



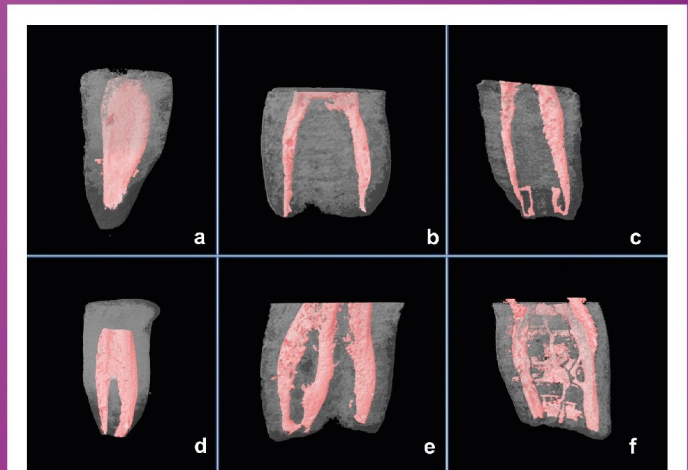
CUMHURİYET



DENTAL JOURNAL

The Official Journal of Sivas Cumhuriyet University Faculty of Dentistry

**Editor-in-Chief**  
İhsan Hubbezoğlu  
**Co-Editor-in-Chief**  
Burak Buldur



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## Cumhuriyet Dental Journal

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### Aims and Scope

Cumhuriyet Dental Journal (CDJ) is an international journal dedicated to the latest advancement of dentistry. The aim of this journal is to provide a platform for scientists and academicians all over the world to promote, share, and discuss various new issues and developments in different areas of dentistry.

CDJ publishes original research papers, reviews, and case reports within clinical dentistry, on all basic science aspects of structure, chemistry, developmental biology, physiology and pathology of relevant tissues, as well as on microbiology, biomaterials and the behavioral sciences as they relate to dentistry.



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## INDEXING



## CUMHURIYET DENAL JOURNAL

### AUTHOR GUIDELINE

Cumhuriyet Dental Journal (CDJ) is the Official Publication of the Cumhuriyet University, Faculty of Dentistry. CDJ accepts original experimental investigations and review articles concerning topics of clinical relevance to the general dental practitioner. Case reports and technique articles will be very critically reviewed in terms of interest to the general dental practitioner and the supporting data provided.

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**Frequency: Four times a year (March, June, September, and December)**

CDJ is published using an open access publication model, meaning that all interested readers are able to freely access the journal online without the need for a subscription. Manuscripts will be reviewed by the editor, and at least two reviewers with expertise within the scope of the article. In addition, CDJ use double-blind review process (every effort is made to prevent the identities of the authors and reviewers from being known to each other)

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#### **Manuscript Format and Style**

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Manuscript length depends on manuscript type. Paper dimensions should be 8.5 × 11 inches with 2.5 cm margins on all sides. Please use normal, plain font (12-point Times New Roman), justified and number all pages consecutively. Indent or space paragraphs.

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**Original Research Article:** Title, Abstract, Introduction, Materials and Methods, Results, Discussion, Conclusions, Acknowledgements, References, Tables and Figure Legends

**Review Articles:** Although a Review article (particularly following a systematic review) may adhere to the format of the Original Research Article, both Review and Focus Articles need not contain Materials and Methods, Results or Discussion sections, and may instead employ other headings as relevant for the topic addressed.

**Case Report:** Title, Abstract, Introduction, Case Report, Discussion, Conclusions, Acknowledgements, References, Tables and Figure Legends

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##### **Submission site**

Manuscripts should be submitted online through <http://dergipark.gov.tr/cumudj>. Full instructions and support are available on the website, and a *user ID* and *password* can be obtained at the first visit. All parts of the manuscript (Main Document, Tables, Figures and Supplemental Information) must be available in an electronic format: Microsoft Word or generic RTF are recommended for text and tables; and TIFF or EPS for graphics (see under Figures).

## **ELEMENTS OF a MANUSCRIPT**

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- -Title
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- References must be identified in the body of the article with superscript Arabic numerals after punctuation marks.
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Hilton TJ. Direct posterior composite restorations. In: Schwartz RS, Summitt JB, Robbins JW (eds). *Fundamentals of Operative Dentistry*. Chicago: Quintessence 1996:207-228.

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While the peer-reviewed journal literature should be accessible online without cost to readers, it is not costless to produce. However, experiments show that the overall costs of providing open access to this literature are far lower than the costs of traditional forms of dissemination. With such an opportunity to save money and expand the scope of dissemination at the same time, there is today a strong incentive for professional associations, universities, libraries, foundations, and others to embrace open access as a means of advancing their missions. Achieving open access will require new cost recovery models and financing mechanisms, but the significantly lower overall cost of dissemination is a reason to be confident that the goal is attainable and not merely preferable or utopian.

To achieve open access to scholarly journal literature, we recommend two complementary strategies.

**I. Self-Archiving:** First, scholars need the tools and assistance to deposit their refereed journal articles in open electronic archives, a practice commonly called, self-archiving. When these archives conform to standards created by the Open Archives Initiative, then search engines and other tools can treat the separate archives as one. Users then need not know which archives exist or where they are located in order to find and make use of their contents.

**II. Open-access Journals:** Second, scholars need the means to launch a new generation of journals committed to open access, and to help existing journals that elect to make the transition to open access. Because journal articles should be disseminated as widely as possible, these new journals will no longer invoke copyright to restrict access to and use of the material they publish. Instead they will use copyright and other tools to ensure permanent open access to all the articles they publish. Because price is a barrier to access, these new journals will not charge subscription or access fees, and will turn to other methods for covering their expenses. There are many alternative sources of funds for this purpose, including the foundations and governments that fund research, the universities and laboratories that employ researchers, endowments set up by discipline or institution, friends of the cause of open access, profits from the sale of add-ons to the basic texts, funds freed up by the demise or cancellation of journals charging traditional subscription or access fees, or even contributions from the researchers themselves. There is no need to favor one of these solutions over the others for all disciplines or nations, and no need to stop looking for other.

Open access to peer-reviewed journal literature is the goal. Self-archiving (I.) and a new generation of open-access journals (II.) are the ways to attain this goal. They are not only direct and effective means to this end, they are within the reach of scholars themselves, immediately, and need not wait on changes brought about by markets or legislation. While we endorse the two strategies just outlined, we also encourage experimentation with further ways to make the transition from the present methods of dissemination to open access. Flexibility, experimentation, and adaptation to local circumstances are the best ways to assure that progress in diverse settings will be rapid, secure, and long-lived.

The Open Society Institute, the foundation network founded by philanthropist George Soros, is committed to providing initial help and funding to realize this goal. It will use its resources and influence to extend and promote institutional self-archiving, to launch new open-access journals, and to help an open-access journal system become economically self-sustaining. While the Open Society Institute's commitment and resources are substantial, this initiative is very much in need of other organizations to lend their effort and resources.

We invite governments, universities, libraries, journal editors, publishers, foundations, learned societies, professional associations, and individual scholars who share our vision to join us in the task of removing the barriers to open access and building a future in which research and education in every part of the world are that much more free to flourish. Submitting a paper to CDJ is free of charges. In addition, CDJ has not have article processing charges.

### ***PLAGIARISM and ETHICS***

CDJ aims to the highest standards with regard to research integrity and in particular the avoidance of plagiarism, including self-plagiarism. It is therefore essential that authors, before they submit a paper, particular attention should be paid. When submitting a paper on CDJ, authors will be prompted as to whether they have read and agree to these guidelines before proceeding further with their submission. They will be asked specifically for an assurance that the paper contains no element of data fabrication, data falsification or plagiarism (including unacknowledged self-plagiarism). Authors are reminded that, where they draw upon material from another source, they must either put that material in the form of a quote OR write it entirely in their own words (i.e. there is no 'middle way'). In both cases, they must explicitly cite the source, including the specific page number in the case of a quote or a particular point. **CDJ uses Ithenticate: Plagiarism Detection Software.**

For the experimental, clinical and drug human studies, approval by ethical committee and statement on the adherence of the study protocol to the international agreements (Helsinki Declaration revised 2008) are required. In experimental animal studies, the authors should indicate that the procedures followed were in accordance with animal rights and they should obtain animal ethic committee approval. The Ethic Committee approval document should be submitted to the Cumhuriyet Dental Journal together with the manuscript.

The approval of the ethic committee, statement on the adherence to international guidelines mentioned above and that the patients' informed consent is obtained should be indicated in the "Materials and Methods" section and is required for case reports whenever data/media used could reveal identity of the patient. The declaration of the conflict of interest between authors, institutions, acknowledgement of any financial or material support, aid is mandatory for authors submitting manuscript and the statement should appear at the end of manuscript. Reviewers are required to report if any potential conflict of interest exists between reviewer and authors, institutions.

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### **Greetings from the Last Issue of 2019 and Special Thanks to All Members of CDJ!**

In 2019, Cumhuriyet Dental Journal underwent a major renewal with its rich content and increasing international colleagues. As a result of the process I managed with the vision that Cumhuriyet Dental Journal should be a more internationally recognized journal, in 2019 we had the opportunity to publish the articles of many authors from different countries. I hope that the number of authors from other countries in Cumhuriyet Dental Journal will increase gradually next year.

Cumhuriyet Dental Journal received 235 submissions in 2019 and reached the highest level in its publication history. However, our acceptance rate is 26%. For this reason, the review process of our journal has become more selective.

The new policy of our journal to be held since 2020, and revised Author Guideline is now published in our website. The authors, who will also submit new papers to Cumhuriyet Dental Journal, should carefully examine the relevant sections and submit their papers according to the writing rules.

In 2020, Cumhuriyet Dental Journal will welcome new prestigious Associate Editors and Editorial Board members. I would like to also welcome all colleagues who would like to join Cumhuriyet Dental Journal.

I owe many thanks to the people who contributed to the development of Cumhuriyet Dental Journal. First of all, I would like to thank the readers who followed the rich content of our journal. I would like to thank the Authors for their great effort and papers. I have special thanks to the Editorial Board members and Associate Editors. I owe a very special thanks to the Reviewers who have significantly helped the development of Cumhuriyet Dental Journal.

I wish a happy new year in 2020 and also happy readings in the last issue of 2019!

Burak Buldur  
Co-Editor-in-Chief

**The Editor-in-Chief's recommendation of this issue's article to readers;**

**COMPARISON OF MINERAL TRIOXIDE AGGREGATE, ENDOSEQUENCE ROOT REPAIR MATERIAL, AND BIODENTINE USED FOR REPAIRING ROOT PERFORATIONS: A SYSTEMATIC REVIEW**

I am pleased to inform you that I have chosen this article by Alghamdi and Alhajdali<sup>1</sup> as Editor-in-Chief's Choice for the last issue of 2019.

Calcium silicate-based cements are widely used in dental procedures and pediatric dentistry. There are several advantages of these cements which lead to increase the clinical outcome of dental treatment.

This systematic review confirmed that there none of the materials discussed had all the major properties higher than the others, this way it is required the enforcement of further studies aimed at selecting the best characteristics of the material suggested in the root perforation treatment.

Happy readings in the last issue of 2019!

Burak Buldur

Co-Editor-in-Chief

**REFERENCE:**

1. Alghamdi F, Alhajdali E. Comparison of Mineral Trioxide Aggregate, Endosequence Root Repair Material, And Biodentine Used for Repairing Root Perforations: A Systematic Review. Cumhuriyet Dent J 2019;22:4:469-476.



## SHOULD WE USE THE DIGITAL MODELS IN PEDIATRIC DENTISTRY?

### ABSTRACT




**Objectives:** This study aims to compare the measurements of the extraction spaces in the plaster models and digital dental models and to investigate the usability of the digital models in preventive and interceptive appliances in pediatric dentistry clinics.

**Materials and Methods:** In the dental models of 56 patients whose first molar teeth were extracted in the early period, the dental arch length of the extraction spaces were measured with a digital caliper in the plaster model (Control Group), with computer software in the digital model (Experimental Group). Measured values were compared using a t-test and statistical significance levels were determined.

**Results:** In plaster models and digital models, the measured mean arc length of the extraction spaces were 6.94 mm and 6.83 mm, respectively. There was no statistically significant difference between the two measurement methods ( $p>0.05$ ).

**Conclusions:** The use of digital models is recommended in pediatric dentistry clinics due to shorter chair time in pediatric patients, effective treatment planning/follow-ups, reducing treatment costs and other advantages.

**Key Words:** Dental model, dental occlusion, primary tooth.

 Sibel ÇAYÖNÜ<sup>1</sup>  
 \*Akif DEMİREL<sup>2</sup>  
 Şaziye SARI<sup>2</sup>

ORCID IDs of the authors:  
S.Ç. 0000-0002-9378-2499  
A.D. 0000-0002-1433-0452  
Ş.S. 0000-0003-2202-5148

<sup>1</sup> Pediatric Dentist, Topraklık Dental Hospital, Ankara, Turkey,

<sup>2</sup> Department of Pediatric Dentistry, Faculty of Dentistry, Ankara University, Ankara, Turkey,

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## **INTRODUCTION**

Despite the increasing number of patients consulting to the pediatric dentistry clinics due to modern advances in dental caries prevention and the preservation of natural dentition; premature loss of primary teeth is still a common problem.<sup>1-4</sup> This is considered to be the most important cause of dental and skeletally malocclusion.<sup>5,6</sup> In these cases, it is necessary to use of space maintainers in order to keep the existing space in the dental arch and prevent the development of malocclusion by tilting the adjacent and opposite teeth.<sup>7-14</sup> The space maintainers or other orthodontic appliances should be specially designed considering the dental occlusion, condition of the existing teeth and future dental requirements for each case. Therefore, the preparation stage of these appliances and the quality of dental models are quite critical for the treatment planning and the follow-up procedures.<sup>14</sup>

Orthodontic and pedodontic archives are needed for the diagnosis and treatment planning of occlusion and space management in pediatric dental clinics and the models are considerable parts of these dental records. Especially in orthodontic treatment planning and space management, the measurement of dental spaces are required and the spaces can be measured using compasses, ruler or caliper.<sup>15</sup> The distance between the two dental points to be measured is determined by the compass and measured on the ruler. However, in these measurements, when the tip of the compass is placed on the ruler, the exact distance cannot be measured, causing the values to be rounded up to the nearest integer, therefore, measurements cannot be performed with great precision.<sup>15</sup> A digital caliper is also used in the dental plaster model in order to make more accurate measurements. In this method, when the caliper is placed between two points to be measured, the numerical distance is displayed digitally. However, the possibility of incorrect placement of the caliper makes it difficult to measure correctly. Additionally, the storage of the dental models can be troublesome and if it is not properly maintained, its breakage can be a major disadvantage.<sup>16</sup> The conventional measurements cause the wear and tear of the dental models and these conditions affect the accuracy of the

measurements.<sup>17</sup> Also, the risk of bacterial contamination and transportation of the models are the other disadvantages of conventional measurements.<sup>18,19</sup>

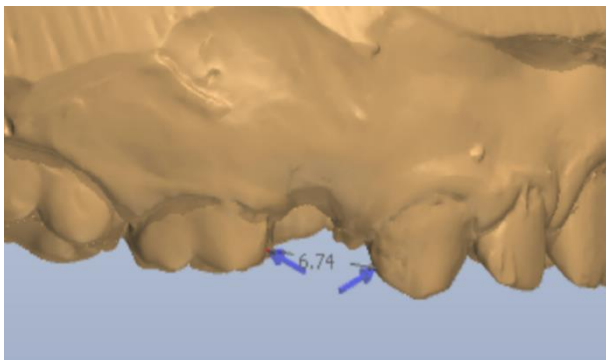
With the development of the technology, dental plaster models have been replaced by digital models that facilitate diagnosis and interdisciplinary planning, which is accelerating with the development of 3D scanners.<sup>20,21</sup> On the digital models created, the points to be measured can be determined more easily by enlarging and zooming the images, measurements can be made very precisely and the images can be used in all parts of the world by keeping them in the storage softwares.<sup>22</sup> The main advantages of the digital dental models are easy storage, accurate and precise measurement, access and sharing of digital files over the web, diagnosis and treatment planning to be completed in a shorter time and prevention of loss of information.<sup>16,22,23</sup> To date, different technologies have been developed to create 3D scanning devices and each has its own limitations, advantages and disadvantages. Today, digital orthodontic model forming systems are Cerec, Cadent Itero System, E4D, Lava COS and Trios.<sup>23</sup>

The digital models are obtained directly or indirectly by any of mentioned three-dimensional scanning systems.<sup>21,23</sup> The models created by directly mouth scanning provide many advantages on patients with cleft-palate-lip, gag reflex, mouth breathing, probable aspiration risk of the impression material, sedation requirements.<sup>19,21</sup> However, in case of the absence of a scanning device in the clinic, the digital model can be obtained indirectly by scanning previously obtained measurements or dental plaster models.<sup>16,23-26</sup> This study aimed to evaluate whether the indirectly obtained digital models are as reliable as dental plaster models and their utility in pediatric dental clinics. The null hypothesis tested in this study was that there would be no difference between the measurements of the extraction spaces in the plaster models and the digital dental models.

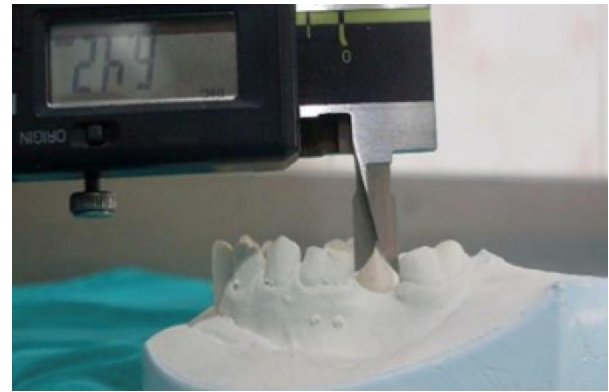
## **MATERIALS AND METHODS**

The study protocol was approved by the Ethics Committee of Ankara University (36290600/24 21.03.2017). Fifty-six patients (30 male, 26

female) aged 7-8 who have lower (n=28) or upper (n=28) primary first molar tooth extraction (due to dental caries, excessive dentoalveolar abscess, periodontal defects, pathological root resorption, non-restorable tooth structure, etc) in early stages were included in the study. All the patients' occlusion had Class 1 molar relationship. One week after the extraction, the patients were recalled and a dental impression was obtained with hydrocolloid impression material -alginate- and disinfected with 5% NaOCl solution for 15 minutes. After disinfection procedures, the dental models are obtained by using hard dental plaster at the end of 30 min. setting period (Control Group). 3Shape Dental Scanner (Trios, 2017, Denmark) was used to create the digital models (Experimental Group) of the experimental group and the arc length of the extraction space was measured on these models. While the distance between the most convex point of the mesial proximal surface of the primary second molar tooth and the most convex point of the distal proximal surface of primary canine tooth was measured by 3Shape Orthoanalysar software (Figure 1), the same measurement was made using digital caliper in plaster models of the control group (Figure 2).



**Figure 1:** The measurement of the extraction space in the digital dental model with a computer software.



**Figure 2:** The measurement of the extraction space in the plaster dental model with a digital caliper.

Measurements were repeated one week later for both groups and the results were consistent with the kappa test (mean k value=0.90). For statistical analysis, *t-test* was used and p-value <0.05 was considered statistically significant.

### RESULTS

The mean arc length of the extraction space was 6.83 mm for the experimental group and 6.94 mm for the control group. The difference between the two groups was not statistically significant (p=0.529) (Table 1).

**Table 1.** Mean arc length measurement values of the extraction space for the experimental and control groups.

|   | Experimental Group<br>(Digital Models)<br>(n=56) | Control Group<br>(Plaster Models)<br>(n=56) | p value*<br>(t-test) |
|---|--|---|----------------------|
| Mean arc length of the extraction spaces (mm) | 6.83 ± 1.28                                      | 6.94 ± 0.85                                 | 0.529                |

\*p value <0.05 was considered statistically significant.

### DISCUSSION

In orthodontics and pediatric dentistry, the measurements of extraction spaces, arc length, interdental and other spaces are important steps in treatment planning and follow-up periods. To date, although these measurements mostly carried out by

using plaster models, the use of digital models has to become widespread today.<sup>15</sup> However, disadvantages such as difficulties of storage of the plaster models, the possibility of fracture<sup>16</sup> and damage to the plaster model during measurements<sup>17</sup> made the digital models popular.<sup>20,21</sup> In the studies

used plaster models and digital models, it has been reported that digital models can be preferred due to accuracy and repeatability in the measurements of tooth dimensions and interdental spaces.<sup>27-29</sup> In this study, it was investigated whether the measurements made in digital models are as reliable as the plaster models and their utility. The effect of measurement differences in plaster and digital dental models on providing acceptable occlusion and ideal teeth alignment is not well-known, therefore, the differences between the two methods need to be investigated.

In the plaster dental models, spaces and diameters are usually measured by using compasses, calipers or rulers.<sup>15</sup> However, digital caliper measurements are known to be more reliable than the other techniques.<sup>15,16</sup> In digital models, the points to be measured can be determined more easily by enlarging and zooming the images, so that the measurements can be carried out more precisely. The created measurement images can be stored and re-used in the computer software and shared with other clinicians or multidisciplinary departments.<sup>22</sup> In this study, while the measurements were performed with digital caliper and computer software in plaster dental models and the digital models, respectively.

In both plaster and digital models, interdental spaces, extraction spaces or distances between points including different dental structure can be measured.<sup>27-29</sup> On the other hand, in pediatric dentistry, it is valid that the space maintainers can be applied in case of loss of space instead of applying immediately in the extraction of first primary molars. In this regard, the models of the cases planned to be followed without applying the space maintainer were used and the measurements of the extraction spaces in the plaster and digital dental models were compared in this study. According to the results of this study, it was found that there was no statistical significance between the plaster and digital dental models ( $p>0.05$ ).

Considering the advantages of digital scanning systems, the use of digital models in diagnosis, treatment planning and follow-ups is recommended in pediatric dentistry clinics. Pediatric dentistry clinics are the first departments

where the treatment decisions regarding primary teeth extraction, space maintainers and other appliances and space management procedures are taken. Especially, digital scanners and models are beneficial for pediatric dentists and patients when patient monitoring is required to avoid unnecessary treatments and costs. Also, in pediatric dentistry clinics, intraoral 3D scanner devices should be used due to reducing chair time and lack of cooperation, especially in young children. In addition, the limitation of this study was the measurements of extraction space were performed extraoral on the dental models. Therefore, it is possible to conclude that there is need for further studies about intraoral scanning systems. The null hypothesis that there are no differences among the measurements of the plaster and digital dental models was accepted.

## CONCLUSIONS

The use of digital scanning systems and models should be encouraged in pediatric dentistry clinics.

## ACKNOWLEDGEMENT

None

## CONFLICT OF INTEREST STATEMENT

No potential conflict of interest was reported by the authors.

## *Pedodontide Artık Dijital Modelleri Kullanalım mı?* **ÖZ**

**Amaç:** Alçı modeller ile dijital dental modellerde yapılan çekim boşluğuna ait ölçümlerin karşılaştırılması ve dijital modellerin pedodonti kliniklerinde koruyucu ve durdurucu uygulamalarda kullanılabilirliğinin araştırılması amaçlanmıştır. **Gereç ve Yöntemler:** Süt 1. azı dişi erken çekilen 56 hastanın dental modellerinde, çekim boşluğuna ait dental ark boyunun alçı modelde (Kontrol Grubu) dijital kumpas ile, dijital modelde (Deney Grubu) ise bilgisayar yazılımı kullanılarak ölçümü yapılmıştır. Ölçüm değerleri t-test kullanılarak karşılaştırılmış ve istatistiksel anlamlılık düzeyleri saptanmıştır. **Bulgular:** Alçı modellerde ve dijital modellerde çekim boşluğuna ait ark boyu ortalaması sırasıyla 6.94 mm. ve 6.83 mm. olarak ölçülmüştür. İki ölçüm metodu arasında istatistiksel anlamlı bir farklılık saptanmamıştır ( $p>0,05$ ). **Sonuçlar:** Pedodonti kliniklerinde çocuk hastalarda çalışma zamanını kısaltmak, efektif tedavi planı ve takibi oluşturmak, tedavi maliyeti azaltmak ve

sağladığı diğer avantajlar nedeniyle dijital modellerin kullanımı önerilmektedir. **Anahtar Kelimeler:** Diş modeli, süt dişi, diş oklüzyonu.

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## ROOT CANAL MORPHOLOGY OF MANDIBULAR PRIMARY MOLARS: A MICRO-CT STUDY

### ABSTRACT




**Objectives:** Frequency of typical and non-typical root and canal morphology of primary teeth, which in clinical practice cannot be detected using 2D radiographic images, should be known by clinicians to decrease failures arising from complexity of root canal morphologies. The aim of this in vitro study was to evaluate morphologic variations in mandibular primary molars' root canal systems.

**Materials and Methods:** Primary mandibular 1<sup>st</sup> (n=17) and 2<sup>nd</sup> (n=33) molars were scanned using micro-CT. 3D root models were obtained and root canal morphologies were evaluated according to a modified Vertucci classification. Type 1 and Type 4 canal morphologies were evaluated as 'normal' and all other types and 'non-typical' canal morphology were evaluated as 'abnormal' root canal morphology.

**Results:** Most common root canal morphology among mandibular primary 1<sup>st</sup> molars were Vertucci Type 4 morphology for both mesial and distal roots (47% and 41.2% respectively), and non-typical morphology for both the mesial and distal roots (45.7% and 21.2% respectively) of mandibular primary 2<sup>nd</sup> molars.

**Conclusions:** Wide range of morphologic variations and frequency of non-typical morphology could be seen especially among mandibular primary 2<sup>nd</sup> molars and use of disinfectant irrigants and root canal fillings with high antibacterial efficacies are important in order to decrease failures arising from these inaccessible areas.

**Key words:** Microcomputed tomography, root canal, tooth deciduous.

 Meryem ZİYA<sup>1</sup>  
 \*Burcu Nihan YÜKSEL<sup>1</sup>  
 Şaziye SARI<sup>1</sup>

ORCID IDs of the authors:  
M.Z. 0000-0003-4756-3348  
B.N.Y. 0000-0002-8133-6627  
Ş.S. 0000-0003-2202-5148

<sup>1</sup> Department of Pediatric Dentistry, Faculty of Dentistry, Ankara University, Ankara, Turkey

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## INTRODUCTION

It was clearly known that fundamentally the main philosophy of a root canal treatment is filling all the spaces from which pulp removal was done in the root canal following shaping and cleaning procedures.<sup>1-3</sup> From this point of view, it is necessary to have an idea about all the internal morphological diversity in the root canal system where the pulp is likely to occupy space.<sup>1</sup>

Root canal treatment consists of access cavity preparation and determination of working length, followed by chemo-mechanical preparation and obturation of root canal system in both dentitions.<sup>4,5</sup> Although root canal treatment applications of primary and permanent teeth are similar primary teeth require a different approach due to both continuous change in the location and size of the apical opening and morphologic variations as an outcome of secondary dentin deposition.<sup>5-7</sup> Both situations cause a limitation on the chemo-mechanical preparation and hermetically obturation of the root canals.<sup>8,9</sup> Leaving untreated canals due to a lack of detecting all root canals was reported as the major cause of failures in root canal therapy.<sup>8,9</sup> Therefore, a thorough knowledge about root canal morphology and variations in the root canal systems is needed in order to alter any possible complication during each of these stages and improve the success of treatment.<sup>10</sup>

Root canal treatment of primary incisors and canines are performed more easily as they generally exhibit only one root and root canal.<sup>5</sup> However, in cases where a root exhibits two root canals, especially as in the mesial roots of mandibular primary molars, deposition of secondary dentin in multi-rooted teeth causes small fins, lateral fibrils and connecting branches between canals and formation of a non-typical morphologic structure which cannot be clinically detected<sup>5</sup>, leading to failures in an effective chemo-mechanical preparation and obturation of the root canals during treatment.<sup>11</sup>

In clinical practice, complexity of the root canal morphology and variations such as lateral canals, apical ramifications or interconnecting branches cannot be detected through the radiographs.<sup>12</sup> Root canal morphology of primary teeth has been

evaluated in vitro with different methods such as; material injection<sup>13-16</sup>, dye perfusion<sup>17</sup>, digital radiographs<sup>18</sup>, longitudinal and transverse cross-sectioning<sup>18-19</sup>, clearing technique<sup>10,19-21</sup>, Scanning Electron Microscopy (SEM)<sup>22</sup>, Cone-Beam Computed Tomography (Cone-beam CT)<sup>23</sup> and micro-CT.<sup>9,24</sup> Micro-CT, which is recently used in many different fields in dentistry, is a fast, reproducible, non-invasive and non-destructive method, providing more accurate and complete results when compared to other CT methods.<sup>25-27</sup> In addition, the resolution of voxels ranging from 1-50 µm ensures that even very small and complex objects can be displayed. Micro-CT has been proven to be a very effective method in imaging and studying the complex structure of root canal morphology.<sup>28,29</sup>

Most of the morphologic studies in primary teeth, in which root canal morphology was defined according to number of only the main root canals and connecting branches, lateral fibrils were evaluated separately, seem to lack in defining morphologic variations in a systematic classification as in permanent teeth. Despite all the studies systematically trying to define the diversity of canal configurations, it is seen that there may still be too many atypical canal systems and there is still no classification system suitable for all tooth types.<sup>30</sup> Considering the limitations of evaluation methods used in studies and limited number of studies carried out using a systematic classification, a detailed description of the variations in root canal morphology of primary teeth is still lacking in the literature. Therefore, the present study aimed to evaluate the morphological variations seen in the root canal systems of the mandibular primary molars within a systematic classification by using micro-CT.

## MATERIALS AND METHODS

The study protocol was approved by the research ethics committee of Ankara University Faculty of Dentistry (36290600/23-2015).

### *Sample Selection*

Mandibular primary molars extracted for several reasons were selected from among patients attending pediatric clinic. According to the Power Analysis; to obtain results with maximum 20%

error, with 5% Type 1 error and 80% power level, at least 49 teeth were required.

Total of 50 mandibular primary molars [first molars (n=17) and second molars (n=33)] were selected according to the inclusion criteria which was determined as teeth could be included in the study when the apical root resorption level did not exceed 1/3 of total root length. Soft tissues and debris on the surface of the extracted teeth were removed and cleaned with a toothbrush and scaler under tap water. Specimens were stored in a 0.2% thymol solution until used.

#### ***Micro-CT Scanning and Reconstruction***

Each tooth was slightly dried and mounted on a wax block and scanned in a micro-CT scanner (Bruker Micro-CT 1172, Kontich, Belgium) at a pixel resolution of 12.5  $\mu\text{m}$ . The X-ray tube was operated at 100 kV and 100 mA, and the scanning was performed by 360° rotation around the vertical axis with a rotation step of 0.4°, using a 0.5-mm-thick aluminum filter. Each image was composed from the average of three poses obtained from each tooth. Duration for each pose is 1750 ms. Depending on the size of the specimen, an average of 1400 cross section was obtained. Images of each specimen were reconstructed with Nrecon software (Nrecon v. 1.7.1.0, Bruker\_microCT) providing axial cross sections of the inner structure of the samples.

#### ***Qualitative Analysis***

Data analysis and reconstructions of three-dimensional models were done based on CTAn software (CTAn, v.1.13, Bruker\_microCT), imaging was done using CTVOx software (CTVOx,

Bruker\_microCT) and solid state of models were imaged on CTVol software (CTVol, Bruker\_microCT). Using CTAn software (CTAn, v.1.13, Bruker\_microCT), choosing certain intervals on gray scale (0-255), separate models of root canal space and tooth structure were obtained. These models were then combined together using CTVol software (CTVol, Bruker\_microCT). For a more accurate morphologic evaluation, models of mesial and distal roots of each tooth were obtained separately. Color settings were selected to show the root canal space (pink-red) in the 3D images of the tooth structures.

#### ***Morphologic Classification***

Using models obtained via micro-CT, root canal morphologies of mesial and distal roots of mandibular primary first and second molars were determined according to Vertucci Classification.<sup>4</sup> Root canal morphologies non-classifiable with Vertucci's Classification, were recorded as non-typical root canal morphology that was earlier defined by Sarı and Aras.<sup>20</sup> According to this, single canal in a root (Vertucci Type 1) and two separate canals in a root (Vertucci Type 4) were evaluated as 'normal' canal morphology, while all other Vertucci canal types and non-typical root canal structures were all together categorized as 'abnormal' canal morphology. Frequency distribution of root canal morphology types of mandibular primary molars' mesial and distal roots were shown with numbers and percentages.

## **RESULTS**

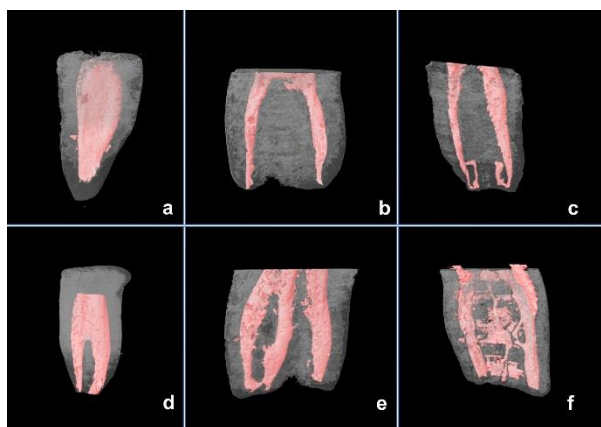
Data obtained from the evaluation of root canal morphology according to modified Vertucci Classification are given in Table 1.

**Table 1.** Root Canal Morphology of Mandibular Primary Molars (n=50)

| Tooth                | Root   | Root Number | Canal Types  |             |              |               |              |              |           |              |               | Normal Canal Morphology (Type 1 & Type 4) | Abnormal Canal Morphology (Other canal types) |
|----------------------|--------|-------------|--------------|-------------|--------------|---------------|--------------|--------------|-----------|--------------|---------------|---|---|
|                      |        |             | Type 1       | Type 2      | Type 3       | Type 4        | Type 5       | Type 6       | Type 7    | Type 8       | Non-Typical   |   |   |
| Primary First Molar  | Mesial | 17          | -            | -           | -            | 8<br>(%47)    | 1<br>(%5.9)  | 2<br>(%11.8) | -         | 1<br>(%5.9)  | 5<br>(%29.4)  | 8<br>(%47)                                | 9<br>(%53)                                    |
|                      | Distal | 17          | 3<br>(%17.6) | -           | 2<br>(%11.8) | 7<br>(%41.2)  | 3<br>(%17.6) | -            | -         | 2<br>(%11.8) | -             | 10<br>(%58.8)                             | 7<br>(%41.2)                                  |
| Primary Second Molar | Mesial | 35*         | 4<br>(%11.4) | 1<br>(%2.9) | -            | 10<br>(%28.6) | 2<br>(%5.7)  | -            | -         | 2<br>(%5.7)  | 16<br>(%45.7) | 14<br>(%40)                               | 21<br>(%60)                                   |
|                      | Distal | 33          | 2<br>(%6.1)  | 3<br>(%9.1) | 1<br>(%3)    | 5<br>(%15.1)  | 6<br>(%18.2) | 2<br>(%6.1)  | 1<br>(%3) | 6<br>(%18.2) | 7<br>(%21.2)  | 7<br>(%21.2)                              | 26<br>(%78.8)                                 |

\* 2 out of 33 second primary molars have two mesial roots and one distal root each.

Images of 3D modelled roots and root canals used in determining root canal morphology are shown in Figure 1.



**Figure 1.** Images obtained from the 3D models a) Distal root of mandibular primary 1<sup>st</sup> molar (Vertucci Type 1). b) Distal root of mandibular primary 1<sup>st</sup> molar (Vertucci Type 4). c) Mesial root of mandibular primary 1<sup>st</sup> molar (Non-typical). d) Mesial root of mandibular primary 1<sup>st</sup> molar (Vertucci Type 5). e) Distal root of mandibular primary 2<sup>nd</sup> molar (Vertucci Type 8). f) Mesial root of mandibular primary 2<sup>nd</sup> molar (Non-typical)

In Table 1, the frequency distribution of root canal types for mesial and distal roots of mandibular primary first and second molars are given in numbers and percentages. The frequency of ‘normal’ and ‘abnormal’ canal morphologies are also given in Table 1.

Three roots were observed in 2 out of 33 mandibular primary second molars [one distal and two separate mesial roots (mesiobuccal and mesiolingual), while all other primary molars (n=48) had two roots.

Vertucci Type 4 (47% and 41.2% respectively) was the most common root canal morphology for both mesial and distal roots of

mandibular primary first molars while it was the non-typical morphology for primary second molars for both roots [mesial (45.7%), distal (21.2%)]. Frequency of ‘Abnormal’ canal morphologies were enrolled 53% and 41.2% in mesial and distal roots of first molars respectively, and 60% and 78.8% in mesial and distal roots of second molars.

### DISCUSSION

Although detailed descriptions of root canal morphology of primary molars have been already reported<sup>5,14,15,18,19</sup>, most of these studies seem to lack in defining morphologic variations in a systematic classification in which only the prevalence of one or two root canals were reported and connecting branches, lateral fibrils were evaluated separately. So, the present study has aimed to analyze the detailed images of the root canal morphology and determine the frequency of complex morphologies of primary molars. Vertucci’s Classification<sup>4</sup> which form the basis of the classification systems used in permanent teeth, has identified a total of eight configurations that were more complex than described in earlier studies.<sup>30</sup> The classification system has been modified by Sari and Aras<sup>20</sup> in order to define the non-typical root canal variations in primary teeth.

Although new suggestions have come up to define the diversity of canal systems,<sup>30</sup> the recommendations generally focus on permanent teeth. A more recent study<sup>31</sup> describes new root and canal classification system for the primary dentition in a similar way as in permanent dentition<sup>30</sup>, however it is reported that the presence

of physiological root resorption and the presence of permanent successors close to the roots of primary teeth may be a challenge to implement this system. In addition, the numbering procedure appears to be somewhat complex and requires clinical experience. For this reasons, it is considered appropriate to use the modified type<sup>20</sup> of Vertucci system used in primary teeth in this present study.

Primary incisors and canines have been reported to have less complex root canal morphologies compared to primary molars.<sup>19,32</sup> Root canal number of both mandibular and maxillary molars vary between 2-5<sup>8,10,20,32,34</sup>, resulting with greater morphologic variations in their root canal systems, which has been reported to be most frequent especially in mandibular primary molars.<sup>13,20,34</sup> Regarding this, the present study was carried out on mandibular primary first and second molars.

The present study has shown that 47.1% of mesial roots of mandibular primary first molars have Vertucci Type 4 canal morphology. This result takes part in the range 24-100%, which was reported in previous studies.<sup>8-10,13,20,23,24,33</sup> Vertucci Type 4 was the most common also in distal roots of mandibular primary first molars (41.2%). The only study that we can compare the findings of the canal morphology we have obtained from the present study within the systematic classification is a study using clearing technique, carried out by Sarı and Aras,<sup>20</sup> in which Vertucci Type 1 was the most common root canal morphology reported for distal roots of first molars (70%), while only 9.3% of them had Type 4 morphology. The difference between the research findings is thought to be caused by the difference in the methods used in the study. Hence, the clearing technique used in that study has some limitations; due to weakened tooth structures during demineralization process, the weakened tooth could be bent during the handling process of the specimen, causing small structures like accessory canals or isthmuses to be affected even by a slight distortion. This morphological distortion could result in closure or narrowing of accessory canals.<sup>35</sup> It is more appropriate to compare our findings with the results of micro-CT studies<sup>9,24</sup> carried out with limited number of

samples, which have reported 50-60% of two root canals in distal roots of mandibular primary first molars.

In this present study, the most common canal morphology determined in both mesial and distal roots of mandibular primary second molars was non-typical morphologic structure (45.7% and 21.2% respectively). In the single study, in which canal types non-classifiable with Vertucci's classification were considered as non-typical canal morphology, it has been emphasized the prevalence of non-typical canal morphology in both mesial and distal roots to be (28% and 8% respectively).<sup>20</sup> Additionally, Vertucci Type 4 was the most common (48.6%) canal morphology among mesial roots of mandibular primary second molars reported also by the single reference study.<sup>20</sup> In other studies where only canal numbers were reported without systematic classification, the frequency of the occurrence for two canals in mesial roots of second molars was given between 32% and 100%.<sup>8-10,13,23,24,33</sup> In the literature variations like apical ramifications, interconnecting branches and lateral fibrils in the mesial roots of mandibular molars were frequently reported.<sup>9,13</sup> According to findings reported by a study<sup>13</sup>, canal morphology characterized with these variations was the most frequent (36%) in mesial roots of mandibular primary second molars.<sup>8</sup> Other studies evaluating only the main root canal(s) and not considering variations between the main root canals in determining canal morphology, have reported a higher prevalence of two root canals (85.7%-100 %) compared to our findings.<sup>8-10,20,23,24,33</sup>

In this present study, non-typical canal morphology was found to be the most frequent (21.2%) in the distal roots of the second molars. Type 1 canal morphology was the most common for distal roots reported by Sarı and Aras<sup>20</sup>, which is followed by Type 4 morphology with 18% and non-typical morphologic structure with 8%. In the present study, findings for Type 1 canal morphology were much less compared to results reported by Sarı and Aras<sup>20</sup>, while similar results for type 4 canal morphology (15.1%) was obtained. The difference between the results is thought to be

due to the number of samples used in the study or the methods used to determine the morphology. Prevalence of one root canal and two root canals in distal root of mandibular primary second molars varied between 21-88.9% and 11.4-100% respectively among studies.<sup>8-10,23,24,29-33</sup> Morphologic variations that was reported among distal roots of mandibular primary second molars<sup>8-10</sup> have suggested that on contrary to only one or two root canals reported among studies, non-typical root canal morphology may also be as prevalent, however, prevalence of this morphologic structure may vary among investigation methods. Additionally, perhaps due to the fact that the evaluations were not made in a systematic classification, the canals which can be considered as non-typical according to the modified Vertucci classification,<sup>20</sup> are determined as one or two canals. For this reason, it is possible to say that the modified classification used in the present study has given a more specific information about canal morphology.

Prevalence of 'normal' canal morphology defining Vertucci Type 1 and Type 4, and 'abnormal' canal morphology, defining all other canal types and non-typical morphological structure, were nearly equal in mesial and distal roots of first molars and mesial roots of second molars ('Normal' canal morphology; 47%, 58.8% and 40% respectively and 'Abnormal' canal morphology; 53%, 41.2% and 60% respectively). The difference in the frequency was more significant only in distal roots of second molar teeth ('Normal' canal morphology 21.2% and 'Abnormal' canal morphology 78.8%). This may be due to wide range of morphologic variations arising from random secondary dentin deposition in the wide, ribbon-like distal roots of second molars.

As a general assessment of 'abnormal canal morphology' recorded in the present study, morphological changes were most likely to appear as ramifications in the apical region. Thus, studies have reported that, in the apical region, continual secondary dentin deposition may develop small fins and connecting branches between canals and divide them into two or more canals.<sup>9,13,15,20</sup>

## CONCLUSIONS

Wide range of morphologic variations and frequency of non-typical morphology especially among mandibular primary 2nd molars, which in clinical practice cannot be detected using 2D radiographic images, should be considered during root canal treatment. Depending on these results, it is suggested that, during root canal treatment, use of disinfectant irrigants and root canal fillings with antibacterial efficacies are important in order to decrease failures arising from these inaccessible areas. Further studies are essential to create a general classification system including all diversities for the root canal system in primary teeth and more research are needed to evaluate the effect of variations among primary teeth root canal morphology on success of root canal filling.

