fabricating complete dentures for the patients that have severely atrophic mandible. The technique helps us in placing the teeth and shaping the polished surfaces of the prostheses.[3] Therefore patients with partial glossectomy, mandibular resections or motor nerve damage, etc. can take advantage of this neutral zone treatment option.[1] In previous studies various techniques, for recording the neutral zone which maintains the polished surface of mandibular dentures, have been described.[4-6] Tissue conditioners, resilient lining materials, impression compounds, soft waxes and, silicones have been recommended for recording the neutral zone.[3]

Many studies exist in the literature that compared patient comfort in complete dentures fabricated with the neutral zone concept and dentures fabricated with conventional techniques. It has been shown that neutral zone dentures are functionally more stable than conventional ones.[3,6] Considering that conventional and neutral zone techniques can make differences in terms of soft tissue functions, it is important to evaluate and compare the changes of the soft tissue in the lower anterior facial region between these two techniques. To the best of our knowledge, there is no study encountered in the literature comparing conventional and neutral zone techniques with respect to soft tissue changes in a clinical report.

The aim of the present case report was to compare the functional ease of use, soft tissue measurements, and volumetric differences on the face of the patient, whose complete dentures were fabricated either with conventional or neutral zone impression techniques.

Clinical Report

A 70-year-old female patient was referred with the complaint of functional disability of her mandibular complete denture. The clinical examination revealed a misfit between the base of the mandibular denture and the denture-bearing mucosa, combined with phonetic problems originating from the maxillary denture and advanced mandibular ridge resorption associated with loss of vertical dimension (Figure 1). For the treatment of the patient, it was decided to fabricate a new complete denture with the neutral zone impression technique to improve the stability and functions of the prosthesis. The dentures fabricated with the neutral zone technique will improve the oral functions of the patient however we do not have any information about the esthetical changes that will be affected. Therefore, it was decided to prepare one more complete denture according to conventional technique. Thus, the soft tissue changes and volumetric differences of the patient could be seen.

After clinical examination, primary impressions for both the two dentures were made using a high-viscosity irreversible hydrocolloid impression material (Alginmax, major Prodotti Dentari S.P.A, Italy). Then two close-fitting custom-made impression trays were prepared with autopolymerizing polymethylmethacrylate resin (Orthoresin; Dentsply, Milford, DE). After that secondary impressions were prepared with zinc oxide eugenol impression material (SS White Mfg, Gloucester, England). The obtained impressions were poured with dental stone (Lab Stone Heraeus Kulzer, South Bend; IN). Acrylic resin plates were fabricated both for the upper and lower jaws for both dentures. Different from the conventional technique, the occlusal rim was adjusted only to the upper plate to support the upper lip to provide occlusal plane, and phonetics when recording the neutral zone impression. Midline, canine lines, and smile line were marked at the clinical try-in appointment for both dentures. After checking the mandibular base plate in the mouth, the patient was ready to start for neutral zone impression with tissue conditioner material (Visco-gel, Dentsply Ltd., Weybridge, U.K.)

The patient head table was adjusted in an upright position with the head supported comfortably, before starting the neutral zone impression. The patient was told to wear her previous maxillary denture to do the functional movements more comfortably than the maxillary occlusal rim. The mandibular base plate was placed to the ridge and the tissue conditioner in fluid viscosity was put bilaterally to the molar region of the mandible. When the tissue conditioner was getting hardened to simulate the physiological functions the patient was asked to swallow, speak, suck, purse lips, pronounce vowels, and slightly protrude the tongue several times. These actions are repeated for 10 minutes until the material is completely hardened. After the shaping of the molar region, the same procedure was applied both to the premolar and incisal regions (Figure 2). When the impression was completed, the base plate was removed and adapted to the master stone model. The impression of the neutral zone on the master model was removed, and locating grooves were prepared (Figure 3). A dental stone index of the model and the impression was taken to preserve the space of the neutral zone record. The tissue conditioner was removed and melted laboratory wax was poured into the negative space that belongs to the neutral zone. Occlusal vertical dimension and intermaxillary relationship record was transferred to a semi-adjustable articulator (Hanau Wide Vue II, Buffalo, NY). The artificial teeth were aligned according to the records of the neutral zone. Functional bite records were checked after the confirmation of centric relation in the clinic. After receiving the opinion of the patient about the esthetic and functional status of the try-in teeth, the

dentures were finished in the conventional technique. After the final clinical adjustments, both dentures were delivered to the patient. The extraoral and intraoral photos of the patient with conventional and neutral zone dentures are shown in Figures 4 and 5.

The measurements of soft tissue changes were applied using conventional lateral cephalograms (L) respectively taken with conventional technique (L1), neutral zone technique (L2), and with no dentures (L3) (Figure 6). Dolphin software (Dolphin Imaging Systems, Chatsworth, Calif) was used for the measurement of soft tissue changes on L. These measurements were made by one orthodontist (E.A.) previously calibrated.

