Official Publication of
Istanbul University
Faculty of Dentistry

European Oral Research

Volume 55 ▪ Issue 3 ▪ September 2021

ISSN print 2630-6158 ▪ ISSN online 2651-2823

EOR

eor.istanbul.edu.tr

Istanbul University Press

European Oral Research

Official Publication of
Istanbul University
Faculty of Dentistry

INDEXING AND ABSTRACTING

WEB OF SCIENCE - EMERGING SOURCES CITATION INDEX (ESCI)
PUBMED Central
PROQUEST
EBSCOHOST
DIRECTORY OF OPEN ACCESS JOURNALS (DOAJ)
OPEN AIRE
CHEMICAL ABSTRACTS
GOOGLE SCHOLAR
TUBITAK ULAKBIM TÜRKİYE ATIF DİZİNİ

European
Oral Research

Official Publication of
Istanbul University
Faculty of Dentistry

OWNER

Prof. Dr. Gülsüm AK
Department of Division of Clinical Sciences, Istanbul University, Faculty of Dentistry, İstanbul, Turkey

RESPONSIBLE MANAGER

Prof. Dr. Handan ERSEV
Department of Endodontics, İstanbul University, Faculty of Dentistry, İstanbul, Turkey

CORRESPONDENCE ADDRESS

Istanbul University, Faculty Of Dentistry
Prof. Dr. Cavit Orhan Tütengil Sokak No. 4 Vezneciler-Fatih-İSTANBUL
Phone: +90 (212) 440 00 00 / 12100
E-mail: eor@istanbul.edu.tr
https://dergipark.org.tr/tr/pub/eor
https://iupress.istanbul.edu.tr/tr/journal/eor/home

PUBLISHER

İstanbul University Press
İstanbul University Central Campus,
34452 Beyazit, Fatih / İstanbul, Turkey,
Phone: +90 (212) 440 00 00

PRINTED BY

İlbey Matbaa Kağıt Reklam Org. Müc. San. Tic. Ltd. Şti.
2. Matbaacılar Sitesi 3NB 3 Topkapı/Zeytinburnu, İstanbul, Turkey
E-mail: www.ilbeymatbaa.com.tr
Certificate No: 17845

The publication languages of the journal is English.

This is a scholarly, international, peer-reviewed and open-access journal published triannually in January, May and September.

Publication Type: Periodical

European Oral Research

Official Publication of Istanbul University Faculty of Dentistry

EDITORIAL MANAGEMENT BOARD

European Oral Research

Official Publication of Istanbul University Faculty of Dentistry

EDITORIAL ADVISORY BOARD

European Oral Research

Official Publication of Istanbul University Faculty of Dentistry

AUTHOR GUIDELINES

General information

European Oral Research (Eur Oral Res) is an open access, peer-reviewed international title and it is the official scientific publication of Istanbul University Faculty of Dentistry. Eur Oral Res does not charge authors or authors' institutions for submitting, processing or publication of articles. There is no fee for extra pages or color images.

Basic and clinical research papers, case reports and review articles on the main topics of oral and maxillofacial surgery, oral diseases, oral and dental health, oral and maxillofacial radiology, biomaterials, restorative dentistry, periodontics, pedodontics, orthodontics, endodontics, prosthodontics, oral biology, epidemiology, geriatric dentistry and dental education may be submitted for consideration. Letter to the Editor section is also available for authors who wish to comment on previously published articles in the journal. Authors should take collective responsibility for their work and for the content of their publications. Editor-in Chief or the Editorial Board reserves the right to change the format, grammar or sentence structure of any part of the manuscript to comply with the guidelines to fit the standard format and style of Eur Oral Res and scientific journals in general.

Who is an author ?

Eur Oral Res adheres to the International Committee of Medical Journal Editors (ICMJE) standards to define the qualifications of authorship and recommends that it should be based on the following 4 criteria: substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND drafting the work or revising it critically for important intellectual content; AND final approval of the version to be published; AND agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Before you begin

Disclaimer and copyright

Submission of a manuscript implies that the work described has not been published before; that it is not under consideration for publication anywhere else entirely or partially, nor will it be submitted for consideration of publication anywhere else until a final publication decision concerning this manuscript has been made by the Editor(s) of the Journal; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities at the institute where the work has been carried out. The publisher cannot be held legally responsible should there be any claims for compensation.

Eur Oral Res provides free access to and allows free download of its contents from the journal's website (http://eor.istanbul.edu.tr/tr/_). Both anonymous or registered users can read and/or download articles for personal use. Unless otherwise indicated, the articles and journal content are licensed under Creative Commons License Attribution-NonCommercial 4.0 International (CC BY-NC 4.0) license (https://creativecommons.org/licenses/by-nc/4.0/). Users must give appropriate credit, provide a link to the license, and indicate if changes were made. Users may do so in any reasonable manner, but not in any way that suggests the journal endorses its use. The material cannot be used for commercial purposes. If the user remixes, transforms, or builds upon the material, he/she may not distribute the modified material. No warranties are given. The license may not give the user all of the permissions necessary for his/her intended use. For example, other rights such as publicity, privacy, or moral rights may limit how the material can be used.