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## CONFLICT OF INTEREST STATEMENT

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

## *Alt Süt Azı Dişlerinin Kanal Morfolojileri: Bir Mikro-BT Çalışması*

### ÖZ

**Amaç:** Süt dişlerinin kök kanal sistemlerinde sık rastlanan ancak klinik pratiğinde 2D radyografiler üzerinden tespit edilmesi mümkün olmayan morfolojik varyasyonlar, morfolojiden kaynaklanan başarısızlığın en aza indirgenebilmesi için klinik uygulamalarda hekim tarafından dikkate alınmalıdır. In-vitro olarak gerçekleştirilen bu çalışmada, alt süt azı dişlerin kök kanal morfolojilerindeki varyasyonların çeşitliliğinin değerlendirilmesi amaçlanmıştır. **Gereç ve Yöntemler:** 50 adet alt süt azı diş, [süt 1. azı (n=17), süt 2. azı (n=33)] yüksek çözünürlüklü mikro-BT ile taranmıştır. Mikro-BT ile örneklerin 3D görüntü ve modellerinin elde edilmesini takiben Vertucci sınıflamasına atipik kanal morfolojisi de ilave edilerek kanallardaki morfolojik varyasyonlar gruplandırılmıştır. Tip 1 ve Tip 4 kanal tipi 'normal' morfoloji, bunun dışında kalan tüm kanal tipleri ise 'anormal' morfoloji olarak değerlendirilmiştir. Elde edilen veriler sayı ve yüzde (%) ile gösterilmiştir. **Bulgular:** Alt süt 1. azı dişi için, mesial ve distal kökte en sık Tip 4 morfoloji (sırasıyla %47 ve %41,2); süt 2. azı dişi için ise, mesial ve distal kökte en sık atipik morfolojik yapı (sırasıyla



%45,7 ve %21,2) tespit edilmiştir. **Sonuçlar:** Atipik morfolojik varyasyonların süt azı dişlerinde çok geniş bir aralıkta ve sıklıkta görüldüğü ve özellikle alt çene süt ikinci azı dişlerinde gözlemlendiği dikkate alınarak, bu dişlerle ilgili planlanan endodontik tedaviler sırasında ulaşılamayan alanlardan kaynaklanan başarısızlığın en aza indirgenebilmesi için antibakteriyel etkinliğe sahip olan kanal dezenfektanı ve kanal dolgu materyali kullanımının önemli olduğu sonucuna varılmıştır. **Anahtar kelimeler:** Taurodontizm, Mikrobilgisayarlı Tomografi, Süt dişi, Kalıcı diş.

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## COMPARISON OF ORTHODONTIC TREATMENT WITH DIFFERENT PREMOLAR EXTRACTION MODALITIES IN TERMS OF SOFT TISSUE PROFILE

### ABSTRACT


**Objectives:** To evaluate the differences in changes in the soft tissue profile and dentoskeletal parameters between different premolar extraction and non-extraction treatment modalities.

**Materials and Methods:** Fifty patients with skeletal Class I malocclusion were divided into three groups. Group 1 consisted of 17 patients (mean age:16.76±1.68 years) treated with maxillary and mandibular first premolar extractions; Group 2 consisted of 16 patients (mean age:15.81±1.19 years) treated with maxillary and mandibular second premolar extractions, and Group 3 consisted of 17 patients (mean age:16.29±1.15 years) treated with non-extraction protocol. From the pre-treatment (T0) and post-treatment (T1) cephalometric radiographs, 13 measurements for dentoskeletal and 15 for soft tissue parameters were assessed. To determine changes due to treatment, and to compare differences among the groups, the Wilcoxon Signed-Rank and Kruskal-Wallis tests were performed, respectively.


**Results:** Mx1-SN, Mx1-FH, Mx1-NA, IMPA and Md1-NB values decreased significantly in Groups 1 and 2 compared to Group 3 ( $p<0.001$ ). According to the vertical reference line (VRL-li) and E-plane (E-LL), the lower lip showed a statistically significant change (retraction) in Group 1 and 2, compared to the non-extraction group ( $p<0.05$ ). The mean change value for the upper and lower lip thicknesses in Groups 1 and 2 was greater than in Group 3 ( $p<0.05$ ). Groups 1 and 2 did not show a significant difference in any dentoskeletal or soft tissue measurements.

**Conclusions:** Soft tissue profile change following extraction treatment was similar regardless of the extracted teeth. However, extraction treatment modalities resulted in significant profile changes especially in the lower lip compared to the non-extraction control group.

**Keywords:** Soft tissue, tooth extraction, vertical dimension.

 Ezgi ATİK<sup>1</sup>

 \*Hande GÖRÜCÜ COŞKUNER<sup>1</sup>

 Tülin TANER<sup>1</sup>

ORCID IDs of the authors:

E.A. 0000-0002-5912-4505

H.G.C. 0000-0001-7426-6731

T.T. 0000-0003-1358-0633

<sup>1</sup> Department of Orthodontics, Faculty of Dentistry, Hacettepe University, Ankara, Turkey

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## **INTRODUCTION**

Orthodontic treatments with extraction are treatment approaches that significantly affect the patient's profile and aesthetics.<sup>1</sup> One of the most controversial issues in the field of orthodontics is whether to use treatment alternatives with extraction or non-extraction. The choice between extraction and non-extraction treatment modalities has a considerable impact on numerous parameters, such as treatment stability, vertical dimension, arch width, soft tissues and facial convexity.<sup>2-5</sup> One of the most important reasons for this discussion is the possible effect of these two different treatment methods on the soft tissue. In many studies<sup>6-8</sup>, it has been emphasized that orthodontic treatments, including premolar extractions, may cause lip retraction, and a significant change in the facial profile compared to non-extraction treatment approaches. However, contrary to this view, other authors have stated that the withdrawal treatments will not have a negative effect on the soft tissue profile.<sup>9-12</sup>

Another effect area of orthodontic treatments on the face is the change in vertical height. According to the "wedge-type effect," extracting permanent posterior teeth may reduce the vertical dimension of the face by counterclockwise rotation of the mandible, through the forward movement of the posterior teeth.<sup>13</sup> It has been hypothesized that second premolar extractions allow the molar teeth to move more forward when compared to first premolar extractions, thus resulting in a greater decrease of the facial vertical dimension.<sup>14-16</sup>

In the literature, several studies have compared various extraction treatment protocols to non-extraction treatment.<sup>2,17-20</sup> However, there is a limited number of studies comparing the extraction of the four first premolar teeth with the four second premolar teeth regarding the amount of soft-tissue and vertical facial height changes performed during treatment.<sup>21-22</sup> Thus, in this retrospective observational study, we aimed to determine whether the removal of four first premolar teeth resulted in any differences in the soft tissue profile and vertical facial height change compared to the removal of four second premolar teeth using a non-extraction control group. The

null hypothesis of the present study was that there is no statistically significant difference in soft tissue and vertical facial height changes when comparing the extraction of four first premolars to four second premolars in patients treated with different extraction modalities.

## **MATERIALS AND METHODS**

The treatment records of 50 patients with skeletal Class I malocclusion were selected from the archive of the Department of Orthodontics, Faculty of Dentistry, Hacettepe University. The Institutional Ethics Committee of Hacettepe University (GO 19/21) approved the study design. The inclusion criteria for this retrospective study were as follows: 1) Lateral cephalometric images of patients who had been treated with four first or second premolar extraction with moderate anchorage mechanics or without extraction; 2) Use of a transpalatal arch and a Nance appliance in the maxilla and a lingual arch in the mandible for anchorage in patients who underwent extraction treatment; 3) Images of patients with high-quality pre- and post-treatment lateral cephalometric films exhibiting relaxed lips and teeth in occlusion, taken using the same cephalostat; 4) Images of patients with skeletal Class I malocclusion and moderate maxillary and mandibular crowding at the beginning of treatment; 5) Images of patients over 14 years of age; and 6) Images of patients with no craniofacial anomalies, and all teeth, excluding third molars, were present.

The dental files of 115 patients, who had been treated with extraction of either the four first or four second premolars, were reviewed. Of 115 records, 33 patients treated with the extraction protocol were included for this study. The final sample size per group was determined by the maximum number of cases eligible according to the strict inclusion criteria.

Group 1 consisted of 17 patients (12 female, 5 male) with a mean age of 16.76 years, who had been treated with four first premolar extractions. Group 2 consisted of 16 patients (10 female, 6 male) with a mean age of 15.81 years, who had been treated with four second premolar extractions. Group 3 consisted of 17 patients (13 female, 4

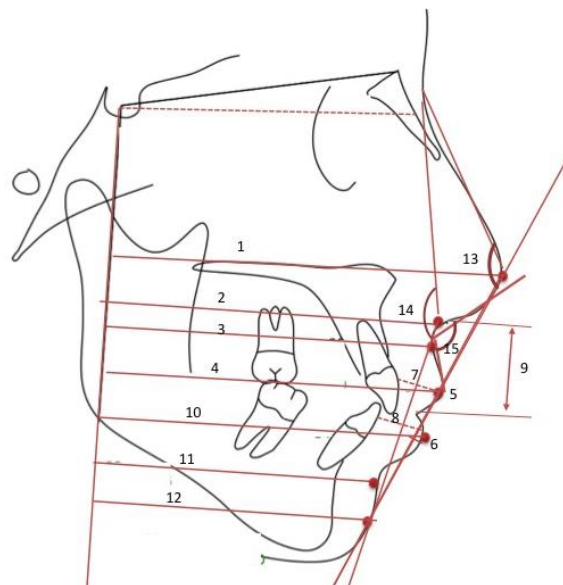
male) with a mean age of 16.29 years, with moderate maxillary and mandibular anterior crowding, and had been treated with the non-extraction treatment to serve as a control group. We aimed to make the clinical and demographic characteristics of the control group similar to the extraction groups. All patients were treated with pre-adjusted Roth prescription 0.018-inch appliances in both arches. In the extraction groups, premolar teeth were extracted to relieve crowding, the reduction of incisor protrusions, and/or the reduction of lip protrusion. In these groups, moderate anchorage mechanics, including the Nance appliance and a transpalatal arch for the maxilla; a lingual arch for the mandible was preferred. Crowding was initially alleviated by retraction of the canines, and the remaining space was closed by reciprocal traction of the posterior segment.

In the extraction groups, space closure was performed with sliding mechanics combined with a stainless steel (SS) archwire of 0.016×0.016-inch. The canine teeth in Group 1 and the canine and first premolar teeth in Group 2 were retracted through an archwire, using coil springs with sliding mechanics. After the canine teeth were retracted, the anchorage devices were removed, and 0.016×0.022-inch SS archwires with T-loops were used to close the remaining spaces. In the non-extraction group, crowding was eliminated by the expansion of the arches and/or proclination of the incisors. In the cases needing expansion, a Quad-Helix was used as an expansion device.

Lateral cephalometric radiographs were taken with a cephalostat (Promax; Planmeca, Helsinki, Finland) before (T0) and after (T1) treatment. Each subject's pre-treatment and post-treatment lateral cephalograms were traced using the Quick Ceph Studio software (Quick Ceph Systems, San Diego, Calif) by one examiner (E. A.) and were reviewed twice by another investigator (H. G-C.) for accurate landmark identification. Twenty-five subjects were randomly selected and retraced 2 weeks later by the same investigator to evaluate intra-examiner reliability.

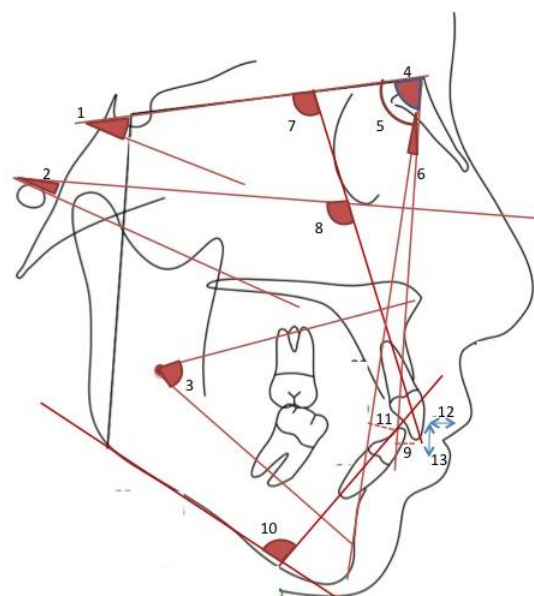
For the analysis of soft tissue measurements, two reference lines were created: a horizontal

reference line through Sella seven degrees inferior to the Sella-Nasion line, and a vertical reference line (VRL) through Sella perpendicular to SN-7°. <sup>23</sup> Twelve linear and 3 angular measurements were constructed for soft tissue measurements (Figure 1).



**Figure 1. a. Linear measurements of the soft tissue:** 1- VRL-prn; 2- VRL-sn; 3- VRL-A; 4-VRL-ls; 5-E-UL; 6-E-LL; 7-U1-ls; 8-L1-ls; 9-upper lip length; 10-VRL-li; 11-VRL-B; 12-VRL-pog. **b. Angular measurements of the soft tissue:** 13-na-prn-pog; 14-na-sn-pog; 15-nasolabial angle.

For skeletal and dental measurements, 9 angular and 4 linear cephalometric variables were recorded (Figure 2).



**Figure 2. a. Vertical measurements of the hard tissue:** 1- GoGnSN; 2-FMA; 3-ANS-Xi-Pm. **b. Other skeletal and dental measurements of the hard tissue:** 4-SNA; 5-SNB; 6-ANB; 7-Mx1-SN; 8-Mx1-FH; 9-Mx1-NA (mm); 10-IMPA; 11-Md1-NB (mm); 12-Overjet (mm); 13-Overbite (mm).

#### **Statistical Analysis:**

Statistical analyses were performed using SPSS software for Windows (version 21; IBM, Chicago, IL, USA). The descriptive data were presented as

frequency (%) or mean ± standard deviation. Group comparability regarding gender and cervical vertebral maturation stage (CVMS) was evaluated with chi-square analysis. Since all data were non-normally distributed, the Kruskal-Wallis nonparametric test was used to compare of the groups regarding age, treatment duration, maxillary crowding amount, mandibular crowding amount, and initial cephalometric measurements. Also, for the comparison of difference related to hard and soft tissue measurements between T0 and T1, the Kruskal-Wallis test was used. The Bonferroni Dunn Test was used to reveal the group that created the difference.

The Wilcoxon Signed Rank test was used to determine the significance of cephalometric measurement changes from T0 to T1 within the groups. Intra-examiner reliability was measured with Intraclass Correlation Coefficient (ICC) analysis. The significance level was set at p<0.05 for all the tests.

**RESULTS**

The ICC values were between 0.892 and 0.996, which were within acceptable limits. The pre-treatment demographic characteristics are shown in Table 1.

**Table 1.** Demographic characteristics of the sample in different groups.

| Variables                         | Group 1 (N=17)                      | Group 2 (N=16)                      | Group 3 (N=17)                      | p-value                               |
|-----------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|---------------------------------------|
|                                   | Mean ± SD or<br>Number (frequency%) | Mean ± SD or<br>Number (frequency%) | Mean ± SD or<br>Number (frequency%) |                                       |
| <b>Age (years)</b>                | 16.76 ±1.68                         | 15.81±1.19                          | 16.29±1.15                          | 0.118 <sup>a</sup>                    |
| <b>Gender</b>                     |                                     |                                     |                                     |                                       |
| Female                            | 12 (70.6%)                          | 10 (62.5%)                          | 13 (76.5%)                          | 0.680 <sup>b</sup>                    |
| Male                              | 5 (29.4%)                           | 6 (37.5%)                           | 4 (23.5%)                           |                                       |
| <b>CVMS period</b>                |                                     |                                     |                                     |                                       |
| CVMS 4                            | 3 (17.6%)                           | 7 (43.8%)                           | 3 (17.6%)                           | 0.115 <sup>b</sup>                    |
| CVMS 5                            | 6 (35.3%)                           | 7 (43.8%)                           | 5 (29.4%)                           |                                       |
| CVMS 6                            | 8 (47.1%)                           | 2 (12.5%)                           | 9 (52.9%)                           |                                       |
| <b>Treatment duration (years)</b> | 2.18±0.64                           | 2.03±0.50                           | 1.16±0.43                           | <0.001 <sup>a</sup><br>(1-3)<br>(2-3) |
| <b>Maxillary crowding (mm)</b>    | 5.65±1.22                           | 5.44±1.39                           | 5.53±0.51                           | 0.891                                 |
| <b>Mandibular crowding (mm)</b>   | 5.18±1.27                           | 4.94±1.03                           | 4.85±0.95                           | 0.789                                 |

a: Kruskal-Wallis test, b:chi-square test  
 p<0.05 is statistically significant.  
 (1-3): p value for difference between Group 1 and 3,  
 (2-3): p value for difference between Group 2 and 3.

There were no significant differences in any demographic characteristics, cephalometric or model measurements among the 3 groups except for treatment duration, which was significantly

lower in Group 3 compared to Groups 1 and 2 (p<0.001). Skeletal, dental, and soft tissue measurement differences at pre-treatment among the 3 groups are shown in Table 2.

**Table 2.** Pre-treatment (T0) differences between the groups with respect to hard and soft tissue cephalometric measurements.

| Variables             | Group 1 (N=17) | Group 2 (N=16) | Group 3 (N=17) | p-value                              |
|-----------------------|----------------|----------------|----------------|--------------------------------------|
|                       | Mean ± SD      | Mean ± SD      | Mean ± SD      |                                      |
| ANB°                  | 3.28±1.01      | 2.59±0.99      | 3.29±0.83      | 0.085                                |
| Mx1-SN°               | 106.39±6.35    | 106.87±6.22    | 105.55±6.48    | 0.898                                |
| Mx1-FH°               | 116.46±5.69    | 116.11±5.03    | 115.49±6.46    | 0.962                                |
| Mx1-NA (mm)           | 6.94±2.36      | 6.44±1.60      | 5.37±2.24      | 0.228                                |
| IMPA°                 | 103.98±6.36    | 96.24±7.12     | 99.91±8.39     | 0.017*                               |
| Md1-NB (mm)           | 8.14±1.61      | 7.08±2.51      | 6.74±2.05      | (1-2=0.013)<br>0.083                 |
| Overjet (mm)          | 3.73±1.61      | 3.44±1.24      | 3.98±0.93      | 0.309                                |
| Overbite (mm)         | 1.00±1.53      | 1.18±1.09      | 2.20±1.04      | 0.012*<br>(1-3=0.017)                |
| GoGnSN°               | 33.56±5.10     | 37.36±4.89     | 32.45±5.06     | 0.017*<br>(2-3=0.017)                |
| FMA°                  | 23.77±4.93     | 28.10±3.32     | 22.49±4.82     | 0.001*<br>(1-2=0.006)<br>(2-3=0.001) |
| ANS-Xi-Pm°            | 48.68±4.92     | 49.02±3.51     | 45.84±3.19     | 0.066                                |
| VRL-prn (mm)          | 97.22±6.88     | 94.75±3.55     | 94.28±4.99     | 0.386                                |
| VRL-sn (mm)           | 82.43±7.70     | 80.09±5.11     | 80.38±4.00     | 0.461                                |
| VRL-A' (mm)           | 80.64±7.34     | 78.28±4.91     | 77.93±4.03     | 0.444                                |
| VRL-ls (mm)           | 82.65±7.95     | 80.74±5.68     | 79.55±5.25     | 0.518                                |
| E-UL (mm)             | -3.26±1.94     | -3.36±2.35     | -3.93±2.44     | 0.542                                |
| E-LL (mm)             | -1.28±2.19     | -0.87±2.02     | -2.20±1.84     | 0.142                                |
| U1-ls (mm)            | 11.32±1.71     | 11.80±2.20     | 12.15±2.24     | 0.631                                |
| L1-li (mm)            | 12.27±2.30     | 13.31±1.39     | 12.68±2.37     | 0.324                                |
| upper lip length (mm) | 21.52±2.29     | 22.24±3.61     | 19.75±1.99     | 0.032*<br>(2-3=0.052)                |
| VRL-li                | 79.69±8.88     | 78.14±5.46     | 77.04±5.86     | 0.693                                |
| VRL-B' (mm)           | 71.49±9.18     | 69.44±5.76     | 68.71±6.28     | 0.726                                |
| VRL-pog' (mm)         | 70.44±10.37    | 68.64±6.07     | 68.56±7.34     | 0.929                                |
| na-prn-pog°(mm)       | 127.35±3.44    | 129.56±2.87    | 126.52±4.10    | 0.068                                |
| na-sn-pog°(mm)        | 157.78±5.14    | 159.54±5.70    | 156.18±4.82    | 0.244                                |
| nasolabial angle      | 103.87±13.39   | 105.56±11.76   | 105.81±9.02    | 0.922                                |

Kruskal-Wallis test was used for intergroup comparisons, and Bonferroni Dunn test for post-hoc analysis.

(1-3): p value for difference between Group 1 and 3, (2-3): p value for difference between Group 2 and 3.

Significant differences in skeletal and dental measurements were found in IMPA°, overbite (mm), GoGnSN°, and FMA° values ( $p < 0.05$ ). Pre-treatment soft tissue measurements did not differ among the groups, except for upper lip length, which was higher in Group 2 compared to Group 3 ( $p = 0.052$ ).

Table 3 shows the mean values for pre-treatment and post-treatment skeletal and dental measurements and the significance of changes during treatment in each group. Mx1-SN°, Mx1-FH°, Mx1-NA (mm), IMPA°, Md1-NB (mm)

values decreased significantly in Groups 1 and 2, while these values significantly increased in Group 3 ( $p < 0.05$ ). Overbite was significantly increased in Groups 1 and 2, but was significantly decreased in Group 3 ( $p < 0.05$ ). Vertical skeletal variables (GoGnSN°, FMA°, and ANS-Xi-Pm°) did not show significant changes from pre-treatment to the post-treatment period in all groups. The decreases in maxillary and mandibular incisor inclinations in Groups 1 and 2 were significantly different from the increase of these values in Group 3 ( $p < 0.05$ ).



**Table 3.** Pre-treatment (T0) and post-treatment (T1) skeletal and dental measurements, changes during treatment in each group, and intergroup comparisons.

| Variables        | T0/T1 | Group 1<br>(N=17)<br>Mean±SD | p-value<br>(Group 1<br>T1-T0) | Group 2<br>(N=16)<br>Mean±SD | p-value<br>(Group 2<br>T1-T0) | Group 3<br>(N=17)<br>Mean±SD | p-value<br>(Group 3<br>T1-T0) | Intergroup<br>comparison<br>p-value |
|------------------|-------|------------------------------|-------------------------------|------------------------------|-------------------------------|------------------------------|-------------------------------|-------------------------------------|
| Mx1-SN°          | T0    | 106.39±6.35                  |                               | 106.87±6.22                  |                               | 105.55±6.48                  |                               | <0.001*                             |
|                  | T1    | 98.60±7.85                   | <0.001*                       | 100.07±5.85                  | 0.003*                        | 110.92±4.88                  | <0.001*                       | (1-3<0.001)                         |
|                  | T1-T0 | -7.79±3.92                   |                               | -6.80±6.29                   |                               | 5.37±4.71                    |                               | (2-3<0.001)                         |
| Mx1-FH°          | T0    | 116.46±5.69                  |                               | 116.11±5.03                  |                               | 115.49±6.46                  |                               | <0.001*                             |
|                  | T1    | 109.28±7.41                  | <0.001*                       | 110.16±5.16                  | 0.007*                        | 120.69±3.84                  | <0.001*                       | (1-3<0.001)                         |
|                  | T1-T0 | -7.18±4.30                   |                               | -5.95±6.41                   |                               | 5.20±4.40                    |                               | (2-3<0.001)                         |
| Mx1-NA<br>(mm)   | T0    | 6.94±2.36                    |                               | 6.44±1.60                    |                               | 5.37±2.24                    |                               | <0.001*                             |
|                  | T1    | 2.91±2.49                    | <0.001*                       | 4.30±2.03                    | 0.004*                        | 6.61±2.05                    | 0.001*                        | (1-3<0.001)                         |
|                  | T1-T0 | -4.04±2.71                   |                               | -2.14±2.13                   |                               | 1.24±0.80                    |                               | (2-3=0.001)                         |
| IMPA°            | T0    | 103.98±6.36                  |                               | 96.24±7.12                   |                               | 99.91±8.39                   |                               | <0.001*                             |
|                  | T1    | 94.97±7.31                   | <0.001*                       | 90.46±7.56                   | 0.011*                        | 103.94±8.42                  | 0.004                         | (1-3<0.001)                         |
|                  | T1-T0 | -9.01±4.68                   |                               | -5.78±7.49                   |                               | 4.03±4.91                    |                               | (2-3=0.002)                         |
| Md1-NB<br>(mm)   | T0    | 8.14±1.61                    |                               | 7.08±2.51                    |                               | 6.74±2.05                    |                               | <0.001*                             |
|                  | T1    | 5.10±1.73                    | <0.001*                       | 5.71±2.29                    | 0.012*                        | 8.42±1.86                    | <0.001*                       | (1-3<0.001)                         |
|                  | T1-T0 | -3.04±1.34                   |                               | -1.38±1.74                   |                               | 1.69±1.15                    |                               | (2-3=0.001)                         |
| Overjet<br>(mm)  | T0    | 3.73±1.61                    |                               | 3.44±1.24                    |                               | 3.98±0.93                    |                               |                                     |
|                  | T1    | 3.47±0.69                    | 0.469                         | 3.13±0.94                    | 0.395                         | 3.36±1.01                    | 0.028*                        | 0.751                               |
|                  | T1-T0 | -0.27±1.40                   |                               | -0.32±1.44                   |                               | -0.62±1.02                   |                               |                                     |
| Overbite<br>(mm) | T0    | 1.00±1.53                    |                               | 1.18±1.09                    |                               | 2.20±1.04                    |                               | <0.001*                             |
|                  | T1    | 2.00±0.98                    | 0.012*                        | 1.90±0.80                    | 0.013*                        | 1.29±0.91                    | 0.004*                        | (1-3<0.001)                         |
|                  | T1-T0 | 0.99±1.43                    |                               | 0.72±0.92                    |                               | -0.91±0.97                   |                               | (2-3=0.001)                         |
| GoGnSN°          | T0    | 33.56±5.10                   |                               | 37.36±4.89                   |                               | 32.45±5.06                   |                               |                                     |
|                  | T1    | 33.39 ± 5.21                 | 0.619                         | 37.91±4.19                   | 0.315                         | 32.92±5.38                   | 0.236                         | 0.433                               |
|                  | T1-T0 | -0.17±1.96                   |                               | 0.55±2.49                    |                               | 0.47±1.46                    |                               |                                     |
| FMA°             | T0    | 23.77±4.93                   |                               | 28.10±3.32                   |                               | 22.49±4.82                   |                               |                                     |
|                  | T1    | 23.07±5.08                   | 0.266                         | 27.80±3.02                   | 0.517                         | 23.18±5.16                   | 0.084                         | 0.110                               |
|                  | T1-T0 | -0.70±2.23                   |                               | -0.30±1.90                   |                               | 0.68±1.62                    |                               |                                     |
| ANS-Xi-<br>Pm°   | T0    | 48.68±4.92                   |                               | 49.02±3.51                   |                               | 45.84±3.19                   |                               |                                     |
|                  | T1    | 48.03±5.04                   | 0.443                         | 49.36±3.57                   | 0.315                         | 45.71±3.87                   | 0.766                         | 0.456                               |
|                  | T1-T0 | -0.65±2.45                   |                               | 0.34±1.35                    |                               | -0.14±1.86                   |                               |                                     |

Wilcoxon signed rank test was used for intragroup comparisons, Kruskal-Wallis test was used for intergroup comparisons, and Bonferroni Dunn test for post-hoc analysis. (1-2): p value for difference between group 1 and 2, (1-3): p value for difference between group 1 and 3, (2-3): p value for difference between group 2 and 3. p<0.05 is statistically significant.

The pre-treatment (T0) and post-treatment (T1) soft tissue measurements, changes during treatment in each group, and intergroup comparisons are shown in Table 4. In relation to the E-plane, the upper lip was significantly retracted (p=0.027) in Group 1, while the lower lip was significantly retracted in both Group 1 (p=0.003) and Group 2 (p=0.008). Upper lip thicknesses (U1-ls) showed a statistically significant increase from T0 to T1 in both Groups

1 and 2 (p≤0.001). Lower lip thickness (L1-li) significantly increased (p=0.032) in Group 1, whereas it was decreased in Group 3 (p=0.029). Soft tissue measurements related to the lower lip and pogonion (VRL-li, VRL-B', VRL-pog') significantly moved anteriorly in Group 3 (p<0.05). VRL-li distance significantly decreased (p=0.01) in Group 1. The nasolabial angle significantly decreased (p=0.039) in Group 3.

**Table 4.** Pre-treatment (T0) and post-treatment (T1) soft tissue measurement changes during treatment in each group, and intergroup comparisons.

| Variables               | T0/T1 | Group 1<br>(N=17)<br>Mean±SD | p-value<br>(Group 1<br>T1-T0) | Group 2<br>(N=16)<br>Mean±SD | p-value<br>(Group 2<br>T1-T0) | Group 3<br>(N=17)<br>Mean±SD | p-value<br>(Group 3<br>T1-T0) | Intergroup<br>comparison<br>p-value |
|-------------------------|-------|------------------------------|-------------------------------|------------------------------|-------------------------------|------------------------------|-------------------------------|-------------------------------------|
| VRL-prn<br>(mm)         | T0    | 97.22±6.88                   |                               | 94.75±3.55                   |                               | 94.28±4.99                   |                               |                                     |
|                         | T1    | 96.95±7.13                   | 0.501                         | 95.50±4.93                   | 0.162                         | 94.62±5.31                   | 0.286                         | 0.128                               |
|                         | T1-T0 | -0.27±1.29                   |                               | 0.75±1.93                    |                               | 0.34±1.34                    |                               |                                     |
| VRL-sn<br>(mm)          | T0    | 82.43±7.70                   |                               | 80.09±5.11                   |                               | 80.38±4.00                   |                               |                                     |
|                         | T1    | 82.59±7.10                   | 0.868                         | 80.51±5.66                   | 0.477                         | 80.65±4.32                   | 0.266                         | 0.859                               |
|                         | T1-T0 | 0.17±2.35                    |                               | 0.42±3.00                    |                               | 0.27±1.15                    |                               |                                     |
| VRL-A'<br>(mm)          | T0    | 80.64±7.34                   |                               | 78.28±4.91                   |                               | 77.93±4.03                   |                               |                                     |
|                         | T1    | 79.99±7.06                   | 0.213                         | 78.68±5.11                   | 0.570                         | 78.30±4.39                   | 0.133                         | 0.160                               |
|                         | T1-T0 | -0.65±1.66                   |                               | 0.40±2.05                    |                               | 0.37±1.44                    |                               |                                     |
| VRL-ls<br>(mm)          | T0    | 82.65±7.95                   |                               | 80.74±5.68                   |                               | 79.55±5.25                   |                               |                                     |
|                         | T1    | 81.66±8.21                   | 0.113                         | 80.44±5.19                   | 0.649                         | 80.57±5.12                   | 0.130                         | 0.091                               |
|                         | T1-T0 | -0.99±2.32                   |                               | -0.30±2.37                   |                               | 1.02±2.16                    |                               |                                     |
| E-UL (mm)               | T0    | -3.26±1.94                   |                               | -3.36±2.35                   |                               | -3.93±2.44                   |                               |                                     |
|                         | T1    | -4.04±2.45                   | 0.027*                        | -4.19±1.92                   | 0.062                         | -3.65±2.36                   | 0.897                         | 0.151                               |
|                         | T1-T0 | -0.78±1.24                   |                               | -0.83±1.62                   |                               | 0.28±1.61                    |                               |                                     |
| E-LL (mm)               | T0    | -1.28±2.19                   |                               | -0.87±2.02                   |                               | -2.20±1.84                   |                               | <0.001*                             |
|                         | T1    | -2.78±2.64                   | 0.003*                        | -2.41±2.12                   | 0.008*                        | -1.39±1.74                   | 0.052                         | (1-3=0.001)                         |
|                         | T1-T0 | -1.50±1.69                   |                               | -1.54±1.70                   |                               | 0.81±1.60                    |                               | (2-3=0.002)                         |
| U1-ls (mm)              | T0    | 11.32±1.71                   |                               | 11.80±2.20                   |                               | 12.15±2.24                   |                               | <0.001*                             |
|                         | T1    | 13.01±1.77                   | <0.001*                       | 13.80±2.00                   | 0.001*                        | 11.37±1.83                   | 0.063                         | (1-3<0.001)                         |
|                         | T1-T0 | 1.69±1.22                    |                               | 2.00±1.82                    |                               | -0.78±1.74                   |                               | (2-3<0.001)                         |
| L1-li (mm)              | T0    | 12.27±2.30                   |                               | 13.31±1.39                   |                               | 12.68±2.37                   |                               | 0.003*                              |
|                         | T1    | 12.87±2.37                   | 0.032*                        | 13.97±1.29                   | 0.075                         | 11.97±1.35                   | 0.029*                        | (1-3=0.010)                         |
|                         | T1-T0 | 0.60±1.00                    |                               | 0.66±1.27                    |                               | -0.71±1.48                   |                               | (2-3=0.009)                         |
| Upper lip<br>length(mm) | T0    | 21.52±2.29                   |                               | 22.24±3.61                   |                               | 19.75±1.99                   |                               |                                     |
|                         | T1    | 21.83±2.79                   | 0.351                         | 22.79±2.40                   | 0.210                         | 20.38±1.89                   | 0.185                         | 0.702                               |
|                         | T1-T0 | 0.31±1.21                    |                               | 0.56±1.65                    |                               | 0.62±1.75                    |                               |                                     |
| VRL-li (mm)             | T0    | 79.69±8.88                   |                               | 78.14±5.46                   |                               | 77.04±5.86                   |                               | 0.001*                              |
|                         | T1    | 78.07±8.73                   | 0.010*                        | 77.23±5.05                   | 0.268                         | 78.55±5.55                   | 0.005*                        | (1-3=0.001)                         |
|                         | T1-T0 | -1.62±2.23                   |                               | -0.91±2.96                   |                               | 1.51±1.71                    |                               | (2-3=0.013)                         |
| VRL-B'<br>(mm)          | T0    | 71.49±9.18                   |                               | 69.44±5.76                   |                               | 68.71±6.28                   |                               | 0.009*                              |
|                         | T1    | 70.57±9.04                   | 0.196                         | 68.02±5.33                   | 0.113                         | 69.97±6.03                   | 0.011*                        | (1-3=0.044)                         |
|                         | T1-T0 | -0.92±2.36                   |                               | -1.42±3.08                   |                               | 1.26±1.67                    |                               | (2-3=0.013)                         |
| VRL-pog'<br>(mm)        | T0    | 70.44±10.37                  |                               | 68.64±6.07                   |                               | 68.56±7.34                   |                               | 0.018*                              |
|                         | T1    | 70.25±10.37                  | 0.569                         | 68.50±5.85                   | 0.572                         | 70.12±7.17                   | 0.010*                        | (1-3=0.047)                         |
|                         | T1-T0 | -0.19±1.43                   |                               | -0.14±2.10                   |                               | 1.57±2.06                    |                               | (2-3=0.039)                         |
| na-prn-pog°             | T0    | 127.35±3.44                  |                               | 129.56±2.87                  |                               | 126.52±4.10                  |                               |                                     |
|                         | T1    | 127.42±4.19                  | 0.638                         | 129.23±4.02                  | 0.556                         | 126.97±3.90                  | 0.507                         | 0.848                               |
|                         | T1-T0 | 0.07±2.14                    |                               | -0.33±2.46                   |                               | 0.45±2.19                    |                               |                                     |
| na-sn-pog°              | T0    | 157.78±5.14                  |                               | 159.54±5.70                  |                               | 156.18±4.82                  |                               |                                     |
|                         | T1    | 158.55±5.55                  | 0.438                         | 159.29±6.60                  | 1.000                         | 157.14±3.95                  | 0.163                         | 0.548                               |
|                         | T1-T0 | 0.77±3.60                    |                               | -0.26±3.11                   |                               | 0.96±3.01                    |                               |                                     |
| nasolabial<br>angle     | T0    | 103.87±13.39                 |                               | 105.56±11.76                 |                               | 105.81±9.02                  |                               |                                     |
|                         | T1    | 104.85±12.44                 | 0.723                         | 106.83±16.19                 | 0.737                         | 102.68±6.31                  | 0.039*                        | 0.246                               |
|                         | T1-T0 | 0.98±7.27                    |                               | 1.27±8.09                    |                               | -3.13±6.66                   |                               |                                     |

Wilcoxon signed rank test was used for intragroup comparisons, Kruskal-Wallis test was used for intergroup comparisons, and Bonferroni Dunn test for post-hoc analysis. (1-2): p value for difference between group 1 and 2, (1-3): p value for difference between group 1 and 3, (2-3): p value for difference between group 2 and 3. p<0.05 is statistically significant.

In relation to the E-plane, the lower lip was retracted by 1.50 mm in Group 1 and 1.54 mm in Group 2, but was protracted by 0.81 mm in Group 3. These changes in Groups 1 and 2 showed a statistically significant difference from Group 3 (p<0.001). According to the vertical reference line (VRL-li), the lower lip was retracted in both extraction groups, and showed a statistically significant change compared to the non-extraction group (an increase of 1.51 mm) (p=0.001). The

mean change values for the upper and lower lip thicknesses were 1.69 mm and 0.60 mm for Group 1, and 2mm and 0.66 mm for Group 2, respectively, both of which were greater than in Group 3 (decrease of 0.78 mm for upper lip thickness and 0.71 mm for lower lip thickness) (p<0.05). The mean soft tissue change values for VRL-B' and VRL-pog' showed a statistically significant increase in Group 3 compared to Groups 1 and 2 (p<0.05). Groups 1 and 2 did not

show a significant difference in any dentoskeletal or soft tissue measurements between each other ( $p > 0.05$ ).

## DISCUSSION

Orthodontists frequently encounter moderate anterior crowding in Class I malocclusions.<sup>24</sup> In cases with 4-9 mm arch size/tooth size discrepancies, non-extraction and extraction treatments are possible, and the treatment plan depends on the hard and soft tissue characteristics of the patient. The extraction option is particularly used to relieve moderate to severe crowding and/or to lessen dental or dentoalveolar protrusion, while non-extraction treatment is usually preferred for minor skeletal and moderate dental crowding.<sup>25</sup> The conflict surrounding the decision whether to extract or not is mostly related to the stability of treatment and its effects on the soft tissue profile. It is thought that the facial profile might be improved by decreasing facial convexity.<sup>26</sup> This fact can be considered as the reason for preferring tooth extraction in borderline cases. Most studies<sup>2,4,12,20,27-29</sup> have compared extraction treatment vs. non-extraction in regard to profile changes. However, there is no consensus as to whether soft tissue profile changes are different from orthodontic treatment with the first premolar extraction from those treated with second premolar extractions. Hence, this retrospective study was conducted to compare both extraction modalities to each other and as well as to a non-extraction control group.

For a meaningful comparison of the effects of extraction vs. non-extraction treatments on the facial profile, a high degree of homogeneity is necessary, especially regarding the growth parameter. The groups in the present study exhibited similar age, sex, and CVMS distribution, and this result can be considered as advantageous to control the confounding factors due to growth status. Also, the groups exhibited almost similar pre-treatment soft tissue characteristics. Therefore, the effect of known confounding factors related to the soft tissue profile was reduced, and any differences among the three groups at the end of the treatment might be mostly attributed to the treatment modality.

However, the initial vertical skeletal parameters differed, especially between Group 2 and 3, and this difference can be related to the factor that the clinicians might have a tendency to extract second premolars instead of first premolars, for reducing the vertical height.

According to the results of the present study, the null hypothesis was accepted, and there were no statistically significant differences in soft tissue, dental and vertical facial height changes when comparing the extraction of four first premolars to four second premolars in Class I patients. Therefore, this study does not support that there is a greater retraction of the upper and lower lips when first premolar teeth are extracted compared to when second premolar teeth are removed, in contrary to the Nance's argument.<sup>30</sup> Omar *et al.*<sup>22</sup> compared first vs. second premolar extraction in soft tissue profile changes. Similar to the results of our study, Omar *et al.*<sup>22</sup> did not observe significant differences between different extraction patterns, such as the nasolabial angle and upper and lower lip position changes. On the other hand, different from the results of the present study, Omar *et al.*<sup>22</sup> found that the amount of retraction of upper and lower incisors achieved in second premolar extraction cases was less than half the amount of retraction achieved in first premolar extraction cases. In the present study, both extraction patterns showed similar significant retraction of the upper and lower incisor teeth. This difference may arise from different anchorage techniques since they used a molar anchorage appliance more frequently in the first premolar extraction group. On the other hand, the same moderate anchorage mechanics were used in both extraction groups in the present study.

The results of the present study indicated that both extraction groups showed statistically significant differences related to dental and soft tissue parameters when compared to the non-extraction control group. Lip retrusion is expected to be further increased in the case of extractions with active incisor retraction.<sup>31</sup> In the present study, the lower lip showed statistically significant retraction instead of upper lip according to E-plane and VRL in both extraction

groups. The significant retraction of lower lips in both the first and second premolar extraction groups, when compared to the non-extraction group, can be attributed to the posterior dentoalveolar movement of both upper and lower anterior segments. Kouli *et al.*<sup>29</sup> evaluated facial profiles and hard tissue changes in matched extraction and non-extraction Class I patients with the use of discriminant analysis validation. Kouli *et al.*<sup>29</sup> concluded that both lips, but especially the lower, were more retruded relative to the nose and chin, compared to the non-extraction group, similar to our results.

Similarly, the distance from the lower lip to the aesthetic line significantly increased more in the extraction group due to incisor retraction compared to the non-extraction group in the study by Kirschneck *et al.*<sup>4</sup> Yashwant *et al.*<sup>28</sup> compared soft tissue changes in Class I borderline patients treated with either extraction or non-extraction modalities. According to their results, upper and lower lips were more retracted, and the thickness of the upper lip increased more in borderline extraction cases. In the present study, both upper and lower lip thicknesses significantly increased in both extraction groups compared to the non-extraction group, and might be related to the loss of tension in the upper and lower lips following the retraction of anterior teeth. According to the results of the present study, the nasolabial angle did not show a significant change in extraction groups, similar to the study by Kirschneck *et al.*<sup>4</sup>, contrary to the results of other studies<sup>20,28,32</sup> that showed a significant increase in this angle. The differences in soft tissue changes, due to extraction or non-extraction treatment between different studies, may depend on the characteristics of the patients studied, sample size, the prescription used, anchorage considerations, and treatment mechanics.

In the present study, we also compared the effect of four premolar extractions, for the skeletal vertical dimension. The main idea behind this supposition is that tooth extractions reduce the vertical dimension based on the wedge-effect concept, by the anterior rotation of the mandible.<sup>33</sup> Also, in the non-extraction treatment protocol, it

could be expected that vertical facial height might increase because of the buccal crown tipping in the posterior area during crowding relief. However, the results of the present study did not show a significant change of vertical height in either the extraction or non-extraction groups. A recent systematic review<sup>34</sup> assessed the effects of orthodontic treatment with four premolar extractions on the skeletal vertical dimension compared to non-extraction treatment. This review concluded that an extraction treatment protocol aiming to reduce vertical dimension does not seem to be an evidence-based clinical approach, as several studies<sup>35-37</sup> indicated no significant differences between extraction and non-extraction treatments. In the study by Beit *et al.*<sup>38</sup>, the extraction of four first premolars showed a slight decrease in the vertical skeletal measurements, whereas non-extraction treatment showed a slight increase. However, Beit *et al.*<sup>38</sup> commented that because of the small-scale intergroup differences, it is open to discuss whether these results are clinically significant. Similar to our study design and results, Kim *et al.*<sup>21</sup> suggested that there was no decrease in facial vertical dimension regardless of the maxillary and mandibular first or second premolar extractions.