The 3D images were taken in a separate room under standard light using the 3dMDface™ (3dMD Ltd, Atlanta, GA, USA) imaging system. Three images were taken with the patient sitting on a chair with adjustable height, without glass or any other equipment on the examined region. The patient wore a cap to expose the front and the external auditory meatus. The patient directed her chin slightly upwards with her eyes open. Three images (I) were taken on the same day respectively with dentures completed by conventional technique (I1), dentures completed by neutral zone technique (I2), and with no dentures (I3). 3dMDvultus™ (3dMD Ltd, Atlanta, GA, USA) software was used to perform superimposition of the images. After the images were manually aligned using the same software, automated superimposition procedures were applied. Two superimpositions were calculated which were respectively, conventional superposed with no dentures and neutral zone superposed with no dentures. We aimed to determine the changes in the facial contour for the same patient after wearing two different dentures. 3dMD histogram images are shown for both superimpositions in Figure 7. The root mean square (RMS) was considered accurate as 0.02 taking into account a reference article.[7] Volumetric and surface area differences were calculated automatically using software difference surface calculations.

On lateral cephalometric analysis total of 15 linear, 3 angular, and 2 ratio measurements are taken to determine soft tissue facial changes after wearing dentures. The results of the sagittal position of the upper and lower lip measurements are presented in Table 1. The upper lip was positioned 5.4 mm anteriorly related to the Sn-Vertical Line in L1 and 3.9 mm in L2, while it was 0.5 mm in L3. According to E-Line, the upper lip was positioned at -8.2 mm in L1 and -11.1 mm in L2, while positioned at -15.3 mm in L3. UL-SnPg' distances were measured respectively 1mm for L1, -0.7 mm for L2, and -4.1 mm for L3. Upper lip thickness to A point was 14.7 mm for L1, 14.1 mm for L2 and, 10.2 mm for L3. Lower lip was positioned 5.9 mm anteriorly related to the Sn-Vertical Line in L1 and 4.6 mm in L2, while it was 1.1 mm in L3. According to E-Line, the lower lip was positioned at -7 mm in L1 and -9.9 mm in L2, while positioned at -14.5 mm in L3. LL-SnPg' distances were measured respectively -0.2 mm for L1, -1.9 mm for L2, and -5.2 mm for L3.

The results of the vertical position of the upper and lower lip were also presented in Table 1. Upper lip length measurements (Sn-Stms) were respectively 20.3 mm, 22.6 mm and, 19.6 mm for L1, L2, and L3; while lower lip length measurements (Stmi-Me') were respectively 40 mm, 43.1 mm, and 37.1 mm for L1, L2 and, L3. Interlabial gap measurements were almost the same for all three groups, which were respectively: 1.5 mm, 1.2 mm, and 1.1 mm for L1, L2 and, L3.

Upper and lower sulcus and chin measurements for the three groups were also presented in Table 1. Subnasale to H-Line measurements were respectively 1.5 mm, -1 mm and, -5.8 mm for L1, L2 and, L3; while inferior sulcus to H-line was respectively 4.3 mm, 4.7 mm, and 2.3 mm for L1, L2 and, L3 groups. Superior sulcus depth according to Holdaway analysis was 5.4 mm, 3.5 mm and, 1.4 mm respectively for L1, L2 and, L3 groups. Soft tissue chin thickness for L1, L2, and L3 were respectively 12.5 mm, 14.1 mm and 13 mm. Soft tissue pogonion was positioned anteriorly 10.9 mm for L1, 12.9 mm for L2 and 15 mm for L3 according to Sn-Vertical line.

Soft tissue vertical measurements and convexity angle measurements are shown in Table 1. Facial convexity degree was higher in L1 (-4.5°) than L2 (-5.9°) and L3 (-16.8°). Nasolabial angle degrees were 97.1°, 96.5° and 104.9° respectively for L1, L2 and L3. H-angle was lower in L3 (-16.6°), than L1 (-0.1°) and L2 (-4.2°). Vertical soft tissue rate measurements were as following: Sn-Stomion/Stomion-Me ratio was 60.1 for L1, 65.2 for L2, and 54.8 for L3. G'Sn/SnMe' ratio was almost the same for L1 (1), L2 (0.9), and L3 (1.1).

3dMD software volume and surface area comparison was presented in Table 2. According to the results of the software comparison, the area difference between conventional (I1) and no denture (I3) images showed 2033.640 cm^2, and the volumetric difference between conventional (I1) and no denture (I3) images showed 129.634 ccs. The root mean square difference between I1 and I3 was 2.61 (-53.17- 56.01). Neutral zone (I2) superposition with no denture (I3) showed 2116. 242 cm^2 area difference, 63.969 cc volumetric difference and 2.44 (-24.30- 19.46) root mean square difference. After the prosthesis had been delivered to the patient, firstly the patient used the neutral zone complete dentures. The recall sessions were arranged for 1 day, 1 week, and 3 weeks later. After eight weeks use of the neutral zone dentures were replaced with conventional dentures, and the same recall sessions were arranged for conventional dentures. However, the patient was referred to the clinic with major complaints after three weeks of use of the conventional dentures and stated that the neutral zone dentures were more comfortable.