Articles may not be published elsewhere, in whole or in part, electronically or in print, without written permission from the Editor-in-Chief. The responsibility of the content(s) and/or opinion(s) provided in the articles which are published in the print and/or online versions of the journal, belong exclusively to their respective author(s). The publisher/editor/editorial board/reviewers cannot be held responsible for errors, scientific or otherwise, in the contents or any consequences arising from the use of information contained therein. The opinions expressed in the articles published in this journal are purely those of their respective authors and in no way represent the opinions of the publisher/editor/editorial board/reviewers of the journal.

Publication ethics and avoiding allegations of plagiarism

Please refer to http://eor.istanbul.edu.tr/en/content/about/publication-ethics-and-publication-malpractice-statement to consult Eur Oral Res Publication Ethics and Publication Malpractice Statement. By submitting their manuscripts to Eur Oral Res, authors also accept that their manuscripts may be screened for signs of plagiarism using any means necessary and available; including, but not limited to, the use of plagiarism detection software.

Ethical approvals

Experimentation involving human subjects should be conducted in full accordance with the Helsinki Declaration of World Medical Association (http://www.wma.net/en/30publications/10policies/b3/index.html) and legal requirements of the country where the research had been carried out. Manuscript must include a statement indicating that the informed consent was obtained from all participants. A statement confirming that the study has been reviewed and approved by an ethical or advisory board should also be included. The patient's privacy should not be violated. Identifying information such as names, initials, hospital numbers, unnecessary details in photographs should be omitted from the submission. When detailed descriptions, photographs and/or videos of faces or identifiable body parts that might permit a patient to be identified must be included in the submission, authors must obtain written

European Oral Research

Official Publication of Istanbul University Faculty of Dentistry

informed consent for its publication from the patient or his/her parent/guardian.

Experiments on laboratory animals must comply with the Guidelines of the European Communities Council Directive 2010/63/EU and with local laws and regulations. A statement confirming that the study has been reviewed and approved by an ethical or advisory board should also be included (http://www.eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32010L0063).

Clinical trials

Randomized controlled clinical trials should be reported in full accordance with the guidelines available at http://www.consort-statement.org. The CONSORT checklist must be provided in the submission documents. Following free public clinical registries can be used to register clinical trials: http://www.clinicaltrials.gov, http://clinicaltrials.ifpma.org/clinicaltrials/, http://isrctn.org/ . Registration number and project name will be published in the article.

DNA Sequences and Crystallographic Structure Determinations

Manuscripts reporting protein or DNA sequences and crystallographic structure should provide Genbank or Brookhaven Protein Data Bank accession numbers, respectively.

Submission forms

Authors who are willing to submit their manuscripts to Eur Oral Res are required to complete and sign Manuscript Submission Form, Copyright Transfer Agreement form and Disclosure of Potential Conflict of Interest Form. Please send these forms electronically when submitting your manuscript. Article evaluation process cannot be started until all documents are received.

All three documents are available for download at:

http://dishekimligi.istanbul.edu.tr/wp-content/uploads/2015/04/JIUFD-submission-forms.zip

Manuscript preparation

Language

Authors should write their manuscripts in US English. Spelling and phrasing should conform to standard usage and be consistent throughout the paper. Authors whose native language is not English are encouraged either to consult with a native speaker or to collaborate with a colleague whose English skills are more advanced. Authors may also use professional translation services at their own expense. Please note that using language editing services does not imply that the article will be selected for peer-review or be accepted by Eur Oral Res.

Style and format

Use A4 page format in Microsoft Word® software, custom margins (top & bottom 3 cm, left& right 2.5 cm), Times New Roman Font, Font size 12, double line spacing for main text and single line spacing for "References" section. US English grammar check option should be enabled. Words and abbreviations in Latin should be written in italics: "*et al., in vivo, in vitro, in utero, in situ, ad libitum, clostridium perfringens, Staphylococcus aureus*".

Unit abbreviations

Abbreviations used for units, prefixes, and symbols should comply with the International System of Units (SI) (http://physics.nist.gov/Pubs/SP330/sp330.pdf). If this is not possible, SI equivalents must be presented between parentheses. The complete names of individual teeth must be given in the main text (e.g. maxillary right central incisor). FDI 2-digit system should be used in table and figure legends (e.g. 11 for maxillary right central incisor).