From the findings of the present study, the clinical relevance for orthodontists is that during orthodontic treatment, including an extraction protocol, extraction of either the four first or four second premolar teeth does not show different impacts on the facial profile and vertical parameters when using the same moderate anchorage mechanics. Therefore, the clinician should consider other possible factors, such as tooth prognosis, morphology, or size, while deciding whether to extract the first or second premolar teeth.

Some study design bias factors need to be considered when interpreting the results of this study. The possible growth changes in the nose, lips, and chin were not measured. Because of the nature of the study, the premolar extraction pattern was not determined randomly. It would be better to perform well-conducted randomized clinical trials with large sample sizes to increase

the confidence of evidence regarding the effect of different extraction patterns on soft tissue profiles.

## CONCLUSIONS

- The change in soft tissue profile and dentoskeletal parameters following extraction treatment was similar, regardless of whether the first premolar or second premolar teeth were extracted.
- Maxillary and mandibular incisor inclinations significantly decreased in extraction groups, compared to the non-extraction group.
- In relation to the E-plane, the lower lip was retracted by 1.50 mm and 1.54 mm for Groups 1 and 2, and protracted by 0.81 mm for Group 3, respectively.
- The lower lip was retracted by 1.62 mm and 0.91 mm, according to the VRL in Groups 1 and 2, respectively, and protracted by 1.51 mm in Group 3.

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None

## CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

### *Farklı Premolar Çekimli Ortodontik Tedavi Yöntemlerinin Yumuşak Doku Profili Bakımından Karşılaştırılması*

## ÖZ

**Amaç:** Farklı premolar çekimli ve çekimsiz tedavi yöntemleri arasında yumuşak doku profili ve dentoiskeletsel parametrelerdeki değişikliklerin karşılaştırılmasıdır. **Gereç ve Yöntemler:** İskelet Sınıf I maloklüzyona sahip 50 hasta üç gruba ayrıldı. Grup 1, maksiller ve mandibular birinci premolar çekimiyle tedavi edilen 17 hastadan (ortalama yaş:16,76±1,68 yıl); Grup 2, maksiller ve mandibular ikinci premolar çekimiyle tedavi edilen 16 hastadan (ortalama yaş:15,81±1,19 yıl) ve Grup 3, çekimsiz protokol ile tedavi edilen 17 hastadan (ortalama yaş:16,29±1,15 yıl) oluşuyordu. Tedavi öncesi (T0) ve tedavi sonrası (T1) lateral sefalometrik radyograflar değerlendirildi. Dentoiskeletsel parametreler için 13, yumuşak doku parametreleri için 15 ölçüm yapıldı. Tedaviye bağlı yumuşak doku ve dentoiskeletsel değişiklikleri belirlemek ve gruplar arasındaki farklılıkları karşılaştırmak için sırasıyla Wilcoxon İşaretli Sıra ve

Kruskal-Wallis testleri uygulandı. **Bulgular:** Mx1-SN, Mx1-FH, Mx1-NA, IMPA ve Md1-NB değerleri Grup 1 ve 2'de Grup 3'e göre anlamlı olarak azaldı ( $p < 0,001$ ). Vertikal referans çizgisine (VRL-li) ve E-düzlemine (E-LL) göre, alt dudak Grup 1 ve 2'de çekimli olmayan gruba kıyasla istatistiksel olarak anlamlı bir değişiklik (retraksiyon) gösterdi ( $p < 0,05$ ). Grup 1 ve 2'deki üst ve alt dudak kalınlıkları için ortalama değişim değeri Grup 3'ten daha fazlaydı ( $p < 0,05$ ). Dentoiskeletsel ve yumuşak doku ölçümleri bakımından Grup 1 ve 2 arasında istatistiksel olarak anlamlı bir farklılık bulunmadı. **Sonuçlar:** Çekimli tedavi sonrası yumuşak doku profilindeki değişiklik, birinci veya ikinci premolar dişlerin çekilip çekilmediğine bakılmaksızın benzerdi. Ancak, çekimli tedavi yöntemleri, çekimsiz kontrol grubuna göre özellikle alt dudak bölgesinde önemli profil değişikliklerine neden oldu. **Anahtar Kelimeler:** Dikey boyut, diş çekimi, yumuşak doku.

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## STRUCTURAL ALTERATIONS OF ZIRCONIA DEPENDING ON SINTERING PARAMETERS AND EFFECTS ON BOND STRENGTH AFTER DIFFERENT SURFACE TREATMENTS

### ABSTRACT

**Objectives:** Zirconia having different physical and optical properties is obtained after the application of different sintering parameters. This study aims to investigate structural variations after administering different sintering protocols and to evaluate the effect of different surface treatments on shear bond strength.

**Materials and Methods:** Eighty translucent zirconia specimens (7x3 mm) were divided into two different sintering groups (1480 °C for 180 min; 1510 °C for 30 min), then divided into four subgroups according to surface treatments: control, sandblasted, Er-YAG, and Nd-YAG (n:10). One specimen from each group was analyzed with XRD and one from each subgroup was analyzed with SEM. Ceramics (5x3 mm) were fired onto the zirconia for shear bond strength test using universal testing machine and the failure mode was determined by using stereomicroscope. Translucency and contrast ratio were measured by using spectrophotometer, and biaxial flexural strength test performed by employing universal testing machine on specimens with a diameter (15x1,3 mm) from each sintering group (n:10). Data was analyzed by using two-way ANOVA and Bonferroni Post hoc tests (p<.05).

**Results:** The short sintering group showed higher biaxial flexural strength (943.87±48.69 MPa). The highest surface roughness values were obtained in short sintering groups and within the groups Nd-YAG application was found the most effective method (4.11±0.28 Ra). The highest bond strength value was obtained in sandblasted short time sintered group (29.71±2.52 MPa). The translucency and contrast ratio showed no significant difference.

**Conclusions:** Although a physically stronger zirconia is obtained by short sintering process, long-term sintered zirconia forms a more durable bond strength with ceramics. Sandblasting improve the ceramic-zirconia bond strength may have more benefits than the use of Er-YAG, and Nd-YAG lasers.

**Keywords:** Shear bond strength, sintering, surface treatment, zirconia.

 \*Mehmet Emre COŞKUN<sup>1</sup>  
 Fehim ÇELENK<sup>2</sup>

ORCID IDs of the authors:  
M.E.C. 0000-0002-2430-5170  
F.Ç. 0000-0002-7484-2855

<sup>1</sup> Department of Prosthodontics, Faculty of Dentistry, Cumhuriyet University, Sivas, Turkey

<sup>2</sup> Division of Prosthodontics, Alanya Public Health Center, Antalya, Turkey.

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## **INTRODUCTION**

Despite the long-term success of metal-ceramic restorations, high aesthetic expectations of patients have increased the demand for full-ceramic restorations. Zirconia, among the full-ceramic restoration materials, has gained the popularity in prosthetic rehabilitation, due to the superior mechanical properties; high flexural strength (700-1200 MPa) and fracture toughness (7-10 MPa m<sup>1/2</sup>).<sup>1-4</sup> However, the main problem of using zirconia is optical, since it is not as translucent as natural tooth structure or glass ceramics.<sup>5</sup> Two different proceedings have been carried out to overcome the esthetic problem of zirconia. The first one is using the zirconia as a framework and veneered with feldspathic ceramic. However, using the layered zirconia, it has been stated that chipping problem of the veneering ceramic is 15.2% for the posterior restorations after five-years usage.<sup>6</sup> The latter method is to make a full anatomical monolithic restoration, yet these restorations are aesthetically unsatisfactory when compared to the anterior teeth because of the opacity. Whichever technique is chosen to fabricate a prosthetic restoration that can mimic the natural teeth appearance to achieve better esthetic results, the main problem to be solved is making the zirconia more translucent.

In order to compensate the opacity of zirconia, sintering parameters have become a major interest in dentistry especially after the invention of state of art processing furnace that shorten the sintering cycles. The applied maximum temperature and dwelling time plays a decisive role on grain size, translucency, surface roughness (SR), porosity, and density.<sup>4,7,8</sup>

The grain size of the zirconia that effects the physical and optical properties, increases with the increasing temperature and dwelling time.<sup>9-11</sup> The increased grain size improves the translucency<sup>12,13</sup> however, expansion of the grain size deteriorates the mechanical strength and it was reported that the sintering exceed 1550 °C decreased the flexural strength remarkably.<sup>9</sup> Furthermore, the increasing grain size as a result of higher sintering temperature and dwelling time has a reducing effect in surface roughness (SR). The importance of enhanced SR

has been emphasized in literature for providing mechanical retention to get adequate bond strength between ceramic and zirconia.<sup>14</sup> In literature different surface treatment methods such as sandblasting, acid etching, and laser applications (Er:YAG, Nd:YAG, CO<sub>2</sub>, femtosecond) have been applied to improve the bond strength.<sup>15-24</sup> It's often to perform the Er:YAG laser on cavity preparation and ceramic surface treatments because of its wavelength consistency with water.<sup>17</sup> Furthermore, it is mentioned that the Nd:YAG laser is an effective method in order to improve the resin-ceramic bond strength.<sup>18</sup>

Most of the studies in the literature aim to determine the effect of different sintering parameters on optical and physical properties of the material. However, there is limited study on the effect of altering physical properties on shear bond strength (SBS) between zirconia and ceramic. So, this study aimed to investigate the effect of different sintering parameters on the optical properties in terms of translucency parameter (TP), contrast ratio (CR) and as well as mechanical properties such as fracture resistance, surface roughness (SR), and grain growth of zirconium. Further, the study evaluated the effect of mechanical properties altered by different sintering protocols on SBS between zirconia and ceramic after the application of different surface treatment modalities. The hypothesis was that the high sintering temperature would enhance the mechanical and optical properties but not influence the bond strength.

## **MATERIAL AND METHODS**

Specimens were prepared from partially sintered state zirconia (Optimadent; Upcera, China) using Computer-aided design (CAD) (DWOS; Dental Wings, Canada) and computer-aided manufacturing (CAM) (D40; Yenadent, Turkey). Specimens' surfaces were polished using P600, P800, and P1200 grid silicon carbide paper (English abrasives; Atlas, Turkey) sequentially. All specimens were randomly divided into two groups according to the sintering protocols (n:40). Long sintering (Ls) protocol was performed at 1480 °C for 180 min (MOS 160/1; Protherm, Turkey) and short sintering (Ss) protocol was at 1510 °C for 30

min (inFire HTC Speed; Sirona Dental, USA). Each group was divided into four subgroups (n:10) according to the surface treatments as below:

**Control group:** No surface treatment was applied.

**Sandblasting group:** Samples surfaces were treated with 110 nm Al<sub>2</sub>O<sub>3</sub> (Metoxide; Metoxide frits and chemicals, Germany) at 5 bar pressure for 15 sec from a distance of 10 mm using a sandblaster (Mikrotek; Turkey).

**Nd-YAG group:** Samples were irradiated with a Nd:YAG laser ( $\lambda = 1.064$  nm) (Smarty A10; Deka, Italy). The following settings were used: 150 mJ of energy, 10 Hz frequency, 1.5 W power, and 2.94  $\mu$ s pulse duration for 15 sec. The contact-type laser optical fiber was aligned perpendicular to the sample, maintaining contact between the fiber and specimens.

**Er-YAG group:** Surface irradiation were performed by using Er:YAG laser ( $\lambda = 2.940$  nm) (Smart 2940D Plus; Deka, Italy) in pulsed mode 2.94  $\mu$ s for 15 sec with 1.5 W power, 10 Hz frequency, 150 mJ energy, and under water cooling (5 ml/min). The optical hand piece was held perpendicular to the surface at a distance of 10 mm.

#### ***Biaxial flexural strength tests***

Piston-on-three ball technique was used in order to investigate the biaxial flexural strength. Three stainless steel balls with 3.2 mm diameter were placed in a 10 mm diameter circle at an angle of 120 degree on the surface and twenty specimens (diameter 15, thickness 1.3 mm) from each sintering group were placed onto these balls (n:10). Tests were performed with a universal testing machine (Lloyd LF Plus; Ametek, UK) with a 0.5 mm/min crosshead speed.

#### ***Characterization***

The characterization of the zirconia specimens was performed using different techniques. One specimen (diameter 15, thickness 1.3 mm) from each sintering group was analyzed by x-ray diffraction (XRD) (AXS D8 Advance; Bruker, USA) to identify the crystalline phase with monochromatic CuK $\alpha$ . Then, the analyses were evaluated using a software program (Jade 6.0; Materials Data Inc). In addition, one specimen from each surface treatment group was analyzed

using scanning electron microscopy (SEM) (LEO 440; Computer Controlled Digital, UK) for detailed characterization. Photomicrographs of every surface treated specimens were obtained at 50x, 2500x, 10000x magnification.

#### ***Surface roughness tests***

After surface treatments, all specimens from all groups with the dimension (diameter 7 mm, thickness 3 mm) were cleaned ultrasonically with isopropanol for 10 min and then air dried (n:80). Roughened surfaces were measured with profilometer (Surftest SJ-301; Mitutoyo, Japan) 6 times and the average roughness values (Ra,  $\mu$ m) of the specimens were determined.

#### ***SBS tests***

Zirconia specimens (diameter 7 mm, thickness 3 mm) were placed into the holes compatible with the specimens' dimension on the custom-made metal mold base part, and ceramic (Cerabien ZR; Kuraray Noritake Dental Inc, Japan) veneering application was performed onto zirconia specimens through the second layer of the mold having cylindrical holes (diameter 5, thickness 3 mm). Then, they were fired according to the manufacturer's instructions. All specimens were immersed in distilled water at 37 °C for 24 h and SBS tests were performed with a universal testing machine (Lloyd LF Plus; Ametek Company, UK) with a 1 mm/min crosshead speed. The fractured zirconia surfaces were analyzed by stereomicroscope (Stemi DV4; Carl Zeiss, Germany). The failure mode of the specimens was categorized as adhesive, cohesive, and mix.

#### ***Optical measurements***

For optical measurements 10 specimens (diameter 10 mm, thickness 1 mm) were fabricated for each sintering group (n:10). Measurements were performed with a spectrophotometer (Easyshade advance; Vita Zahnfabrik, Germany) according to the CIELab\* scale under the D65 light. Black background (CIE L\* = 1.1 a\* = 13.8 b\* = 52.2) and white background (CIE L\* = 17.6 a\* = 2.0 b\* = 6.6) were used in measurements. The measurements were performed three times for each sample. TP was calculated according to the formula  $TP = [(L_B - L_W)^2 + (a_B - a_W)^2 + (b_B - b_W)^2]^{1/2}$ , and the CR was calculated as  $Y_B / Y_W$ , where Y is  $[(L+16) / 116]^3 \times$

100, in both formulas B and W subscripts represent black and white backgrounds, respectively.<sup>23</sup>

### Statistical analysis

Statistical analysis was performed with SPSS 22.0 software (SPSS; SPSS Inc, USA). Shapiro-Wilks test was used to assess the homogeneity of variance and the data showed normal distribution. Two-way

ANOVA test followed by the Bonferroni Post hoc comparisons were performed with a significant difference ( $p < .05$ ).

### RESULTS

The identified crystalline phase of the zirconia specimens which were sintered by using different protocols was depicted in Figure 1.

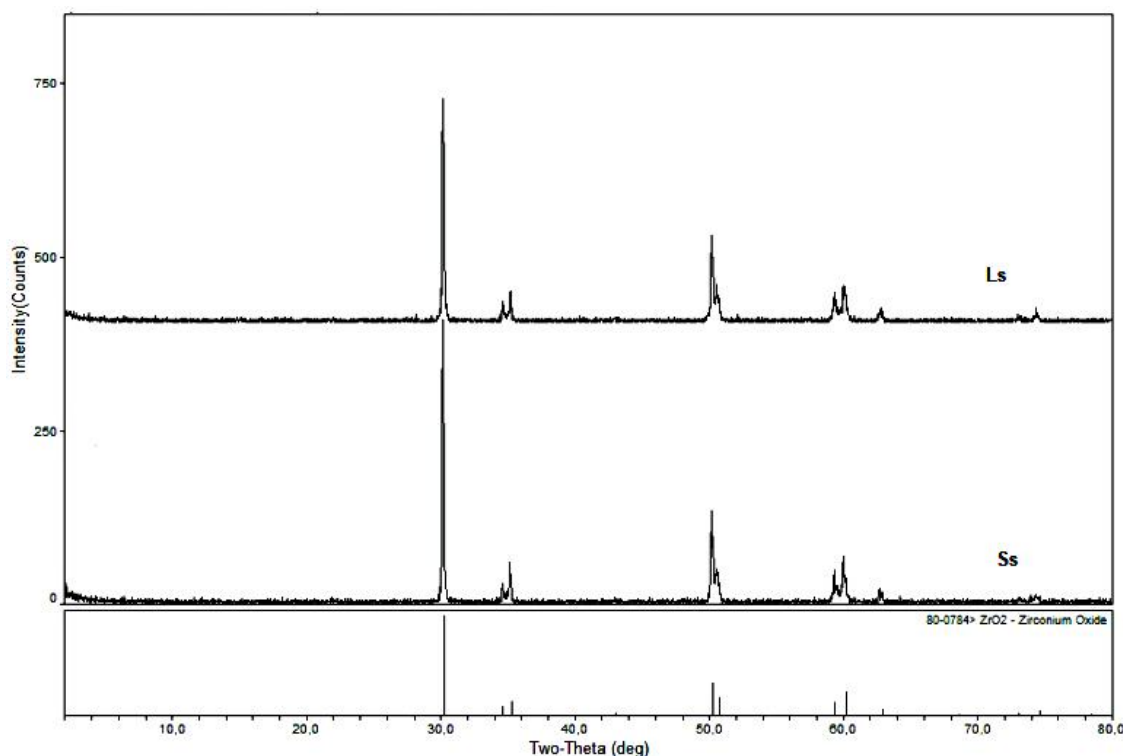


Figure 1. XRD analysis of the zirconia specimens

According to the XRD analyzes, the peak values for the spectra of both long and short sintered samples overlapped with 80-0784 ICSD tetragonal zirconia codes and besides short sintering group has larger grain size (1116 Å, 111 nm) than Ls group (555 Å, 55 nm)

The mean BFS of Ss group ( $943.872 \pm 48.69$  MPa) was higher than the Ls group ( $779.651 \pm 52.34$  MPa) and the difference between each group was statistically significant ( $p < .05$ ) (Table 2).

Table 2. Mean values and standard deviations of TP, CR and BFS

|    | TP & Sd           | CR & Sd            | BFS & Sd (MPa)       |
|----|-------------------|--------------------|----------------------|
| Ss | $2.63 \pm 0.67^a$ | $0.93 \pm 0.016^b$ | $943.87 \pm 48.69^x$ |
| Ls | $2.85 \pm 0.42^a$ | $0.92 \pm 0.015^b$ | $779.65 \pm 52.34^y$ |

<sup>a</sup>TP, translucency parameter; CR, contrast ratio; BFS, biaxial fracture strength. Means with same superscripts letters are not significantly different ( $p > .05$ ).

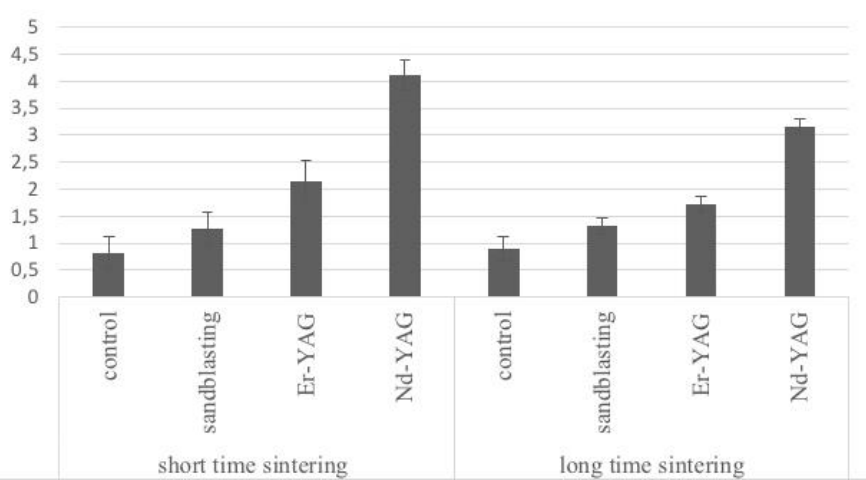
Regarding the SR, all surface treatments increased the Ra values. The highest mean surface roughness was determined in Ss Nd:YAG group

( $4.11 \pm 0.28$  Ra) and the lowest was measured Ss control group ( $0.83 \pm 0.29$  Ra) (Table 1, Fig.2.).

**Table 1.** Surface roughness (Ra,µm), shear bond strength (MPa), biaxial flexural strength (MPa), standard deviations, and failure modes

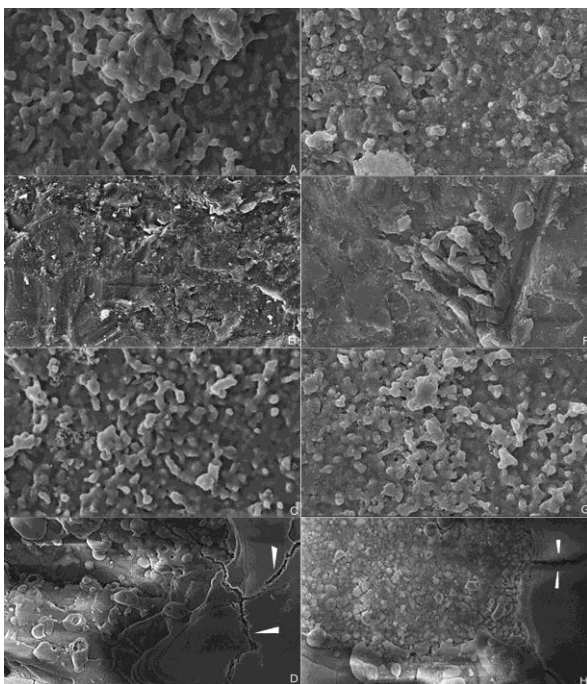
|           | Surface Treatment   | SR & Sd                | SBS & Sd (MPa)                | Mode of Failure |          |     |
|-----------|---------------------|------------------------|-------------------------------|-----------------|----------|-----|
|           |                     |                        |                               | adhesive        | cohesive | mix |
| <b>Ss</b> | <b>control</b>      | 0.83±0.29 <sup>x</sup> | 20.82±1.40 <sup>a,c</sup>     | 8               | 0        | 2   |
|           | <b>sandblasting</b> | 1.27±0.30 <sup>y</sup> | 22.30±1.40 <sup>a,b</sup>     | 5               | 2        | 3   |
|           | <b>Er-YAG</b>       | 2.15±0.38              | 24.44±2.20 <sup>b,d,e,f</sup> | 4               | 2        | 4   |
|           | <b>Nd-YAG</b>       | 4.11±0.28              | 26.54±1.88 <sup>d,g,h,i</sup> | 1               | 3        | 6   |
| <b>Ls</b> | <b>control</b>      | 0.90±0.23 <sup>x</sup> | 24.23±1.58 <sup>c,e,g,l</sup> | 8               | 1        | 1   |
|           | <b>sandblasting</b> | 1.33±0.14 <sup>y</sup> | 29.71±2.52 <sup>k</sup>       | 3               | 4        | 3   |
|           | <b>Er-YAG</b>       | 1.73±0.15              | 26.85±1.78 <sup>f,h,l</sup>   | 3               | 4        | 3   |
|           | <b>Nd-YAG</b>       | 3.17±0.14              | 27.92±2.04 <sup>i,k</sup>     | 2               | 4        | 4   |

\*Ss, short sintering; Ls, long sintering; SR, surface roughness; Sd, standard deviation; SBS, shear bond strength. Means with same superscripts letters are not significantly different ( $p>.05$ )

**Figure 2.** The mean and standard deviation of surface roughness (Ra)

The difference of surface roughness between all groups showed statistically significant. Only the differences between sandblasting groups and

control groups showed no statistically significant difference. The SEM images of all zirconia samples showed in Figure 3.

**Figure 3.** SEM images of zirconia specimens after different surface roughness and control groups: (A) Ls control, (B) Ls sandblasting, (C) Ls Er-YAG, (D) Ls Nd-YAG, (E) Ss control, (F) Ss sandblasting (10.00 K X); (G) Ss Er-YAG, (H) Ss Nd-YAG (2.50 K X). White lines in D and H figures show the crack regions on zirconia.

Concerning the shear bond strength, Ls groups showed higher bond strength than Ss groups (Table 1, Fig.4). The highest bond strength value was obtained in Ls sandblasting group ( $29.71 \pm 2.52$ ) however the difference between Nd:YAG and

sandblasting group was not statistically significant. In short sintering group, the highest bond strength value was obtained in Nd:YAG group, but no statistically significant difference was detected between Nd:YAG and Er:YAG group.

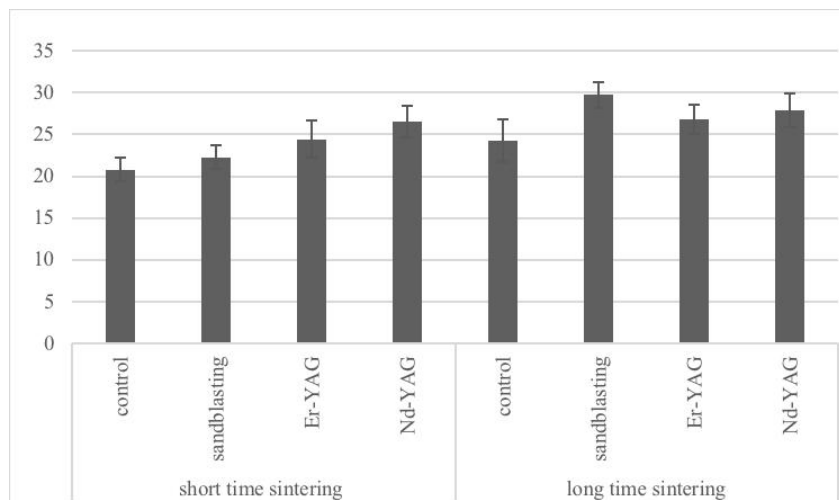


Figure 4. The mean and standard deviation of shear bond strength (MPa)

The TP and CR mean values were depicted in Table 2. Regardless of the sintering type, TP and CR values did not show statistically significant difference.

## DISCUSSION

This study aimed to reveal the effects of different sintering processes on overall properties of zirconia. The sintering process is applied in order to get the desired microstructural properties of the zirconia such as particle size and pore distribution. In this study, the sintering parameters applied in the first group (Ls group) were designed in accordance with the manufacturer's instructions, while the second group (Ss) was planned as a shorter time application at higher temperature. The results obtained from this study showed that the increasing sintering temperature with a short application time enhance the mechanical properties while deteriorating the bond strength between ceramic and zirconia. The optical properties did not show any alterations due to the changing sintering conditions. Therefore, the tested hypothesis was partially rejected.

Grain size of the zirconia which is characterized by the sintering temperature and dwelling time, is the major determinant on optical and mechanical properties of the material<sup>13</sup>. The increasing temperature leads to the growth of the grains.<sup>12</sup> In

the present study, two sintering protocols with different temperatures and dwelling times were tested on zirconia. In terms of grain size, higher molecular expansion was detected in Ss group, in which higher temperature and shorter dwelling time was applied. According to the results of this study, it was determined that the increasing dwelling time was not as effective as the temperature increase on the growth of grain size, and it may be summarized that the temperature was the main actor for characterization. Furthermore, Ebeid *et al.*<sup>26</sup> in his research mentioned that the granular expansion of zirconia occurred only after the 4 hours sintering at the same temperature. However, no dwelling time at that duration was applied in this research.

Concerning the optical properties of zirconia, Sen *et al.*<sup>8</sup> reported that the higher temperature increased the grain size which made TP higher. However, in the current study, no statistical difference regarding the TP and CR was found between sintering groups, while the grain size was different. Such a result may be attributed to the fact that the change in grain size was not sufficient to make a difference with regard to TP and CR.

Regarding the BFS, high temperature applied zirconia specimens (Ss group) showed a significant increase. The reason for the increased flexural

strength was the increase of grain size, which means reduction of the pores in zirconia's structure. Although this result is in agreement with Aydogdu, *et al.*<sup>10</sup> that aimed to reveal the effect of different sintering temperatures and dwelling times on biaxial flexural strength of zirconia, and mentioned that the temperature increase with a decreasing time enhanced the strength of the zirconia. However, in another research Ebeid, *et al.*<sup>26</sup> mentioned that the changing sintering parameters (time/temperature) did not alter the mechanical properties of zirconia. Furthermore, another study conducted by Stawarczyk, *et al.*<sup>9</sup> emphasized that the temperature increase up to 1550 had favorable effect on mechanical properties of zirconia, besides the exceed of this temperature decreased the fractural strength. So, there is an inconsistency among the studies however, it is obvious that the higher sintering temperature leads to the expansion of grain size. For this reason, to obtain a higher strength with the increasing temperature seems logical.

The changes in the surface texture of the zirconia after sintering process are clearly indicated in many researches<sup>26,27</sup> in the literature. In sintering process, the initial heating is occurred from the surface than in time, varies depending on the thickness, penetrate to the inner portion of the material and increase the grain size whereby the pores between grains decreased. In the case of applying the same temperature, the factor that determines the surface topography is the dwelling time, and vice versa. However, in this study both time and temperature were different and within the limitations of this study no significant difference was obtained because of the small temperature difference between the groups.

It is injudicious not to expect that all the changes of physical properties of zirconia have no effect on zirconia-ceramic bond strength. The final physical properties of zirconia effect the effectiveness of the surface treatments. Each type of laser, a form of energy, was found to be proper in order to get a high surface roughness as Er-YAG and Nd-YAG roughen the surface higher than that of the sandblasting and control group in both sintering groups (Table 2). Sandblasting, the

mechanical surface treatment method, was not be able to form a rough surface ( $R_a$ ,  $\mu\text{m}$ ) like laser treatments. Although in literature it was shown that the laser applications improve the surface roughness<sup>15,18</sup>, there is no consensus on the comparison of the effect of different surface treatments on ceramic-zirconia bond strength. Several studies<sup>19,20</sup> in the literature indicated that the sandblasting was more effective in order to increase SBS between ceramic and zirconia however, Korkmaz, *et al.*<sup>21</sup> emphasized that the sandblasting had no effect on bond strength, besides Kirmali, *et al.*<sup>22</sup> obtained the mean in vitro SBS of veneering ceramic on sandblasted zirconia only higher than the control group. In all studies only one sintering protocol was used which determine the final physical properties of the material and the difference of this research from the mentioned studies is the testing the different surface treatment on differently sintered zirconia.

In the current study, both type of laser application found to be the appropriate method in order to change the surface topography despite the diversity of physical properties of the zirconia determined by sintering. However, the results of this study indicated that, the same surface treatment application had different effect on Ss and Ls groups. The difference in hardness of the zirconia samples after the sintering process had an effect on the effectiveness of the surface treatments applied on them. In the case of the samples having a harder structure (Ss group), the laser efficiency was found to be higher and the mechanical method, sandblasting, was made a little difference which was not significant between Ls and Ss groups. Besides, the Nd:YAG laser will be more useful than Er:YAG laser for surface roughening of zirconia. It should be pointed out that the Nd:YAG ablation caused destructive effect on the zirconia surface due to the high temperature gradient which caused phase transformation (t $\rightarrow$ m).<sup>17</sup> High temperature increases during the Nd:YAG application melted the zirconia surface and then cracks (in Fig. 2 G and H between white lines) were formed after sudden cooling that deteriorated the bond strength of ceramic-zirconia. In literature some studies mentioned the formation of microcracks on zirconia after Nd:YAG

treatment<sup>17,18</sup> which is inconsistent with the results of this study. Another issue that needs to be mentioned is the formation of black carbonation zones on the surface of zirconia as a result of Nd:YAG application and this discoloration negatively affect the esthetic requirements.

Regarding the shear bond strength, the results of the current study showed that the bond strength was not directly proportional to the surface roughness and the shear bond values of all Ls groups were higher than the same treated Ss groups. In literature, several studies determined that the surface treatments like sandblasting<sup>23,24</sup> and laser applications<sup>18</sup> caused crystallographic transformation (t→m). The thermal expansion coefficient (TEC) of monolithic zirconia is  $7.5 \times 10^{-60}$ , tetragonal zirconia is  $10.8 \times 10^{-60}$  and that of the ceramic is  $9.5 \times 10^{-60}$ <sup>19</sup>. The increase of the TEC difference between ceramic and zirconia decreased the SBS which shorten the life span of the zirconia restoration and that means the bond strength between ceramic and tetragonal zirconia is higher than the bond strength between ceramic and monolithic zirconia. Applying the higher temperature in sintering process increases the flexural strength and makes the zirconia harder. However, according to the recent study, easier phase transformation occurs when the zirconia becomes harder and deteriorates the bond strength of zirconia-ceramic. Furthermore, the crystallographic transformation as a result of laser applications on zirconia was higher than that of sandblasting.

The assessment of SBS can not be completed without the evaluation of failure mode. The mode of failure gives crucial and precise information about bonding effectiveness. Lower bond strength represented by adhesive failure and it is not preferred. Kim, *et al.*<sup>20</sup> and Fischer, *et al.*<sup>23</sup> declared the ineffectiveness of sandblasting on bond strength between ceramic and zirconia, and mentioned mix and cohesive failure modes on control groups. Furthermore, they emphasized that sandblasting caused the phase transformation that decreased the bond strength. On the contrary, in the light of the results of the current study, without any

surface treatments the SBS value was the lowest and the failure mode was adhesive.

## CONCLUSIONS

Initially, pre-sintered zirconia produced in the same process with the same content has the same properties, but after the application of different sintering processes, a material having different properties is obtained. Within the limitations of this laboratory research such as the difficulty of applying ceramic onto zirconia at the same diameter; it was concluded that;

The applied higher sintering temperature increased the strength of zirconia. Laser; form of energy applications caused phase transformation which deteriorate the shear bond strength between zirconia and ceramic. Sandblasting; mechanical surface treatment is the appropriate method in order to increase zirconia-ceramic bond strength.

## ACKNOWLEDGEMENTS

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## CONFLICT OF INTEREST STATEMENT

The authors report no conflict of interest.

### *Zirkonyanın Sinterleme Parametrelerine Bağlı Oluşan Yapısal Değişimleri ve Farklı Yüzey İşlemleri Sonrasında Bağlantı Üzerine Etkileri*

**Amaç:** Zirkonya farklı sinterleme parametreleri sonucunda farklı fiziksel ve optik özellikler kazanır. Bu çalışmanın amacı, farklı sinterleme protokollerinin zirkonyada oluşturduğu fiziksel değişiklikleri ve uygulanan farklı yüzey işlemlerinin bağlantı üzerine etkilerini araştırmaktır. **Gereç ve Yöntemler:** Seksen translusent zirkonya (7x3 mm) farklı sinterleme protokollerine göre (1480 °C' de 180 dk; 1510 °C' de 30 dk) 2 farklı gruba ayrıldı ve sonrasında uygulanan yüzey işlemlerine göre (kontrol, kumlama, Er-YAG ve Nd-YAG) 4 farklı alt gruba ayrıldı. Her gruptan birer örnek XRD incelemesine tabii tutulurken, her alt gruptan birer örneğin SEM analizi yapıldı. Bağlantı dayanım testi için zirkonya yüzeylerine seramik (5x3 mm) pişirildi ve üniversal test cihazında testler gerçekleştirildi, kırılma şekilleri steriomikroskop kullanılarak belirlendi. Biaxial bükülme direnci üniversal test cihazında 15x1,3 mm boyutlarındaki örneklerde gerçekleştirildi. Elde edilen veriler iki yönlü ANOVA ve Bonferroni Post hoc

testleri kullanılarak değerlendirildi. ( $p < .05$ ) **Bulgular:** En yüksek bükülme direnci kısa süreli sinterleme grubunda tespit edilmiştir ( $943,87 \pm 48,69$  MPa). Yüzey pürüzlülüğü bakımından en yüksek değerler kısa süreli sinterleme gruplarında bulunurken, gruplar içerisinde Nd-YAG uygulaması en etkili yöntem olarak bulunmuştur ( $4,11 \pm 0,28$  Ra). Translülentlik ve kontrast oranları arasında önemli bir farklılık bulunmamıştır. **Sonuç:** Kısa süreli sinterleme işlemiyle fiziksel olarak daha kuvvetli bir zirkonya elde ediliyor olsa da uzun süreli sinterleme işlemiyle daha kararlı seramik bağlantısı elde edilmektedir. Kumlama işlemi seramik-zirkonya bağlantısını artırmada Er-YAG ve Nd-YAG uygulamalarına nazaran daha etkilidir. **Anahtar Kelimeler:** Zirkonya, sinterleme, makaslama bağlantı dayanımı, yüzey işlemi.

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## PROLIFERATION OF MESENCHYMAL STEM CELLS IN CARBONATE APATITE-CHITOSAN SCAFFOLDS IN BONE TISSUE ENGINEERING TECHNIQUES

### ABSTRACT



**Objectives:** Chitosan is a popular bone graft material. However, chitosan also has a weakness for cell adhesion and the lack of sufficient bone formation capabilities. To improve it, we tried to develop a chitosan scaffolds that combine with carbonate apatite (CA), which has excellent biocompatibility properties of the tissues of the human body. We tried to find the most appropriate amount of Carbon Apatite (CA) to be combined with Chitosan scaffold (ChSs) to produce a good scaffolds structure, as well as to evaluate CA-ChSs from the standpoint of cell proliferation using mesenchymal stem cells (MSCs).

**Materials and Methods:** Porous chitosan matrix was made by using the lyophilization technique. ChSs containing chitosan powder was made by the following procedure. 100, 150, 200, and 400 mg of chitosan powder was dissolved in 5 ml of 2% acetic acid at room temperature. ChSs containing 200 mg of chitosan powder was chosen to make CA-ChSs. Ultraviolet radiation was then performed for 2 hours. CA-ChSs structure was observed by a scanning electron microscope (SEM). Proliferation of MSCs in CA-ChSs evaluated at days 1, 3, 5 and 7.

**Results:** This study demonstrated that CA-ChSs containing 200 mg of chitosan powder and 50 mg CA has a three-dimensional structure that is porous and attachment powder CA in the pores and absorbance values were increased from the examination day 1 until day 7. Cell proliferation using MSCs in CA-ChSs are better, as the absorbance value of CA-Chss with 50 mg CA content was significantly higher than ChSs. These findings also confirm that MSCs has good viability and biocompatibility in bone tissue engineering techniques.

**Conclusions:** Based on these results, it is expected CA-ChSs may be candidates for bone graft material in tissue engineering techniques.

**Keywords:** Chitosan, carbonic apatite, mesenchymal stem cell, scaffold.

 \*Aqsa Sjuhada OKI<sup>1</sup>  
 Maretaningtias Dwi ARIANI<sup>2</sup>

ORCID IDs of the authors:  
A.S.O. 0000-0003-4427-2561  
M.D.A. 0000-0002-7473-2015

<sup>1</sup> Department of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga, Indonesia.

<sup>2</sup> Department of Prosthodontics, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia

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## **INTRODUCTION**

The need for bone repair has increased with increasing life expectancy. Bone defects caused by tooth extraction, trauma and other pathological conditions have limitations in spontaneous bone repair. In this regard, strategies for improving bone defects include autografts, allografts and xenografts. Autografts have become the ideal choice to fill the bone defects.<sup>1</sup> However, this method has several significant disadvantages that may cause complications in the healing process, require additional surgical measures, cause pain to donors and limited supply of bone donors. On the other hand, allografts and xenografts have risks to immunogenic reactions and disease transmission from liquids and donor tissues.<sup>2</sup> Given these weaknesses, it encourages increased use of alternative bone graft materials to develop the ideal bone graft material in tissue engineering techniques.<sup>3</sup>

In tissue engineering techniques, there are three important pillars, namely cells, which play a role in the regeneration of new tissues; scaffolds, providing matrices for cells; and signaling molecules, which can enhance attachment, cell proliferation and differentiation. For the success of tissue engineering techniques, it is important to optimize the three pillars.<sup>4</sup> Platelet-rich plasma have been developed as grafting material in bone regeneration, it resulted excellent primary stability and radiographic integration.<sup>5</sup>

Scaffolds play an important role in tissue engineering techniques to support bone regeneration. Currently, several natural and synthetic materials have been used to develop three-dimensional scaffolds that play an important role as artificial extracellular matrix, supporting cell proliferation and differentiation as well as maintaining its function. Particular attention has been given to scaffolds polymers for in vitro research on cell-scaffolds interactions and in vivo studies of bone graft materials.<sup>6</sup>

Chitosan is an interesting topic in the development of bone graft material because it has the desired characteristics with regard to its potential as a cell-scaffolds. Chitosan is an amino polysaccharide (poly-1,4-D-glucosamine), which

is a deacetylation product of chitin. Chitosan is considered a good candidate for bone graft material because it has high biocompatibility and biodegradability, non-toxic and has good adsorption properties. Chitosan may increase proliferation and differentiation in osteoblast cell cultures.<sup>7</sup> Chitosan has other useful characteristics, including bacteriostatic, hemostatic, anti-cholesterol and as enzymatic degradation. The properties of chitosan matrix, such as microstructure, crystallinity and mechanical strength, can be varied by changing the concentration, molecular weight and degrees of chitosan deacetylation. Furthermore, chitosan can be made into various forms and has been widely applied in the biomedical field.<sup>8</sup>

To make suitable scaffolds a bone graft material, current efforts are focused on improving mechanical strength and biological properties through the incorporation of bioceramic materials, such as carbonate apatite (CA). CA and other calcium phosphate (CaP) are important for the repair of bone tissue because they have similar properties to minerals in bone.<sup>9</sup> As already well known that the main content of bone inorganic material is CA which contains about 7% carbonate of total weight. CA is easily soluble in acidic conditions and has thermodynamic properties in neutral conditions. Therefore, CA is expected to be a substitute for ideal bone material, which has both osseointegrative and bioresorbable properties.<sup>10</sup>

The specific properties of chitosan and CA can be combined to create a carbonate apatite-chitosan scaffolds (CA-ChSs) as a new bone graft material with unique structural and mechanical properties. CA-Chss can act as bioactive materials that can affect cell proliferation and differentiation. In addition to scaffolds that play an important role in bone tissue engineering techniques, the selection and use of cells also affects the success of tissue engineering techniques. A previous study has found that CA-ChSs is biocompatible and supports mouse osteoblast-like cell proliferation (MC3T3-E1).<sup>11</sup> However, there has never been a mesenchymal stem cell culture (MSCs) in CA-ChSs.

Mesenchymal stem cells have been proven to be the most promising craniomaxillofacial application for a therapeutic support in bone engineering, as compared to the use of conventional autologous and allogenic bone grafts.<sup>12</sup>

Stem cells are recommended as a treatment option to repair tissue damage, including bone defects. In this case, the MSCs present in the bone marrow receive special attention because of their differentiated ability to be bone, nerve, carotid and muscle tissue.<sup>13</sup>

Therefore, it is necessary to conduct a study to determine the interaction of MSCs in CA-ChSs and to evaluate the proliferation and differentiation of MSCs in scaffolds.

## MATERIALS AND METHODS

This study was cleared by the faculty's Ethical Clearance Commission. The porous chitosan matrix was prepared using lyophilization technique. ChSs containing 100, 150, 200, and 400 mg of chitosan powder were prepared by the following procedure. One hundred, 150, 200, and 400 mg of chitosan powder (98.7% deacetylation grade, YSK, Japan) were dissolved in 5 ml of 2% acetic acid at room temperature, shaken for 15 min, then neutralized with 15 ml 0.1 M NaOH solution. After centrifugation at 1500 rpm for 10 minutes, the excess water is removed, then the chitosan gel is packed into the mold (diameter: 5 mm, height: 2 mm). Molds were frozen at -80°C for 2 hours and dried in a dry-freeze machine at -54°C for 24 hours. To make CA-ChSs, ChSs were selected containing 200 mg of chitosan powder. After neutralization, 50, 100, 200 and 300 mg of 0.06 M CA were added to the chitosan gel containing 200 mg of chitosan powder.