Figure 1: Extra oral photos of edentulous patient.

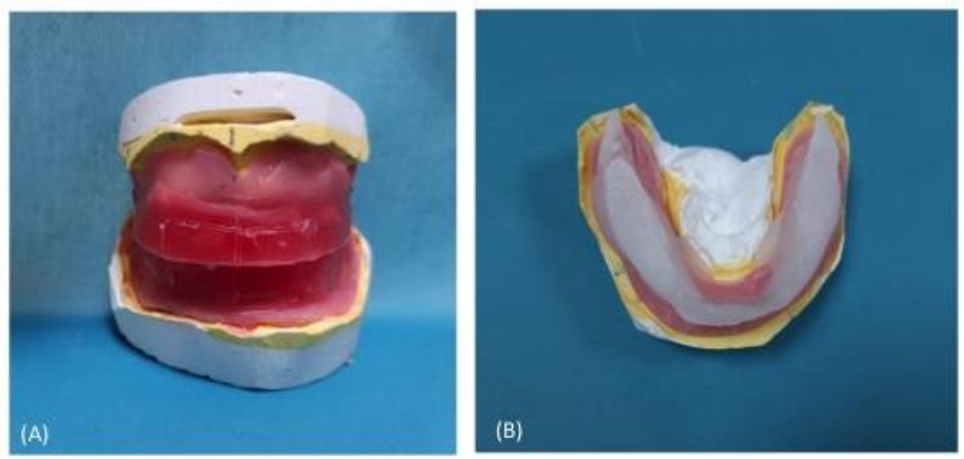

Figure 2: **A:** Occlusal rims for conventional denture; **B:** Mandibular base plate with tissue conditioner for neutral zone impression.

Figure 3: Laboratory stages of neutral zone impression.

Figure 4: Extra oral and intraoral photos of patient after wearing denture that fabricated with conventional technique.

Figure 5: Extra oral and intraoral photos of patient after wearing denture that fabricated with neutral zone technique.

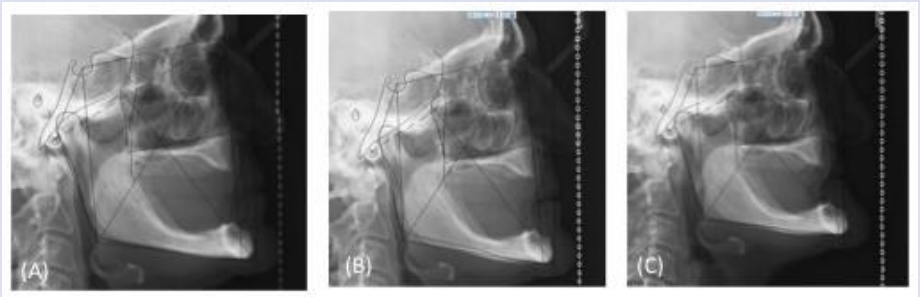

Figure 6: **A:** Lateral cephalogram taken with conventional technique; **B:** Lateral cephalogram taken with neutral zone technique; **C:** Lateral cephalogram of edentulous patient.

*Figure 7: **A:** Conventional technique superposed histogram; **B:** Neutral zone technique superposed histogram*

Table 1. Mean values of the soft tissue measurements for conventional technique (L1), neutral zone technique (L2), and edentulous (L3).