Title page

Main title of the manuscript should not exceed 150 characters (including spaces) and it should be written in Times New Roman font, in bold capital letters of 12 font size. A running title not more than 50 characters (including spaces) written in lower case letters must also be provided below the main title. Names, surnames and affiliations of all authors should appear below the running title. Use superscript numbers "1,2,3" for authors from different institutions, do not use any numbers if all authors are from the same department. Name, surname, postal address, phone, fax and e-mail of the corresponding author should be mentioned separately. If the paper has been previously presented in a scientific meeting either orally or as a poster, the title of the manuscript should be followed by an asterisk (*), which refers to a footnote indicating the name of the organization, location and date of its presentation. Please do not use page numbers for this page.

Abstract & Keywords page

This page should start with main and running titles of your manuscript. It should not contain author names, affiliations or any citations. This section must describe the main objective(s) of the study, explain how the study was done without giving too much methodological detail and summarize the most important results and their significance. It should be as clear and concise as possible. Start numbering from this page on and place it at the lower right-hand corner of the page footer. Abstracts should not exceed 250 words for original research papers and should be structured to include Purpose, Materials and Methods, Results, Conclusion headings written in bold letters. Abstracts of case reports and review articles are limited to 150 words and should be unstructured.

If not absolutely necessary, do not include names of statistical tests or software in the Materials and Methods. In Results section, Provide p values between parentheses at the end of the sentence before the period. If p value is lower than 0.05 or 0.01 or higher than 0.05 (not significant), then provide its exact value using a maximum of three digits after the decimal point. If it is lower than 0.001, then use only less-than sign, e.g. p=0.078, p=0.048, p=0.009, p<0.001.

European Oral Research

Official Publication of Istanbul University Faculty of Dentistry

A maximum of five keywords should follow the abstract, preferably chosen from the Medical Subject Headings (MESH) terms (http://www.ncbi.nlm.nih.gov/mesh).

In-text citations

Eur Oral Res recommends the use of reference management software to ensure that the citations are correctly formatted. In-text citations should be numbered consecutively in the order of appearance with Arabic numerals between parentheses and be placed immediately after the author(s)' name(s).

Articles with two authors are cited using their last names separated by "and":

"John and James (1) demonstrated...".

For articles with three or more authors, use the first author's last name followed by "et al.": "James *et al*. (2) showed...".

Multiple quotations used within the same sentence should be cited immediately after each author(s)' name(s).

"John and James (1) and James *et al*. (2) have suggested…"

If author(s)' name(s) are not to be used, the citation number(s) should be placed at the end of the sentence before the period. Use number ranges for consecutive citations.

"The validity and reliability of dental anxiety scales have been evaluated previously (7) or (4, 6, 8) or (12-19)."

Original research articles

Original research articles are limited to 15 pages including main text, references, tables and figures. They should be organized into the following sections:

- Title page
- Abstract & Keywords
- Introduction
- Materials and Methods (or Subjects and Methods)
- Results
- Discussion
- Conclusion
- Acknowledgements (optional)
- Source of funding
- Conflict of Interest
- References

Introduction should provide a concise account of the research problem and introduce the reader to the pertinent literature. The objective(s) and/or hypothesis of the study should be clearly stated in the last paragraph. Writing style of this section should allow the readers outside the field of your expertise to understand the purpose and significance of the study.

Materials and Methods section should describe the study population/sample or specimens, the variables and the procedures of the study with sufficient detail to ensure reproducibility. Standard methods already published in the literature could be briefly described and the original reference should be cited. If your research includes direct involvement with human subjects, use 'Subjects and Methods' heading instead of 'Materials and Methods'.

Feel free to use sub-headings written in italic letters (but not bold) to improve readability of your manuscript such as Population characteristics, Immunohistochemical staining, Experimental protocols or Light microscopy evaluation.

Include ethical approvals for clinical trials and animal studies in the first paragraph of this section. Provide the name of the responsible organization, year of approval and project number:

This project has been reviewed and approved by the Ethical Committee of Istanbul University, Faculty of Medicine (2012/891-1085).

Include Brand name, Manufacturer, City, (state abbreviation for USA), Country details for each material used in the experimental protocol:

DNA was extracted using a MagNA Pure-Compact DNA Isolation Kit (Roche Diagnostics GmbH, Mannheim, Germany)

Bone grafts were fixed with 2 mm bioresorbable screws (Inion CPS system, Inion OY, Tampere, Finland).