When the process of making CA-ChSs, acetic acid solution was used to dissolve chitosan powder and then neutralized with NaOH solution. To remove alkaline salts, ions or some toxic substances in CA-ChSs, desalinations were carried on. Desalination was performed by the following procedure, 25 cm plastic cellulose was prepared and 100 ml of distilled water was added. ChSs and CA-ChSs are inserted into cellulose plastic, then placed under tap water for 24 hours and frozen at -

80°C for 2 hours. The scaffolding was moved to a freeze dried machine at -54°C for 24 hours. Furthermore, ultraviolet radiation in ChSs and CA-ChSs for 2 hours. The sample is ready to be tested in cell culture.

Scaffolds are randomly selected and macroscopic photos of scaffolds are taken using a digital camera. Microscopic structures and porosity scaffolds were observed using SEM (3D Microscope VE-8800, Keyence, Japan).

Prior to the testing, chitosan and powdered CA samples were mixed with potassium bromide (KBr) then were grinded using mortar to remove the scattering effect of large crystals. The delicate and uniform samples are mechanically compressed to form a translucent pellets that can be passed through the infrared spectrum. The FTIR test produces a transmission graph (%) of the wave number ( $\text{cm}^{-1}$ ).

The compressive strength test is performed by using a sloped CA-ChSs sample which is topped and the bottom is smoothed with sandpaper, the sample side is measured using a sliding range. The sample is placed on the press machine compression unit, then the engine is turned on and set the speed and style to be measured. Load cell slowly lowered then stopped and recorded the amount of force obtained.

Culture MSCs (JCRB Cell Bank, Japan) in the form of cell-line grown in dish. After confluent, the culture was harvested using trypsin versene solution. The yields were replanted in a DMEM medium containing 10% bovine serum albumin, 2% penicillin / streptomycin, incubated in 5%  $\text{CO}_2$  at 37°C. Media is replaced every 3-4 days and done sub culture every 7 days. Then the cell is moved in a small bottle and made with a density of  $2 \times 10^4$  cells/ml, the cell is ready to be used for sample testing.

This test is carried out according to the recommended protocol standard for the MTT assay. Cell proliferation in scaffolds is examined using an MTT assay. 20  $\mu\text{l}$  cell suspension with a density of  $2 \times 10^4$  cells was added to ChSs and CA-ChSs placed in a 24-well tissue culture plate. After 2 hours, 980  $\mu\text{l}$  DMEM was added to each well. Then the cells were incubated in the incubator ( $\text{CO}_2$

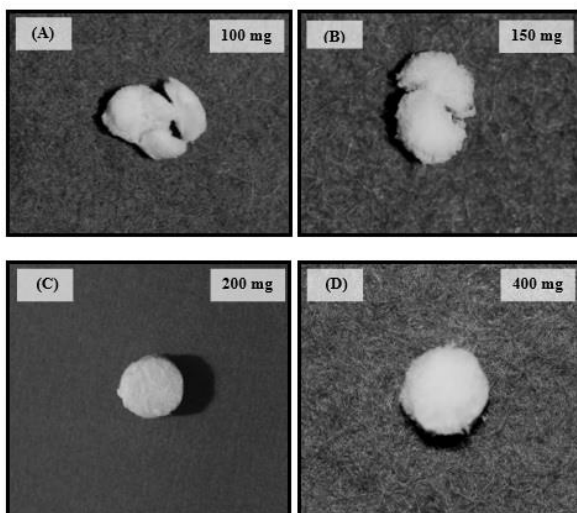
5% at 37°C). Media culture is replaced every 3 days.

On observations of days 1, 3, 5 and 7, ChSs and CA-ChSs were washed using PBS, incubated for 2 h in 500 µl of medium culture containing 50 µl of MTT reagent, the well was introduced into the incubator (CO<sub>2</sub> 5% at 37°C). The media supernatant (110 µl) was transferred to a 96-well culture plate and the absorbance was measured using a microplate reader with a wavelength of 450 nm. The results obtained are expressed in optical density (absorbent). Large absorbents of each well indicate the amount of cell proliferation in a media culture.

Statistical analysis was performed using SPSS. The result of the measurement is tabulated according to each group, then tested one way Anova statistic with 5% significance level. If there is a meaningful difference then proceed with LSD test. This study was conducted in accordance to ethical clearance, which was declared by the faculty ethical committee.

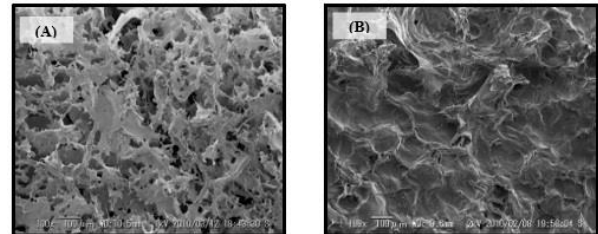
## RESULTS

The macroscopic photos of ChSs are shown in Figure 1. ChSs containing 100 and 150 mg of chitosan powder have fragile and fragile properties (Figs 1A and 1B). In contrast, ChSs containing 200 and 400 mg of chitosan powder have good form and handling properties (Figs 1C and 1D).



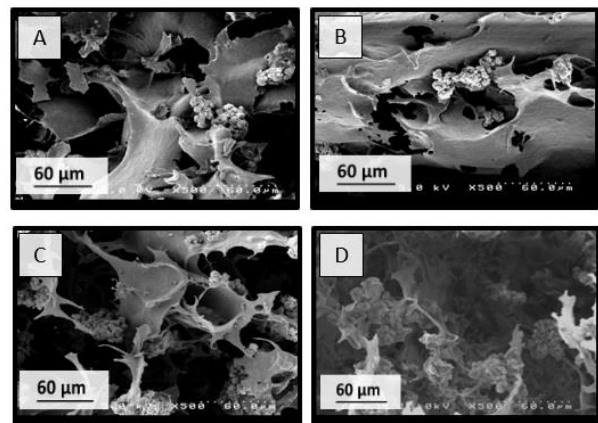
**Figure 1.** Macroscopic photos of ChSs with different amounts of chitosan powder (A) 100 mg; (B) 150 mg; (C) 200 mg and (D) 400 mg.

The results of the test using SEM (Fig. 2A), showed that ChSs with 200 mg of chitosan powder had a three-dimensional structure with many pores. On the other hand, ChSs with 400 mg of chitosan powder have a three-dimensional structure with little pores (Fig. 2B). These results indicate that ChSs with 200 mg chitosan powder is a scaffold that has good handling.



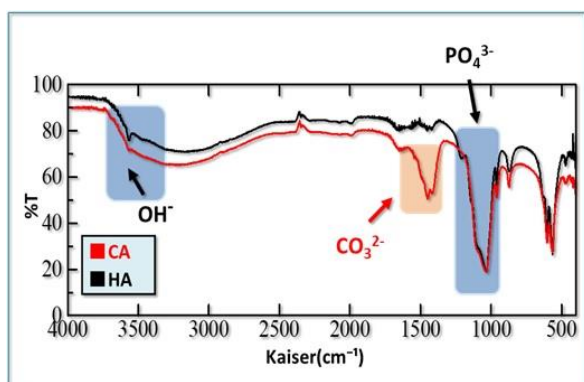
**Figure 2.** Examination of SEM ChSs with content (A) 200 mg and (B) 400 mg chitosan powder.

Figure 3 shows that CA-ChSs made with different amounts of CA powder have a three-dimensional structure with many pores. The pore size ranges from 50-200 µm. In general, CA-ChSs with 50 mg of CA has a three-dimensional structure with the most CA bonds in its pores (Fig 3A).



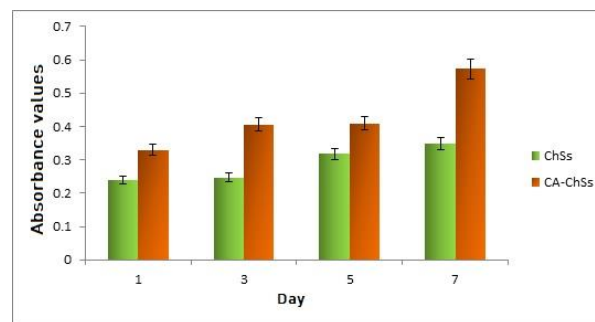
**Figure 3.** SEM CA-ChSs examinations with different amount of CA powder (A) 50 mg, (B) 100 mg, (C) 200 mg and (D) 300 mg.

The FT-IR CA spectrum shows the peak of transmittance for phosphate groups at 1000-1100 cm<sup>-1</sup> and 500-600 cm<sup>-1</sup>, carbonate groups at 1400-1500 cm<sup>-1</sup>, and hydroxyl groups at 3500-3600 cm<sup>-1</sup>. On the other hand, the HA spectrum does not have a peak on the carbonate group, while the peak of the phosphate and hydroxyl groups can be observed (Fig 4).



**Figure 4.** FT-IR spectrum. CA (red) and HA (black) synthesized in this study.

The absorbance value of CA-Chss with 50 mg CA content was significantly higher than ChSs (Fig 5).



**Figure 5.** Proliferation test results of ChSs dan CA-ChSs.

Cell proliferation test results in ChSs and CA-ChSs are shown in Table 1.

**Table 1.** The absorbance value of ChSs and CA-ChSs ( $p < 0.05$ ).

| Sampel  | Day 1        | Day 3        | Day 5        | Day 7        |
|---------|--------------|--------------|--------------|--------------|
| ChSs    | 0.239 ± 0.02 | 0.247 ± 0.06 | 0.318 ± 0.05 | 0.348 ± 0.09 |
| CA-ChSs | 0.330 ± 0.04 | 0.406 ± 0.07 | 0.409 ± 0.08 | 0.572 ± 1.01 |

## DISCUSSION

In bone tissue engineering techniques, scaffolds have an important role in supporting bone regeneration. To date, much effort has been made to develop a scaffold with a three-dimensional structure that provides the necessary support as an extracellular matrix, allowing cells to proliferate and differentiate. A good scaffold should be non-toxic, biodegradable, biocompatible and has high porosity and mechanical properties.<sup>14</sup>

In the present study, we developed a scaffold that combines chitosan with CA. First, an evaluation of the amount of chitosan powder used in the preparation of ChSs to determine the optimal concentration of chitosan powder using SEM examination. Based on SEM, ChSs with 200 mg of chitosan powder have good handling properties and three-dimensional structure with pore size about 50-200  $\mu\text{m}$ . From the results of this examination, it can be concluded that the optimal concentration of chitosan powder that can be used in the manufacture of ChSs is 200 mg.<sup>15</sup>

Current efforts are focused on improving the ability of ChSs in bone formation by combining ChSs with a calcium phosphate substance, such as hydroxyapatite (HA),  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) and carbonate apatite (CA).<sup>7</sup> HA and  $\beta$ -TCP have some disadvantages such as degradation or

dissolution rate that is difficult to predict. HA made by sintering process with high temperature has non-degradable properties. However, it has been reported that the combination of calcium hydroxyapatite (IP / CHA) has systemic pores and almost all pores are interconnected and have good bone-building abilities in in vivo studies. On the other hand, the crystallinity of  $\beta$ -TCP is too low, so in some cases it will be absorbed before it gets enough new bone formation. Among calcium phosphate, it has been reported that CA has an appropriate absorption time and excellent bone-building ability. In this study, selected and combined chitosan which is a biodegradable material and CA to obtain an appropriate period of absorbance and improve bone-building ability.<sup>16</sup>

The properties of a scaffold are also influenced by fabrication techniques. Several techniques have been developed to create scaffolds, including solvent-casting techniques, sol-gel techniques and lyophilization techniques. However, it has been reported that in solvent-casting techniques, the presence of pyrogen salts may affect the cell due to loss of water-soluble biomolecules and non-uniform deformation induction. In sol-gel technique, there are some disadvantages such as difficulty in proper control

of pore structure and hydrolysis and condensation process. In this study, lyophilization techniques were adopted to make scaffolds, since the technique is simpler and easier than any other method described previously and by using lyophilization techniques, the porous nature of CA-ChSs can be obtained from the freeze dried process.<sup>17</sup>

Today, one promising network engineering strategy is to use a scaffold that has a three-dimensional porous structure to facilitate regeneration of bone tissue. As a scaffold for tissue engineering techniques should have a porous structure with pore size ranging from 40-300  $\mu\text{m}$  to allow for migration and cell vascularization and growth factor. In this study, CA-ChSs produced good handling properties and had a three-dimensional porous structure with a pore size of about 50-200  $\mu\text{m}$ .<sup>18</sup>

When deciding to use CA-ChSs in clinical applications, for example in cases of bone augmentation of buccal defects from implant treatment or to fill the bone socket after tooth extraction, the CA-ChSs should be sterilized before use. Since some interventions on oral tissue may lead to bleeding, perilesional edema, and mucosal atrophy, the sterilization process was extremely required to avoid any possible complications during the procedures. It is also expected to improve chronic traumatism in periodontal diseases.<sup>19,20</sup> There are several methods of sterilization that have been in use, including gamma irradiation, steam autoclaving, ethylene oxide and radio frequency plasma glow discharge. Since CA-ChSs is an organic polymer composite, gamma irradiation is a suitable sterilization method for CA-ChSs to maintain its chemical structure.<sup>15</sup>

After the development of a scaffold that has a porous structure, then the selection of the right cells can also affect the success of network engineering techniques. In this study, MSCs cells were cultured on CA-ChSs for cell proliferation measurements, because in the application of bone tissue engineering techniques, stem cells were usually used to confirm their responsibility for bone formation.<sup>13,21</sup>

From cell proliferation test results, indicating that by combining ChSs and CA powders, cell

proliferation capability was higher than in ChSs. It can be said that with the addition of CA powder into ChSs it can increase the surface area of the scaffold so that the cell can attach more. Furthermore, CA-ChSs made from 200 mg of chitosan powder plus 50 mg of CA powder showed cell proliferation ability significantly higher than other groups.<sup>14,22</sup>

Furthermore, a histologic study of CA-ChSs is required to ensure its property in bone regeneration. Based on the results of this study, it can be concluded that CA-ChSs has a porous three-dimensional structure that is interconnected with each other, has good handling properties as well as the ability to support MSCs proliferation so that CA-ChSs is very likely to be used as material for bone tissue engineering.<sup>15</sup>

## **CONCLUSIONS**

From this study it can be concluded that 200 mg of CA is the most effective amount to be combined with ChSS to produce an excellent scaffold on the standpoint of three dimensional structure with little pores. CA-ChSs developed from 200 mg of chitosan plus 50 mg of CA powder demonstrated the highest ability of cell proliferation among the groups.

## **ACKNOWLEDGEMENTS**

None

## **CONFLICT OF INTEREST STATEMENT**

None

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## INFLUENCE OF CAVITY DESIGN ON CALCIUM HYDROXIDE REMOVAL FROM ROOT CANAL IRREGULARITIES

### ABSTRACT

**Objectives:** Conservative endodontic cavity (CEC) design has recently developed to maintain the stability of tooth and provided an alternative to traditional endodontic cavity (TEC) design. The purpose of this study was to assess the influence of cavity design on calcium hydroxide (Ca(OH)<sub>2</sub>) removal from artificial grooves in the coronal and apical parts of root canals.

**Materials and Methods:** Fourty extracted human mandibular premolars with single canals were randomly assigned to CEC or TEC groups (n=20). Following cavity preparation, the root canals were instrumented with ProTaper Universal rotary system up to F3 file and then, each tooth was sectioned longitudinally. Two standardized grooves were prepared in the coronal and apical parts of 1 root half. Ca(OH)<sub>2</sub> was placed into the grooves and the root halves reassembled. After 1 week, each root canal was enlarged with a #40 H-file. Irrigation was performed with the sonic activation of 5 mL 2.5% sodium hypochlorite and 5 mL 17% ethylenediaminetetraacetic acid solutions using the medium size tip (25/04) of EndoActivator System at medium speed for 30 seconds between each 2.5 mL irrigant. The remaining Ca(OH)<sub>2</sub> in the grooves was evaluated using a stereomicroscope with x25 magnification and the images were scored using a 4-scoring scale by 2 examiners. Data were analyzed using the Mann-Whitney U and Wilcoxon tests.

**Results:** Ca(OH)<sub>2</sub> remnants were found in both groups. There was no significant difference between the CEC and TEC groups in terms of Ca(OH)<sub>2</sub> removal efficacy (p>0.05). The grooves in the coronal and apical parts of the roots presented similar amount of Ca(OH)<sub>2</sub> remnants in both groups (p>0.05).

**Conclusions:** Based on the present findings, the cavity design had no effect on the removal of Ca(OH)<sub>2</sub> from root canal irregularities.

**Keywords:** Calcium hydroxide, cavity preparation, endodontics, minimally invasive, root canal.

\*Selen KÜÇÜKKAYA EREN<sup>1</sup>  
Emel UZUNOĞLU ÖZYÜREK<sup>1</sup>

ORCID IDs of the authors:  
S.K.E. 0000-0001-5023-1454  
E.U.Ö. 0000-0001-5032-9996

<sup>1</sup> Department of Endodontics, Faculty of Dentistry, Hacettepe University, Ankara, Turkey.

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## INTRODUCTION

Calcium hydroxide (Ca (OH)<sub>2</sub>) is a commonly used intracanal medicament in patients undergoing root canal treatment with multiple visits for disinfection purposes.<sup>1,2</sup> Ca (OH)<sub>2</sub> has various advantages including its biocompatible, antibacterial, regenerative and therapeutic properties.<sup>3</sup> However, complete removal of Ca (OH)<sub>2</sub> from the root canal system is recommended before permanent root canal obturation, because the remnants may negatively influence the sealer penetration into the dentine tubules.<sup>4,5</sup> Furthermore, Ca (OH)<sub>2</sub> remnants may impair the physical properties of root canal sealers by accelerating their setting reaction, decreasing working time, reducing flow and increasing film thickness.<sup>6,7</sup>

Appropriate access cavity preparation is of great importance for successful root canal treatment. The traditional endodontic cavity (TEC) design includes the complete removal of pulp horns and pulp chamber roof to obtain straight-line access to the canal orifices.<sup>8</sup> The tooth structure removal during access cavity preparation can decrease the resistance of endodontically treated teeth to fracture.<sup>9,10</sup> Recently, conservative endodontic cavity (CEC) design has been introduced to decrease the amount of tooth structure removal. In this minimally invasive approach, some of the chamber roof and pericervical dentine are preserved.<sup>10</sup> Pericervical dentine is located 4 mm below and 4 mm above the alveolar crest, is responsible for distribution of functional mechanical stresses inside tooth and its presence plays an important role for long-term survival.<sup>11</sup>

In several studies, mechanical aspects of cavity design were investigated and CECs were found to increase the fracture strength of teeth compared to TECs.<sup>12,13</sup> Moreover, the effect of CEC design on root canal localization, root canal transportation, root canal instrumentation and debridement efficacy has been assessed.<sup>8,14</sup> However, it is not known whether the Ca (OH)<sub>2</sub> removal from root canals might be affected by the cavity design. Therefore, the purpose of the current study was to assess the effect of TEC and

CEC designs on the Ca (OH)<sub>2</sub> removal from simulated irregularities in the coronal and apical parts of root canals. The null hypothesis was that there would be no influence of the cavity type on the removal of Ca (OH)<sub>2</sub> from irregularities located either in the coronal or apical parts of root canals.

## MATERIALS AND METHODS

The present study included 40 freshly extracted single-rooted human mandibular premolar teeth with round canals, closed apices, similar length and dimensions after Hacettepe University Non-interventional Clinical Research Ethics Board approval (no: GO 18/397). Inclusion criteria included teeth devoid of caries, resorption, fracture, and defects. The presence of a single-root canal for each tooth was confirmed with periapical radiographs.

The teeth were numbered and randomly allocated (using the website <http://www.random.org>) into 2 groups (n=20) according to the following cavity designs:

a) The CEC Group: The access cavities in this group were prepared with a mosquito 392 bur (Spring Health Diamonds, St Louis Park, MN, USA) in a high-speed handpiece under water cooling. CECs were prepared as described earlier.<sup>12</sup> Accordingly, initial access was created at 1 mm buccal to the central fossa, and then the cavities were enlarged apically without performing buccolingual and mesiodistal enlargement. Lingual shelf, pericervical dentine and a part of the pulp chamber roof were maintained (Figure 1A).

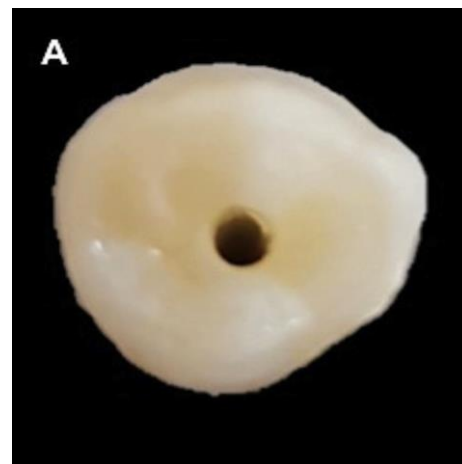
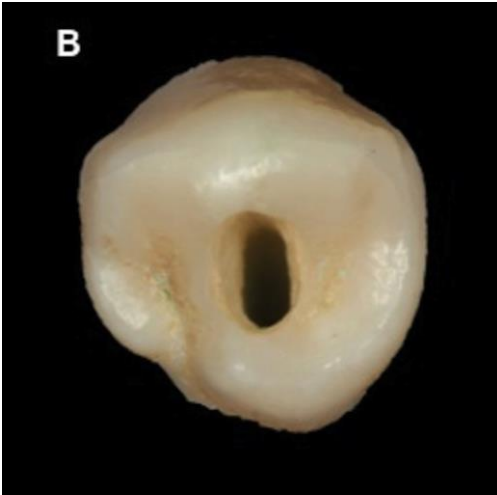


Figure 1. Photographs demonstrating the cavity designs (A) CEC design.

b) The TEC Group: Each access cavity in this group was prepared using an LA Axxess diamond bur (SybronEndo, Glendora, CA, USA) in a high-speed handpiece under water cooling. The pulp chamber roof was removed and outline of each cavity was modified until straight-line access to root canals could be obtained (Figure 1B).



**Figure 1.** Photographs demonstrating the cavity designs (B) TEC design.

Following the access cavity preparation, working length (WL) was determined by introducing #15 K-file to the canal foramen and subtracting 0.5 mm. A periapical radiograph was taken to confirm the accuracy of the WL. Then, the root canals were prepared with ProTaper Universal rotary system (Dentsply Maillefer, Ballaigues, Switzerland) up to F3 file. A 30-G side-vented needle (Max-i-probe, Dentsply, Rinn, Elgin, IL, USA) was inserted until 1 mm short of the WL and irrigation was performed with 3 mL 2.5 % NaOCl solution between each instrument. The teeth were placed in tubes (Eppendorf-Elkay, Shrewsbury, MA, USA) with a silicone material (Optosil; Heraeus Kulzer, Hanau, Germany) to obtain an individual mold for each tooth.<sup>15</sup> Each tooth was then removed from its mold and split longitudinally into 2 halves with a disc (Multicut diamond disc/354, Edenta Ag Dental Products, Hauptstrasse, Switzerland), allowing subsequent reassembling. A previously described protocol was applied for the preparation of grooves to simulate root canal irregularities.<sup>16</sup> Briefly, two longitudinal grooves (0.5-mm deep, 0.2-mm wide and 3 -mm long) were prepared in the coronal or apical root canal wall of one tooth half. The groove in the apical part was created at 2 mm

from the apex. The groove in the coronal part was created at 9 mm from the apex.<sup>16</sup> Debris was removed from the grooves using a toothbrush. Final irrigation was performed using 5 mL 17% EDTA for 60 seconds and 5 mL 2.5% NaOCl for 60 seconds, respectively. Each irrigant was delivered with a 30-G side-vented needle (Max-i-probe, Dentsply) inserted 1 mm short of the WL. Ca(OH)<sub>2</sub> was prepared by mixing the powder (Merck, Darmstadt, Germany) and distilled water at a powder to liquid ratio of 1:1.5, and the standardized grooves were filled with Ca (OH)<sub>2</sub>.<sup>17</sup> Thereafter, the specimens were carefully reassembled, secured with sticky wax under an operating microscope and positioned in their silicone molds. A temporary filling material (Cavit; ESPE, Seefeld, Germany) was placed in the access cavities.

After 7-day storage at 37°C and 100% humidity, each root canal was instrumented with an F4 file (ProTaper Universal, Dentsply Maillefer) at the WL. Then, a 40 H-file was used in a circumferential filing action. Then, the root canals were flushed with 5 mL 2.5% NaOCl for 60 seconds and 5 mL 17% EDTA for 60 seconds. Both irrigants were sonically activated using the medium size tip (25/04) of EndoActivator System (Dentsply, Tulsa Dental Specialties, Tulsa, OK, USA) at medium speed for 30 seconds between each 2.5-mL irrigant. The sonic tip of EndoActivator System was placed at 2 mm from the WL and activated. Five mL of distilled water was applied as a final irrigant. Finally, the root canals were dried with paper points.

The tooth halves were removed from the eppendorf tubes and separated. The remaining amount of Ca (OH)<sub>2</sub> in the grooves was evaluated using a stereomicroscope with x25 magnification (Olympus BX43; Olympus Co, Tokyo, Japan). Each image was scored using a previously described evaluation scale.<sup>16</sup> Accordingly, score 0, the groove is completely empty; score 1, less than 50% of the groove is filled with Ca (OH)<sub>2</sub>; score 2, more than 50% of the groove is filled with Ca (OH)<sub>2</sub>; and score 3, the groove is entirely covered with Ca (OH)<sub>2</sub> (Figure 2).

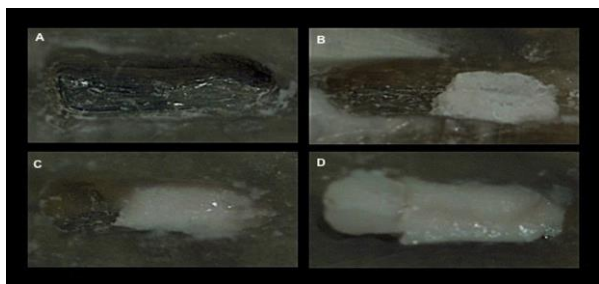


Figure 2. Representative images of scores (A) Score 0, (B) Score 1, (C) Score 2 and (D) Score 3.

$$\text{Percentage of score reduction} = \frac{\text{score before Ca(OH)}_2 \text{ removal} - \text{score after Ca(OH)}_2 \text{ removal}}{\text{score before Ca(OH)}_2 \text{ removal}} \times 100\%$$

Data were statistically analysed using the Mann-Whitney U and Wilcoxon signed rank tests at the 95% level of confidence (p<0.05). The analyses were performed using a software program (SPSS 23 software; IBM SPSS Inc,

Table 1 Score of Ca (OH)<sub>2</sub> before and after removal (n=20)

| Groups      | Before | After              |      |
|-------------|--------|--------------------|------|
|             |        | Mean               | SD   |
| TEC-Coronal | 3.00   | 2.06 <sup>a*</sup> | 1.25 |
| TEC-Apical  | 3.00   | 2.59 <sup>b*</sup> | 0.71 |
| CEC-Coronal | 3.00   | 1.59 <sup>a+</sup> | 1.23 |
| CEC-Apical  | 3.00   | 2.24 <sup>b+</sup> | 0.66 |

Different superscript letters indicate significant differences between the cavity designs for the same root part. Different superscript symbols indicate significant differences between the root parts for each cavity design.

Figure 3 shows the distribution of percentage score reduction of Ca(OH)<sub>2</sub> remnants in the grooves of the samples for each group. Ca(OH)<sub>2</sub> remnants were found in both groups. No significant difference was found between the CEC and TEC groups in terms of Ca(OH)<sub>2</sub> removal efficacy (p>0.05). The grooves in the coronal and apical parts of the roots presented similar amount of Ca(OH)<sub>2</sub> remnants in both groups (p>0.05).

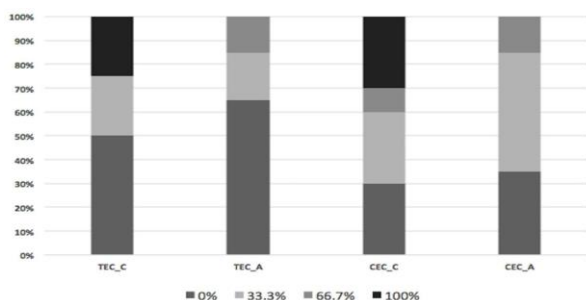


Figure 3. The distribution of percentage score reduction of Ca(OH)<sub>2</sub> remnants in the grooves of the samples for each group.

## DISCUSSION

The use of minimally invasive procedures in endodontics has become increasingly popular due to the maintenance of tooth structure and stability.<sup>18</sup> In the present study, the effect of TEC and CEC designs on Ca(OH)<sub>2</sub> removal from root

The scoring was performed independently by two calibrated endodontists which were blinded to the groups. When a disagreement occurred, the two examiners reevaluated the image and final decision was given on consensus. Calculation of the percentage of score reduction was performed according to the following formula:<sup>16</sup>

Chicago, IL, USA).

## RESULTS

The results regarding the amount of Ca (OH)<sub>2</sub> are presented in Table 1.

canals were compared. It has been well established that the removal of Ca(OH)<sub>2</sub> from irregular canal walls is difficult.<sup>16</sup> To simulate such irregularities, artificial grooves were prepared similar to previous studies.<sup>16,19</sup> This model also allowed to standardize the groove location and size and the amount of Ca(OH)<sub>2</sub> placed in the groove.

Based on the present findings, the null hypothesis was accepted because the cavity design had no significant effect on the removal of Ca(OH)<sub>2</sub> from irregularities located either in the coronal or apical parts of root canals. Similar to this result, no difference was found between the CEC and TEC designs in the amount of tissue remnants in the root canals after root canal preparation and irrigation procedures in a previous study.<sup>8</sup> On the other hand, the CEC design was associated with more remaining obturation material in a previous study that evaluated the removal of root canal filling materials from oval shaped canals.<sup>20</sup> The different results in the studies can be related to the anatomical variations among the teeth used. In the present study, teeth with single and round canals were used. The



influence of cavity design on  $\text{Ca(OH)}_2$  removal from root canals could be different in cases of teeth with more complex anatomy.

A variety of irrigation regimens and techniques are used for the removal of intracanal medicaments.<sup>16,17,21</sup> The most commonly described method for the  $\text{Ca(OH)}_2$  removal is preparation of the root canal using a master apical instrument at the WL and irrigation using NaOCl and EDTA.<sup>16</sup> In the present study, the root canals were enlarged to the next file size and the irrigants were sonically activated to enhance the  $\text{Ca(OH)}_2$  removal.<sup>21-24</sup> Several methods were used to evaluate the remaining amount of  $\text{Ca(OH)}_2$  in root canals including digital photographs<sup>25</sup>, stereomicroscopy<sup>26</sup>, scanning electron microscopy<sup>27</sup> and micro-computed tomography.<sup>28</sup> In the current study, a stereomicroscope was used to evaluate the amount of  $\text{Ca(OH)}_2$  remnants in root canals. This method requires the sectioning of roots for evaluation. The destructive nature of the process is a limitation, however sectioning the roots before  $\text{Ca(OH)}_2$  placement allowed the irregularities to be completely filled and prevented  $\text{Ca(OH)}_2$  losses that could occur during root cleavage. The stereomicroscope images were evaluated using a previously described scoring method,<sup>16</sup> which is widely used in recent studies.<sup>15,17,19,29,30</sup> Imaging software programs can also be used to calculate the percentage ratio of  $\text{Ca(OH)}_2$  covered surface area to the total area.<sup>31</sup> However, a scoring scale was used as it could be difficult to automatically select the areas covered with  $\text{Ca(OH)}_2$  using a software due to the similar color of the medicament and dentine.

Complete removal of  $\text{Ca(OH)}_2$  from root canals could not be achieved, similar to previous studies.<sup>29,30</sup> There was no significant difference between the grooves in the coronal and apical root canal thirds in terms of  $\text{Ca(OH)}_2$  removal. Previously, some studies reported superior  $\text{Ca(OH)}_2$  removal in the apical root part compared to the coronal part<sup>29,30</sup>, while others found  $\text{Ca(OH)}_2$  remnants mainly in the apical region.<sup>32,33</sup> In addition to the differences in cavity designs, variations in test parameters such as final diameter of root canal preparation, irrigant volume, irrigant

activation method, needle size and needle penetration depth may be the cause of conflicting results in the literature. Although the difference was not significant, the percentage of score reduction was higher both in coronal and apical irregularities of the samples which had CECs. This may be attributed to the close contact of the Endoactivator tip with the crown dentine which could enhance the effect of sonication through the root canal. The closer proximity between the tip and dentine may lead to higher hydrodynamic shear stresses and improve fluid exchange within the root canal.<sup>34</sup>

## CONCLUSIONS

In conclusion, the cavity design had no effect on the  $\text{Ca(OH)}_2$  removal from root canal irregularities. In both cavity types, it was not possible to obtain complete removal of  $\text{Ca(OH)}_2$ . Further techniques should be developed to remove  $\text{Ca(OH)}_2$  from the root canal more efficiently. To adapt the CEC design in clinical practice, it must not be less effective than the TEC design. In the light of the present findings, the CEC design can be an alternative to its traditional counterpart. However, more studies are required to assess the effect of access cavity design on the long-term clinical success.

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## CONFLICTS OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

### *Kavite Tasarımının Kalsiyum Hidroksitin Kök Kanal Düzensizliklerinden Uzaklaştırılmasına Etkisi*

**Giriş:** Konservatif endodontik kavite (KEK) tasarımı son zamanlarda dişlerin mekanik stabilitesini korumak için geliştirilmiştir ve geleneksel endodontik kavite (GEK) tasarımına bir alternatif olarak yerini almıştır. Bu çalışmanın amacı, kavite tasarımının, kalsiyum hidroksitin ( $\text{Ca(OH)}_2$ ) kök kanallarının koronal ve apikal kısımlarında hazırlanan yapay oluklardan uzaklaştırılmasına olan etkisini araştırmaktır. **Gereç ve Yöntemler:** Kırk adet çekilmiş tek kanallı insan alt küçük azı dişleri, rastgele KEK ve GEK gruplarına

ayrıldı (n=20). Kaviteeler hazırlandıktan sonra, kök kanalları ProTaper Universal döner eğe sistemi ile F3 eğesine kadar genişletildi ve her diş uzunlamasına kesildi. Kök parçalarından birinin koronal ve apikal kısımlarında 2 adet standart oluk hazırlandı. Ca(OH)<sub>2</sub> olukların içine yerleştirildi ve kök yarılıarı yeniden birleştirildi. Bir hafta sonra, her bir kök #40 H-tipi el eğesi ile genişletildi. İrrigasyon 5 mL %2,5'lük sodyum hipoklorit ve 5 mL %17'lik etilendiamintetraasetik asit solusyonlarının, EndoActivator sisteminin orta hızda, orta boy uç (25/04) ile her 2,5 mL irrigant arasında 30 saniye süreyle kullanılmasıyla gerçekleştirilen sonik aktivasyonu ile yapıldı. Oluklarda kalan Ca (OH)<sub>2</sub> miktarı bir stereomikroskop kullanılarak x25 büyütme ile değerlendirildi ve görüntüler 2 araştırmacı tarafından 4-skorlu skala kullanılarak skorlandı. Veriler Mann-Whitney U ve Wilcoxon testleri kullanılarak analiz edildi. **Bulgular:** Her iki grupta da Ca(OH)<sub>2</sub> kalıntıları bulundu. KEK ve GEK grupları arasında Ca(OH)<sub>2</sub>'in uzaklaştırılma etkinliği açısından anlamlı fark yoktu (p>0,05). Köklerin koronal ve apikal kısımlarındaki oluklar her iki grupta da benzer miktarda Ca(OH)<sub>2</sub> kalıntıları gösterdi (p>0,05). **Sonuçlar:** Çalışmanın bulgularına göre, kavite tasarımının Ca(OH)<sub>2</sub>'in kök kanal düzensizliklerinden uzaklaştırılması üzerinde etkisi yoktu. **Anahtar kelimeler:** Endodonti, kalsiyum hidroksit, kavite preparasyonu, kök kanalı, minimal invaziv.

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## COMPARISON OF PERIODONTITIS DIAGNOSES ACCORDING TO 1999 AND 2017 CLASSIFICATIONS: AN ORIGINAL ARTICLE

### ABSTRACT

**Objectives:** Classification systems of periodontitis have changed several times over the past 30 years as new information gathered about the pathophysiology of the disease rendered previous systems inadequate for classifying the diagnoses of all patients. Although the 1999 classification system was widely used in clinical practice and scientific studies, it had significant limitations leading to the reclassification introduced in 2017. In this context, the aim of this study is to evaluate how individuals diagnosed with periodontitis under the 1999 system were reclassified according to the 2017 system.

**Materials and Methods:** Participants diagnosed with periodontitis according to 1999 classification and who had not received periodontal treatment in the last six months were included in the study. The patient assessment procedure consisted of a comprehensive periodontal diagnosis through periodontal charting and full-mouth radiography.


**Results:** A total of 315 subjects (133 males and 182 females) were included in the study. According to the new classifications, 42 patients (17.94%) previously diagnosed with generalized chronic periodontitis according to 1999 classifications, were now classified as healthy with reduced periodontium, and 11 (4.7%) patients were classified as gingival inflammation with reduced periodontium. 63 (26.92%) patients were classified as SIII-GC and 33 patients (14.14%) as SIV-GC.

**Conclusions:** The new classification system is based on not only disease severity but also the dimensions of an individual's disease which include complexity and risk factors.

**Keywords:** Classification, diagnosis, disease, periodontitis.

 \*Fatih KARAASLAN<sup>1</sup>

 Ahu DİKİLİTAŞ<sup>1</sup>

 Esra Özge AYDIN<sup>1</sup>

ORCID IDs of the authors:  
F.K. 0000-0002-9899-3316  
A.D. 0000-0003-4130-2526  
E.Ö.A. 0000-0001-8166-560X

<sup>1</sup> Department of Periodontology, Faculty of Dentistry, Usak University Usak Turkey

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## **INTRODUCTION**

Periodontitis is a microbially associated and host mediated multifactorial inflammatory disease characterized by loss of periodontal attachment.<sup>1</sup> Periodontitis classification has been modified several times in the last 30 years in accordance with emerging scientific findings.<sup>2</sup> The periodontitis classification system widely accepted in 1999 was used in both clinical practice and scientific research, despite its important deficiencies, such as the lack of pathobiology-based distinctions, diagnostic imprecision, and difficulties in practice.<sup>3</sup> The analysis of these important shortcomings prompted the reclassification of periodontitis in the 2017 workshop.<sup>4</sup>

The result of the 2017 workshop provides a current and future-oriented classification of the periodontal status of patients. Three types of periodontitis have been defined in the new classification: a) periodontitis, b) necrotizing periodontitis, and c) periodontitis as a manifestation of systemic disease. A staging and grading system was created to replace the term “aggressive periodontitis.” Periodontitis was reclassified into four stages (I, II, III, and IV) according to severity of the disease, and three grades (A, B, and C) to differentiate disease susceptibility.<sup>5</sup> Importantly, the terms “clinical health” and “intact and reduced periodontium” were defined.<sup>6</sup>

The 2017 classification system addresses unresolved problems in the earlier system and provides a “future-proof” system for classifying periodontitis. This study evaluates how individuals diagnosed with periodontitis under the 1999 system are to be reclassified using the 2017 parameters.

## **MATERIALS AND METHODS**

This study was conducted from February 2019 to August 2019 at the Faculty of Dentistry of Usak University. Participants were informed about the purpose of the investigation and informed consent forms were signed. The study was conducted according to the Helsinki Declaration’s norms, and ethical approval was granted by the Local

Ethical Committee of Usak University (Registration No: 226-04).

A comprehensive periodontal diagnosis was undertaken in the patient assessment procedure through periodontal charting and full-mouth radiography. Participants who had been diagnosed with periodontitis according to 1999 classifications and who had not received periodontal treatment in the previous six months were included in the study. The patients diagnosed under 1999 parameters as having chronic or aggressive periodontitis were reviewed by a calibrated periodontologist (AD). Patients’ periodontitis was reported as localized if  $\leq 30\%$  of the sites were affected—otherwise, as generalized.<sup>7,8</sup>

The same patients were re-evaluated according to the 2017 classification system by another calibrated investigator (FK) who was blind to their 1999 classifications. The patients were diagnosed as having periodontitis under these criteria: their interdental clinical attachment loss (CAL) was detectable at  $\geq 2$  non-adjacent teeth; their buccal or oral CAL was  $\geq 3$  mm with pocketing  $> 3$  mm detectable at  $\geq 2$  teeth;<sup>5</sup> and the observed CAL could not be attributed to non-periodontitis causes. Periodontitis patients who do not have a probing pocket depth (PPD) of 4 mm or more, and with bleeding on probing (BoP) lower than 10%, were diagnosed with reduced periodontium; those with BoP higher than 10% were diagnosed as having reduced periodontium with gingival inflammation. If periodontitis patients had a PPD of 4 mm or more, periodontitis needs to be assessed as to stage and grade.<sup>9,10</sup>

The stages are based primarily on the worst-affected tooth’s interdental CAL. The complexity score depends on the difficulty of treating the case, considering factors like deep probing depths, furcation involvement, and vertical defects. Subclassification of stages was reported as localized if  $< 30\%$  of teeth were affected—otherwise, as generalized.<sup>5,10</sup>

Grade is based on the assessment of bone loss at the worst-affected tooth as a function of age. It is measured as radiographic bone loss in a

percentage of root length divided by the patient's age. Grades A and B can be modified if the patient smokes or is diabetic.<sup>5,10</sup>

Data analysis was performed by using the software Statistical Package version 17.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were used to evaluate the data in this study.

## RESULTS

A total of 315 patients (133 males and 182 females) were included in the study. According to 1999 classifications, 234 (74.28%) of these patients suffered from generalized chronic periodontitis, 38 (12.06%) from generalized

aggressive periodontitis, 21 (6.68%) from localized chronic periodontitis, and 22 (6.98%) from localized aggressive periodontitis.

Under the new classification system, 42 patients (17.94%) diagnosed with generalized chronic periodontitis in the 1999 system were reclassified as healthy and reduced periodontium, and 11 (4.7%) were reclassified as gingival inflammation with reduced periodontium. Sixty-three patients (26.92%) were classified as SIII-GC (stage III, grade C) and 33 (14.14%) as SIV-GC (Table 1).

**Table 1.** The new classification of patients with chronic periodontitis

| 1999 C<br>n/ % | 2017 C n/ %  |             |            |             |             |             |              |               |            |               | Total<br>n/ % |
|----------------|--------------|-------------|------------|-------------|-------------|-------------|--------------|---------------|------------|---------------|---------------|
|                | RP           | RPG         | N-RPG      | SII-<br>GB  | SII-<br>GC  | SIII-<br>GA | SIII-<br>GB  | SIII-<br>GC   | SIV-<br>GB | SIV-<br>GC    |               |
| <b>GCP</b>     | 42/<br>17.94 | 11/<br>4.7  | -          | 19/<br>8.11 | 22/<br>9.4  | 6/ 2.56     | 31/<br>13.24 | 63/<br>26.92  | 7/ 2.99    | 33/<br>14.14  | 234/<br>100   |
| <b>LCP</b>     | 5/<br>23.80  | -           | 2/ 9.53    | -           | -           | -           | 8/<br>38.09  | 6/<br>28.58   | -          | -             | 21/<br>100    |
| <b>Total</b>   | 47/<br>18.43 | 11/<br>4.31 | 2/<br>0.78 | 19/<br>7.45 | 22/<br>8.62 | 6/ 2.35     | 39/<br>15.29 | 69 /<br>27.05 | 7/ 2.74    | 33 /<br>12.98 | 255 /<br>100  |

C: Classification

RP: Reduced periodontium

RPG: Reduced periodontium with gingival inflammation

N-RPG: Non periodontitis caused reduced periodontium with gingival inflammation

LCP: Localized chronic periodontitis

GCP: Generalized chronic Periodontitis

Five (23.80%) patients who were diagnosed with localized chronic periodontitis using 1999 parameters were reclassified as healthy and reduced periodontium. Two patients (9.53%) were classified as reduced periodontium and gingival inflammation from non-periodontitis causes (Table 1).