Measurement	1.Conventional (L1)	2.Neutral zone (L2)	3.Edentulous (L3)	Norm Value
Upper lip anterior to Sn Vertical (mm) (ULA-Sn Vertical)	5.4 mm	3.9 mm	0.5 mm	3 mm
Upper Lip-E (mm)	-8.2 mm	-11.1 mm	-15.3 mm	-6 mm
Upper lip protrusion (mm) (UL-SnPg')	1 mm	-0.7 mm	-4.1 mm	3 mm
Upper Lip Thickness to A point (mm)	14.7 mm	14.1 mm	10.2 mm	17mm
Lower lip anterior to Sn Vertical (mm) (LLA-Sn Vertical)	5.9 mm	4.6 mm	1.1 mm	1 mm
Lower Lip-E (mm)	-7 mm	-9.9 mm	-14.5 mm	-2 mm
Lower lip protrusion (mm) (LL-SnPg')	-0.2 mm	-1.9 mm	-5.2 mm	2mm
Upper lip length (mm) (Sn-Stms)	20.3 mm	22.6 mm	19.6 mm	21 mm
Lower Lip length (mm) (Stmi-Me')	40 mm	43.1 m	37.1 mm	40 mm
İnterlabial gap (mm)	1.5 mm	1.2 mm	1.1 mm	2 mm
Subnasale to Holdaway Line (mm)	1.5 mm	-1 mm	-5.8 mm	4.2 mm
Inferior sulcus to Holdaway Line (mm)	4.3 mm	4.7 mm	2.3 mm	4 mm
Superior sulcus depth (mm)	5.4 mm	3.5 mm	1.4 mm	3 mm
Chin Thickness (mm) (Pg-Pg')	12.5 mm	14.1 mm	13 mm	13.9 mm
Soft tissue pogonion to Sn Vertical (mm) (Pog'-Sn Vertical)	10.9 mm	12.9 mm	15 mm	-3 mm
Facial convexity (°) (G'-Sn-Po')	-4.5°	-5.9°	-16.8°	12°
Nasolabial angle (°)	97.1°	96.5°	104.9°	102°
H-Angle (Pg'UL-Pg' Na') (°)	-0.1°	-4.2°	-16.6°	10°
Sn-Stomion/Stomion-Me (%)	60.3	65.2	54.8	50
Soft tissue Face Height (G'-Sn:Sn-Me') (%)	1	0.9	1.1	1

Descriptons:

ULA: Upper Lip Anterior; **Sn**: Subnasale; **Sn Vertical**:Line Passing Through Subnasale And Perpendicular To The Horizontal Plane; **E:**E Line Passing Through Nasal Tip And Soft Tissue Pogonion; **UL**:Upper Lip; **SnPg'**:Line Passing Through Subnasale And Soft Tissue Pogonion; **A**:A Point; **LLA**:Lower Lip Anterior; **LL**:Lower Lip; **Sn-Stms**:Distance Between Subnasale And Stomion Superior; **Stmi-Me'**:Distance Between Stomion Inferior And Soft Tissue Menton; **Pg-Pg'**:Distance Between Hard Tissue Pogonion And Soft Tissue Pogonion; **Pog'-Sn Vertica**l: Distance Between Soft Tissue Pogonion And Sn-Vertical Line; **G'-Sn-Po'**:Angle Between Soft Tisue Glabella, Subnasale And Soft Tissue Pogonion; **Pg'UL-Pg' Na'**: Angle Between Line Passing Through Soft Tissue Pogonion And Upper Lip And Line Passing Through Soft Tissue Pogonion And Soft Tissue Nasion; **Me**: Menton; **G'Sn:Snme'**:Ratio Between Soft Tissue
Glabella- Subnasale and Subnasale-Menton Distances.

Table 2. 3dMD sofware volume comparison and surface differences.

Volume comparison and Surface Difference Measurements	Conventional (I1) superposed with no denture (I3)	Neutral zone (I2) superposed with no denture (I3)
Area Difference	2033.640 cm^2	2116.242 cm^2
Volumetric Difference	129.634 cc	63.969 cc
RMS (Min.-Max.)	2.61 (-53.17- 56.01)	2.44 (-24.30- 19.46)

RMS: Root Mean Square; **Min.:** Minimum; **Max.:** Maximum.

Discussion

The forces that are caused by soft tissues during mastication, speaking, and swallowing affects the stability of the dentures.[5] The denture stability is an important factor for the patients who have complete dentures, especially with inadequate tissue support and severe ridge resorption.[5,6] The neutral zone technique provides the maximum stability by determining the peripheral borders, tooth position, and external contours of the dentures.[6,8,9]

In the present case, the mandibular ridge of the patient was severely resorbed and she was carrying an uncomfortable conventional denture, which was fabricated a year ago. For this reason, it was decided to fabricate a new denture according to the neutral zone technique to increase patient satisfaction. However, the fabrication technique of the neutral zone dentures was different from conventional ones so somehow changes could be expected in the soft tissues of the patient's face. Therefore, in the present case, it was decided to evaluate the soft tissue changes that can be caused by two different fabricating dentures.

It has been reported that because of soft tissue loss and reduction of face height, horizontal and vertical lip contour changes could be observed in edentulous patients.[10] The cephalometric soft tissue measurements of the present case showed that upper and lower lips were positioned anteriorly in both conventional (L1) and neutral zone techniques (L2) on the sagittal plane. These results could be attributed to the fact that the placement of the prosthesis in the mouth helps direct lip position with big linear measurements. These results are in accordance with the other studies in the literature, in which a larger displacement of upper and lower lips after inserting a complete denture was encountered.[11,12] However from a clinical perspective, anterior positioning of the upper and lower lips was more pronounced in L1 than in L2. This result may be related to the fact that, the mandibular anterior teeth were placed more lingually in the neutral zone technique records, while the maxillary anterior teeth were placed closer to the position of the natural anterior teeth, as reported in a study.[13] Upper and lower lip lengths were found lower in L1 compared to L2 in the present case, which can be considered clinically significant. This result may be due to the fact that the tips of the lips were more deviated because of the positioning of the anterior teeth more anteriorly in the conventional technique. In accordance with the result of more pronounced upper anterior teeth proclination and upper lip protrusion, subnasale to H-Line measurement and superior sulcus depth measurements were also more pronounced in the conventional technique when compared to the neutral zone technique.

As the teeth in the dentures and the base of the prosthesis supported tissues around the mouth, facial convexity, and Holdaway angles increased in dentures fabricated with conventional (L1) and neutral (L2) techniques compared to edentulous (L3) cephalogram. This result is similar to the findings of Toyoshima *et al.*[14] and Pucciarelli *et al.*[15] Also, these convexity angle increases were more evident in L1 than in L2.

When comparing conventional and neutral zone techniques procedures with regard to the volumetric difference of superimposed images, the volumetric difference between conventional (I1) and no denture (I3) images was more pronounced compared to Neutral zone (I2) superposition with no denture (I3). This volumetric difference is an expected result that reflects all the differences in cephalometric soft tissue measurements between the two techniques discussed above.

During the control time of both conventional and neutral zone complete dentures, the patient's mastication, comfort, and speech were evaluated and compared. With the neutral zone denture, the patient could do these functions more comfortably, but with the conventional denture these functions, especially mastication, were limited. Also, there was seen major stomatitis that made the prosthesis uncomfortable with conventional technique. Stomatitis was seen over the crest of the lower ridge.

It has been reported that there are several factors affect denture stability such as flange contour and the influence of tooth position. When fabricating dentures, the polished surfaces and teeth positions may be determined according to the neutral zone. As the musculature will vary for every patient, dentists should not insist on placing the teeth over the crest of the ridge. Although the prosthesis made with the conventional technique showed superiority in terms of soft tissue changes in the present case report, it should be kept in mind that positioning artificial teeth in the neutral zone achieves more advantages.

Conclusions

An alternative mandibular denture impression technique, Neutral zone, has been suggested for the severely atrophic ridges. It has been recommended especially in edentulous cases where dental implants are not possible. It provides denture stability in muscular balance during function. Although the technique is simple, the chair time and laboratory costs increase. In the present case, although the dentures fabricated with the conventional technique provide superior esthetical soft tissue changes, especially in the upper and lower lip area, it should be keept in mind that the neutral zone denture provides better stability, retention, and normal muscle function.

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Osteosarcoma of Maxilla – An Unusual Entity: Case Report and Review of Literature

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ABSTRACT

Osteosarcoma of the jaw is a rare malignancy. It affects the mandible more often than the maxilla. There have been rare cases of osteosarcomas reported in the maxilla; however, given the lack of specific signs and a diverse range of radiographic features, the diagnosis is often difficult and requires a multidisciplinary approach. This is a case report of a 56-year-old female presenting with a well-defined, dome-shaped firm swelling having radiographic features of extensive destruction of the buccal cortical bone in the maxillary right posterior region. Osteosarcoma of the jaw is difficult to diagnose and manage due to the high frequency of errors in biopsy results, the few specific radiological characteristics, and difficulties in appropriate resection because of the proximity to vital structures. Over the years, the survival rate of patients has greatly improved, due to systematic approach and refined surgical and reconstructive techniques. Due to the aggressiveness of this disease, early diagnosis of the lesion is required.

Key words: Diagnostic Imaging, Maxillary Neoplasms, Osteosarcoma, Osteogenic Sarcoma, Bone Neoplasm.

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Introduction

Osteosarcoma (OS) or osteogenic sarcoma is a rare entity that accounts for approximately 6-10% of jaw bone lesions.[1] In the human skeleton excluding the jaw bones, OS is the second most common malignant bone tumor with tumors of the bone marrow being first. It follows an aggressive course and can cause considerable morbidity and mortality as local recurrence is common.[2] OS displays a bimodal distribution with two peaks, one in the first or second decade and the other in the fourth decade.[1,3]

OS is classified into two types – primary and secondary. The etiology of the primary type is not clearly known and may be due to genetic influences or other environmental factors. In the secondary type, craniofacial osteogenic sarcomas occur in older patients with skeletal Paget's disease, fibrous dysplasia of bone, Li-Fraumeni syndrome, and as a late sequela to cranial irradiation. Environmental factors such as ionizing radiation and chromic oxide (a radioactive scanning agent), have been incriminated as possible causes. Genetic mutations in tumor suppressor gene p53 and mutated retinoblastoma gene have been claimed to be amongst other etiologic factors.[4]

In a study by Nissanka *et al.,* most patients related the occurrence of the tumor to previous dental treatment, most commonly dental extractions. The reason for this is most likely the rapid growth of tumors immediately following trauma, a phenomenon usually seen in skeletal OS.[5]