Of the patients diagnosed with generalized aggressive periodontitis according to 1999 classification, 21 (55.27%) were classified as SIV-GC, and 13 patients (59.09%) diagnosed with localized aggressive periodontitis according to 1999 classifications were classified as SIII-GC (Table 2).

**Table 2.** The new classification of patients with aggressive periodontitis

| 1999 C<br>n/ % | 2017 C n/ % |           | Total n/ % |
|----------------|-------------|-----------|------------|
|                | SIII-GC     | SIV-GC    |            |
| <b>GAP</b>     | 17 / 44.73  | 21/ 55.27 | 38/ 100    |
| <b>LAP</b>     | 13/ 59.09   | 9/ 40.91  | 22/ 100    |
| <b>Total</b>   | 30/ 50.0    | 30/ 50.0  | 60/ 100    |

C: Classification

GAP: Generalized aggressive periodontitis

LAP: Localized aggressive periodontitis

According to 1999 classification, the mean age of generalized chronic periodontitis patients was  $47.42 \pm 10.67$ , and the mean age of localized aggressive periodontitis patients was  $22.75 \pm 3.40$ .

The age and gender distribution of patients diagnosed according to 1999 classification is shown in Table 3.

**Table 3.** The age and gender distribution of patients who diagnosed according to 1999 classification

| 1999 C<br>n/ % | Gender       |                |               | Age         |
|----------------|--------------|----------------|---------------|-------------|
|                | Male<br>n/ % | Female<br>n/ % | Total<br>n/ % | Mean± sd.   |
| <b>GAP</b>     | 14 /10.52    | 24/ 13.18      | 38/ 12.06     | 36.33±6.86  |
| <b>GCP</b>     | 99/ 74.43    | 135/ 74.17     | 234/ 74.28    | 47.42±10.67 |
| <b>LAP</b>     | 12/ 9.02     | 10/ 5.49       | 22/ 6.98      | 22,75±3.40  |
| <b>LCP</b>     | 8/ 6.03      | 13/ 7.16       | 21/ 6.68      | 42,00±6.08  |
| <b>Total</b>   | 133/ 100     | 182/ 100       | 315/ 100      | 43.99±9.39  |

C: Classification  
 GAP: Generalized aggressive periodontitis  
 GCP: Generalized chronic periodontitis  
 LAP: Localized aggressive periodontitis  
 LCP: Generalized chronic periodontitis

According to 2017 classification, the mean age of reduced periodontitis patients was 42.05 ± 7.57, and the mean age of SIV-GB patients was 52.0 ±

6.34. The age and gender distribution of patients diagnosed according to 2017 classification is shown in Table 4.

**Table 4.** The age and gender distribution of patients who diagnosed according to 2017 classification

| 2017 C         | Gender       |                |               | Age         |
|----------------|--------------|----------------|---------------|-------------|
|                | Male<br>n/ % | Female<br>n/ % | Total<br>n/ % | Mean± sd.   |
| <b>RPG</b>     | 4 /3.01      | 7 /3.84        | 11 /3.49      | 53.00±8.49  |
| <b>N-RPG</b>   | 1 /0.75      | 1 /0.54        | 2 /0.63       | 52.19±5.32  |
| <b>RP</b>      | 20 /15.03    | 27 /14.83      | 47 /14.92     | 42.05±7.57  |
| <b>SII-GB</b>  | 11 /8.27     | 8 /4.39        | 19 /6.03      | 51.00±14.00 |
| <b>SII-GC</b>  | 10 /7.51     | 12 /6.59       | 22 /6.98      | 53.50±5.97  |
| <b>SIII-GA</b> | 2 /1.50      | 4 /2.19        | 6 /1.90       | 37.00±4.69  |
| <b>SIII-GB</b> | 16 /12.03    | 23 /12.63      | 39 /12.38     | 46.17±9.58  |
| <b>SIII-GC</b> | 46 /34.62    | 53 /29.12      | 99 /31.42     | 40.13±10.60 |
| <b>SIV-GB</b>  | 2 /1.50      | 5 /2.74        | 7 /2.22       | 52.00±6.34  |
| <b>SIV-GC</b>  | 21 /15.78    | 42 /23.13      | 63 /20.03     | 42.70±18.02 |
| <b>Total</b>   | 133 /100     | 182 /100       | 315 /100      | 43.99±9.39  |

C: Classification  
 RPG: Reduced periodontium with gingival inflammation  
 N-RPG: Non periodontitis caused reduced periodontium with gingival inflammation  
 RP: Reduced periodontium

According to 1999 classification, 55.0% of smokers were diagnosed as generalized chronic periodontitis, and 35.41% of diabetics were

diagnosed as localized chronic periodontitis. Distribution of smokers and diabetic patients is shown in Table 5.

**Table 5.** Distribution of smoker and diabetic patients who diagnosed according to 1999 classification

| 1999 C       | Smoker patients n/ % | Diabetic patients n/ % |
|--------------|----------------------|------------------------|
| <b>GCP</b>   | 33 /55.0             | 31 /64.59              |
| <b>LCP</b>   | -                    | 17 /35.41              |
| <b>GAP</b>   | 19 /31.66            | -                      |
| <b>LAP</b>   | 8 /13.34             | -                      |
| <b>Total</b> | 60 /100              | 48 /100                |

C: Classification  
 GAP: Generalized aggressive periodontitis  
 GCP: Generalized chronic periodontitis  
 LAP: Localized aggressive periodontitis  
 LCP: Generalized chronic periodontitis

According to 2017 classification, 31.66% of smokers were diagnosed as SIII-GC and 37.5% of diabetics were diagnosed as SII-GC. Distribution

of smokers and diabetic patients is shown in Table 6.

**Table 6.** Distribution of smoker and diabetic patients who diagnosed according to 2017 classification

| 2017 C       | Smoker n /%    | Diabetic n /%  |
|--------------|----------------|----------------|
| SII-GC       | 5 /8.34        | 18 /37.5       |
| SII-GB       | 6 /10.0        | -              |
| SIII-GB      | 11 /18.34      | 7 /14.58       |
| SIII-GC      | 19 /31.66      | 9 /18.75       |
| SIV-GB       | -              | -              |
| SIV-GC       | 12 /20.0       | 9 /18.75       |
| RP           | 7 /11.66       | 5 /10.41       |
| <b>Total</b> | <b>60 /100</b> | <b>48 /100</b> |

C: Classification

RP: Reduced periodontium

## DISCUSSION

The 2017 classification system was developed in order to accommodate advances in knowledge derived from both biological and clinical research. The new classification characterizes periodontitis using a staging and grading system.<sup>11</sup> Periodontal health, gingival health, and gingival diseases on an intact and reduced periodontium are clearly defined for the first time.<sup>12</sup>

Of patients diagnosed with generalized chronic periodontitis or with localized chronic periodontitis according to 1999 classification, 17.94% and 23.80%, respectively, were re-diagnosed as having periodontitis-caused reduced periodontium with clinical gingival health under the 2017 classification system. Determining the current disease status of a patient who received periodontal therapy in the past is important in the new classification system.<sup>13</sup> A successfully-treated periodontitis patient may appear to be healthy, but a periodontitis patient remains a periodontitis patient for life. Clinical gingival health may be found in a patient with a history of periodontitis who was successfully treated and is currently stable.<sup>14</sup> Although patients are clinically healthy, periodontal stability requires careful maintenance and continued risk-factor control, because the disease may progress at any time.<sup>12,15</sup>

Of the patients diagnosed with generalized chronic periodontitis according to 1999 classification, 4.7% were re-diagnosed as having reduced periodontium with gingival inflammation. Dental plaque-induced gingivitis may arise on a reduced periodontium in a currently stable

periodontitis patient in whom clinical inflammation has been eliminated. Therefore, patients should be closely monitored during periodontal maintenance for any reactivation of periodontitis.<sup>4,16</sup>

Of the patients diagnosed with localized chronic periodontitis according to 1999 classification, 9.53% were re-diagnosed as having reduced periodontium with gingival inflammation from non-periodontitis causes, due to attachment loss caused by orthodontic treatment. Alveolar bone loss or attachment loss due to causes other than periodontitis was classified as reduced periodontium in a non-periodontitis patient, according to the new classification.<sup>17</sup>

Chronic periodontitis patients were classified into different stages according to the new classification system. Patients can be diagnosed in a more detailed and precise way in the new classification, because the old classification is based solely on severity, while staging is now taken into account using complexity factors that affect treatment success, in addition to the standard dimensions of severity and extent.<sup>3</sup> Although chronic periodontitis patients were diagnosed as to different stages, there were no patients diagnosed as SI. This may be due to the absence of SI patients in this sample group, or because patients who would have been designated as SI were not diagnosed as having periodontitis in the 1999 classifications, because the worst-affected teeth guided the 2017 classifications, whereas diagnosis of periodontitis was based on

the mean CAL of the entire dentition in the 1999 classification.<sup>3,13</sup>

Chronic periodontitis patients were classified into different grades; 2.35% of chronic periodontitis patients were diagnosed as Grade A, which assumes a slow rate of progression, while 48.65% were diagnosed as Grade C, which indicates a high rate of disease progression. This means that almost half of chronic periodontitis patients are at risk for further progression of the disease and possibly poor outcomes of treatment. The reason for the high number of Grade C patients, despite slow to moderate rates of disease progression in patients with chronic periodontitis, can be explained as follows: if the patient has risk factors associated with greater disease progression or is less responsive to bacterial reduction therapies, the grade score was raised. The high rate of patients with chronic periodontitis being diagnosed as Grade C affects the intensity of therapy, secondary prevention after therapy, and careful maintenance therapy.<sup>18,19</sup>

Although chronic periodontitis patients were classified into different grade levels, all patients with aggressive periodontitis were diagnosed as Grade C, which is a predictor of adverse future disease progression in the absence of intervention for risk-factor control and treatment. While the severity of disease varies from slight to severe in individuals with chronic periodontitis, all individuals with aggressive periodontitis exhibit severe CAL.<sup>20</sup> According to the new classification, individuals with aggressive periodontitis will experience more rapid and severe destruction than individuals with chronic periodontitis. This is also consistent with the definition of the clinical features of aggressive periodontitis in the 1999 classification.<sup>3,21</sup>

Recognized risk factors for severe destruction at an earlier age and that negatively affect treatment response have not been included in previous periodontitis classification systems, but have been used to classify a patient who is a smoker or a patient with diabetes mellitus.<sup>22,23</sup> Improved knowledge of how risk factors affect periodontitis indicate that risk factors should be considered in the classification of periodontitis.<sup>24,25</sup>

Since including risk factors in the classification system predicts future disease susceptibility, the new classification can be seen as a future-oriented system.

According to 1999 classification, the mean age of generalized chronic periodontitis was highest, and the mean age of localized aggressive periodontitis was the lowest. This result is consistent with the literature that reports chronic periodontitis as being most prevalent in adults, while aggressive periodontitis usually affects people under 30 years of age.<sup>20,26</sup> According to the new classification, systemic conditions, risk factors, and treatment requirements modified the stage and grade of periodontitis rather than age. Thus, making an age-dependent generalization would be inaccurate with the new classification.

In the 1999 classification, the patient who requires initial periodontal treatment and the patient who requires advanced periodontal treatment were diagnosed with periodontitis and this situation was changed with 2017 classification. In the 2017 classification, the stage of the periodontitis increases as the patient's need for periodontal treatment increases. Another difference was that in 1999 classification all individuals with periodontitis were under the same maintenance treatment protocol. In 2017 classification, the necessity of more careful maintenance treatment was demonstrated for individuals with high disease progression rate as well as grade of periodontitis.

Although our study aims to compare the previous and the new classification systems, there are certain limitations. Firstly, individuals were classified as healthy or diseased considering their clinical status, and only HbA1c levels were requested from diabetic individuals. Secondly, different results may be obtained in other selected sample groups due to the nature of periodontitis.

In sum, the new classification is based on not only disease severity but also includes dimensions of an individual's disease, including complexity and risk factors. Thus, factors that influence approaches to therapy and disease outcomes are included in the classification.

## ACKNOWLEDGMENT

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## CONFLICT OF INTEREST STATEMENT

The authors report no conflicts of interest related to this study.

### *Periodontitis Teşhisi Konulan Bireylerin 1999 Ve 2017 Sınıflamalarına Göre Karşılaştırılması*

## ÖZ

**Amaç:** Periodontitisin patofizyolojisi hakkında toplanan yeni bilgiler, önceki sınıflandırmaların tüm hastaların tanılarını koymada yetersiz kaldığı için, periodontitisin sınıflandırma sistemleri son 30 yılda birkaç kez değişti. 1999 sınıflandırma sistemi klinik uygulamada ve bilimsel çalışmalarda yaygın olarak kullanılmasına rağmen, 2017'deki yeniden sınıflandırmaya yol açan önemli sınırlamalara sahipti. Bu bağlamda, bu çalışmanın amacı, 1999 sistemi altında periodontitis tanısı konan bireylerin 2017 sistemine göre nasıl sınıflandırıldığını değerlendirmektir. **Gereç ve Yöntemler:** 1999 sınıflamasına göre periodontitis tanısı alan ve son altı ayda periodontal tedavi görmeyen katılımcılar çalışmaya dahil edildi. Hasta değerlendirme prosedürü, periodontal indekslerin alınması ve tam ağız radyografisi ile kapsamlı bir periodontal tanıdan oluşmaktaydı. **Bulgular:** Çalışmaya toplam 315 birey (133 erkek ve 182 kadın) dahil edildi. Yeni sınıflandırmaya göre, daha önce 1999 sınıflandırmasına göre generalize kronik periodontitis tanısı konan 42 hasta (%17,94), azalmış periodonsiyum ile sağlıklı, 11 (%4,7) hasta ise azalmış periodonsiyum ile dişeti iltihabı olarak sınıflandırıldı. 63 (%26,92) hasta SIII-GC ve 33 hasta (%14,14) SV-GC olarak sınıflandırıldı. **Sonuçlar:** Yeni sınıflandırma sistemi sadece hastalık şiddetini değil, aynı zamanda bireye özgü komplekslik ve risk faktörlerini de içermektedir. **Anahtar Kelimeler:** Sınıflandırma, tanı, hastalık, periodontitis.

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## THE EFFECTS OF DIFFERENT COLORS AND LIGHT SOURCES ON THE BOND STRENGTH OF CEREC FELDSPATHIC BLOCKS BONDED TO LIGHT-CURED RESIN CEMENT

### ABSTRACT

**Objectives:** To evaluate the bonding with resin cement of different colored prefabricated feldspathic ceramic blocks used in the computer aided design and computer aided manufacturing (CAD-CAM) technique when different light sources were used beneath the ceramics.

**Materials and Methods:** Specimens of 2 mm thickness were prepared in nine different colors. All the groups were bonded to composite resin blocks using a light-cured resin cement beneath plasma arc (PAC, 2400 mW/cm<sup>2</sup>), light emitting diode (LED, 1600–1800 mW/cm<sup>2</sup>) and quartz tungsten halogen (QTH, 800–1200 mW/cm<sup>2</sup>) light sources. Following the cementation, all the specimens were kept in distilled water for 24 hours in closed cups before the shear test was performed. The data were analysed by means of a two-way analysis of variance (ANOVA) and the Tukey HSD test (p<0.05).

**Results:** The highest light power was observed in the PAC groups, while the lowest light power was observed in the QTH groups, and the difference was found to be statistically significant (p<0.05). The highest bond strengths were obtained in the S2M and S2T color groups without the discrimination of the light source, and no statistically significant difference was found between these groups. The lowest bond strength was obtained in the S4O color group.

**Conclusions:** This in vitro study found that the bond strength of feldspathic ceramic restorations is directly related to the utilised light source and, further, that the bond strength decreases as the ceramic color becomes darker.

**Keywords:** CAD-CAM, resin cement, bond strength.

 \*Betül YILMAZ EVMEK<sup>1</sup>  
 İbrahim DURAN<sup>2</sup>

ORCID IDs of the authors:  
B.Y.E. 0000-0002-6975-5183  
İ.D. 0000-0001-5075-4327

<sup>1</sup> Department of Prosthodontics, Faculty of Dentistry, Akdeniz University, Antalya, Turkey.

<sup>2</sup> Department of Prosthodontics, Faculty of Dentistry, Ondokuz Mayıs University, Samsun, Turkey.

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## INTRODUCTION

Dental ceramics are the oldest aesthetic materials still used daily in the field of dentistry. Ceramic material has two basic applications in fixed dentures, namely ceramic systems with a metal substructure and all-ceramic systems. Metal-ceramic systems are widely used in fixed prosthetic restorations. However, increasing attention is now being paid to all-ceramic systems due to the disadvantages of the metal substrate blocking light transmission and causing both corrosion and metal reflection at the gingival margin.<sup>1,2</sup>

The superior aesthetic qualities of all-ceramic systems are considered the most important factor behind such systems being preferred by clinicians and patients in recent years. The color and translucency properties of all-ceramic restorations are important determinants of the final aesthetics because they affect the diffuse and specular reflection of light.<sup>3-5</sup>

CAD-CAM restorations are generally preferred over the other restorative options because the prefabricated blocks used in CAD-CAM systems can be produced with a higher degree of homogeneity and fewer errors.<sup>6-14</sup> Resin-based bonding cements, which are widely used nowadays, are preferred because of their high mechanical strength, low solubility and high aesthetic properties in relation to the cementation of all-ceramic restorations. Clinical and laboratory studies comparing adhesive cements have indicated that the use of resin cements in all-ceramic restorations serves to increase the success of such restorations.<sup>15-17</sup>

The mechanical properties of resin-based bonding cements are closely related to the amount of filler used, the structure of the cement and the degree of polymerisation. The polymerisation of the bonding cement is important in terms of the biocompatibility of the resin cement and the reduction of the residual monomer content.<sup>18,19</sup> For the cementation of all-ceramic restorations, light-polymerised, chemically polymerised, and both light and chemically polymerised (dual-cured) resin cements can be used. Resin cements that are polymerised with light are preferred because they

exhibit better color stability in aesthetic applications.<sup>20,21</sup>

This study aimed to investigate the bond strength with different light sources between light-cured resin cement and different colored prefabricated feldspathic ceramic blocks when used in CAD-CAM systems.

The study hypothesised that the use of different colored feldspathic ceramics will affect the bonding strengths of resin cements with different light sources.

## MATERIALS AND METHODS

The study protocol was carried out according to the principles of the Helsinki Declaration, including all amendments and revisions. Collected data were only accessible to the researchers. Feldspathic ceramic blocks (Cerec Blocs, Sirona Dental Systems, Bensheim, Germany) were used 12 x 14 x 18 mm in size and in nine different colors (S2-T, S3-T, S4-T, S2-M, S3-M, S4-M, S2-O, S3-O, S4-O). The sectioning process was conducted by using a precision cutter (IsoMet 1000, Buehler, Lake Bluff, IL, USA) under water cooling at a speed of 300 rpm, and samples were obtained in size of 2x5x6 mm. All ceramic specimens evaluated with a digital caliper (Mitutoyo, Tokyo, Japan) for the required final thickness ( $\pm 0.1$  mm). For nine different colors, three polymerization units (QTH, LED, PAC) and each group has 10 specimens (n=10) a total of 270 ceramic samples were prepared. Then the prepared cylindrical molds were filled with auto-polymerizing acrylic resin (Paladent 20, Heraeus Kulzer, Germany) and sockets were prepared on the top and center of the acrylic molds in a depth of 2 mm and width of 7 mm. The prepared sockets were filled with composite resin filling material (Voco Arebesk, VOCO GmbH, Cuxhaven, Germany) and polymerized 45 seconds with LED (1600 mW/cm<sup>2</sup>). The samples were held on a polishing machine (Minitech 233, Grenoble - France) for 1 minute under running water with 240, 400, 800 and 1200 grit abrasive polishing discs at 500 rpm to provide surface smoothness. The samples were cleaned with distilled water using a 53 kHz ultrasonic cleaner (Kudos, Shanghai, China) for 90

seconds. Adhesive surfaces of the ceramic samples were roughened for 60 seconds with 9.5% hydrofluoric acid gel (9.5% Buffered Hydrofluoric Acid Gel, BISCO, Schaumburg, U.S.A.) in accordance with the manufacturer's instructions, then washed with 20 seconds with air-water spray and dried for 20 seconds with oil-free air. Silane (Monobond S, Ivoclar Vivadent, Schaan, Liechtenstein) was applied to the bonding surfaces of the ceramic samples for 60 seconds and air-dried for 2-5 seconds. After the silane application a bonding agent (Heliobond, Ivoclar Vivadent, Schaan, Liechtenstein) applied 10 second by a disposable brush and air dried 2-3 second. After surface treatments of the ceramic specimens, light cure medium value (MV 0) resin luting cement (Ivoclar Vivadent, Schaan, Liechtenstein) was used for cementation. The light cure adhesive resin was directly applied to the adhesion surface of the ceramic by being directly squeezed from the tube. The ceramic samples were placed on prepared composite surface with a presser and fixed with a constant pressure of 500 grams by using a cementation apparatus, then excess cement was removed with the help of a brush. An oxygen-blocking agent (Liquid Strip, Ivoclar Vivadent, Schaan, Liechtenstein) was applied to the cementation site for 2 minutes. Polymerization was provided using a QTH light device for 40 second, LED light device for 20 second, and a PAC light device for 3 seconds on the top and center of the ceramics. At the end of the polymerization process with light, ceramic discs were removed from the cementation apparatus and stored in closed dark cups in distilled water for 24 hours. The shear bond

strength between ceramic and composite resin was measured using a universal test machine (Shimadzu AGS-X, Shimadzu Corp., Tokyo, Japan) taking ISO TR 11405 criteria into consideration. The blade, which is placed parallel to the ceramic and composite resin interface, is placed on the bonding interface and applied force with a speed of 0.5 mm/min. The maximum force at which the fracture occurred was recorded at N (Newton). This data was then divided to surface area (mm<sup>2</sup>) of the samples to obtain results in MPa (Megapascal's);  $\sigma = P / A$  (P: Force at break N (Newton) A: Bonding area (mm<sup>2</sup>)). The data were analyzed by IBM SPSS V.23 (SPSS Statistics, IBM, Somers, New York, USA). Normal distribution of the data was tested with Shapiro Wilk. Two-way analysis of variance (ANOVA) was used to compare the data. The homogeneity of variance was assessed by the Levene test and the multiple comparison of different groups were performed by Tukey HSD. The results were presented as mean  $\pm$  standard deviation. Significance level was taken as  $p < 0.05$ .

**RESULTS**

Following the two-way analysis of variance (ANOVA) in which different light sources and different color groups were assessed, the light and color effects were found to be statistically significant in relation to the bond strengths ( $p < 0.001$ ). The color interaction with the light polymerisation systems exerted a statistically significant effect on the bond strength ( $p < 0.001$ ) (Table 1).

**Table 1.** Two way ANOVA. The color interaction with light polymerization system.

|                    | Sum of Squares | df | Mean Square | F      | p           |
|--------------------|----------------|----|-------------|--------|-------------|
| Light Source       | 864.47         | 2  | 432.23      | 252.73 | $p < 0.001$ |
| Color              | 7253.93        | 8  | 906.61      | 530.11 | $p < 0.001$ |
| Light Source*Color | 182.38         | 16 | 11.39       | 6.6    | $p < 0.001$ |

According to the results of the Tukey HSD test comparing the light sources, there was a statistically significant difference between the bond strengths ( $p < 0.001$ ). The average value in the QTH light group was 16.10 MPa, while the average

in the LED light group was 18.16 MPa and in the PAC light group was 20.48 MPa. The highest value was obtained in the PAC group, while the lowest value occurred in the QTH light group. Further, according to the results of the Tukey HSD test

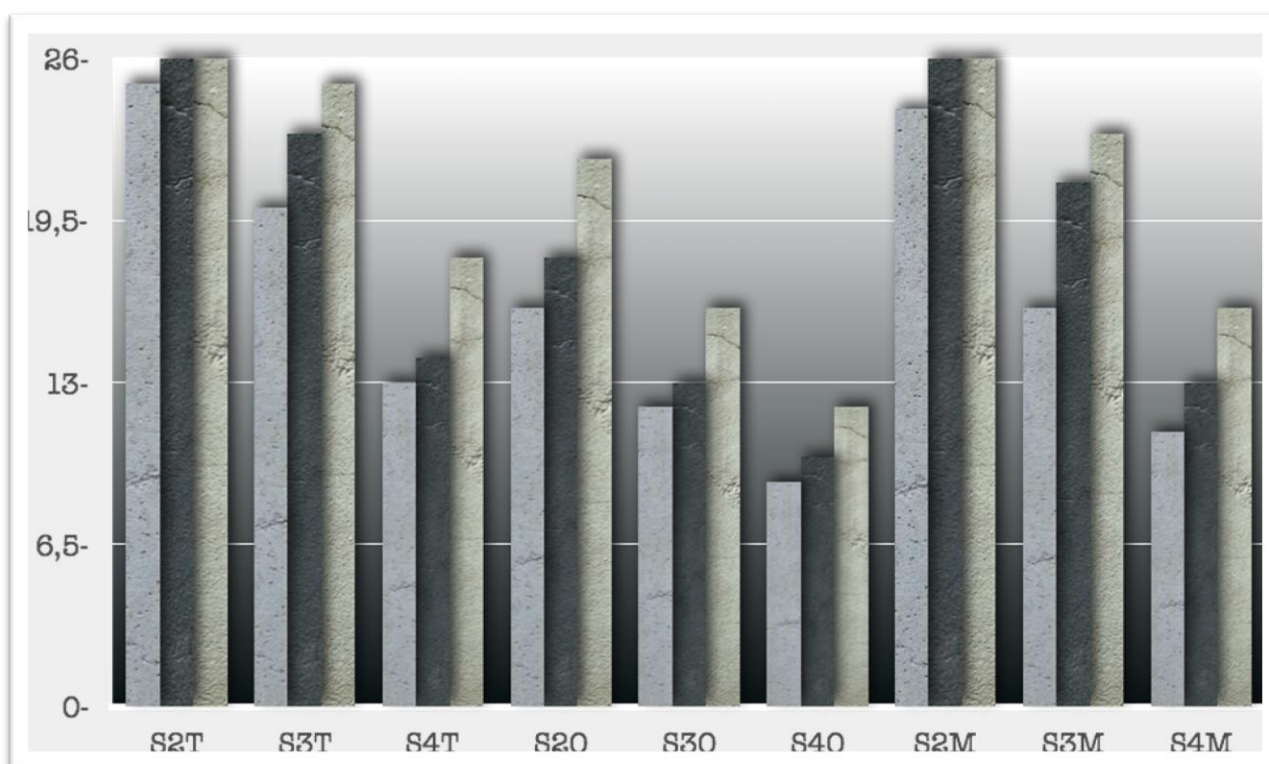
comparing the colors for all the polymerisation units, there was a statistically significant difference between the groups ( $p < 0.001$ ). The maximum bond strength values were obtained in relation to the S2T and S2M colors, and there was no statistically significant difference between the groups. This was followed by the S3T, S3M, S2O and S4T colors, and there was a statistically significant difference among them. There was no significant difference

between the S3O and S4M colors. In terms of the S4O color, the lowest bond strength was obtained and a statistically significant difference was found between it and all the other color groups. A comparison of the mean and standard deviation values concerning the bond strengths for each color for the QTH, LED and PAC light sources is presented in Table 2 and Figure 1.

**Table 2.** Comparison of bond strength in light sources for each color ( $p < 0.05$ ).

| Color | QTH                     |   | LED                     |   | PA                       |   | p      |
|-------|-------------------------|---|-------------------------|---|--------------------------|---|--------|
| S2T   | <sup>a</sup> 24.7 ± 1.7 | A | <sup>a</sup> 25.5 ± 2.0 | A | <sup>a</sup> 25.8 ± 1.4  | A | 0.332  |
| S3T   | <sup>b</sup> 20.2 ± 1.7 | A | <sup>b</sup> 22.6 ± 1.3 | B | <sup>ab</sup> 24.8 ± 1.2 | C | <0.001 |
| S4T   | <sup>d</sup> 12.6 ± 0.9 | A | <sup>d</sup> 14.4 ± 1.3 | B | <sup>d</sup> 17.6 ± 1.5  | C | <0.001 |
| S2O   | <sup>c</sup> 15.7 ± 1.3 | A | <sup>c</sup> 18.4 ± 1.4 | B | <sup>c</sup> 21.8 ± 1.3  | C | <0.001 |
| S3O   | <sup>d</sup> 12.2 ± 1.0 | A | <sup>d</sup> 12.9 ± 1.2 | A | <sup>e</sup> 15.7 ± 1.1  | B | <0.001 |
| S4O   | <sup>e</sup> 8.6 ± 0.8  | A | <sup>e</sup> 10.1 ± 1.3 | B | <sup>f</sup> 12.3 ± 0.8  | B | <0.001 |
| S2M   | <sup>a</sup> 23.8 ± 1.2 | A | <sup>a</sup> 25.7 ± 1.6 | B | <sup>a</sup> 26.3 ± 1.6  | B | 0.002  |
| S3M   | <sup>c</sup> 16.1 ± 1.3 | A | <sup>b</sup> 21.0 ± 1.2 | B | <sup>b</sup> 23.9 ± 1.0  | C | <0.001 |
| S4M   | <sup>d</sup> 11.0 ± 1.4 | A | <sup>d</sup> 12.9 ± 1.0 | B | <sup>de</sup> 16.2 ± 1.1 | C | <0.001 |

A, B, C: There is no difference between light sources with the same character for each color



**Fig. 1.** Mean standard deviation graph of light sources defining colors

## DISCUSSION

The hypothesis of the present in vitro study was accepted. The bond strengths of the CAD-CAM feldspathic porcelain samples of nine different colors and using three different light sources were evaluated and found to be different.

In clinical applications, the adhesive cementation of all-ceramic restorations can be

performed using resin-based adhesive cements that are polymerised by light, by chemicals or by both. The advantages offered by the light-cured resin cements are that they can be easily cleaned, have different opacity and color choices, have a long working time and allow the physician to polymerise when he wants.<sup>22-25</sup> Dual-cured resin cements allow for the completion of the polymerisation with chemical initiators when the

light transmission is limited. The polymerisation starts with light and then continues chemically, even if there is not enough light transmission. The most important disadvantage of dual-polymerised resin cements when compared to light-polymerised resin cements is that the color stability is worse. This is due to the aromatic tertiary amine present in the dual-polymerised resin cement, which reacts with benzoyl peroxide to initiate the chemical reaction. To achieve acceptable color stability, the initiation of the polymerisation by means of reacting with camphorquinone represents a better approach.<sup>20,21,26,27</sup> However, the use of dual-cured resin cements is recommended when the restoration thickness exceeds 2 mm<sup>28,29</sup>, since the degree of polymerisation of light-cured resin cements determines the extent of the restoration. The thickness of the ceramic used in this study was set at 2 mm because that value represents the highest thickness at which resin cement can be successfully polymerised by light, which we wanted to test.

The color, thickness and light application time of the restoration are important factors that all affect each other during cementation. In light-curing resin systems in particular, the darkening of the restoration color significantly reduces the polymerisation depth of the resin. Duran and Güler<sup>30</sup> used 12 different colors and three different thicknesses of zirconium samples, and they reported that when the color increased, the light transmission decreased. Palta *et al.*<sup>31</sup> showed that, for all the monolithic zirconia specimens, the light transmission values decreased in the order of 0.5>1.0>2.0 mm in all the color groups and in the order of A1>A2>A3>A4 in all the thickness groups. The researchers, therefore, noted that the light transmission was affected by the color and the thickness of the monolithic zirconia.

Soares *et al.*<sup>32</sup> reported that the effect of the ceramic restoration shade was less significant than that of the thickness when they compared different thicknesses (1 mm, 2 mm and 4 mm) and different shades (A1, A2, A3, A3 and A3.5). Peixoto *et al.*<sup>33</sup> found a significant difference in the light transmittance between the lightest and darkest colors of each group in the A, B and C color groups

of the Vita color scale at 1.5 mm and 2 mm ceramic thicknesses. In this study, the highest bonding values were obtained in the S2M and S2T color groups regardless of the light device used, and no statistically significant difference was found between these groups. The lowest resistance was obtained in the S4O color group. Kılınc *et al.*<sup>34</sup> used four different colors and four different ceramic thicknesses to test the light transmission, and they concluded that when the color became darker, the passage of light was reduced. Cardash *et al.*<sup>35</sup> found that the light transmittance decreased as the color became darker when using ceramic samples of 2 mm thickness. In this study, parallel results were obtained, since as the color became darker, the bonding values decreased.

In studies using samples of feldspathic and compressible ceramics, the thickness and color of all the ceramic restorations are referred to as the light-polymerising agent, which affects the degree of polymerisation. Generally, in the case of thicker and darker restorations, the power of the available light source is of the utmost importance in terms of achieving the optimal polymerisation of the material. The minimum light output power required to achieve the activation of camphorquinone is 280–300 mW/cm<sup>2</sup>.<sup>36</sup>

In this study, light sources with output powers ranging from 800–2400 mW/cm<sup>2</sup> were used. With regards to the comparison of the different color groups for the QTH light device, the highest bond strengths were obtained in the S2T and S2M groups, and no statistically significant difference was found between these groups. The lowest value was obtained in the S4O groups. For the LED light device, the S2T and S2M groups showed the highest bond strengths, and there was no statistically significant difference between them. The next highest bond strengths were found in the S3T and S3M groups, and there was no significant difference between them. The S4O groups showed the lowest bonding resistance. In terms of the PAC device, the S4O groups showed the lowest bond strength, while the S2T, S3T and S2M groups showed the highest bond strengths, and there was no statistically significant difference between any of these groups. The polymerisation efficiency was

increased for more color groups when the output power was increased. Further, when compared to the other light sources, in the case of all the colors, the PAC polymerisation unit showed the highest light transmission values.

Duran and Güler<sup>30</sup> evaluated the light transmission using halogen, LED-1, LED-2, Bluephase and PAC light units. There were statistically significant differences identified among the transmitted light powers. The highest light transmission of all the groups was observed with the PAC light unit, while the lowest was observed with the halogen lamp. The Bluephase light unit exhibited higher light transmission than the LED-2, while the LED-2 exhibited higher transmission than the LED-1. Rasetto *et al.*<sup>36</sup> examined the polymerisation of resin cement under a ceramic veneer, and they found that high output power lamps demonstrated more effective polymerisation under a ceramic veneer than conventional halogen lamps. The inadequate polymerisation of resin cement under a restoration may adversely affect the mechanical properties and the dimensional stability of that restoration.<sup>37,38</sup> In this study, the bonding values were found to be higher in the light devices with high output power.

The bond strength of the feldspathic ceramic samples used in this study was directly related to the light source. The PAC light source showed the highest values in this regard. The conventional QTH lamp, which had a lower value than the other polymerisation units in terms of the output power, proved to be the polymerisation unit that gave the lowest bonding value under the ceramic. This study further found that the bonding value decreased as the color became darker, without distinguishing the light device. The findings of similar prior studies support these results.<sup>30-32,34</sup> According to the results of this study, light units with higher output power may be preferred in order to compensate for the reduced light transition observed in relation to high chroma values. Where light devices with higher output power cannot be used, rather than light-cured resin cements, it may be advisable to use dual-cured resin cements, in which the polymerisation begins with light and then continues chemically. To confirm the findings of

this study concerning the feldspathic ceramics used in the CAD-CAM system and to extend them to other ceramic types currently used in the clinic, additional studies are needed in this area.

## CONCLUSIONS

Bearing in mind the limitations of this study, it can be asserted that the bond strength of feldspathic ceramic restorations is directly related to the light source and, further, that the bond strength decreases as the ceramic color becomes darker. Based on these results, it appears that the use of dual-polymerised resin systems may prove safer than the use of light-cured resin systems, which are polymerised by light in dark restorations of 2 mm thickness. Further studies are needed to understand that this study on feldspathic ceramics used in CAD-CAM system gives similar results to other types of ceramics currently used in the clinic.

## ACKNOWLEDGMENTS

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## CONFLICTS OF INTEREST STATEMENT

The authors report no conflicts of interest.

### *Farklı Renklerdeki Cerec Feldspatik Blokların Farklı Işık Kaynakları Kullanılarak Rezın Simanla Olan Bağlantısının Değerlendirilmesi*

## ÖZ

**Amaç:** Bu çalışmanın amacı farklı renklerdeki CAD-CAM üretim tekniğinde kullanılan prefabrike feldspatik seramik blokların, farklı ışık kaynakları altında rezın simanla olan bağlantısının değerlendirilmesidir. **Materyal ve Metot:** Çalışmada 2mm ( $\pm 0,1$  mm) kalınlıkta ve 9 farklı renk grubunda Cerec feldspatik seramik örnekler hazırlandı. Bütün örnekler Plazma ark ( $2200 \text{ mw/cm}^2$ ), LED ( $1450 \text{ mw/cm}^2$ ) ve halojen ( $1000 \text{ mw/cm}^2$ ) ışık kaynakları kullanılarak ışıkla sertleşen rezın simanla polimerize edildi. Simantasyondan 24 saat sonra makaslama testi uygulandı. Elde edilen sonuçlar istatistiksel olarak iki yönlü varyans analizi (ANOVA) ve Tukey HSD testi kullanılarak değerlendirildi ( $p < 0,05$ ). **Bulgular:** Çalışmanın sonuçları istatistiksel olarak değerlendirildiğinde, gruplar içerisinde plazma ark en yüksek ortalama değere sahipken, en düşük değer

halojendedir. Işık cihazı ayırt edilmeksizin en yüksek bağlantı dirençleri S2M ve S2T renk gruplarında elde edilmiş ve bu gruplar arasında istatistiksel olarak anlamlı bir farklılık bulunamamıştır. En düşük bağlantı direnci ise S4O renk grubunda elde edilmiştir. **Sonuç:** Yapılan bu in vitro çalışmanın sonucunda feldspatik seramik restorasyonların bağlantı direncinin kullanılan ışık kaynağı ile doğrudan ilişkili olduğu ortaya çıkmış ve seramik rengi koyulaştıkça bağlantı direnci düşmüştür. Çıkan sonuçlar ışığında, 2 mm kalınlığındaki koyu renkli restorasyonlarda ışıkla polimerize olan rezin siman sisteminin yerine dual olarak polimerize olan rezin sisteminin kullanımının daha güvenli olabileceği söylenebilir. **Anahtar Kelimeler:** CAD-CAM, rezin siman, bağlanma dayanımı

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## ASSESSMENT OF THE EFFECTIVENESS OF OZONE THERAPY AND AN ANTIBACTERIAL BONDING AGENT ON THE CAVITY DISINFECTION OF DECIDUOUS TEETH: AN IN VIVO STUDY

### ABSTRACT






**Objectives:** The aim of this study is to evaluate antibacterial effectiveness of 30 second ozone therapy applied via OzonyTronX, Clearfil Protect Bond (a MDPB containing bonding agent) and Dycal (Ca(OH)<sub>2</sub> containing cavity lining material) on *Streptococcus mutans* in deciduous teeth.

**Materials and Methods:** 40 primary molars were obtained from ten patients whose ages ranged between 5 and 11. Dentin samples which were collected before the treatment and after a period of four weeks following the implementation of materials were microbiologically evaluated and material's antibacterial effectiveness were compared.

**Results:** Differences between the antibacterial effectiveness of the materials were found to be statistically significant according to the results of covariance analysis with randomized block design (p<0.05). Antibacterial effectiveness of the groups on *S. mutans* is Group 2 (ozone therapy) > Group 3 (CPB) > Group 1 (Dycal).

**Conclusions:** Although CPB, which is an antibacterial self etching system, and ozone therapy do not increase the duration of clinical treatments, they can be efficient solutions for the restorative treatment of primary teeth.

**Key Words:** Ozone, MDPB, Calcium hydroxide, Anti-bacterial agents.

 Esra YEŞİLÖZ GÖKÇEN<sup>1</sup>  
 \*Merve AKSOY<sup>2</sup>  
 Ayşe Işıl ORHAN<sup>3</sup>  
 Berrin ÖZÇELİK<sup>4</sup>  
 Firdevs TULGA ÖZ<sup>5</sup>

ORCID IDs of the authors:

E.Y.G. 0000-0001-7144-976X

M.A. 0000-0003-1577-0289

A.I.O. 0000-0002-9559-0706

B.Ö. 0000-0003-0525-1186

F.T.Ö. 0000-0002-8731-5907

<sup>1</sup> Pediatric Dentist at Private Practice, Mc Lean, Virginia, USA.

<sup>2</sup> Ministry Of Health, Topraklık Oral Health Center, Ankara, Türkiye.

<sup>3</sup> Yıldırım Beyazıt University, Faculty of Dentistry, Department of Pedodontics, Ankara, Türkiye.

<sup>4</sup> Gazi University Faculty Of Pharmacy, Department of Pharmacy, Ankara, Türkiye.

<sup>5</sup> Ankara University Faculty Of Dentistry, Department of Pedodontics, Ankara, Türkiye.

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## INTRODUCTION

Removing infected dentine is a routine protocol for the treatment of dental caries but there is no certain way to be sure if there are bacterial remains on the cavity surface or not. Even if affected dentine has removed, bacterial component may have still existed by the depth of 0.1-2.4 mm from cavity floor, through the pulp tissue.<sup>1-4</sup> To prevent pulp tissue from bacterial invasion which may still exist in cavity after treatment or comes from microleakage between restorative filling materials and dentine tissue; cavity cleansing systems have been considered to be used in dental practice for a long time ago.<sup>5,6</sup>

Different kind of treatment methods can be used for this purpose and applying calcium hydroxide [Ca(OH)<sub>2</sub>] based cement on cavity floor is the oldest and most useful one of them. This material is a good choice due to its antibacterial activity and hard tissue forming capacity and thanks to that it is a gold standard for antibacterial treatment practices.<sup>7,8</sup>

On the other hand, Imazato<sup>9</sup> has developed MDPB (12-meth-acryloyloxydodecylpyridinium bromide) which is a resin monomer that has a strong antibacterial activity and doesn't effect the junctions between materials and dentine and inhibit bacterial growth.<sup>10,11</sup> MDPB is a compound of an antibacterial agent, quarternary ammonium with a methacryloyl group, and exhibits strong antibacterial activity against oral *Streptococci*. Among *Streptococcus* species, *Streptococcus mutans* is considered the chief etiological agent for causing dental caries. After that, this material started to be used in dentistry under the trade name of Clearfil Protect Bond.<sup>12</sup>

Other choice is ozone treatment that has been using in dentistry due to its antibacterial activity, new tissue forming capacity and the ability of stimulating healing. Ozone gas is the most effective anti-oxident agent in nature. It has been using due to its healing effect in medicine and there is no reported reverse effect and negative effect to bonding strengths and general health.<sup>13</sup> Previous

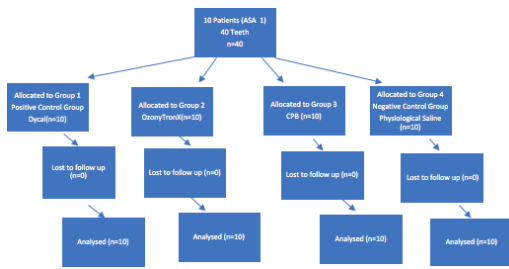
studies have shown that ozonized water and ozone gas reduce the total cultivable microbiota significantly in vitro.<sup>14-17</sup> Baysan *et al.*<sup>18</sup> found a significant reduction in *S. mutans* and *Streptococcus sobrinus* in the ozone-treated side of the root caries lesions compared with the control side.