OS has no specific clinical signs. Given the lack of specific signs and the diverse nature of the radiographic features, like sunray spicules, "hair-on-end" trabeculae or Codman's triangle, internal osseous structure taking up the appearance of granular or sclerotic-appearing bone, cotton balls, wisps, or honeycombed internal structures in areas with adjacent destruction of the preexisting osseous architecture, it can lead to misleading interpretation.[6] This lesion presents itself with the features seen in most malignant tumors, such as bone destruction with no periosteal reaction and irregular subperiosteal new bone formation mass.[7] The diagnosis is often difficult and requires a multidisciplinary team including a molecular biologist. Nearly 20% of all metastases occur in the lung, making it the most frequent site.

In the orofacial region, the mandible is more commonly affected than the maxilla with a ratio of 1.5:1, with males showing a predilection for occurrence in the mandible and females in the maxilla.[8,9] In mandible, the body is most commonly involved followed by angle, symphysis, ascending ramus, and the antrum.[4] This article reports a case of OS in the posterior maxilla.

Case report

A 58-year-old female developed a painless swelling on the maxillary right posterior region since one month for which she had visited a local dentist, who thought it to be associated with the maxillary right first molar, which was subsequently removed. However, not only did the swelling not subside, but instead there was a rapid increase in size, following which she was referred to our facility. Her past medical history revealed that she had undergone anti-tubercular therapy for extra-pulmonary tuberculosis, which lasted 2 years. On examination, a single, well-defined, dome-shaped, firm swelling with a sessile base of size 5 x 4 cm was noted attached to the alveolus. Its superior margin was located 2 cm below the inferior orbital margin and its inferior margin was at the level of the alveolar ridge. Antero-posteriorly, the extent ranged from the distal aspect of tooth 13 to the mesial aspect of tooth 16 (Figure 1) displaying a surface appearance that was partly erythematous and partly white with the surrounding tissues appearing normal. There was no bleeding or pus discharge seen (Figure 2).

Considering the location, the absence of tenderness, and its rapid growth, a provisional diagnosis of "Aggressive, non-odontogenic neoplasm of the maxillary right posterior region" was given.

A cone beam computed tomography (CBCT) was advised which revealed a single, unilateral, radiolucent area present in the region pertaining to teeth numbers 15 and 16 with extensive bone destruction of the buccal cortex and minimal perforation of the palatal cortex. Alteration in the trabecular architecture and sclerotic bone formation was noted posterior to the lesion and extended to the area of the maxillary tuberosity and appeared to blend into the surrounding cortical plate with radiopaque but irregular margins confirming the presence of an aggressive bony lesion (Figure 3).

An incisional biopsy was taken which showed the presence of spindle-shaped cells with pleomorphic vesicular nuclei, coarse chromatin, distinct nucleoli, and moderate cytoplasm arranged in sheets. Besides, malignant osteoid formation was also noted (Figure 4) leading to the final diagnosis of "OS of the maxillary right posterior region". Further investigations with Positron Emission Tomography (PET) were performed which showed a metabolically active primary malignant mass lesion in the right maxillary region and no evidence of other metastasis (Figure 5). It was decided that surgical intervention was the best available treatment option and she had undergone subtotal right maxillectomy and the histopathologic report came out with the pathologic stage classification of pT_1N_0 OS. A PET scan was taken post-surgically which showed the area was not metabolically active and there was no evidence of metastasis (Figure 5).

Figure 1. Swelling was seen on the maxillary right posterior region.

Figure 2. Swelling was presented with surface appearance that was partly erythematous.

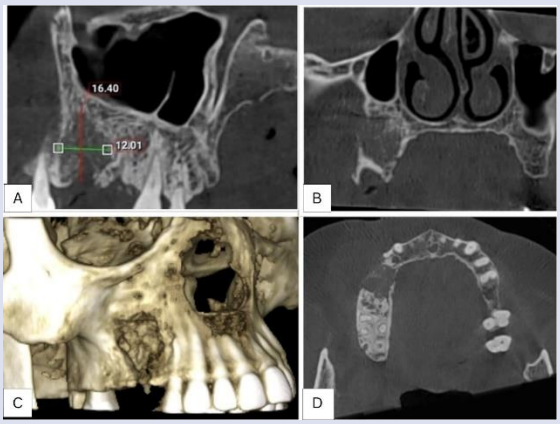

Figure 3. CBCT images showing osteolytic areas on the maxillary right posterior region
***A:** Sagittal view **B:** Coronal view **C:** Three-dimensional view **D:** Axial view*

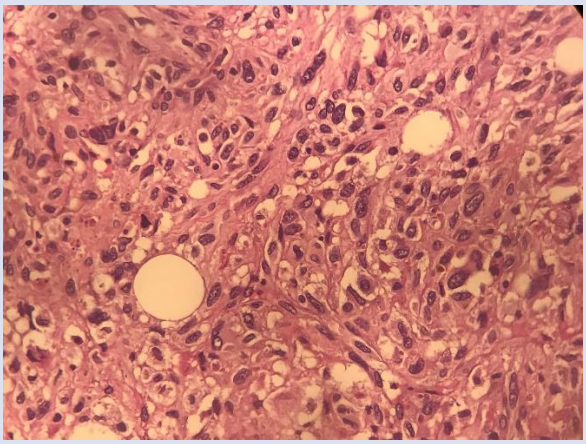

Figure 4. Histopathological picture showing the presence of malignant osteoid formation.

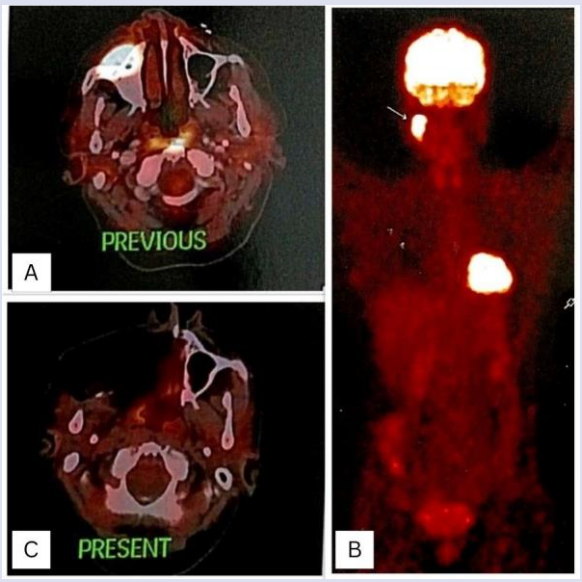

*Figure 5. PET images **A, B:** Preoperative, **C:** Postoperative*

Discussion

OS is a heterogeneous group of primary malignant neoplasms in which mesenchymal cells produce osteoid or immature bone. More than half of all OS arise in the long bones of the limbs, particularly in the region of femur, tibia, and pelvis.[10] Craniofacial OS accounts for only 1% of all head and neck malignancies.[11] Jaws are the fourth most commonly afflicted site but constitute only approximately 6 to 7% of all skeletal cases of OS.[12,13] OS of jaw bones have some distinct features from those of long bones such as older age at presentation with local recurrences being difficult to control, which leads to death of the patients.[14]

OS usually affects the long bones in growing children and young adults due to the rapid growth in their bones.[14] Jaw lesions typically occur with a peak in the fourth decade, about 10 years later on average than the occurrence in long bones which shows bimodal distribution. Mean age according to Garrington *et al.,* ranges from 34 to 36 years.[15] However, this case represents the lesion in a patient of age 58 years.

The occurrence of the disease in males is twice as frequent as in females. This has been attributed to a longer period of skeletal growth and an additional volume of bone in men, though neither has been confirmed.[4] Some authors have reported an equal predilection.[12,13] This article represents the lesion in a female patient.

Clinically, OS presents with myriad symptoms which include bony swelling, facial deformity, loosening, and/or separation of teeth. Paresthesia, toothache, and regional pain are mostly related to regional compression by the growing mass.[5,15] These tumors rarely present with symptoms of ulceration, epistaxis, or visual problems.[6] They grow rapidly causing expansion of the cortical plates with displacement and resorption of roots which were also observed in this case. They easily invade adjacent structures due to their invasive growth pattern with mucosal ulceration and pathologic fractures being common. Sensory abnormalities are encountered when the peripheral nerve is involved.[16] However, in our case, no sensory deficits or pathologic fractures were noted and the only clinical presentation was an intraoral hard tissue swelling with expansion of the buccal cortical plate.

Radiographic features show sunray spicules (ossific laminae radiating in sun-burst pattern from the affected bone surface) or “hair-on-end” trabeculae may be seen because the tumor grows very rapidly, new bone formation tends to occur in a straight line, at an angle of 90º to the bone surface. The lesion involves the periosteum directly or by extension if the periosteum is elevated due to rapid expansion and maintains its osteogenic potential only at the periphery and a Codman’s triangle at the edges is formed, which is rare in the maxilla.[17]

OS may be entirely radiolucent, mixed radiolucent-radiopaque, or quite radiopaque.[6] The Garrington sign, which is the symmetric widening of the periodontal ligament of one or more teeth on a periapical radiograph, is also an early radiologic feature and should raise suspicion for OS.[10] Garrington *et al*., in their analysis of 56 OS cases, reported the presence of a “sun ray” effect in about 25% of cases. However, these features are not specific to OS; hence, radiologic impressions can often be misleading and histopathology is considered the gold standard diagnostic modality. In addition, Magnetic resonance imaging (MRI) can demonstrate invasion of the surrounding soft tissue and peripheral mineralization.[3,10,15] In this case, CBCT images showed the lesion was radiolucent with ill-defined margins representing extensive bone loss on maxillary right posterior regions.