There is a previous study in which antibacterial effectiveness of 30 seconds and 60 seconds ozone therapy applied via OzonyTronX, Clearfil Protect Bond (a MDPB containing bonding agent) and Dycal [Ca(OH)<sub>2</sub>] on *S. mutans* in deciduous teeth were evaluated in vitro.<sup>19</sup> But there is no any other study, in which these materials are compared applying deciduous teeth in -vivo. Therefore, the aim of this study is to compare antibacterial effectiveness of CPB (contains MDPB) and ozone therapy against *S.mutans* in deciduous teeth, in-vivo.

## MATERIALS AND METHODS

The study protocol was carried out according to the principles of the Helsinki Declaration, including all amendments and revisions. Collected data were only accessible to the researchers. Informed consent was obtained from all participants. Patients or their legal representatives gave their informed consent prior to any treatment of the teeth. The study was reviewed and approved by the institutional ethics board of Ankara University Faculty of Dentistry (no: 118/2; date:16.05.07).

A power analysis (Power and Precision software, Biostat, Englewood, NJ, USA) was conducted in order to determine an appropriate sample size based on previous studies. It indicated that detection of differences could be obtained with at least 10 teeth at a power of 0.8. Thus, this study was conducted using 40 deciduous teeth in total. 40 primary molars were obtained from ten patients whose ages ranged between 5 and 11 and whose all primary second molars were decayed. All decayed deciduous second molars were divided into four groups randomly according to CONSORT guideline and in that way, it could be possible to evaluate all groups in one patient's mouth (Figure 1).



**Figure 1.** Consort Diagram

**Group 1: Dycal (Dentsply/Caulk, Denstply International Inc. Milford, DE, ABD) -positive control group**

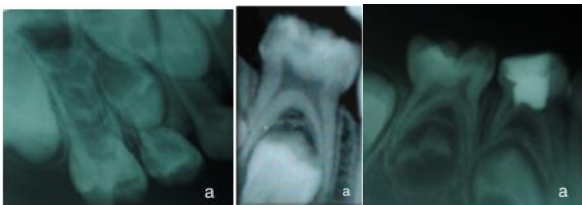
**Group 2: OzonyTronX (Mymed, Almanya)-30 second ozone therapy.**

**Group 3: CPB (Clearfil Protect Bond (CPB), Kuraray, Europe).**

**Group 4: Physiological Saline (PS)- Negative control group.**

In clinical examinations before treatment, the patients who are healthy as ASA classification (ASA 1) and whose teeth didn't have the symptoms of irreversible pulp degenerations, pathological or physiological mobility and positive response to percussion and palpation test were incorporated into study.

In radiological examinations, the criterias to select the appropriate patients for the study were; to have a healthy dentinal tissue between pulp and caries dentine and absence of external or internal root resorption. Also, surround bone tissue's health and condition of periodontal space and lamina dura were considered (Figure 2a, 3a, 4a).



**Figure 2a)** The radiography of right upper deciduous second molar.  
**3a)** The radiography of left inferior deciduous second molar.  
**4a)** The radiography of left inferior deciduous second molar.

Treatment sessions were held in an air conditioned clinic which has only one dental unit and rubber dam was used to avoid bacterial contamination.

All teeth surfaces except cavitated areas were cleaned with savlon solutions and washed with saline solution. After that, superficial surfaces of necrotic dentin were removed with sterile steel round burs (Figure 2b, 3b, 4b).



**Figure 2b)** After first session, removing superficial decayed dentine tissue.

**3b)** After first session, removing superficial decayed dentine tissue.

**4b)** After first session, removing superficial decayed dentine tissue.

Cavities were prepared by a depth of 2,5 mm to make enough place for compomer restorative materials. Remained dentin was cleaned with physiological saline solution and dried with cotton pellets. For microbiological assessment, dentine samples were collected with cooled burs from the middle of cavity floor. The samples were transferred to Gazi University Faculty of Pharmacy in Reduced Transport Fluid<sup>20</sup> within 2 hours.

### Study Groups and Control Groups:

#### Group 1:

Dycal was applied with sterile round handpieces on the remaining decayed dentine (Figure 2c).



**Figure 2c)** Applying Dycal on cavity floor.

After that, sterile sponge (VDW, Munich, Germany) was placed on the material (Figure 5) and cavity floor and the cavity were covered with

blue compomer (Twinky Star, Voco, Cuxhaven, Germany) (Figure 6a) which was polymerized with a light cure (Ultralume 5; Ultradent, S. South Jordan, UT, USA) during 20 second.

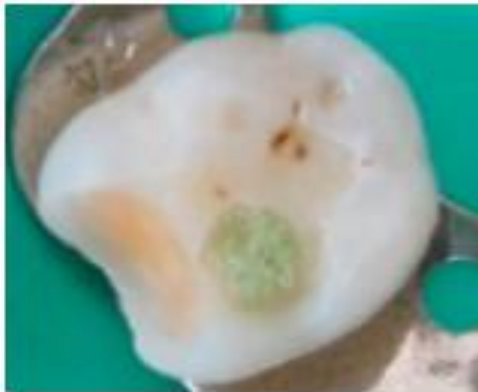


Figure 5. Placing sterile sponge on cavity floor.



Figure 6a) Dycal

**Group 2:**

OzonyTronX was applied for 30 seconds in forth degree with CA Prob (Figure 3c).



Figure 3c) Performing Ozone treatment.

After that, sterile sponge and pink compomer (Twinky Star, Voco, Cuxhaven, Germany) (Figure 6b) were used to fulfill the cavity.



Figure 6b) Ozony TronX Group

**Group 3:**

CPB, which contain MDPB was applied as one drop on the remaining decayed dentin, on cavity floor (Figure 4c).

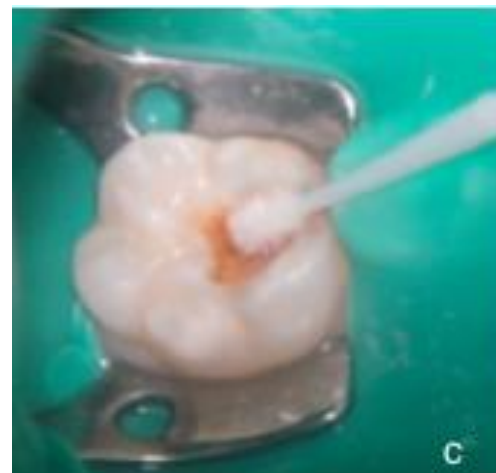


Figure 4c) Applying CPB on cavity floor.

After a period of 20 second, bonding agent was used and polymerized for 10 second. (Ultralume 5; Ultradent, S. South Jordan, UT, USA) Sterile sponge was placed on the cavity floor and green compomer (Twinky Star, Voco, Cuxhaven, Germany) was used to cover the cavity (Figure 6d).



Figure 6d) CPB Group

**Group 4:**

For negative control group, cavity floor was washed with P.S and sterile sponge was placed. Than, Orange compomer (Twinky Star, Voco,

Cuxhaven, Germany) was used for cavity restoration (Figure 6c).



Figure 6c) Control Group

After a period of four weeks, compomer restorations were removed from the cavities by means of a sterile diamond bur without contacting the dentine on cavity walls. Then, the dentine samples were collected for a second time by sterile steel round burs. A total CFU count was obtained through a culture (MSA plates) of dentine samples collected from each group. Counts below 20 CFU were below the limits of detection and were recorded as 0 (undetectable). All microbiological processes were carried out by a microbiologist experienced in oral microbiology for microbiological assessments. The samples were transferred to Gazi University Faculty of Pharmacy within 2 hours.

Finally, decayed dentine was removed completely and final restorations were performed (Figure 7).



Figure 7. After second session, complete cavity cleaning.

## RESULTS

The analyses of previous study's results were performed in Ankara University Faculty of Statistics by using "Statistical Package for the Social Sciences" software (SPSS 11.5 for windows, SPSS Inc., Chicago, Illionis, USA). Duncan Test was performed to determine the differences between groups and Kovaryans Analyses were performed before treatment. Wilcoxon test was used before and after treatment. P-values of less than 0.05 were considered to be statistically significant.

Bacterial assessment of collected tissue before treatment were shown in Table 1 and Figure 8 ( $p < 0.05$ ).

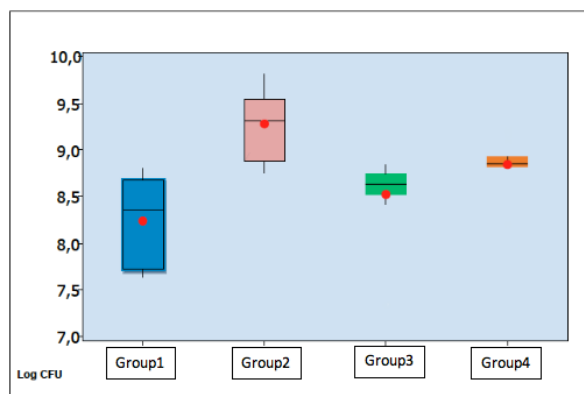


Figure 8. Microbial counts (log CFU/g dentine) in the cavities of all groups before treatment.

Table 1: Bacterial assessment (Log CFU) of collected tissue before treatment

| Group               | N  | Mean | Median | SD    | Min   | Max   |
|---------------------|----|------|--------|-------|-------|-------|
| Group 1: Dycal      | 10 | 8.23 | 8.355  | 0.465 | 7.633 | 8.799 |
| Group 2: OzonyTronX | 10 | 9.27 | 9.310  | 0.367 | 8.748 | 9.820 |
| Group 3: CPB        | 10 | 8.51 | 8.633  | 0.438 | 8.322 | 8.845 |
| Group 4: Control    | 10 | 8.83 | 8.854  | 0.261 | 8.704 | 8.904 |

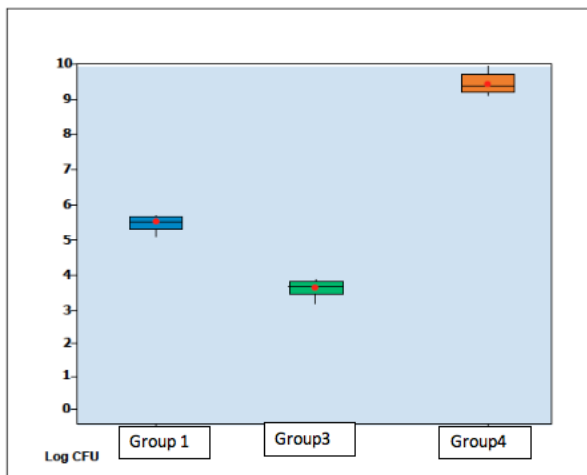
Differences between the antibacterial effectiveness of the materials were found to be statistically significant according to the results of the

covariance analyses with randomized block design. Bacterial assessment of collected tissue after treatment were shown in Table 2 ( $p < 0.05$ ).

**Table 2:** Bacterial assessment (Log CFU) of collected tissue after treatment

| Group                   | N         | Mean         | Median       | SD           | Min          | Max          |
|-------------------------|-----------|--------------|--------------|--------------|--------------|--------------|
| <b>Group 1: Dycal</b>   | <b>10</b> | <b>5.463</b> | <b>5.516</b> | <b>0.222</b> | <b>5.079</b> | <b>5.699</b> |
| <b>Group 3: CPB</b>     | <b>10</b> | <b>3.621</b> | <b>3.648</b> | <b>0.219</b> | <b>3.204</b> | <b>3.863</b> |
| <b>Group 4: Control</b> | <b>10</b> | <b>9.445</b> | <b>9.388</b> | <b>0.266</b> | <b>9.113</b> | <b>9.934</b> |

Differences between group 1 were statistically significant from group 2, group 3, group 4. Group 2 was statistically significantly different from group 3 and group 4. Group 3 was statistically significantly different from group 4. Bacterial assessment of the collected tissue after treatment were Group 4 (control) > Group 1 (Dycal) > Group 3 (MDPB) > Group 2 (OzonyTronX).

**Figure 9.** Microbial counts (log CFU/g dentine) in the cavities of all groups after treatment.

As result, end of the treatment, an increase was seen in the amount of microorganisms of group 4 while all the others showed decrease. Which means all the materials, except saline solution which was control group, has an effect on eliminating bacterial growth in dentinal tissue and they can be used as cavity cleansing systems in dental practices. Previous study shows that Ozone is the most effective way to protect dentinal tissue from bacterial remains.

## DISCUSSION

It is always questionable if cavity preparation and removing infected dentine is a sufficient way to prevent pulp tissue from bacterial invasion or not.<sup>21-23</sup> To prevent the pulp tissue, lots of cleansing systems and antibacterial agents have been used and Ca(OH)<sub>2</sub> is one of them and the most acceptable one due to its antibacterial effect and remineralization activity.<sup>24</sup> Thus, Dycal was

After treatment, in ozone group, the bacterial counts were below 20 CFU, below the limits of detection and were recorded as 0 (undetectable). Hence, we couldn't show the values of bacterial assessment of ozone group in the chart (Figure 9).

chosen as positive control group. Antimicrobial monomer, MDPB has been developed in recent years, which has very strong bactericidal activity against oral micro-organisms and reported against *S. mutans*.<sup>12</sup>

Application of ozone (OzonyTronX) has also been proposed due to its antibacterial activity.<sup>25,26</sup> In that study, it was aimed to evaluate the antibacterial activity of these two materials in vivo. Sterile saline was chosen as negative control group. Patients whose ages differs between 5 and 11, whose all primary molars are still exist and who is systematically healthy were chosen for the study. Thanks to that method, it was possible to compare all the groups in one patient's mouth. Rubber dam, which is a standard way to protect tooth from oral microflora in dental treatments, was used to avoid bacterial contamination.<sup>27</sup> The superficial surfaces of necrotic dentin were removed only to determine the antibacterial effectiveness of test materials.<sup>23</sup>



Dentin samples were collected by the burs assembled to a slowly rotating micromotor.<sup>1</sup> The burs were kept at -25 °C degree to eliminate the formation of heat.<sup>28,29</sup> Collected samples was at a quantity to fill the grooves of the burs. This is also an appropriate and standard way which is used in lots of studies.<sup>1,22,27</sup>

Sterile sponges were applied to prevent dentin in second session. Compomer, which is most common restorative material using in pediatric dentistry was chosen as the restorative material to distinguish between groups.<sup>30,31</sup>

The period takes between two treatment session was decided as four weeks which is suitable for the studies that is about antibacterial activity of materials.<sup>32</sup> After four weeks period, patients asked about pain and sensitivity. The remaining caries were cleaned and final restorations were performed.

As a result of the study, except saline group, in all the others, there were a decrease in bacterial population in treated samples.

These results are similar to previous study which was held on cavity models in vitro. In which, a tooth cavity model was designed on cylindrical cavities created in 90 deciduous second mandibular molars. In that study, the antibacterial effectiveness of ozone therapy -60 seconds, ozone therapy -30 seconds, MDPB, MDP and Ca(OH)<sub>2</sub> were compared. *S. mutans* suspensions were inoculated in the cavities. The teeth distributed into six study group (five studying groups and one control group). Dentine samples, which were collected from the cavities before and after treatment sessions were microbiologically evaluated and antibacterial effectiveness of ozone therapy -60 seconds found statistically higher than ozone therapy-30 seconds, MDPB, MDP and Ca(OH)<sub>2</sub>.<sup>19</sup>

Hauser-Gersparch *et al.*<sup>33</sup> held a study in which immediate effects of gaseous ozone and chlorhexidine gel were compared on bacteria in cavitated carious lesions in children. 30 second gaseous ozone was applied. In the end of the study, nor ozone gas neither chlorhexidine gel were found to be efficient in reducing microorganisms in open

occlusal carious lesions. This results conflict with this study.

Atabek and Oztas<sup>34</sup> held another study to evaluate the efficiency of ozone alone and with a remineralizing solution following application on initial pit and fissure caries lesions in permanent molars. 40 second ozone gas was applied. In the end, compatibly with this study results, ozone treatment either alone or combined with a remineralizing solution was found to be effective for remineralization of initial fissure caries lesions.

## CONCLUSIONS

The findings of this study indicate that ozone treatment could be considered to exert an antibacterial effect in the treatment of deciduous teeth. Conceiving that Ozone Therapy Systems could not be found easily in every dental offices, MDPB containing CPB could be a good choice for cavity disinfection process. However, further research on the long-term effects of ozone on micro-organisms, and a more detailed comparison of ozone with dentine-bonding systems and Ca(OH)<sub>2</sub>, is necessary.

## ACKNOWLEDGEMENTS

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## CONFLICTS OF INTEREST STATEMENT

All authors declare no conflict of interest.

### *Ozon Tedavisi ve Bir Antibakteriyel Bonding Ajanın Süt Dişi Kavite Dezenfeksiyonundaki Etkinliğinin İn-Vivo Olarak Değerlendirilmesi*

#### ÖZ

**Amaç:** Bu çalışmada, OzonyTronX cihazı ile 30 s ozon tedavisi, MDPB içerikli CPB ve Ca(OH)<sub>2</sub> içerikli bir pat olan Dycal'ın *S. mutans* üzerine anti bakteriyel etkinliğinin süt dişlerinde in vivo koşullarda değerlendirilmesi amaçlanmıştır. **Gereç ve Yöntemler:** Yaşları 5 ile 11 arası değişen 10 çocuk hastanın dentin çürüğü bulunan 40 adet II. süt azı dişi çalışmaya dahil edilmiştir. Tedavi öncesi toplanılan dentin örnekleri ile materyallerin uygulanmasının ardından 4 hafta sonra toplanılan dentin örnekleri mikrobiyolojik olarak değerlendirilmiş ve anti bakteriyel etkinlikleri karşılaştırılmıştır. **Bulgular:** Grupların *S. mutans* üzerinde antibakteriyel etkinliklerinin başarı sıralamaları şu şekildedir: Grup 2 (Ozon tedavisi)>

*Grup 3 (CPB) > Grup 1(Dycal). Sonuçlar: Çalışma sonucunda; anti bakteriyel bir self-etching sistem olan CPB'nin ve ozon tedavisinin klinik uygulamalara ek bir süre getirmediği gibi, süt dişlerinin restoratif tedavilerinde etkin bir çözüm olabileceği kanısına varılmıştır. Anahtar Sözcükler: Anti bakteriyel etki, kalsiyum hidroksit, ozon, MDPB, kavite dezenfeksiyonu.*

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## EFFECT OF DIFFERENT SURFACE TREATMENTS ON THE REPAIR OF AGED BULK-FILL COMPOSITES: AN IN VITRO STUDY

### ABSTRACT

**Objectives:** The aim of this *in vitro* study was to evaluate the efficiency of different surface treatments on the microtensile bond strength ( $\mu$ TBS) of aged bulk-fill composite.

**Materials and Methods:** Sixty bulk-fill resin-based composite (RBC) specimens in 5 x 5 x 5 dimensions were prepared. After the aging by thermal cycling for 5000 times between 5 and 55°C, substrate surfaces were abraded with SiC abrasive papers. Specimens were divided into 6 groups according to the surface treatment protocol: no surface treatment (control), control + Single Bond Universal (SBU; 3M ESPE) application, phosphoric acid etching (PA) + SBU, hydrofluoric acid etching (HF) + SBU, aluminum oxide air abrasion (AIO) + SBU, and tribochemical silica coating (TSC) + SBU. Surface roughness values were measured in five different directions using a contact profilometer (n=10). Then, specimens were repaired with a conventional RBC. After the repair, bonded specimens were cut into 1 mm<sup>2</sup> beams and  $\mu$ TBS values were determined until failure at a crosshead speed of 0.5 mm/min. Specimen surfaces after surface treatments were observed by SEM. Data were analyzed using ANOVA and Tukey tests ( $p<0.05$ ).

**Results:** One-way ANOVA revealed significant difference ( $p<0.001$ ) among the surface treatments. The lowest repair  $\mu$ TBS values were observed for the control group. SBU application alone significantly improved repair  $\mu$ TBS values ( $p<0.001$ ). The highest  $\mu$ TBS values were obtained for the AIO and TSC, and HF followed. The surface roughness ranking for the five surface treatment protocols was as follows: TSC > AIO > HF > PA = Control.

**Conclusions:** Aged bulk-fill RBCs can be successfully repaired if effective and safe repair protocol is chosen. The highest  $\mu$ TBS values were obtained for the AIO and TSC. The use of universal adhesive alone is promising to facilitate the repair of bulk-fill RBCs.

**Keywords:** Composite resins, dental restoration repair, dental adhesives, surface properties.

 Soner ŞİŞMANOĞLU<sup>1</sup>

ORCID IDs of the authors:  
S.Ş. 0000-0002-1272-5581

<sup>1</sup> Department of Restorative Dentistry, Faculty of Dentistry, Altınbaş University, Istanbul, Turkey.

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## INTRODUCTION

The application of resin-based composite (RBC) restorations in the posterior region increases in parallel with the development of restorative materials. One of the most important problems of RBCs is polymerization shrinkage and its associated stress. Due to the polymerization shrinkage, loss of adhesion may occur at the tooth-restoration interface, which results in nano-leakage causing secondary caries.<sup>1,2</sup> In order to prevent the undesirable effects of polymerization shrinkage, the use of incremental technique is required when applying the RBC to the cavity. According to this technique, maximum of 2-mm thick RBC layers are applied to the cavity.<sup>3</sup> The polymerization of each layer separately with light and the polymerization time varying between 20 to 40 seconds prolong the duration of the restorative procedure. On the other hand, the application of RBCs is a process requiring technical precision and adequate isolation. The adaptation and light-curing of each layer with incremental technique increases the risk of contamination, which may adversely affect restoration success.<sup>4</sup> Thus, the use of RBCs is time-consuming and requires more technical precision, especially in the posterior region. Therefore, the demand for relatively easy-to-use materials, such as amalgam, which can be placed to the cavity as a single bulk increment to overcome the application difficulty, the risk of contamination, the possibility of leaving air gaps between the increments, and the time consumption of the incremental technique<sup>4</sup> led to the development of bulk-fill RBCs. This novel material that exhibits controlled polymerization shrinkage, acceptable degree of conversion and micro hardness<sup>5</sup> when placed in a single bulk increment have been introduced with a wide range of products to the dental market.<sup>6</sup>

Bulk-fill RBCs are a new type of composite produced by the customization of resin monomers, photo-initiators and filler particles. The most important feature of bulk-fill RBCs is that they can be used as bulk with a depth of cure up to 5 mm.<sup>7</sup> Unlike bisphenol A-glycidyl methacrylate (Bis-GMA) based resin composites, higher molecular weight monomers are used to compensate the degree of conversion expected to decrease at the claimed layering depth.<sup>8,9</sup> The approach of reducing the size and increasing the proportion of filler particles in conventional RBCs is

modified in bulk-fill RBCs<sup>10,11</sup>, and composites with reduced filler ratios are produced.<sup>12</sup> Due to the increased filler particle size, the filler and resin matrix interface is reduced, which results in less scattering and more penetration of the curing light. Moreover, adequate polymerization depth of bulk-fill RBCs in deep cavities is also achieved by increasing their translucency.<sup>10</sup> However there are some concerns on their clinical performance regarding the bond strength.<sup>13</sup>

RBCs have a limited longevity with an average of 2.2% annual failure rate regardless of their extensive use.<sup>14</sup> In a recent meta-analysis, researchers reported that there was no significant difference between bulk-fill RBCs and conventional RBC regarding their clinical performances.<sup>15</sup> Many researchers have suggested that composite restorations should be repaired, and in such cases, the bond strength is within clinically acceptable limits that after the repair.<sup>16-20</sup> Total replacement may result in the removal of sound dental substances and unnecessary expansion of the existing preparation. In deep cavities, a total replacement may lead to unnecessary trauma to the dental pulp. In addition, restoration repair is less time-consuming and cost-effective than total replacement. The most common restorative material in dental practice is conventional composites.<sup>21</sup> In this case, bulk fill composite materials may need to be repaired with conventional composites. As bulk-fill composites are increasingly preferred, there is a need for a procedure for repairing these modified RBCs. Therefore, the aim of this *in vitro* study was to evaluate the efficiency of different surface treatments on the microtensile bond strength ( $\mu$ TBS) of aged bulk-fill RBC repair using a conventional RBC. The null hypotheses were: (1) type of surface treatment protocol would not influence the repair  $\mu$ TBS values and (2) type of surface treatment protocol would not influence the surface roughness values of bulk-fill RBC.

## MATERIALS AND METHODS

### Specimen preparation

This *in vitro* study was performed in accordance with the Helsinki Declaration. Sixty specimens were prepared for this *in vitro* study in a Teflon®

mold (5 x 5 x 5 mm). The chemical compositions of the materials are given in Table 1.

**Table 1.** Material, batch number, composition, and application of the universal adhesive

| Restorative   | Composition                             | Filler Type   |  |
|---|---|---|--|
| Filtek™ Bulk Fill Restorative (3M ESPE, St. Paul, MN, USA) #N681830         | UDMA, Bis-GMA, EBPADMA, Procrylat resin | Filler loading is 42.5% by weight and 64% by volume.<br>Zirconia/silica, ytterbium Trifluoride                  |  |
| Filtek Ultimate Universal Restorative (3M ESPE, St. Paul, MN, USA) #N817010 | Bis-GMA, UDMA, TEGDMA, Bis-EMA, PEGDMA. | Filler loading is 78.5% by weight and 63.3% by volume.<br>20 nm silica particles and 4-11 nm zirconia particles |  |
| Adhesive  | pH                                      | Composition   | Application  |
| Single Bond Universal (3M ESPE, St. Paul, MN, USA) #80516B                  | 2.7                                     | 2-HEMA, 10-MDP, dimethacrylate resins, Vitrebond™ copolymer, silane, filler, ethanol, water, initiators.        | 1. Apply the adhesive to the prepared tooth and rub it in for 20 s.<br>2. Gently air dry the adhesive for approximately 5 s to evaporate the solvent.<br>3. Light cure for 10 s. |

Abbreviations: 2-HEMA, 2-hydroxyethyl methacrylate; 10-MDP, 10-methacryloyloxydecyl dihydrogen phosphate; Bis-EMA, ethoxylated bisphenol-A dimethacrylate; Bis-GMA, bisphenol A glycidyl methacrylate; EBPADMA, ethoxylated bisphenol A dimethacrylate; PEGDMA, poly(ethylene glycol) dimethacrylate.

The bulk-fill RBC was loaded into the molds as a single increment (5 mm bulk) and covered with a Mylar strip to obtain flat surface. Then, the bulk-fill RBC was light-cured using a light emitting diode (LED) curing unit (Valo Grand; 1000 mW/cm<sup>2</sup>; Ultradent, South Jordan, UT, USA) for 20 s, and the light intensity was controlled before each specimen. After the specimen removal from the mold, the specimen was further polymerized for 20 s/all surfaces to ensure adequate polymerization since the objective of the current study was not to measure the depth of cure. All samples were kept in distilled water at 37°C for 24 h, and then polymerized specimens were aged by thermal cycling for 5000 cycles between 5 and 55°C with a dwell time of 30 s and a transfer time of 5 s. The aged bulk-fill RBC surfaces were wet-ground flat with 320-grit abrasive paper<sup>22</sup>, and divided into 6 groups according to the surface treatment protocols before repair (n=10).

Group 1: No treatment, control group.

Group 2: Single Bond Universal (SBU; 3M ESPE, St. Paul, MN, USA) adhesive was applied on non-treated bulk-fill RBC surfaces using the self-etch mode according to the manufacturer's recommendations.

Group 3: Bulk-fill RBC surfaces were etched with 37% phosphoric acid (PA, Scotchbond Etchant; 3M ESPE, St. Paul, MN, USA) for 20 s, and rinsed thoroughly.

Group 4: Hydrofluoric acid (HF; 9%, Ultradent) was used for etching the bulk-fill RBC surfaces for 20 s, and rinsed thoroughly.

Group 5: Bulk-fill RBC surfaces were air-abraded using aluminum oxide (AlO) particles (Cobra; 50 µm, Renfert GmbH, Hilzingen, Germany). Air abrasion procedures were performed using a sandblaster (Basic Quattro IS, Renfert GmbH, Hilzingen, Germany) 10 mm above from the RBC surface at 2.5 bar pressure.

Group 6: Tribochemical silica coating (TSC) was performed on the bulk-fill RBC surfaces with silica coated aluminum oxide sand (CoJet Sand; 30 µm, 3M ESPE, Seefeld, Germany) using the sandblaster 10 mm above from the RBC surface at 2.5 bar pressure.

After each surface treatment protocol, the specimens were cleaned in an ultrasonic bath with distilled water for 5 min and air-dried with an air syringe.

**Surface Roughness Measurement**

A contact profilometer (Surtronic S128, Taylor Hobson Ltd., Leicester, England) with a 5 µm diamond stylus was used for the surface roughness measurements. Five consecutive measurements were taken from different directions, with a 0.25 mm cut-off length. The average surface roughness values (Ra) were then recorded in µm (n=10).

**Repair of the Bulk-fill RBC**

After the completion of surface roughness measurements, SBU was applied on surface-treated bulk-fill RBC surfaces according to the manufacturer’s recommendations and light cured for 10 s. A conventional RBC (Filtek Ultimate Universal A3.5; 3M ESPE, St. Paul, MN, USA) was used as a repair material, and A3.5 shade was chosen to distinguish the bulk-fill RBC from the repair RBC. The bulk-fill RBCs were placed in a Teflon® mold (10 x 5 x 5 mm) treated surface facing upwards for repair protocol. The repair RBC (5 x 5 x 5 mm) was applied in 2-mm thick increments. Each increment was polymerized for 20 s, and specimens were kept in distilled water at 37°C for 24 h following the repair.

**Microtensile Bond Strength Testing**

Repaired specimens were longitudinally sectioned using a low-speed saw (Isomet; Buechler, Lake Bluff, IL, USA) to obtain 1 mm<sup>2</sup> beams. Beams in the outer parts of repaired specimens were discarded. Consequently, beams were fixed to jigs of a microtensile testing device (MOD Dental, Esetron Smart Robototechnologies, Ankara, Turkey) with cyanoacrylate adhesive. The tensile load was applied until failure at a crosshead speed of 0.5

mm/min, and recorded in MPa. Failure modes were evaluated by a stereomicroscope at 30x magnification. The failure modes categorized as adhesive failure (A), cohesive failure within substrate (C<sub>sub</sub>), cohesive failure within repair (C<sub>rep</sub>), or mixed failure (M). Pretest failures were excluded from the statistical analysis of the µTBS values.

**Scanning Electron Microscopy Observation**

For scanning electron microscope (SEM) observations, two samples were prepared for each of the five surface treatment groups as described before (in total, 10 specimens). The specimens were sputter-coated with gold (Polaron SC7620 sputter coater, ThermoVG Scientific), and were examined under a SEM (JEOL 5500; JEOL Inc., Peabody, MA, USA) at 10 kV accelerating voltage. Observations were performed under x1000 magnification.

**Statistical Analysis**

The mean values and standard deviations were determined, and analyzed using a one-way ANOVA, to evaluate the effects of surface treatment protocols on the repair µTBS and surface roughness values. Pairwise analyzes were performed using Tukey HSD post-hoc analysis. Data analyzes was performed by SPSS for Windows v22 software (IBM Corp., Armonk, NY, USA), and results were considered as statistically significant for *p*<0.05.

**RESULTS**

**Microtensile Bond Strength Test**

Mean µTBS values and standard deviations are given in Table 2.

**Table 2.** Mean repair µTBS values and failure mode distribution according to the surface treatments

| Treatment              | Mean ± SD                | n  | Failure Modes (%) |                  |                  |      |
|------------------------|--------------------------|----|-------------------|------------------|------------------|------|
|                        |                          |    | A                 | C <sub>Sub</sub> | C <sub>Rep</sub> | M    |
| No treatment (Control) | 12.1 ± 3.47 <sup>a</sup> | 31 | 87.1              | 0                | 0                | 12.9 |
| Control + SBU          | 25.8 ± 4.36 <sup>b</sup> | 36 | 77.8              | 10               | 0                | 22.2 |
| PA + SBU               | 26.8 ± 3.72 <sup>b</sup> | 38 | 58                | 10.5             | 0                | 31.5 |
| HF + SBU               | 33.3 ± 2.23 <sup>c</sup> | 36 | 55.6              | 11.1             | 11.1             | 22.2 |
| AIO + SBU              | 42.9 ± 3.79 <sup>d</sup> | 38 | 26.4              | 21               | 21               | 31.6 |
| TSC + SBU              | 45.5 ± 3.83 <sup>d</sup> | 37 | 24.4              | 32.4             | 10.8             | 32.4 |

Different superscripts indicate significant differences (*p*<0.05). A, Adhesive failure; C<sub>sub</sub>, Cohesive failure within substrate; C<sub>rep</sub>, Cohesive failure within repair; M, Mixed failure.

One-way ANOVA exhibited significant differences (*p*<0.001) among five surface treatment protocols regarding the µTBS values (Table 2). The significantly lowest repair µTBS values were

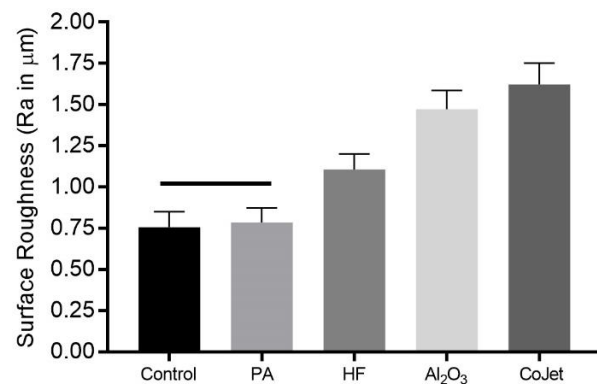
achieved by the control group (12.1 ± 3.47 MPa), in which no universal adhesive was applied. On the other hand, universal adhesive application only (Group 2) was significantly increased repair µTBS

values ( $p < 0.001$ ). The highest  $\mu$ TBS values were obtained for the Group 6 ( $45.5 \pm 3.83$  MPa) and the Group 5 ( $42.9 \pm 3.79$  MPa;  $p < 0.05$ ), which did not show significant difference ( $p > 0.05$ ), and HF etching (Group 4;  $33.3 \pm 2.23$  MPa) was followed them. Although the PA etching (Group 3) slightly increased the repair  $\mu$ TBS values compared to the Group 2. There was no significant difference between Group 2 ( $25.8 \pm 4.36$  MPa) and Group 3 ( $26.8 \pm 3.72$  MPa), and showed lower repair  $\mu$ TBS values than Group 4 ( $p < 0.05$ ).

In terms of failure mode, most of the groups exhibited adhesive failure mode (Table 2). Although cohesive failure was not observed in the control group, there was a clear increase in the number of cohesive and mixed failure modes after surface treatment protocols. The highest adhesive failure rate was observed for Group 6 (30% for  $C_{Sub}$  and 10% for  $C_{Rep}$ ) and Group 5 (20% for  $C_{Sub}$  and 20% for  $C_{Rep}$ ).

### Surface Roughness Measurement

The surface roughness values according to the surface treatment protocols are shown in Figure 1.

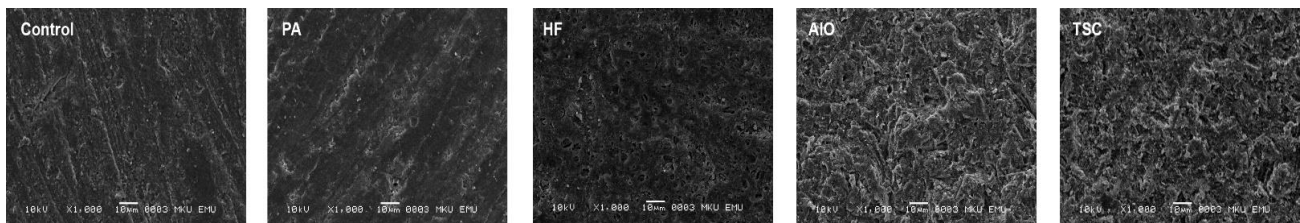


**Figure 1** Surface roughness means and standard deviations according to five different surface treatments. Horizontal line above the columns indicates nonsignificant difference ( $p > 0.05$ )

PA etching (Group 3) of the bulk-fill RBC surfaces did not differ significantly from the control group ( $p = 0.975$ ). However, other surface treatments resulted in rougher surfaces than the control group and Group 3 ( $p < 0.001$ ). The highest surface roughness values were found in the TSC treatment ( $p < 0.05$ ). The ranking of the five surface treatment protocols regarding the surface roughness from highest to lowest were as follows: TSC ( $1.62 \pm 0.13$ ) > AIO ( $1.47 \pm 0.11$ ) > HF ( $1.11 \pm 0.09$ ) > PA ( $0.78 \pm 0.09$ ) = Control ( $0.78 \pm 0.10$ ).

### SEM Observation

SEM images of the bulk-fill RBC surfaces according to the surface treatments are presented in Figure 2.



**Figure 2** SEM images of the bulk-fill RBC after five different surface treatment protocols. Control, No treatment group; PA, Phosphoric acid etching; HF, Hydrofluoric acid etching; AIO, Aluminum oxide air abrasion; TSC, Tribochemical silica coating.

The characteristic image of the surface grinding with 320-grit SiC paper can be identified (Figure 2, Control). Distinct deep grooves and smear debris produced by the grinding motion can be noticed. SEM image (Figure 2, PA) showed that the smear on the bulk-fill RBC surface was slightly removed after PA etching. HF etching effectively removes smear debris on the bulk-fill RBC surface and exposes filler particles (Figure 2, HF). The increase in surface roughness and prominent ridges after AIO air abrasion and TSC treatments were observed in SEM images (Figure 2, AIO and TSC).

### DISCUSSION

The RBC restorations have been reported to have a successful clinical performance with a failure rate ranging between 1.6%<sup>23</sup> to 2.2%<sup>14</sup> per year. The repaired RBC restorations is shown to have an annual failure rate of 5.7% during 4 years of clinical use.<sup>24</sup> In a recent meta-analysis, Veloso *et al.*<sup>15</sup> reported that there was no significant difference in clinical performance between bulk-fill and conventional RBCs. The clinical adequacy of the bond strength between the old and new materials is crucial for durability of the repair. Therefore, in this *in vitro* study, the effect of

different surface treatments on the repair of bulk-fill RBCs was evaluated. The results of the present study showed that the lowest repair  $\mu$ TBS values were found in the no treatment (control) group. Surface treatments effectively improved bond strength values in agreement with the previous studies.<sup>16–20,25,26</sup> Thus, the first hypothesis – that type of surface treatment protocol would not influence the repair  $\mu$ TBS values – was rejected, as there was a significant difference between the repair  $\mu$ TBS values between the surface treatment protocols. The second hypothesis – that type of surface treatment protocol would not influence the surface roughness values – was also rejected, because some surface treatments significantly differed from each other in terms of surface roughness values.

The bonding of the RBC increments to each other is achieved by covalent chemical bonds between the unreacted monomers of the first polymerized increment and the newly added one.<sup>27</sup> The oxygen inhibited layer, which is partially polymerized and has a low viscosity also favors to bonding.<sup>28</sup> Therefore, the conditions that affect the unreacted layer will affect the bond strength. However, RBCs are unstable after polymerization and continue to interact with the oral environment.<sup>29</sup> This interaction causes water diffusion through polymers, causing hydrolytic degradation, which results in leakage of unreacted monomers from the repairable surface.<sup>30</sup> It has been reported that aging process affects repair bond strength values, and an aging protocol should be performed before *in vitro* repair of RBCs to better mimic clinical conditions.<sup>18</sup>

Various methods such as thermal cycling, boiling, water storage, acid challenges are preferred for aging.<sup>20</sup> However, there is still no consensus on the best aging protocol. Thermal cycling properly simulates *in vivo* conditions.<sup>31</sup> This process also induces stress to a restoration due to aging, and thermal challenges.<sup>32</sup> Based on the ISO TR 11450 standard, 500 cycles must be performed for thermal cycling.<sup>33</sup> However, De Munck *et al.*<sup>33</sup> reported that a thermal cycling of 10000 times is similar to approximately 1-year fatigue in the oral environment. Therefore, thermal

cycling, which is preferred in other studies<sup>25,34</sup> was used for aging in this study.

In the present study, universal adhesive application alone doubled the repair  $\mu$ TBS values in comparison to control group, even resulting in a cohesive failure pattern (Table 2). The increase in repair  $\mu$ TBS values after the application of adhesive resin is most likely due to the inability of the repair RBC to penetrate into the substrate microstructure as a result of its high viscosity.<sup>35,36</sup> In addition, as mentioned above, it is inevitable to experience a reduction in bonding potential due to the reduction of unreacted monomers of the substrate surface as a result of aging.<sup>36,37</sup> The application of adhesive resin enhances the mechanical interlocking by infiltration to the irregular surface structure obtained after surface treatments<sup>34,38</sup>, as well as chemical bonding to the organic matrix and exposed filler particles.<sup>16,17,19,25</sup> In a study comparing the efficacy of repair with or without adhesive resin application, the application of adhesive resin was reported to increase repair bond strength values<sup>39</sup>, in consistent with the present study.

On the other hand, the universal adhesive used (Single Bond Universal) in the present study contains 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), polyalkenoic acid copolymer, and prehydrolyzed silane in its chemical formulation (Table 1). The 10-MDP monomer is known to improve bonding to metals, hydroxyapatite and filler particles of RBCs.<sup>40,41</sup> Fonseca *et al.*<sup>42</sup> reported that phosphate esters increase the hydrolytic stability more effectively than silane coupling agents by directly bonding to the surface hydroxyl groups of zirconia. Considering zirconia filler presence in the composition of both substrate and repair RBCs (Table 1), 10-MDP may be one of the reasons for the increase in repair  $\mu$ TBS values. Bond strengths are reported to be similar to the original monolithic composite when SBU is used for repair of the aged RBC substrates.<sup>43</sup> In another study, researchers were concluded that Silane containing universal adhesive alone is suggested as a promising material for the simplification of the repair procedure.<sup>16</sup>

PA etching is the most commonly used repair protocol in clinical practice for the preparation of both the surrounding dental hard tissues and the substrate surface to be repaired.<sup>20</sup> Although it has been reported that the PA etching treatment in composite-composite repair increases the total surface area of the substrate,<sup>35</sup> studies have shown that PA etching does not enhance the repair bond strength values.<sup>20,25</sup> Ayar *et al.*<sup>20</sup> reported that the effect of PA etching is thought to be limited only by removal of debris present on the substrate surface after mechanical surface treatments. In the present study, PA etching did not cause a significant improvement in both surface roughness and repair  $\mu$ TBS values. SEM images show no change in surface topography after PA etching, only debris on the substrate surface is removed (Figure 2, Control and PA). This finding is similar to other studies in the literature.<sup>19,20,25</sup> On the other hand, HF etching of bulk-fill RBC surfaces yielded better repair  $\mu$ TBS values compared to PA did, in parallel with a previous study.<sup>20</sup> However, the use of HF etching intraorally might be very dangerous and protective measures should be taken.<sup>44</sup>

Air abrasion is one of the surface treatments successfully applied to the surface of various dental materials such as direct and indirect RBCs, ceramics and metal alloys.<sup>26</sup> This treatment not only cleans the substrate, it also increases the surface tension and surface area by roughening the substrate surface, thereby improving the interaction between the substrate and the repair material.<sup>42</sup> This interaction has been reported to be more successful, especially when silica coated aluminum oxide particles (CoJet sand) are used.<sup>26</sup> When sandblasting performed with CoJet sand, silica particles tribochemically forms a silicate ceramic layer according to the manufacturer. This layer has surface irregularities that provide mechanical anchoring to repair RBCs, and the silica coating provides a chemical anchoring through the interaction of the silica coating with the monomers of the repair RBCs.<sup>26</sup>

Atalay *et al.*<sup>25</sup> reported that the highest repair  $\mu$ TBS values were obtained by air-abrasion with aluminum oxide particles. In the present *in vitro* study, no significant difference was observed for

surface roughness or repair  $\mu$ TBS values between AIO and TSC treatments. This result is in an agreement with the previous studies.<sup>19,45</sup> In SEM images, it was observed that the both treatments produce similarly rough surfaces (Figure 2, AIO and TSC). When both surface roughness and repair  $\mu$ TBS findings are evaluated, it might be considered that the improvement in repair  $\mu$ TBS values due to the air abrasion protocols was based on micromechanical retention achieved by roughening of the substrate surface. Furthermore, it was observed that the roughness patterns differ according to surface treatment protocol (Figure 2). In a previous study, it was indicated that bond strength might also depend on the roughness pattern.<sup>46</sup> In this study, 320-grit SiC abrasive paper was used to provide coarse diamond bur abrasion in a standardized way.<sup>22</sup> Evaluation of the control SEM image revealed that 320-grit SiC paper abrasion consists of parallel grooves representing on the characteristic of the grinding motion. The same parallel grooves were also evident in the SEM image of PA etched substrate surface. After HF etching, it was clearly observed that the substrate surface is more irregular and the filler particles are exposed. After air abrasion treatments, there are peaks of different heights and dimensions. It could be suggested that this type of roughness pattern obtained after air abrasion treatments may be more favorable for micromechanical retention than the parallel grooves.

The repaired restorations are exposed to chemical and mechanical degradation in the oral environment.<sup>18</sup> This degradation may adversely affect the success of the repair. The main limitation of the present study is that no additional aging protocol is applied after the repair. Further studies using different repair materials with post repair aging protocol should be conducted to improve the repair prognosis and raise awareness of the repair treatment among clinicians.

## CONCLUSIONS

Within the limitations of the present *in vitro* study, the following conclusions can be drawn. According to the profilometry findings, TSC and AIO treatments produced more rough surfaces compared to the other surface treatments.

Furthermore, etching with PA did not provide significant roughness compared to the control group. The highest repair  $\mu$ TBS values were obtained by air abrasion treatments, but no significant difference was observed between AIO and TSC. On the other hand, silane-containing universal adhesive application alone doubled the repair  $\mu$ TBS values compared to the control group. The use of this material is promising to facilitate the repair of bulk-fill RBCs.

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#### CONFLICT OF INTEREST STATEMENT

None.

#### *Farklı Yüzey İşlemlerinin Yaşlandırılmış Bulk-Fill Kompozitlerin Tamirine Olan Etkisi: In Vitro Çalışma*

#### ÖZ

**Amaç:** Bu in vitro çalışmanın amacı, farklı yüzey işlemlerinin, yaşlandırılmış bulk-fill kompozit reçine kompozit tamirinin mikrogerilme bağlanma dayanımı üzerindeki etkilerini değerlendirmektir. **Gereç ve Yöntemler:** Hazırlanan bulk-fill kompozit bloklar ( $n=60$ ,  $5 \times 5 \times 5$  mm), 5 ile  $55^\circ\text{C}$  arasında 5000 defa yaşlandırma döngüsü sonrasında, uygulanacak yüzey işlemi protokolüne göre 6 gruba ayrılmıştır: yüzey işlemi yok (kontrol), universal adeziv uygulaması (SBU; 3M ESPE), fosforik asit (PA) + SBU, hidroflorik asit (HF) + SBU, alüminyum oksit kumlama (AIO) + SBU ve tribokimyasal silika kaplama (TSC) + SBU. Yüzey pürüzlülük değerleri beş farklı yönde ölçüm yapılarak kontakt profilometre ile belirlenmiştir ( $n=10$ ). Tamir işleminden sonra, örneklerden  $1 \text{ mm}^2$ 'lik çubuklar elde edilmiş ve mikrogerilme bağlanma değerleri kopma olana kadar  $0,5 \text{ mm/dak}$  hızda kaydedilmiştir. Yüzey işlemlerinden sonra numune yüzeyleri SEM ile gözlenmiştir. Veriler, tek yönlü ANOVA ve Tukey testleri kullanılarak analiz edilmiştir ( $p<0,05$ ). **Bulgular:** Tek yönlü ANOVA analizi sonucunda yüzey işlemleri arasında önemli farklılıklar tespit edilmiştir ( $p<0,001$ ). En düşük tamir bağlanma dayanımı değerleri, kontrol grubunda gözlenmiştir. Tek başına universal adeziv uygulaması tamir bağlanma dayanımı değerlerinde anlamlı bir artış sağlamıştır ( $p<0,001$ ). En yüksek bağlanma dayanımı değerleri AIO ve TSC işlemleri için

elde edilmiştir ( $p>0,05$ ) ve HF onları takip etmektedir. Beş farklı yüzey işleminin oluşturduğu yüzey pürüzlülükleri;  $\text{TSC}>\text{AIO}>\text{HF}>\text{PA}=\text{Kontrol}$  şeklindedir. **Sonuçlar:** Etkili ve güvenli bir onarım protokolü seçildiğinde, bulk-fill kompozitlerin başarılı bir şekilde onarılabilir. En yüksek mikrogerilme bağlanma dayanımı değerleri AIO ve CoJet işlemleri sonucunda elde edilmiştir. Universal adezivler, bulk-fill kompozit tamiri süreçlerini kolaylaştırmak adına umut vericidir. **Anahtar Kelimeler:** Bileşik rezinler, dental restorasyon onarımı, dental adezivler, yüzey özellikleri.

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## ADHESION OF *CANDIDA ALBICANS* AND *CANDIDA PARAPSILOSIS* TO DIFFERENT RESTORATIVE MATERIALS

### ABSTRACT






**Objectives:** The aim of this study is to compare the susceptibility of seven different restorative materials (three conventional composite resins, two bulk-fill composite resins, one giomer, and one high viscosity glass ionomer material) to adhere *Candida albicans* and *Candida parapsilosis*.

**Materials and methods:** In this study, thirty cylindrical specimens of each material were made according to instructions of the manufacturers. The surface roughness of the specimens was assessed using a profilometer. Thereafter, the specimens were incubated with a reference strain of *Candida albicans* (ATCC 64548) and *Candida parapsilosis* (ATCC 22019). The proliferated colonies counted as CFU/ml. One-way analysis of variance (ANOVA) was used to evaluate the surface roughness and the adhesion value of the materials. Tukey's post-hoc test was used for subsequent pairwise comparisons.

**Results:** There was a statistically significant difference between the groups in terms of the surface roughness of the materials ( $p<0.05$ ). The high viscosity glass ionomer material exhibited significantly higher surface roughness values while X-trafil (a bulkfill composite resin) had the lowest surface roughness values. Also, there was a significant difference between *Candida* adhesion values of the materials ( $p<0.05$ ).

**Conclusions:** There was no significant relationship between surface roughness and adhesion of *Candida albicans* and *Candida parapsilosis*. Involvement was seen more for *Candida albicans* compared to *Candida parapsilosis* in all restorative materials.

**Keywords:** Bulk fill composites, candida, dental materials, glass ionomer.

 Soley ARSLAN<sup>1</sup>  
 Ayşe Nedret KOÇ<sup>2</sup>  
 Burhanettin AVCI<sup>1</sup>  
 \*Hacer BALKAYA<sup>1</sup>  
 Nazire Nurdan ÇAKIR<sup>3</sup>

ORCID IDs of the authors:  
S.A. 0000-0003-4487-2049  
A.N.K. 0000-0002-1736-9707  
B.A. 0000-0002-0529-0508  
H.B. 0000-0001-9180-5610  
N.N.Ç 0000-0003-3137-7954

<sup>1</sup> Department of Restorative Dentistry, Faculty of Dentistry, Erciyes University, Kayseri, Turkey.

<sup>2</sup> Department of Microbiology, Faculty of Medicine, Erciyes University, Kayseri, Turkey.

<sup>3</sup> Department of Restorative Dentistry, Faculty of Dentistry, Nuh Naci Yazgan University, Kayseri, Turkey.

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## INTRODUCTION

Dental caries is an infectious pathology of hard dental tissues which has a localized and transmissible destruction process.<sup>1</sup> Cariogenic microorganism eradication is one of the most important critical factors for preventing primary or residual caries lesions.<sup>2</sup> Previous studies show that *Streptococcus mutans*, *Lactobacillus acidophilus*, *Actinomyces viscosus*, and *Lactobacillus rhamnosus* are the most frequent cariogenic microorganisms.<sup>3,4</sup> Moreover, it was reported that the existence of *Candida* spp. in the oral cavity is associated with the caries lesion progress.<sup>5</sup>

*Candida albicans* (*C. albicans*) is the most common fungus in the human oral cavity and *C. albicans* is normally present in the oral cavity in 20-40% of healthy individuals. *Candida parapsilosis* (*C. parapsilosis*) represents less than 10% of the *Candida* species in the oral cavity.<sup>6-8</sup> It is known that yeast cells have the adhesion potential to host surfaces such as mucosa and non-biological surfaces.<sup>6</sup> In dentistry, studies have focused on the attachment of dentures and the base materials due to adhesion of fungal cells to materials such as resin, glass or metal.<sup>9-11</sup> However, it is not known whether dental restorative materials are potential sources of fungal infections since very few studies have been performed on these materials.

Composite resins that were developed as an aesthetic alternative to amalgam in the 1960s are commonly used as direct restorative materials in modern dentistry. The manufacturers developed distinctive composites to improve the physical and mechanical properties of traditional composites of resin matrices and filler materials. A new restorative approach involves the use of high-viscosity bulk-fill composites. The use of bulk-fill composite resins for posterior restorations reduces the time and effort required for stratification and eliminates the possibility of voids between the layers by allowing 4 mm curing in one step without affecting the polymerization shrinkage and mechanical properties in the negative direction.<sup>12,13</sup>

In addition to composite resins, glass ionomer cements (GIC) have also been used in posterior

restorations. Chemical bonding to tooth structures, decay-inhibiting effects due to fluoride release, remineralization, thermal expansion similar to dental structures, low toxicity and biocompatibility have made GIC a clinically preferred restorative material compared to resin composites.<sup>14</sup> Besides the many advantages of GIC, they have some disadvantages such as low wear resistance, low fracture toughness, and they are also greatly influenced by dehydration and initial moisture contamination. To reduce the moisture sensitivity of GIC in early stages of hardening, to increase their hardness and abrasion resistance, and to enable them to be used in areas exposed to chewing forces, bulk-fill glass hybrid restorative systems, which combine the main advantages of a highly viscous GIC with a nano-filled, light-curing varnish, were presented to the market.<sup>14</sup>

Giomer is a fluoride-releasing hybrid composite restorative material and it contains active glass ionomer particles (pre-reacted glass ionomer - PRG) formed as a result of the acid-base reaction in the aqueous medium between the fluoroaluminasilicate glass particles and the polyalkenoic acid.<sup>15</sup>

In the present study, it was aimed to compare the adhesion of the oral fungal pathogens *C. albicans* and *C. parapsilosis* to bulk-fill composite resin, a conventional composite resin, a giomer, and a high viscosity glass ionomer material. The null hypotheses of this study were that

1. the composition and chemistry of the materials would not affect *Candida* adhesion to the materials.
2. the surface roughness would not affect *Candida* adhesion to the materials.

## MATERIALS AND METHODS

### *Preparation of the samples*

This *in vitro* study was performed in accordance with the Helsinki Declaration. In this study, seven different restorative materials (three conventional hybrid composite resins, three bulk-fill composite resins, one giomer, and one high viscosity glass ionomer material) were used. The used materials, lot numbers, and their compositions are given in Table 1.

**Table 1.** Materials and compositions

| MATERIALS   | COMPOSITIONS  |
|---|---|
| <b>Arabesk N</b><br><b>VOCO GmbH, Cuxhaven, Germany</b><br><b>Batch 1338579</b><br><b>Clearfil Majesty Esthetic</b><br><b>Kuraray, Okuyama, Japan</b><br><b>Batch 00051D</b><br><b>Beautifil-Bulk</b><br><b>Shofu, Kyoto, Japan</b><br><b>Batch PN2034</b><br><b>X-tra Fil</b><br><b>VOCO GmbH, Cuxhaven, Germany</b><br><b>Batch 1612535</b><br><b>Filtek Bulk Fill Posterior Restorative</b><br><b>3M ESPE, St. Paul, MN, USA</b><br><b>Batch N719528</b><br><br><b>Equia Forte Fil</b><br><b>GC, Tokyo, Japan</b><br><b>Batch 160512A</b><br><br><b>Filtek Ultimate</b><br><b>3M ESPE, St. Paul, MN, USA</b><br><b>Batch N618541</b> | Bis-GMA, UDMA, TEGDMA, barium aluminium silicate glass, lithium aluminium silicate glass-ceramic, highly dispersed silicon dioxide<br>Bis-GMA, TEGDMA, hydrophobic aromatic dimethacrylate, Silanated barium glass filler, prepolymerized organic filler<br>Bis-GMA, TEGDMA, inorganic glass filler, aluminuoxide, silica, pre-reacted glass ionomer filler, DL-camphorquinone<br><br>Bis-GMA, UDMA, TEGDMA, Barium boron aluminum silicate glass<br><br>AUDMA, UDMA and DDMA, Zirconia / silica and ytterbium trifluoride filler.<br><br>Powder: 95% strontium fluoroalumino-silicate glass, 5% polyacrylic acid.<br>Liquid: 40% aqueous polyacrylic acid<br>EQUIA Forte Coat: 40%-50% methyl methacrylate, 10%-15% colloidal silica, 0.09% camphorquinone, 30%-40% urethane methacrylate, 1%-5% phosphoric ester monomer<br><br>Bis-GMA, UDMA, TEGDMA, PEGDMA, Bis-EMA, 20 nm silica particuls, 4 - 11 nm zirkonyum particuls |

Bis-GMA; Bisphenol A-Glycidyl Methacrylate, UDMA; Urethane dimethacrylate, TEGDMA; Triethylene glycol dimethacrylate, PEGDMA; polyethyleneglycoldimethacrylate, Bis-EMA; ethoxylatedbisphenol-A dimethacrylate, DDMA; 1,12-dodecane dimethacrylate, AUDMA; Aromatic dimethacrylate.

All materials, Arabesk N (AN) (VOCO GmbH, Cuxhaven, Germany); Clearfil Majesty Esthetic (CME) (Kuraray, Okuyama, Japan); Beautifil-Bulk (BB) (Shofu, Kyoto, Japan); X-tra fil (XF) (VOCO GmbH, Cuxhaven, Germany); Filtek Bulk Fill Posterior Restorative (FBF) (3M ESPE, St. Paul, USA); Equia Forte Fil (EF) (GC, Tokyo, Japan); Filtek Ultimate (FU) (3M ESPE, St. Paul, USA) are applied in accordance with the manufacturer's instructions. For each test group, thirty disc-shaped samples (8 mm in diameter, 2 mm in height) (n=30) were prepared using a special teflon mold with calibrated circular holes. In the composite resin and giomer groups, the restorative materials were placed in molds on glass, their surfaces were covered with a mylar strip and another glass was pressed on top of the materials. All samples were polymerized on both sides for 40 s using a LED light source (VALO, Ultradent, UT, USA, 395-480 nm, 1000 mW/cm<sup>2</sup>). In the EF group, glass ionomer material was placed in the mold on glass, its surface was covered with a mylar strip and another glass was pressed from top of the material. After the

material hardened, Equia Forte Coat (GC) was applied to the surfaces of the samples and it was cured for 20 s. Thereafter, the specimens were removed from the molds and the excesses in the specimens were removed with Sof-Lex XT discs (3M ESPE, St Paul, USA), and the surfaces of the specimens were polished using aluminum oxide/diamond-abrasive-impregnated (Enhance/PoGo; Dentsply Caulk, Milford, USA). Specimens were stored in distilled water for further processing. The surface roughness of each disc-shaped specimens was measured with a profilometer (Mitutoyo SJ-301, Mitutoyo Corporation, Tokyo, Japan.). Roughness measurements were performed in three regions of each specimen and the arithmetic mean of the values was taken.

#### **Adhesion testing for *C. albicans* and *C. parapsilosis***

*C. parapsilosis* (ATCC 22019) standard strain was used in the adhesion test. A suspension of the *C. parapsilosis* strain was prepared in 2.5 to 5x10<sup>6</sup> CFU / ml in sterile saline from a 24-hour culture on

a sabouraud dextrose agar (SDA) medium. From this suspension, 100µl of SDA-containing plaque medium was taken and composite discs were placed on top of it. Disc-shaped specimens were incubated at 37°C for 24 hours and then they were washed with 15 ml of SF. The washed specimens were taken in tubes of 1 ml SF and treated with an ultrasonic sonicator three times for 10 seconds at 30W. This suspension was spread on the supernatant by inoculating 100 µl of both 1:10, 1:100 and 1:1000 dilutions directly onto the SDA medium. These media were incubated at 37°C for 24 hours. The growth colonies on SDA were then counted as CFU / ml (R). The method of the application of *C. albicans* (ATCC 64548) to the samples was identical to the application of *C. parapsilosis* (ATCC 22019).

**Scanning electron microscopy (SEM)**

In order to observe the surface roughness of the specimens under a scanning electron microscope

(SEM), one sample from each group was prepared. The samples were examined with a scanning electron microscope (magnification 3000x and 5000x, GeminiSEM 500, Zeiss, Germany).

**Statistical analysis**

The data were analyzed using statistical software (SPSS Statistics 22.0; SPSS Inc, IL, USA). The distribution of the data in this study was evaluated by the Shapiro Wilk test. One-way analysis of variance (ANOVA) was used to evaluate the surface roughness and the adhesion value of the materials. Tukey’s post-hoc test was used for subsequent pairwise comparisons. The significance level was accepted as p<0.05.

**RESULTS**

**Surface roughness**

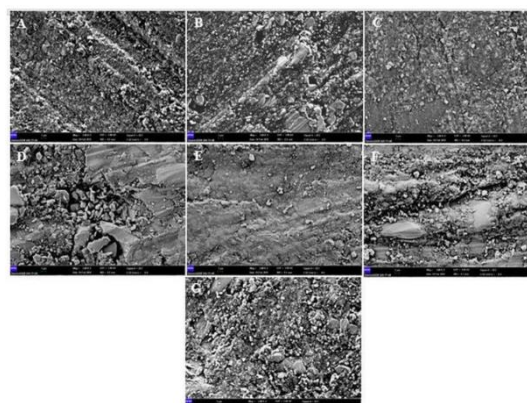
The surface roughness values of the groups are shown in Table 2.

**Table 2.** Surface roughness, *Candida albicans* and *Candida parapsilosis*, adhesion values of the materials (Mean ± SD (Standard Deviation))

| Materials                 | Surface roughness Mean (± SD) | <i>Candida albicans</i> Mean (± SD)                         | <i>Candida parapsilosis</i> Mean (± SD)                      |
|---------------------------|-------------------------------|---|--|
| Arabesk N                 | 0.51 (± 0.13) <sup>a</sup>    | 1.1×10 <sup>3</sup> (± 1.4×10 <sup>3</sup> ) <sup>a</sup>   | 0.8 ×10 <sup>3</sup> (± 0.7 ×10 <sup>3</sup> ) <sup>a</sup>  |
| Clearfil Majesty Esthetic | 0.56 (± 0.14) <sup>ab</sup>   | 29 ×10 <sup>3</sup> (± 20 ×10 <sup>3</sup> ) <sup>b</sup>   | 28 ×10 <sup>3</sup> (± 28 ×10 <sup>3</sup> ) <sup>b</sup>    |
| Beautiful-Bulk            | 0.66 (± 0.21) <sup>b</sup>    | 16 ×10 <sup>3</sup> (± 11 ×10 <sup>3</sup> ) <sup>ab</sup>  | 8.5 ×10 <sup>3</sup> (± 8 ×10 <sup>3</sup> ) <sup>a</sup>    |
| X-tra Fil                 | 0.47 (± 0.12) <sup>a</sup>    | 2.5 ×10 <sup>3</sup> (± 1.8 ×10 <sup>3</sup> ) <sup>a</sup> | 0.2 ×10 <sup>3</sup> (± 0.18 ×10 <sup>3</sup> ) <sup>a</sup> |
| Filtek Bulk Fill          | 0.57 (± 0.16) <sup>ab</sup>   | 58 ×10 <sup>3</sup> (± 36 ×10 <sup>3</sup> ) <sup>c</sup>   | 11 ×10 <sup>3</sup> (± 8.3 ×10 <sup>3</sup> ) <sup>a</sup>   |
| Equia Forte Fil           | 1.13 (± 0.28) <sup>c</sup>    | 63 ×10 <sup>3</sup> (± 32 ×10 <sup>3</sup> ) <sup>c</sup>   | 8 ×10 <sup>3</sup> (± 6.7 ×10 <sup>3</sup> ) <sup>a</sup>    |
| Filtek Ultimate           | 0.63 (± 0.22) <sup>ab</sup>   | 29 ×10 <sup>3</sup> (± 15 ×10 <sup>3</sup> ) <sup>b</sup>   | 4.4 ×10 <sup>3</sup> (± 5 ×10 <sup>3</sup> ) <sup>a</sup>    |

*a,b,c* shows statistical differences in the vertical column

The EF group exhibited significantly higher surface roughness values. A bulk-fill composite resin (XF) had the lowest surface roughness values and there was statistically significant difference between the XF group and BB group and, XF group and EF group (p<0.05). Also, there was a statistically significant difference between BB group and EF group (p<0.05). SEM images of the surface roughness of tested restorative materials are given in Figure 1.



**Figure 1.** SEM images of the surface roughness of Arabesk N (A), Beautiful-Bulk (B), Clearfil Majesty Esthetic (C), Equia Forte Fil (D), Filtek Bulk Posterior Restorative (E), Filtek Ultimate (F) and X-tra fil (G)

### ***C. albicans* adhesion values**

Table 2 shows the comparative adhesion values of *C. albicans* in all test materials. Significant differences between the adhesion values of *C. albicans* were found according to the test results. Although the AN group showed the lowest *C. albicans* adhesion value among all tested materials, there was no statistically significant difference between the AN group and the XF and BB groups ( $p=0.455$ ,  $p=1.00$ ). The EF group showed the highest *C. albicans* adhesion value and there was no statistically significant difference between the EF and the FBF groups ( $p=0.998$ ). Also, there was no statistically significant difference between the CME, BB and FU groups ( $p>0.05$ ).

### ***C. parapsilosis* adhesion values**

Table 2 shows the comparative adhesion values of *C. parapsilosis*. There were significant differences between the *C. parapsilosis* adhesion values according to the test results. The CME group showed significantly higher *C. parapsilosis* adhesion value ( $p<0.05$ ). The XF group showed the lowest *C. parapsilosis* adhesion value among all tested materials and there was a statistically significant difference only between the XF and the CME groups ( $p=0.001$ ), but not with XF and the other groups ( $p>0.05$ ). The CME group showed significantly higher *C. parapsilosis* adhesion than other groups ( $p<0.05$ ). Besides all these, *C. albicans* adhesion was significantly higher when compared to *C. parapsilosis* adhesion ( $p<0.05$ ).

## **DISCUSSION**

This study aimed to compare the adhesion of *C. albicans* and *C. parapsilosis* to traditional composite resins, bulk-fill composite resins, and restorative materials containing glass ionomer. While there was no significant relationship between surface roughness and *Candida* adhesion, the content of restorative materials was found to affect *Candida* adhesion. Therefore, the first hypothesis that the composition and chemistry of the materials would not affect *Candida* adhesion to the materials was rejected and the second hypothesis that the surface roughness would not affect *Candida* adhesion to the materials was accepted.

The human mouth provides different surfaces to which oral microorganisms can adhere.<sup>9</sup> *C. albicans* is the major microbiological factor in fungal infections. Besides, reports show that *C. parapsilosis* is often the second most commonly isolated *Candida* spp. from blood cultures.<sup>16,17</sup> Since we prefer to provide as simple a test pattern as possible, we used culture strains of *C. albicans* and *C. parapsilosis* in this study.

Studies on the adhesion properties of fungal species are commonly focused on denture base and relining material.<sup>10,11,18</sup> Despite their frequent use in clinical dentistry and the fact that they are potential sources for fungal infections, fewer studies have been conducted on composite resin and glass ionomer materials.<sup>9,19</sup> In order to ensure the clinical suitability of microbiological adhesion studies, appropriate materials for clinical dentistry should be used.<sup>20</sup> To the best of our knowledge, this is the first study to compare fungal adhesion to different bulk-fill composite resins.

The number of microorganisms adhering to the restorative material depends on different properties such as surface hydrophobicity, surface roughness, type of matrix, electrostatic forces, the composition of the material, filler size, and configuration of fillers.<sup>6,16,21</sup> It has been proven that the surface roughness has a very important effect for microbial adhesion.<sup>22</sup> The most commonly used parameter to define the roughness of a given surface is the arithmetic mean peak-valley value *Ra*.<sup>23</sup> Therefore, we compared the roughness values of restorative materials using *Ra* value.

Surfaces with *Ra* values below 0.2  $\mu\text{m}$  are considered smooth.<sup>21</sup> Studies have shown that as the value of *Ra* increases, the *Candida* adhesion also increases.<sup>20,22</sup> In our study, the XF and AN groups have numerically the lowest surface roughness values and therefore show the lowest adhesion values. Although the EF group showed a high surface roughness, it showed a similar *Candida* adhesion with some composites. In this study, the low quantity of adhering *Candida* on glass ionomer cements which are known to release significant quantities of fluoride is associated with the antimicrobial effects of fluoride

components.<sup>23,24</sup> These findings support the alternate hypothesis that different types of materials and their composition and chemistry have a significant effect on *Candida* adhesion. However, a statistical correlation analysis between surface roughness and *Candida* adhesion showed no significant correlation in our study.

The capability of *Candida* to persist within the host and to cause infection has been attributed to its different virulence features which include the ability to adhere to host surface, the presence of hyphae, biofilm formation, and the secretion of hydrolytic enzymes.<sup>17</sup> Although *C. albicans* is the most prevalent fungus in humans<sup>25</sup>, the incidence of *C. parapsilosis* has dramatically increased recently.<sup>17</sup> Besides, *C. parapsilosis* has low virulence properties compared to *C. albicans*.<sup>26</sup> The lower virulence of *C. parapsilosis* compared to *C. albicans* can also be attributed to the lack of formation of true hyphae (tube germ).<sup>27</sup> The ability of a microorganism to adhere to host surfaces is the critical first step for successful colonization and subsequent infection of host tissues by a potentially pathogenic *Candida* spp. *C. albicans* adhere to a greater extent to host surfaces than does *C. parapsilosis*.<sup>28</sup> Biofilms are surface-associated communities of microorganisms within the extracellular matrix and seem to contribute to cohesion.<sup>29</sup> Biofilm formation is a potent virulence factor for several *Candida* spp. but *C. parapsilosis* strains produce quantitatively and structurally less complex biofilm than *C. albicans*.<sup>17</sup> Secreted aspartic proteinases (Saps) facilitate the invasion and colonization of host surfaces. Compared to *C. albicans*, *C. parapsilosis* has less Saps activity.<sup>17</sup> Other enzymes that seem to play an important role in the pathogenesis of *Candida* species are phospholipase and lipases. These enzymes affect the adhesion and penetration of host cells. Phospholipase production is concentrated on the tips of hyphae, and the activity is greater when the hypha is in direct contact with the membrane.<sup>29</sup> The extra-cell phospholipases are relevant for *C. albicans*. In *C. albicans*, 10 lipase genes have been identified whereas, in *C. parapsilosis*, two lipase genes have been identified.<sup>17,30</sup> In our study, *C. albicans* may have shown greater adhesion to all tested composite

resin surfaces than *C. parapsilosis* because of the above-mentioned virulence factors.

The limitation of this study was the use of standard species of *C. albicans* and *C. parapsilosis* rather than clinical isolates. Therefore, further studies with clinical isolates are needed.

## CONCLUSIONS

Within the limitation of this study, X-trafil, which is a bulk-fill composite resin, showed similar surface roughness values and similar *Candida* adhesion with Arabesk N (a conventional composite resin). There was no relationship between surface roughness and *Candida* adhesion. Besides, the content of restorative materials was found to affect *Candida* adhesion. The involvement of *C. albicans* was seen more than that of *C. parapsilosis* in all tested restorative materials.

## ACKNOWLEDGEMENTS

None

## CONFLICT OF INTEREST STATEMENT

None

### *Farklı Restoratif Materyallere Candida Albicans ve Candida Parapsilosis Adezyonu*

#### ÖZ

**Amaç:** Bu çalışmanın amacı, yedi farklı restoratif materyalin (üç adet geleneksel kompozit rezin, iki adet bulk-fill kompozit rezin, bir giomer ve bir yüksek viskoziteli cam iyonomer materyal) *Candida albicans* ve *Candida parapsilosis* adezyonuna olan duyarlılığını karşılaştırmaktır. **Gereç ve Yöntemler:** Bu çalışmada her materyalden otuz silindirik örnek üretici talimatlarına göre hazırlandı. Örneklerin yüzey pürüzlülüğü profilometre kullanılarak ölçüldü. Daha sonra örnekler, *Candida albicans* (ATCC 64548) ve *Candida parapsilosis* (ATCC 22019) referans suşu ile inkübe edildi. Materyallerin yüzey pürüzlülük ve adezyon değerlerini değerlendirmek için tek yönlü varyans analizi (ANOVA) kullanılmıştır. Grupların ikili olarak karşılaştırılması için Tukey post-hoc testi kullanıldı. **Bulgular:** Materyallerin yüzey pürüzlülüğü açısından gruplar arasında istatistiksel olarak anlamlı fark bulundu ( $p < 0,05$ ). Bir bulk-fill kompozit rezin olan X-trafil, en düşük yüzey pürüzlülüğü değerlerine sahipken, yüksek viskoziteli cam iyonomer material önemli ölçüde daha yüksek yüzey pürüzlülüğü değerleri gösterdi. Ayrıca materyallerin *Candida* adezyon



değerleri arasında anlamlı fark bulundu ( $p<0,05$ ).  
**Sonuç:** Yüzey pürüzlülüğü ile *Candida albicans* ve *Candida parapsilosis*' in adezyonu arasında anlamlı bir ilişki bulunmazken, tüm restoratif materyallerde *Candida albicans*, *Candida parapsilosis*' ten daha fazla tutulum gösterdi. **Anahtar kelimeler:** Bulk-fill kompozit, candida adezyonu, restoratif material, yüzey pürüzlülüğü.

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## COMPARISON OF MINERAL TRIOXIDE AGGREGATE, ENDOSEQUENCE ROOT REPAIR MATERIAL, AND BIODENTINE USED FOR REPAIRING ROOT PERFORATIONS: A SYSTEMATIC REVIEW

### ABSTRACT

The root perforation, in spite of being taken as a challenging accident in root canal treatment, has to offer favorable results when exposed to appropriate therapeutic conduct and the usage of materials that have convenient properties. The aim of the current review is to collect all updated and available studies including imperative information concerning the use of Mineral trioxide aggregate, EndoSequence root repair material and Biodentine in the treatment of root perforation approaching some of the key properties for treatment success. A search was performed in the two automated databases (Google Scholar and PubMed use English-language literature) for this systematic review, using specific inclusion and exclusion criteria and keywords. The electronic search was done in December 2018 and update in June 2019. Our inquiry uncovered Twenty-two studies that met the exclusion and inclusion criteria. These studies investigated the use of MTA, EndoSequence ERRM and Biodentine in the root perforation that happened during the endodontic treatment. It was confirmed that there is no unanimity in this review concerning the material that shows the best characteristics, once none of the materials discussed had all the major properties higher than the others, this way it is required the enforcement of further studies aimed at selecting the best characteristics of the material suggested in the root perforation treatment.

**Keywords:** EndoSequence root repair material, mineral trioxide aggregate, root canal filling materials, tooth root.

 \*Faisal ALGHAMDI<sup>1</sup>

 Esraa ALJAHDALI<sup>2</sup>

ORCID IDs of the authors:  
F.A.0000-0003-2086-0772  
E.A.0000-0002-2530-3054

<sup>1</sup> Department of Oral Biology, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia.

<sup>2</sup> General Dentistry, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia.

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## **INTRODUCTION**

Root perforation is an unintentional or pathological communication between the pulp cavity and the periodontal tissue arising from iatrogenic, reabsorption or caries.<sup>1,2</sup> It is taken as a big challenge to the most renowned endodontic specialists and clinicians, symbolizing one of the most unpleasant accidents during the endodontic treatment.<sup>3</sup> Pain during the instruments usage and intense and immediate bleeding are mentioned as clinical manifestations; Furthermore, a secondary inflammation and loss of bone insertion represent one of the biggest complications arising from perforation.<sup>4</sup>

The root perforation has as a causal element a pathological change like a large dental caries or root reabsorption, or it may happen through an operative surgical accident.<sup>5-8</sup> There are factors that make it hard to access to the root canal during the endodontic treatment, predisposing to dental perforation as examples: Errors regarding the canal identification, large caries, pulp calculations, badly positioned teeth, internal root reabsorption, excessive abrasion and debility of the dentin displayed in the danger zones.<sup>9</sup> The microorganisms deriving from the root canal, the periodontium or both may colonize the spot where the perforation happened, resulting in the contamination of the area and a probable inflammatory response.<sup>7</sup> As a result of the inflammation, it may cause bone resorption, pain, abscess, suppuration, fistula, and necrosis, which undermine the treatment's efficacy and consequently cause loss of the dental element.<sup>5</sup> The roots cervical third and the pulp-chamber floor are the spots most likely to be contaminated because of the closeness with the oral environment and the consequent facility for the bacteria's colonization in the area.<sup>6,7</sup>

Root perforation constitutes the second most common cause of endodontic flaws.<sup>10</sup> The prognosis is linked to the perforations location, extent, time, presence or absence of contamination, suitable treatment, early diagnosis, and usage of ideal materials.<sup>9, 11</sup> The diagnosis should be performed right away in order to provide proper treatment, favoring the prognosis and preventing bacterial colonization.<sup>10</sup> The treatment may be carried out

through the surgical method, although, since there is a possibility of pocket formation, the non-surgical technique is the most accepted, mainly in areas where the access is hard.<sup>8,12</sup> The treatment has as its aim to offer hermetic sealing and ought to be based immediately through surgical procedures or endodontic path. Because of the possibility of pockets formation in the surgical method, its more advantageous to endodontic therapy, particularly in regions of difficult access.<sup>12,13</sup> The treatment's success is directly associated with the perforations size, location, and level, the usage of suitable materials and techniques, presence or absence of inflammation and repair time.<sup>14</sup> Despite the dental element prognosis in which the root perforation happened being obscure, it is possible to reverse this scenario through good therapeutic practice and using materials that present favorable characteristics.<sup>15</sup>

The mineral trioxide aggregate (MTA) is taken as a gold standard material in the perforations sealing because it holds important properties like high pH, biocompatibility, fixation power even with humidity, periradicular regeneration and osteoinductive capacity.<sup>2,16-19</sup> Nevertheless, it displays some confining disadvantages, which are able to intervene in the clinical practice, expressed through the difficult manipulation and insertion on the spot to be filled, short working time and slow prey time.<sup>20</sup> With the requirement to enhance the physicochemical properties of the MTA and surpassing the limitations displayed, the EndoSequence root repair material (ERRM) was designed (Brasseler USA, Savannah, GA, USA).<sup>21,22</sup> It is a premixed material, its appearance is as a condensed mass or preloaded syringe, has excellent biological and mechanical properties, easy manipulation, highly biocompatible, hydrophilic, radiopacity, osteogenic and insoluble, prescribed for pulp capping and root's repair procedures.<sup>23</sup> The Biodentine (Septodont, Saint-Maur-des-Fossés, France) was manufactured with the goal of assembling the bioactivity and high biocompatibility of calcium silicate.<sup>24</sup> However, does not include aluminate in the formula, which diminishes the potential health risks. It holds properties as low cytotoxicity, excellent sealing ability, compressive resistance, easy handling, besides

keeping the bone-biomaterial interface, so it exhibits clinical indication in root perforation therapy.<sup>2,25</sup>

Overall, the ideal material for the perforation repair success must exhibit proper sealing, having biocompatibility, stimulating the cementogenesis and osteogenesis, being radiopaque, with easy manipulation, not being absorbable, having dimensional stability and not being soluble to tissue fluids.<sup>8,12,21,26,27</sup> Different materials have been designed for the treatment perforation, among these, we can cite the amalgam, zinc oxide, and eugenol cement, calcium hydroxide, resin cement, the hydroxyapatite, and glass ionomer.<sup>28</sup> Consequently, the aim of this review was to collect all updated and available studies including imperative information concerning the use of mineral trioxide aggregate (MTA), biodentine (BD), and Endosequence Root Repair Material (ERRM) in the root perforations treatment that happened during the endodontic treatment.

## **MATERIALS AND METHODS**

This review was reported in accordance with the PRISMA statement.

### ***Focused Question***

“What is the most appropriate repair material to be employed in the root perforation therapy?”

### ***Search Strategy***

The systematic way was performed to look-up for relevant information through several kinds of literature & search engines with great concern to the main question. Such a study was accomplished in December 2018 and applauded with new information until July 2019. A web search was done through PubMed (2008-2018) and Google Scholar (2008-2018) with MesH terms and/or in various combinations (“Endodontics”, “root perforation”, “repair material”, “bioceramic”, “biodentine”, “mineral-trioxide aggregate”, “endosequence”).

### ***Inclusion Criteria***

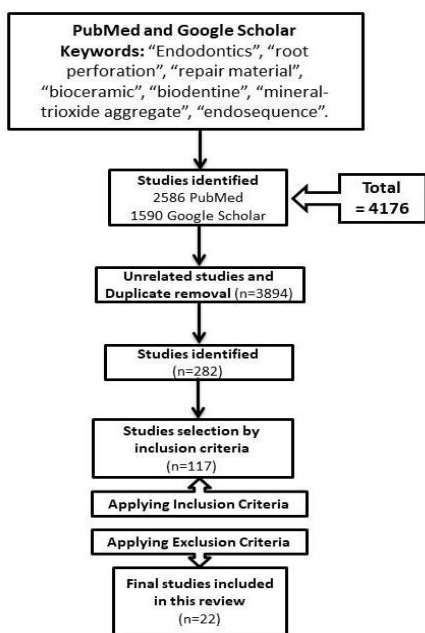
- Native research released in the English language.
- Time framed articles released within 10 years from 2008 - 2018.

- Studies carried out on human and animal subjects.

### ***Exclusion Criteria***

- Articles that described the different repair materials excluding EndoSequence root repair material, Biodentine, and Mineral Trioxide Aggregates.
- Articles that discussed the different clinical applications of repair materials excluding root perforation.
- Review articles.

Relevant articles had been read & assessed by the introduction of the close meaning ideas by the study reviewers. Full articles were obtained for most of the titles and abstracts that met the inclusion criteria, the full text was accessed. From each included article, Study design, interventions and controls, and findings were extracted. Articles used were categorized into two main groups (free & restricted). Free ones have been downloaded directly by the URLs generated from the database. The restricted group has been downloaded by the institutional access of the KAU library. Even though some articles didn't match the main idea, they have been reviewed again & decided to be either relevant or irrelevant. An understanding was there between the authors in relation to the suitability of the chosen articles. Even the reference was examined to identify any studies that haven't been covered by electronic searches. A summary of this review search strategy was summarized in (Figure 1).



**Figure 1.** Flow Chart of the Search Strategy used in this Systematic Review.

## RESULTS

Our exploration uncovered Twenty-two studies which met the exclusion and inclusion criteria. These studies investigated the use of MTA, EndoSequence ERRM and Biodentine in the root perforation that happened during the endodontic treatment on human and animal studies. All the studies included in this systematic review were eleven In-vitro studies<sup>8,10,12,13,20,26,28-32</sup>, two In-vivo studies<sup>2,17</sup>, one Randomized controlled trial study (RCT)<sup>33</sup>, four Retrospective clinical studies<sup>18,34-36</sup>, and four Case reports.<sup>1,7,9,37</sup> In regard to the types of root repair materials performed, fourteen studies were performed with MTA in a combination of other root repair materials such as ERRM and Biodentine.<sup>2,8,10,12,13,17,20,26,28-32,35</sup> The placement of Biodentine alone as a root repair material for root perforation was made in three studies.<sup>1,7,18</sup> On the other hand, five studies, MTA has placed alone.<sup>9,33,34,36,37</sup>

## DISCUSSION

The root perforation prognosis is affected by the chemical and physical properties of the materials used, independent of etiology or placement, the endodontic therapy ought to be performed with materials that display good characteristics.<sup>9,26</sup> This systematic review conducted to summarize, locate, appraise and synthesize all high-quality research evidence taken from 22 articles, which included original studies relevant to a scientific

research question. The question of this review is “What is the most appropriate repair material to be employed in the root perforation therapy?”. All included studies confirmed different repair materials used in the root perforations.