A retrospective analysis of 74 OS cases by Paparella *et al.* revealed varied findings in 25 cases with available

radiologic images. The presumptive clinical radiographic diagnosis in 66.6% of cases was benign lesion (dysplastic, neoplastic), and malignant neoplasia in 33.3% of cases. None of the cases was diagnosed as OS before histologic diagnosis.[15] Similar to these findings, this case also could not secure a definitive diagnosis before the biopsy.

The differential diagnoses considered in this case were non-Hodgkin's lymphoma (NHL), metastatic tumors, central hemangioma, Ewing sarcoma, and OS.

Extranodal NHL can develop in the soft tissues, most frequently the gingiva, palate, or buccal vestibule, or they can appear centrally within the bone. NHL that appears as a growth from the extraction socket is uncommon, however, it has been seen in both HIV-positive and HIV-negative patients.[18] Signs and symptoms including tooth movement, localized edema with ulcer, inexplicable dental discomfort, or vague lytic osseous alterations are frequently present. All of these characteristics fit the circumstances of the current instance.

Most metastatic tumors to the orofacial region are seen in patients aged between 40-70 years. Lung, breast, kidney, and bone malignancies are the most typical initial causes of metastatic tumors in the oral region. The most typical initial site for tumors that spread to the jawbones is the breast. Metastatic lesions to the soft tissues of oral cavity, gingiva is the most frequently affected. It has been demonstrated that gingival metastases are polypoid or exophytic, highly vascularized, and hemorrhagic. The possibility of metastasis was ruled out due to the absence of primary malignancies.[19]

Central hemangioma was considered as it has female predilection. The body of the mandible and posterior area of the maxilla are the most frequent locations for occurrence. An erythematous nodular development in the maxillary gingiva can be a symptom. The central hemangioma's radiographic appearance is not pathognomonic and is a great mimicker. Some lesions have a honeycombed appearance and radiating spicules at the expanded periphery can prove a "sunburst" appearance as in OS.[20] Central hemangioma is ruled as the age of occurrence is younger than our case and because of the radiographic appearance.

In maxilla, the occurrence of Ewing sarcoma is rare. Localized growth is the most common presentation which may be associated with pain and paresthesia. Epistaxis is usually connected with maxillary lesions, although it wasn't present in this case. "Onion skin appearance" on radiographs is mostly found in children and young adults. In our case, a lytic lesion was found, the typical onion skin appearance was not present, and the patient's age did not favor the possibility of an Ewing sarcoma.[21]

OS is typically characterized by slow healing and edema at the tooth extraction socket site. The present case showed a rapid increase in the size of the swelling followed by extraction. This case's clinical and radiographic features, such as swelling, erythematous ulcerated nodules, and complete lack of bone development within the tumor, were in accordance with previous literature.[22]

Wide radical resection is the treatment of choice for OS of jaws with clearance margins of 1.5-2 cm. Maxillectomy is difficult to perform due to the involvement of adjacent structures like maxillary sinus, pterygopalatine fossa, and orbital fossa. A subtotal inferior maxillectomy can be done for selected malignancies located on the alveolar ridge, palate, and involving the antral floor.[23] Obturators are prescribed for the defect created.

The prognostic factors depend on the site, number of metastases, surgical resection of the metastatic disease tumor size, and females with the histologic feature of predominantly chondroblastic pattern.[13,24]

A number of potential prognostic factors have been identified which include the expression of HER2/CerbB2, tumor cell ploidy, specific chromosome gains or losses, loss of heterozygosity of the RB gene, loss of heterozygosity of the p53 locus, and increased expression of p-glycoprotein. The only feature that consistently predicts outcome is the degree of histologic necrosis following induction chemotherapy. Patients with more than 95% necrosis in the primary tumor after induction chemotherapy have a better prognosis than those with smaller amounts of necrosis.[25,26]

It has been noted that patients with skip metastases (≥2 discontinuous lesions in the same bone) have a worse prognosis. Additionally, the prognosis is poor for patients with multifocal OS (>1 bone lesion at diagnosis).[27] The overall 5-year survival rate for the primary OS of the jaws varies from 30 to 40%, and survival rates up to 80% have been reported for patients undergoing early radical resection.[28]

Conclusions

OS is an ancient disease many aspects of which are still incompletely understood. OS of the jaw is difficult to diagnose and manage due to the non-specific clinical and radiological characteristics, high rate of recurrences, and difficulties in appropriate resections because of the proximity to vital structures. However, we acknowledge the need to consider OS in the differential diagnosis when dentists encounter destructive bony lesions in the maxilla as well as the mandible.

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