In the present time, the materials of choice for root perforation repair are the bioceramics, like the mineral trioxide aggregate (MTA), EndoSequence root repair material (ERRM) (Brasseler USA, Savannah, GA, USA) and Biodentine (Septodont, Saint-Maur-des-Fossés, France).<sup>10</sup> Bioceramics are materials made of calcium silicate, they have been widely employed in endodontics as a root repair material, in pulp coating, as cement sealing, and in periapical surgeries, they exhibit relevant characteristics like biocompatibility, dimensional stability, antimicrobial power, and elevated pH.<sup>38</sup> The MTA was the first bioceramic conducted and used in dental perforation treatment. It is primarily formed by tricalcium silicate, silicate oxides, bismuth, and Tricalcium aluminate.<sup>9,13</sup> On the other hand, in spite of the excellent characteristics, the MTA exhibits some limitations, as unpractical handling, granular consistency, long prey time and short work time.<sup>17</sup> With the purpose of enhancing the MTA characteristics and enhancing the reported difficulties, the bioceramic cements ERRM and Biodentine were elaborated.<sup>20</sup> Using calcium silicate, the ERRM is primarily comprised of zirconium oxide, monobasic calcium phosphate, and tantalum oxide, commercially it is obtainable in the consistency of mass that is ready for use, supplying a consistent material and making the Clinical management easier.<sup>26</sup> It is biocompatible, insoluble, hydrophilic and bioactive, does not have aluminum, the prey starts through contact with the humid environment and is able to provide excellent sealing, characteristics that define it as a proper material in the Dental perforations treatment.<sup>10,39-40</sup> The Biodentine bioceramic is comprised of calcium silicate, zirconium oxide, tricalcium silicate, and calcium carbonate, the commercial presentation is powder and liquid.<sup>1,26,28</sup> It exhibits biocompatibility, dimensional stability, excellent sealing capacity, easy manipulation, short prey time, so is suitable for clinical usage in roots repair.<sup>20,29</sup> Moreover, it keeps the bone-biomaterial interface, it displays low cytotoxic capacity and

good fluidity, which makes it easier to insert in the spot to be used.<sup>2</sup>

In this systematic review, few studies claimed that the perforations should be handled promptly with a biocompatible material which generates suitable sealing between the perforation and the adjacent tissues.<sup>1,6,8,10,12</sup> In addition, an in-vitro study conducted in 2016, after they assessed the sealing capacity of MTA and Biodentine declared that there are no considerable differences between the materials, advising for the use of Biodentine like an alternative to MTA in the perforations repair.<sup>29</sup> In another study done in 2017, they noticed that the MTA displayed better sealing in comparison to Biodentine.<sup>2</sup> Also, an in-vitro study conducted by Bampa, *et al.*<sup>31</sup> in 2015, they assessed the MTA ability to seal using three different insertion techniques. The study enabled the observation that irrespective of the technique used it was not possible to prevent the infiltration.<sup>31</sup> That way, they settled the requirement for more studies to be performing in order to enhance the materials sealing property in critical dental spots.<sup>31</sup> One study done in 2014, when comparing the sealing capacity between the Biodentine and Endosequence ERRM noticed that the Endosequence ERRM had better performance.<sup>10</sup> A study conducted by Lagisetti, *et al.*<sup>8</sup> in 2018 compared the Endosequence ERRM to the MTA and settled that there are no statistical differences between them. Also, an in-vitro study done in 2015, compared the MTA with different root repair materials and settled that there are comparable capabilities in sealing the furcal perforations of the primary molars.<sup>32</sup> There are four studies that assert the difficulty in MTA manipulation is a downside of this material.<sup>10,17,26,28</sup> In line with a retrospective study conducted in 2013, they appraised the effect of various endodontic irrigants on the push-out bond strength of Biodentine (Septodont, Saint Maur des Fossés, France) in comparison with different repair materials.<sup>35</sup> They found Biodentine illustrated great work as a perforation repair material even after being exposed to various endodontic irrigants.<sup>35</sup> In addition, another study done in 2017, when they are comparing the MTA to the Biodentine in the furcal perforation repair,

it was noticed an easier usage for Biodentine, because of the decreased prey time of almost 12 minutes which decreases the bacterial contamination risk, in addition to display an easy manipulation and being highly biocompatible, features that define it as positive material.<sup>1</sup> A retrospective study conducted in 2013, they found (73.3%) of cases that which the root perforations repaired with MTA classified as healed.<sup>34</sup> Also, another study done in 2010, they concluded to MTA seems to furnish a biocompatible and long-term effective seal for root perforations in all parts of the root.<sup>36</sup>

Silva, *et al.*<sup>2</sup> in 2017, states that mineralized tissue formation at the spot where the perforation happened is a key indicator regarding treatment success. These results were in agreement with the findings of Rifaey, *et al.*<sup>30</sup>, who provided the osteogenic potential between the ERRM and the MTA, settled that the ERRM promoted better osteoblasts differentiation. Silva, *et al.*<sup>2</sup> noticed that the MTA led to the formation of mineralized tissue with larger thickness and area, in comparison to Biodentine. Nevertheless, Biodentine exhibited good histopathologic outcomes and may be taken as a repair material.<sup>2</sup> Calcium silicate-based materials can have their physical and chemical properties changed when exposed to acidic pH, mainly when local acidosis is prompted by tissue or bacterial inflammation.<sup>2</sup> These results were similar to the findings of other authors, Wang, *et al.*<sup>41</sup> evidenced a decrease in the microhardness of the Endosequence ERRM and MTA in an acid environment. In addition, Deepthi, *et al.*<sup>26</sup>, conducted an in vitro study in which was noticed that MTA and Endosequence ERRM microhardness and microstructure were strongly changed in an acidic environment in comparison to Biodentine, decreasing adhesion to the dentin, materials hardness and sealing capacity. Also, Mancino, *et al.*<sup>18</sup>, claimed that Biodentine offers effective sealing when used in an acid environment. The dental perforation placement is a relevant factor in the perforation prognosis.<sup>18</sup>

Two studies affirmed that the closer to the oral cavity, the harshest prognosis is because of the

bacterial contamination arising from the oral environment.<sup>6,8</sup> In a case report mentioned by Kaushik, *et al.*<sup>7</sup>, Biodentine was the chosen material to a perforation repair situated at the cement-enamel junction due to mechanical properties, short prey time and excellent sealing, after 6 months of follow-up, the patient reported favorable outcomes in the healing of periodontal tissues. However, as a result of the absence of scientific evidence, further studies are required in order to highlight their characteristics over other materials. In accordance with one study done in 2014, they found the coronary situated perforations display unfavorable prognosis, with the Furcal perforation as the worst prognosis when compared to the other spots.<sup>6</sup> Alsulaimani<sup>33</sup>, states that the Furcal perforation is a serious issue in dental practice, being taken as a challenging accident. Also, two studies agree that the prognosis of the furcal perforation is questionable, due to the area displaying smaller dental structures, in addition to being close to the gingival sulcus and for that reason; it is taken as a “danger zone”.<sup>8,10</sup> Alsulaimani<sup>33</sup>, asserts that the size of dental perforation is directly associated with the trauma that can cause to the adjacent tissues, negatively impacting on the prognosis, the smaller the perforation and trauma will be and with easier repair as well. To clarify this, she settled that the periodontal tissues displayed a more favorable response to the MTA when it was put in smaller perforations, the greater the perforation was, and the more critical the treatment would be.<sup>33</sup> With the goal of promoting the recovery of the dental element affected by the perforation, the material of choice must encourage the repair and should be biologically neutral.<sup>17</sup> So toxic materials and pulp tissues ought to be spared.<sup>28</sup> When assessing the cytotoxic effect of MTA, ERRM, and Biodentine to the periodontal ligament fibroblasts, Samyuktha, *et al.*<sup>28</sup>, established that there was not noticed any statistical difference between the 3 materials. Another study conducted in 2016, when assessing the MTA and ERRM biocompatibility in the connective tissue of rats determined that the ERRM was comparatively more irritating, displaying higher biocompatibility after 6 weeks

of usage.<sup>17</sup> In addition, case reports done at 2008, they concluded to the use of MTA to seal small, new furcal root perforation is related to a perfect short-term (i.e., 5 years) clinical outcomes.<sup>37</sup>

## CONCLUSIONS

Through the current systematic review, it may be noticed that there is still no unanimity in this review concerning the most appropriate material to be employed in the root perforation therapy, once among the materials studied none showed all the required properties higher than the others. However, the MTA because of high usage of it among dentists and published different studies about this material as a repair material, while the ERRM and Biodentine were recently used, so they did not present long-term studies. For that issue, it is relevant to the implementation of different studies that have as objective to report using clinical and radiographical evidence, the behavior of the materials available for the dental practice.

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None.

## CONFLICTS OF INTEREST STATEMENT

The authors declare no conflict of interest.

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## INDISCRIMINATE USE OF SMOKELESS TOBACCO LEADING TO ORAL CANCER AT A YOUNG AGE; A CASE REPORT WITH LITERATURE REVIEW ON TOBACCO CONSUMPTION

### ABSTRACT

Oral Squamous cell carcinoma (OSCC) constitutes the sixth most common cancer in world. Oral cancer is known as an elderly disease mostly occurring between the 5<sup>th</sup> and 8<sup>th</sup> decades of life. It is mainly attributed to the use of tobacco and alcohol. Only 1-6% of OSCC is reported to occur below 40 years of age. This report describes a rare occurrence of OSCC in a 35 year old male patient, with a disproportionate history of tobacco chewing since the age of 14years; and literature review about tobacco use among younger generation.

**Keywords:** Oral squamous cell carcinoma, smokeless tobacco, tobacco, young adolescents.

 \*Sajad Ahmad BUCH<sup>1</sup>  
 Subhas G BABU<sup>2</sup>  
 Shruthi RAO<sup>2</sup>

ORCID IDs of the authors:  
S.A.B. 0000-0002-1241-4679  
S.G.B. 0000-0001-9383-7886  
S.R. 0000-0003-3138-6455

<sup>1</sup> Department of Oral Medicine & Radiology, Yenepoya Dental College, Yenepoya (Deemed to be) University, Mangalore, 575018 Karnataka-India.

<sup>2</sup> Department of Oral Medicine & Radiology, AB Shetty Memorial Institute of Dental, Sciences, NITTE (Deemed to be University), Mangalore, 575018 Karnataka-India.

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## INTRODUCTION

Squamous cell carcinoma (SCC) comprises of more than 90% of all malignancies affecting the region of head and neck, and occurs during 5<sup>th</sup> to 8<sup>th</sup> decades of life.<sup>1</sup> OSCC rarely affects people below the age of 40 years; with a consistency of only 1-6% when affecting this age group.<sup>2</sup> OSCC has been predominantly seen in males. It commonly affects the tongue most with a site predilection for its posterior and lateral border.<sup>3</sup> This is followed by lips, floor of the mouth, soft palate, gingiva and buccal mucosa.<sup>2,4</sup> OSCC epidemiology depends greatly on the risk factors. The major risk attributes in the older age groups comprise of tobacco and betel quid use along with synergetic effect of alcohol consumption.<sup>5</sup> In India the cancer of gingivo-buccal complex (alveolar mucosa, buccal mucosa and gingival-buccal sulcus) forms the most common site for oral cancer while the tongue and the floor of the mouth constitute the most frequent sites for oral cancer in the western world.<sup>6</sup> This difference can be attributed to the tobacco habits; In India chewing tobacco habits being more common as compared to the smoking forms in the western world. This case report reinforces tobacco etiology underlying OSCC, with description of a 35 year old adult who had a history of indiscriminate tobacco chewing habits for around 20 years.

## CASE REPORT

A 35-year-old male patient was referred from the department of Otolaryngology for prophylactic dental treatment before a definitive treatment plan for his buccal OSCC could be instituted. The patient was a known case of OSCC of right buccal mucosa. The patient had a history of tobacco and paan chewing since the age of 14 years. Extra oral examination of the patient revealed an ulceroproliferative growth of around 3cmx3cm on right side of the face (Figure 1A). The growth was non tender and presence of induration was confirmed. Upon intra oral examination a large ulceroproliferative growth around 7cm x 3cm was seen on right buccal mucosa extending from the commissure to the right retro molar region (Figure 1B).



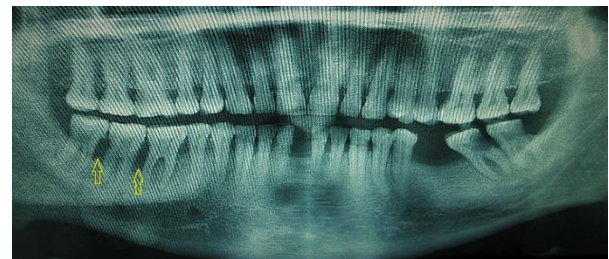
**Figure 1A,** Extra-oral ulceroproliferative growth on right cheek.  
**Figure 1B.** Intra oral image showing extensive involvement of right buccal mucosa with a large proliferative growth.

It was slightly tender and indurated. Extensive generalised tobacco/ paan stains could be seen on all the teeth and mucosal surfaces in oral cavity more so on right side than the left (Figure 2A) and over the entire tongue (Figure 2B).



**Figure 2A.** Massive tobacco/paan stains particularly on right side of mouth  
**Figure 2B.** Tobacco/paan stains covering whole of the dorsal surface of tongue.

Panoramic radiograph revealed generalised interdental bone loss with grossly decayed tooth number 37 (Figure 3).



**Figure 3.** Cropped panoramic radiograph showing extent of bone loss. –Yellow arrows.

There was extensive bone loss with respect to teeth number 46, 47 & 48. The sections used for histopathological assessment in Hematoxylin and Eosin showed dysplastic epithelium proliferating

into connective tissue in the form of sheets and islands (Figure 4).



**Figure 4.** Histopathological slide confirming the diagnosis of oral squamous cell carcinoma.

The features revealed nuclear hyperchromatism, altered nuclear cytoplasmic ratio, individual cell keratinization and keratin pearl formation. Connective tissue showed collagen fibre bundles, chronic inflammatory cells like lymphocytes and plasma cells, muscle fibres, adipose tissue, blood vessels and extravasated RBCs. Extraction of teeth numbers 37, 46, 47 and 48 was carried out followed by thorough periodontal evaluation. The patient was referred back to the department of Otolaryngology for definitive treatment of OSCC after ruling out any further source of infection.

## DISCUSSION

OSCC affects mostly elderly to an extent of 91% and much lower in younger individuals with a range of 0.4 to 3.6%.<sup>7</sup> As is evident from the figures the occurrence of OSCC is not a common finding in young people. OSCC patients are categorised as young if they fall below the age of 40 years, with some authors suggesting the age range as between 20 to 30 years. The age of the patient in present case report was only 35 years. The lateral border of tongue is the most common site for OSCC in patients younger than 40 years; which is similar to OSCC cases among elderly. Our case shows OSCC in buccal mucosa and the reason for the same was exclusive unilateral tobacco chewing habit, with placement of the tobacco in the right mucobuccal fold. The unilateral chewing habit was evident in this case with comparative absence of staining and encrustations on the left side of the oral cavity. In young OSSC patients the

chances of cause effect relationship of tobacco products is quite low due to relatively shorter exposure time. The current case though resembles those of elderly patients with habits, as the patient had a long history of tobacco chewing of around 20 years. This factor exposes the vulnerability of children to the harmful and dangerous culture of our society. The World Bank reports nearly around 99,000 new cases of children and adolescents start smoking habits/day around the world. Reports estimate 5500 adolescents acquire tobacco habits every day in India and add on to the 4 million young tobacco users under the age of 15 years. The children have been victims of easy availability of tobacco products in the Indian markets. India being the second most populous nation in the world, tobacco products have been shown to be prevalent among young boys in the society. India is considered as the second largest consumer and third largest producer of the tobacco products around the globe. In a survey reported among 13-15 year old children nearly 50% reported having first experience of tobacco chewing before the age of 10 years.<sup>8</sup> There are number of tobacco forms available notable among them being, cigarettes, bidi and smokeless tobacco (SLT). SLT does not need to be lit for consumption and is consumed either nasally or placed directly in the mouth. The use of various forms depends on the regional preferences. According to Global Adult Tobacco Survey 2 (GATS-2) 28.6% of the population consume different forms of tobacco, 21.4% use SLT and 10.7% smoke.<sup>9</sup> Tobacco quid chewing leads to six times more risk for oral cancer.<sup>10</sup> India has highest number of oral cancer cases in the world due to SLT use.<sup>11</sup> It is estimated that SLT causes around 50% of all the oral cancer cases. The present case similarly had only tobacco chewing habit with no history of cigarette or bidi smoking. India constitutes the largest global market of SLT (164 million), followed by smokers only (69 million) and having an additional population of 42 million who use both the SLT and the smoked forms.<sup>12</sup> SLT is available in numerous forms namely Naas, Mawa, Gadakhu, Zarda, Betel quid, Paan, Mishri.<sup>13</sup> People in rural areas of India particularly the womenfolk believe SLT as a source of energy and hunger suppressant, as majority of



them depend on hard labour for meagre earnings.<sup>14</sup> The present case belonged to a far flung rural area and was an unskilled worker, spending most of the time farming in other people's land. A disorder of addiction, tobacco use mostly begins during childhood and adolescence. The addictions begins due a myriad of psychosocial factors, like family history of tobacco use, experimentation, influence from peers, easy availability, personality factors, emotional, and psychological issues.<sup>15</sup>

### **CONCLUSIONS**

Application of present policies should be scrutinised particularly in respect to tobacco sales and children purchasing tobacco products. The retail transaction of tobacco products should be banned. Habit counselling should be made mandatory on primary health care level and regional disparities (habit related) should be kept in mind when developing any tobacco counselling programmes. Intense efforts are the need of the hour to target youth for tobacco cessation.

### **ACKNOWLEDGEMENTS**

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### **CONFLICT OF INTEREST STATEMENT**

The authors have no conflict of interest to declare.

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## WILLIAMS-BEUREN SYNDROME- A CASE REPORT

### ABSTRACT

Williams syndrome is a rare neurodevelopmental disorder caused by the spontaneous deletion of genetic material from the region q11.23 of one member of the pair of chromosome 7, so that the person is hemizygous for those genes. Syndrome is a rarely genetic multisystem disorder that occurs equally in all ethnic groups and both sexes. Williams syndrome is a developmental disorder that affects many parts of the body. This condition is characterized by mild to moderate intellectual disability or learning problems, unique personality characteristics, distinctive facial features, and heart and blood vessel (cardiovascular) problems. The diagnosis of this syndrome is based on recognition of the characteristic pattern of dysmorphic facial and physical features. The diagnosis is typically suspected based on symptoms and confirmed by genetic testing. Treatment includes special education programs and various types of therapy. Surgery may be done to correct heart problems. Dietary changes or medications may be required for high blood calcium. Life expectancy is less than that of the general population, mostly due to the increased rates of heart disease. This case report presents the story of 10-year-old girl who suffers from Williams syndrome. Cardiac murmur, mental retardation, severe malocclusion problems, wide mouth, diastema, full lip, periorbital fullness, epicanthal fold, stellate iris pattern, short stature, weaknesses in relational/conceptual language and short term memory observed in this patient. In this study, patient's salivary glands and deciduous anterior tooth were investigated by detailed diagnostic methods (USG, micro-CT). The overall purpose of this case was to diagnose dental manifestations and evaluate physical and clinical characteristics of present case with Williams Syndrome.

**Key words:** Williams Syndrome, salivary glands, tooth, deciduous, heart diseases.

 \*Seren KAYA<sup>1</sup>  
 Kaan ORHAN<sup>2</sup>  
 Firdevs TULGA ÖZ<sup>1</sup>

ORCID IDs of the authors:  
S.K. 0000-0001-7269-2905  
K.O. 0000-0001-6768-0176  
F.T.Ö. 0000-0002-8731-5907

<sup>1</sup> Department of Pediatric Dentistry, Faculty of Dentistry, Ankara University, Ankara, Turkey

<sup>2</sup> Department of Oral, Dental and Maxillofacial Radiology, Ankara University, Ankara, Turkey

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## INTRODUCTION

Williams (Williams-Beuren) syndrome (WS) is a complex systemic disorder that relevant a hemizygous microdeletion of chromosome 7q11.23, encompassing the elastin gene.<sup>1</sup> This syndrome characterized by supravalvular aortic stenosis, distinctive facial features, growth deficiency, mental retardation and learning disabilities.<sup>2-7</sup> This rare congenital disorder was first described in 1961.<sup>2</sup> Williams *et al.* called attention to a syndrome of supravalvular aortic stenosis, mental retardation, and distinctive facial features. Then in 1962, Beuren *et al.*<sup>8</sup> expanded it to include peripheral pulmonary and dental anomalies.

In the great majority of cases, WS is a sporadic event of unknown cause.<sup>9</sup> There are limited data in the literature on syndromes prevalence in the general population but is estimated to be 1 in 20,000 live births. Both sexes affected equally.<sup>10</sup> The diagnosis is based on recognition of the characteristic pattern of dysmorphic facial features, short stature, developmental delay, connective tissue abnormalities affecting the cardiovascular organs, unique cognitive profile, learning difficulties and sometimes transient infantile hypercalcemia.<sup>10,11</sup> A seriously high proportion of this syndrome was reported to have congenital heart disease.<sup>10</sup> WS phenotype consists of a distinct pattern of physical, behavioral, neurologic and cognitive abnormalities evolving from early childhood.<sup>10,1</sup>

This study aimed to evaluate dental manifestations and physical characteristics of the present case with WS.

## CASE REPORT

The procedure performed was in accordance with the ethical standards of the institution and with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained from the patient included in the study and additional written informed consent was obtained from the patient included in the study and additional written informed consent was obtained from the patient for publication of this case report and the accompanying images.

This paper presents a 10-year-old girl who suffers from WS that referred to Ankara University Faculty of Dentistry because of persistent deciduous teeth and routine control. The medical history of the present case showed that the patient had a heart murmur.

In clinical examination; persistent deciduous teeth #51, malpositioned permanent teeth #11 and #21, early eruption, diastema at the front region, severe malocclusion problem, mandibular prognathism, U-shaped arc structure at mandibula (Figure 1) and maxilla (Figure 2), soft tissue hyperplasia and wide mouth structure were detected.



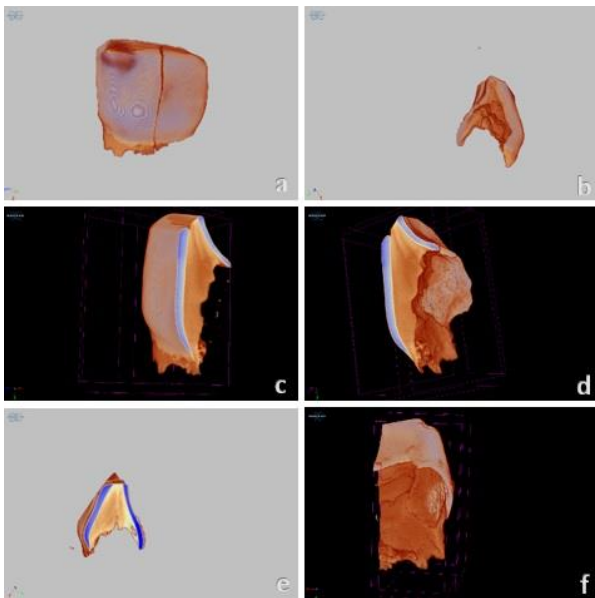
Figure 1. U shaped arc and diastema at the front region intraoral image



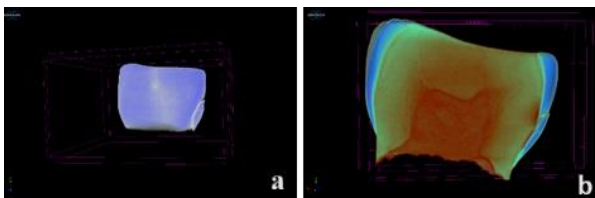
Figure 2. Persistent deciduous teeth and malpositioned permanent tooth intraoral image

Also, plaques on tooth surfaces were detected. But there was no decay, white spot lesion and even fissure coloration detected (dmft 0). There was no intervention at the first appointment, parents just informed about oral hygiene practice. One month later, teeth #51 was extracted. Then, removed tooth and one noncarious extracted tooth #51 from a healthy patient was compared with micro-CT (Figure 3 and Figure 4).





**Figure 3 (A-F)** Micro-CT images of the upper right central primary incisor of the present case. (A) front section of tooth, (B-E) sagittal section of tooth, (F) palatal section of tooth



**Figure 4 (A, B)** Control group's teeth #51 micro-CT image. (A) front section of tooth, (B) vertical section of tooth

According to micro-CT results, the pulp chamber of the patient with WS was detected wider and thinner than the control tooth. Then, the patient was requested to have USG to evaluate the salivary glands. Bilateral parotid and submandibular glands were examined in this examination. Size and contours of bilateral parotid glands were normal. The parenchyma of the glands was homogenous mostly but with heterogeneous foci in some regions of the superficial lobe. Intraparotideal lymph nodes were also observed with a dimension of 1x3 mm in both parotid gland. The dimensions of the bilateral submandibular glands were fairly hyperplastic, but contours were normal. The parenchyma was heterogeneous in both glands. There was no solid or cystic lesion detected in the parenchyma. Especially in the left submandibular gland vascularisation get increased. On both sides, there is a reactive lymph node which is enlarged in the submandibular region.

## DISCUSSION

WS's distinctive facial features are; microcephaly, a broad forehead, medial eyebrow flare, periorbital

fullness, strabismus, stellate iris pattern, flat nasal bridge, malar flattening, along small philtrum, full cheeks and lips a rather pointed chin and a wide mouth. The face becomes more coarse with age.<sup>12</sup>

Syndrome's distinctive physical features reported in some cases are; mild degrees of short stature, kyphoscoliosis, a long neck, inguinal or umbilical herniae, small nails and hallux valgus.<sup>1</sup> From these distinctive features; short stature, a broad forehead, medial eyebrow flare, periorbital fullness, strabismus, stellate iris pattern, pointed chin observed in this case.

Many studies showed that some of the children had mandibular prognathism, temporomandibular joint abnormalities and dental malocclusion as in this case, but did not consider these findings part of the syndrome.<sup>2,13</sup>

Beuren *et al.*<sup>3</sup> reported that two male children demonstrated agenesis of deciduous and permanent teeth, bud-shaped deciduous maxillary molars, small permanent incisors, broad maxillary and small mandibular dental arches with bilateral posterior scissors-bite. And emphasize that the girls had similar but less pronounced dental malformations. But in this case; tooth structure and size differences, agenesis, small mandibular dental arc with posterior scissors-bite were not observed.

The most frequently observed tooth agenesis pattern seems within the three groups; incisors, canines/premolars, and molars. The general dental agenesis pattern seems to be associated with individual innervation pathways.<sup>14</sup> Despite all this, agenesis has not been observed in this case. Some studies have reported some increased frequency of dental abnormalities like hypodontia, microdontia, invagination of maxillary incisors, small and slender roots, increased space between teeth, enamel hypoplasia, high prevalence of dental caries and malocclusion.<sup>10,15,16</sup> These findings were generally reported in medical reviews of limited numbers of WS cases. Except for malocclusion and diastema, no other findings are observed in this case.

45 individuals with WS aged 13 months to 28 years, giving prevalence rates of different dental findings of tooth abnormalities, dental carries and

malocclusion traits.<sup>17</sup> In the other study clinical and radiological examination of 37 individuals with WS 2 to 24 years, it reported that, caries in the deciduous dentition, hypodontia and mineral content in deciduous incisors.

Craniofacial features have reported as dolichocephaly, bitemporal depressions, asymmetry, full cheeks, periorbital fullness, epicanthal folds, stellate or lacy iris patterns, full nasal tip, full lips, long philtrum, wide mouth, and dental malocclusion.<sup>10</sup> Also, periorbital fullness, epicanthal fold, stellate iris pattern, full lip, wide mouth, malocclusion were observed in this case.

In some cases, WS patients have cardiovascular problems which include supraaortic stenosis; failure to thrive in infancy; transient neonatal hypercalcemia.<sup>4,18-20</sup> Hypercalcemia is an infrequent feature when a large series of cases are reviewed.<sup>21</sup> But supraaortic stenosis and arterial narrowing are frequently present.<sup>22</sup> In this case, the only cardiac murmur was observed systematically.

Valve involvement may add the murmur of aortic incompetence to the systolic murmur transmitted to the carotids.<sup>23</sup>

As seen in this case, delayed language and abnormal sensitivities to classes of sounds (hyperacusis) can be observed in the WS.<sup>24,25</sup> They use complex expressive language and they usually have a hoarse voice.<sup>26,27</sup>

WS patients have variable mental retardation. Our patient shows moderate mental retardation. Most patients have mental retardation with an IQ between 50 and 70.<sup>11,28</sup> William syndrome patients usually have a friendly, outgoing personality. Also, our patient was so extrovert and social. According to family history, genetic transition not observed in this case. Already it is stated that WS is rarely familial.<sup>27</sup>

#### ACKNOWLEDGEMENTS

None

#### CONFLICTS OF INTEREST STATEMENT

None

#### Williams-Beuren Sendromu- Vaka Raporu

#### ÖZ

Williams sendromu 7q11.23 kromozomunun silinmesinden kaynaklanan, tüm etnik gruplarda ve her iki cinsiyette eşit ve nadir olarak gözlenen nörogelişimsel multisistemik bir hastalıktır. Vücudun birçok bölümünü ilgilendiren gelişimsel bir hastalık olan sendrom, hafif ile orta derecede zihinsel engellilik veya öğrenme problemleri, farklı kişilik özellikleri, kendine has yüz özellikleri ve kardiyovasküler problemler ile karakterize edilir. Sendromun tanısı, karakteristik dismorfik yüz özellikleri ve karakteristik fiziksel özellikler ile mümkündür. Tanular, genetik testlerle onaylanır. Tedavi, özel eğitim programlarını ve çeşitli terapileri içerir. Kardiyovasküler problemleri düzeltmek amacıyla ameliyat yapılabilir. Bu hastalarda yüksek kan kalsiyum değeri gözlenebildiği için diyet değişiklikleri veya ilaç tedavisi de diğer tedavi seçenekleridir. Yaşam beklentisi, çoğunlukla kalp hastalığı oranlarının artması nedeniyle genel popülasyondan daha azdır. Bu olgu sunumu, Williams sendromu teşhisi konulmuş 10 yaşındaki kızın öyküsünü sunmaktadır. Bu hasta; kardiyak üfürüm, zekâ geriliği, ciddi maloklüzyon problemleri, geniş ağız, diastama, periorbital dolgunluk, epikantal kıvrım, stellat iris paterni, kısa boy, konuşmada zayıflıklar ve kısa süreli hafızaya sahiptir. Bu çalışmada, hastanın tükürük bezleri USG ile ve süt ön dişleri micro-CT ile ayrıntılı olarak incelenmiştir. Bu olgunun genel amacı, sendromun diş hekimliği ile ilgili belirtilerini teşhis etmek ve mevcut vakanın fiziksel ve klinik özelliklerini değerlendirmektir. **Anahtar Kelimeler:** Williams sendromu, tükürük bezleri, diş, süt dişi, kalp hastalıkları.

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## MICRO-CT EVALUATION OF TAURODONTISM IN A DECIDUOUS MOLAR AND A PERMANENT MOLAR: CASE REPORT

### ABSTRACT

Taurodontism is a morphological anomaly of teeth characterized with large pulp chambers and apically displaced pulpal floor. The aim of this case report was to make a comparative evaluation by panoramic radiographies and 3D examinations to locate pulp chamber and root canal morphology of a primary mandibular first molar and the adaptation of root canal filling material of a permanent mandibular first molar, both diagnosed with taurodontism. In Case-1, a mandibular primary first molar was extracted due to physiological root resorption seen in the panoramic radiograph. The micro-CT (Bruker, SkyScan 1174, Belgium) examined the distance of the tooth a (distance between the lowest and the top point of the pulp chamber) and b (distance between the lowest point of the pulp chamber and the apical). The tooth was classified as mesotaurodont by the value of 52.91 obtained with the formula  $a/b \times 100$ . In Case-2, the tooth was extracted because of the large periapical radiolucency observed in the radiography taken from the tooth which was admitted for pain and percussion tenderness. The calculation for subgroup could not be performed due to loss of the "a" value because of the cavity preparation. Although the root canal filling on the radiograph was found to be sufficient and long enough, an empty extra canal and insufficient wall adaptation of root canal filling were detected by micro-CT. Despite the fact that taurodontism can be diagnosed by 2D measurements, it is obvious that a 3-dimensional examination is necessary to obtain decisive data about the root canal cavity morphology especially for endodontic treatment and also to define prevalence and subgroups of anomalies. Although micro-CT is seen as a method that provides more detailed images, it is thought that it should be developed and supported with clinical studies in order to be valid and easy to use in clinical practice.

**Key words:** Taurodontism, microcomputed tomography, primary tooth, permanent tooth.

 \*Burcu Nihan YÜKSEL<sup>1</sup>

 Kaan ORHAN<sup>2</sup>

 Firdevs TULGA ÖZ<sup>1</sup>

ORCID IDs of the authors:

B.N.Y. 0000-0002-8133-6627

K.O. 0000-0001-6768-0176

F.T.Ö. 0000-0002-8731-5907

<sup>1</sup> Department of Pediatric Dentistry, Faculty of Dentistry, Ankara University, Ankara, Turkey

<sup>2</sup> Department of Oral Diagnosis and Radiology, Faculty of Dentistry, Ankara University, Ankara, Turkey

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## INTRODUCTION

Taurodontism has been accepted as a shape alteration defined as the enlargement of the pulp chamber and the apical displacement of the pulpal floor and the bifurcation/trifurcation of the roots.<sup>1</sup>

It is stated that conditions such as calcification delay at pulp chamber floor, odontoblastic deficiency, failure of the invagination level of the Hertwig's epithelial root sheath could be related for the pathogenesis.<sup>2</sup>

In studies conducted in different countries prevalence varies from 0.4%-46.4% for permanent teeth.<sup>3-6</sup> Prevalence studies for primary dentition are limited and frequency was stated as 2.4% for the Turkish population.<sup>7</sup>

Diagnosis have been done with periapical or panoramic radiographs only by visual observations.<sup>8,9</sup> With the evolution of technical equipment, certain dimensions could be done with digital radiographies to locate the points of cementoenamel junction, furcation boundaries and the upper and lower points of pulpal chamber.<sup>5,10</sup> Following years, diagnostic studies were performed to define the subgroups of taurodontism by the calculation of the ratio of the lengths of the crown plus body (CB) to the root.<sup>5,11,12</sup> It was observed that taurodontism occurs by varying degrees that may be classified in increasing order of severity as hypotaurodontism, mesotaurodontism, and hypertaurodontism. As the results of these studies, metric criteria were determined to diagnose the subgroups.

Taurodontism were diagnosed frequently by radiographic techniques.<sup>8-10</sup> Micro-computerized tomography methods (micro-CT) that allows 3-dimensional measurements and images have been began to be used in pediatric dentistry.<sup>13,14</sup>

In this case presentation, comparative evaluation by panoramic radiographies and micro-CT methods was used to locate pulp chamber, root canals and morphologies of a primary mandibular first molar and a permanent mandibular first molar diagnosed with taurodontism.

## CASE REPORTS

### Case 1

A mandibular first primary molar in 10 years-old patient have been diagnosed with deep dentine caries and physiological root resorption. After written informed consent was taken, tooth was extracted due to 2/3 of root growth of the underlying germ was complete and symmetrical first premolar was erupted (Figure 1).

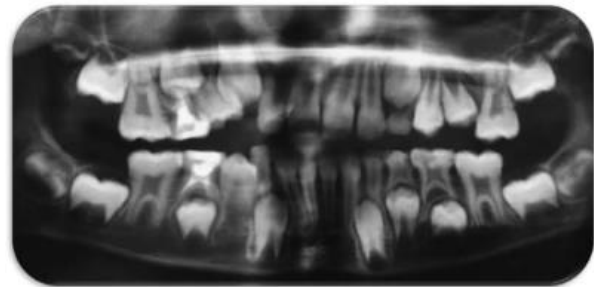


Figure 1: Panoramic radiograph of Case 1.

Radiographic signs of all permanent first molars and left second primary molar have revealed large pulpal chambers which also confirmed the definition for taurodontism. Dental history was not relevant with any medical history. Parents and siblings were also examined radiographically and no signs of taurodontism were detected. Tooth were examined with a micro-CT (Bruker, SkyScan 1174, Belgium) and measurements have been done for the reference points a (distance from the lowest point of pulp chamber roof to the highest point of the floor) and b (distance from the highest point of pulp chamber roof to the apex) (Figure 2a, 2b, 2c, 2d).

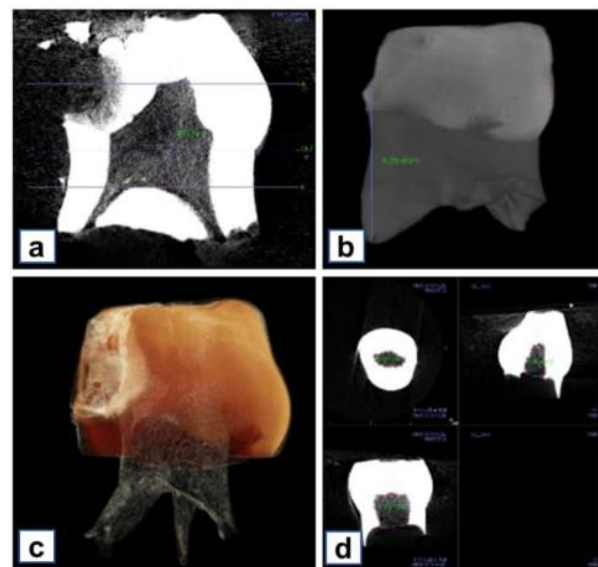


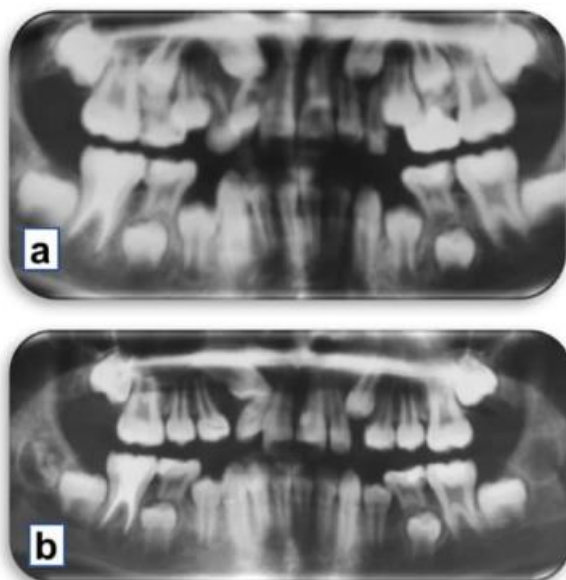
Figure 2a, 2b, 2c, 2d: Images and measurements of Case 1 by micro-CT



According to calculation by  $a/b \times 100$  acquired values as 0-24.9, 25-49.9, 50-74.9, 75-100; teeth were defined respectively as “cynodont (usual), hypotaurodont, mesotaurodont and hypertaurodont”. Shifman and Chanannel (1978) criteria were used for assessment.<sup>9</sup> The value of was calculated as 52,91 for the left mandibular primary molar and classified as mesotaurodont.

### Case 2

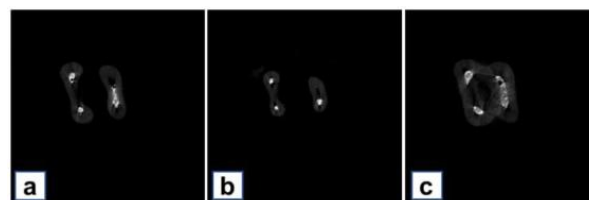
A 13 years-old patient applied with a chief complaint of pain and tenderness to percussion in the right mandibular region. Radiographic examination revealed a periapical radiolucency and a root canal treatment for permanent mandibular right molar (Figure 3a, 3b).



**Figure 3a-3b:** Panoramic radiographs of Case 2 taken 1 year interval.

Parent of the patient told that root canal treatment was a retreatment intervention which was done a year earlier. All permanent first molars and primary mandibular molars was also diagnosed as taurodont. Extraction of tooth was indicated after written informed consent was taken. Tooth were examined with a micro-CT and images were taken.

Despite of the length and the wall adaptation of the root canal treatment could be defined as satisfactory by radiographic observation and the coronal restoration was stated as adequate by visual inspection, an extra canal could be seen as obtained from the sectional images (Figure 4a, 4b, 4c).



**Figure 4a, 4b, 4c:** Images of the sections that presents poor dental wall adaptation of root canal filling for Case 2.

Besides these, especially distal root canal of the tooth could be identified quite elliptical, combination of the gutta percha and the sealer located in the center of the canal space as circular and gaps were detected between the root canal walls and the filling material. Measurements and calculations could not be done because of the preparation and treatment processes. However, morphology of taurodontism could obviously be seen by radiographically.

### DISCUSSION

For making a diagnose and a classification for taurodontism it was reported that certain and constant external attributes (crown, body and root) had to be assessed combined with internal variables.<sup>15</sup> It was reported that external points could be failed because prismatic roots with separate root canals could not be distinguished with the taurodont teeth which may have separate or adjacent root canals. Additionally, space of the pulp chamber which could be counted as internal variable may alter due to calcifications related to environmental changes such as age, caries and also root canals need to be assessed internally. Therefore, biometric measurements will be needed to be made from points that are not affected by environmental factors and that do not change in the developmental process.<sup>2</sup> Subgroups were also defined by studies which have been carried out with numerical values as a result of the biometrical measurements.<sup>5,11,12</sup> In Case 1, the Shifman and Chanannel criteria were used for calculation method to define the subgroup and tooth was classified as mesotaurodont. It was not possible to make a measurement for Case 2 due to preparation and instrumentation process.

Pulp chamber took up a large volume especially for primary teeth even in normal morphology.<sup>14,15</sup> When cavity preparation is planned for any type of restoration, it is desirable

to prevent perforation. In situations where perforation occurs, it is important to locate the pulp chamber and canal orifices to make a better treatment planning. It has been reported that pulp chamber of a taurodont teeth is large compared to cynodont teeth therefore the possibility of perforation was increased.<sup>10</sup> In Case 1, the images supported the superficial and voluminous position of pulpal chamber.

Knowledge of internal root canal anatomy for taurodont teeth could be useful to make a plan including instrumentation techniques and restoration materials.<sup>16,17</sup> For Case 2, the presence of an extra root canal, the difference of the shape of canal anatomy and the failure of the retreatment process displays this necessity.

Diagnosis is usually done by routine radiographs observations.<sup>8-10</sup> It is known that 2D examinations done in the diagnosis stage occasionally may not be useful in the treatment stage. By using micro-CT methods which recently enables 3 dimensional evaluations, detailed sectional measurements and examinations can be made *in vitro*. In both of the cases presented in this case report, micro-CT enabled to make detailed measurements and to obtain data on sectional images. From this point of view, it is clearly seen that sectional examination methods which are applicable *in vivo* should be used by clinicians where clinical conditions are necessary.

## CONCLUSIONS

Diagnosis of taurodontism helps diagnose pathological conditions such as systemic diseases and syndromes accompanying this anomaly, as well as facilitating clinical and operative clarification of diagnosis and treatment. Moreover because of its possible family trait this evaluation can be used for forensic purpose. Even though taurodontism can be diagnosed by 2D measurements on radiographic images, it is obvious that 3 dimensional examinations and volumetric measurements are necessary. Though micro-CT is considered as a method to help diagnosis and treatment with more detailed images in this sense, it is thought that 3D measurement methods should be developed for clinical

application. It was seen that it should be supported by clinical trials.

## ACKNOWLEDGEMENTS

None

## CONFLICT OF INTEREST STATEMENT

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

### *Taurodont Bir Süt ve Daimi Molar Dişin Mikro-CT ile İncelenmesi: Olgu Sunumu*

#### **ÖZ**

*Taurodontizm pulpa odası tabanının apikale doğru yer değiştirmiş olması ile karakterize morfolojik bir dental anomalidir. Bu olgu bildirisinde radyografik olarak taurodontizm tanısı konulan ve çekim endikasyonu olan süt 1. azı ve daimi 1. büyük azı dişinin pulpa odası ve kök kanallarının mikro-CT ile değerlendirilmesinin sunumu amaçlanmıştır. Olgu 1’de, radyografik muayenesinde fizyolojik kök rezorbsiyonu olduğu görülen sol alt süt birinci azı dişin çekimi uygun görülmüştür. Mikro-CT (Bruker, SkyScan 1174, Belgium) ile incelenen dişin a (pulpa odası tavanının en alt noktası ve tabanının en üst noktası arasındaki mesafe) ve b (pulpa odası tavanının en alt noktası ile apikal arasındaki mesafe) mesafeleri hesaplanmıştır. Olgu 1’deki dişte  $a/b \times 100$  formülüyle elde edilen 52,91 sayısal verisine göre süt azı dişi mesotaurodont olarak sınıflandırılmıştır. Olgu 2’de alt sağ daimi birinci büyük azı dişinde ağrı ve perküsyonda hassasiyet nedeniyle başvuran hastadan alınan radyografide geniş periapikal radyolusensi gözleendiği için dişin çekimi uygun görülmüş ve mikro-CT (Bruker, SkyScan 1174, Belgium) ile kök kanal morfolojisi incelenmiştir. Dişin kavite preperasyonu sonucunda a değeri kaybedildiği için hesaplama yapılamamıştır. Alınan radyografide kanal dolgunun kök boyu itibarıyla yeterli ve olması gereken uzunlukta olduğu belirlenmesine karşın mikro-CT’den elde edilen kesitsel görüntülerde ise kanal dolumu yapılmamış ekstra bir kanal boşluğu ve özellikle distal kanalın kesitinin oldukça eliptik olduğu tespit edilmiştir. 2 boyutlu radyografilerle taurodontizm tanısı konulabilmesine rağmen özellikle endodontik tedavi endikasyonu olan dişlerde kök kanal boşluğu morfolojilerinin 3 boyutlu olarak incelenmesi gerekliliği görülmektedir. Mikro-CT daha detaylı görüntüler sağlayan bir yöntem olarak görülmekle birlikte klinik kullanımda geçerliliği ve uygulama kolaylığı olacak şekilde geliştirilmesi ve klinik çalışmalarla desteklenmesi gerektiği*

*düşünülmektedir. Anahtar Kelimeler: Taurodontizm, mikrobilgisayarlı tomografi, süt dişi, kalıcı diş.*

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