Statistical analysis sub-heading must be included as the last paragraph of this section. Authors should provide the name of the statistical software, report which types of descriptive statistics were used to summarize the data, indicate how the distribution of the data was tested for normality assumptions (if applicable), which tests were employed to answer each hypotheses, the confidence interval and p values to determine the level of significance. Consult SAMPL guidelines for more detailed information on statistical reporting in biomedical journals: http://www.equator-network.org/wp-content/uploads/2013/07/SAMPL-Guidelines-6-27-13.pdf

Provide Name, Version, Company, City, (state abbreviation for USA), Country for statistical software:

GraphPad Prism version 3.0 statistical analysis software (GraphPad Software Inc., San Diego, CA, USA)

The following paragraph is a sample for statistical analysis section; please alter the paragraph so that it fits your study:

The collected data from all groups were imported to Statistical Package for Social Sciences (SPSS) for Windows software, version 16.0 (SPSS Inc., Chicago, IL, USA). The standard descriptive methods such as the mean, standard deviation, median, frequency, minimum and maximum were applied to determine the characteristics of the sample. The chi-square test was used to compare the categorical demographic variables among the groups. Because the distribution of the data did not meet the requirements for normality and homogeneity of variances assumptions, the nonparametric Kruskal-Wallis one way analysis of variance by ranks and Mann-Whitney U tests were used for the multiple and pairwise comparisons, respectively. The correlations between at least two continuous variables were examined using Pearson's correlation coefficient. Stepwise regression analysis was performed to understand the statistical dependence of the DFS and MDAS scores in the general population. Covariance analysis was used to determine whether the difference between the mean DFS and MDAS

scores of the groups was statistically significant under a predefined effect. The confidence interval was set to 95% and $p < 0.05$ was considered statistically significant.

Results should be written clearly without subjective interpretation and be supported with tables and figures when necessary. Text should complement any figures or tables but it should not repeat the same information. When reporting your findings, follow the same order you have used in "Materials and Methods" section.

Use a maximum of two digits after the decimal point for descriptive statistics such as mean and standard deviation: "45.66±23.48". If the last digit is 0 then use : "45.6±23.4".

Provide p values between parentheses at the end of the sentence before the period. if p value is lower than 0.05 or 0.01 or higher than 0.05 (not significant) then provide its exact value using a maximum of three digits after the decimal point. If it is lower than 0.001, then use only less-than sign, e.g. (p=0.078), (p=0.048), (p=0.009), (p<0.001).

In the **Discussion** section, authors should state major findings, their meanings and clinical relevance, present any contrasts with the results of similar studies, describe unavoidable limitations in the study design and make suggestions for further research within the limits of their data. When discussing your findings, use the same logical order as in the Results section.

Conclusion should be supported by results and must be consistent with the objectives of the research.

Case reports/Case series

Case reports/Case series should not exceed 7 pages and a maximum of 6 tables or figures. They should make a significant contribution by presenting unusual occurrences of rare entities and/or highlight the need for revision of current therapeutic options. This type of manuscript should be organized as follows:

- Title page
- Abstract & Keywords
- Introduction
- Case report (or case series)
- Discussion
- Conclusion
- Acknowledgements (optional)
- Source of funding
- Conflict of Interest
- References

Narrative or systematic reviews and meta-analyses

Narrative review articles are limited to 10 pages including the main text, references, tables and figures. The manuscripts should summarize the current state of understanding on a particularly important topic in dentistry based on previously published data, preferably written by authoritative figures of that field. A minimum of 50 references must be cited. Authors are encouraged to use headings of their own choosing between Introduction and Conclusion sections.

This type of manuscript should be organized as follows:

- Title page
- Abstract & Keywords
- Introduction
- Conclusion
- Acknowledgements (optional)
- Source of funding
- Conflict of Interest
- References

Authors who are willing to submit a systematic review or a meta-analysis may use the same manuscript design as for the original research articles.

Letter to the Editor

Letters to the Editor are short articles (limited to 500 words and 5 references) in which readers can share their opinions and comment on articles published in the past 12 months. Authors should clearly cite the article to which they are referring. Letters will be evaluated by the Editor-in-Chief and, if accepted for publication, the author(s) of the original paper will be invited to submit a reply.

Acknowledgements

This section is optional. Authors must acknowledge all individuals who do not fulfill the requirements for authorship but who had contributed to the preparation of the manuscript by providing assistance in writing, literature search, data analysis and/or supply of materials.

Source of funding

This section is mandatory to be filled out. Financial support from any institutional, private or corporate sources must be disclosed. Clearly state the name of the funding organization, year and the project number:

"This study has been supported by a research grant from XXX foundation, university, government etc.. (project number: 2012/828128)

If you have no source of funding declaration to make, please write "None declared".

Conflict of Interest

This section is mandatory to be filled out. Any financial or personal activities (royalties, grants, consultancy fee, patent registration, ownership, employment) that could be perceived as potential conflicts of interests must also be disclosed.

Clearly state the names of the author(s) and organization(s) and the type of payment(s):

"Dr. Smith is a consultant for the company X / receives a consultancy fee from the company X." or "Until recently, Dr. Smith was in an employment relationship with the company Z." .

If you have no declaration to make, please write "None declared".

References

Eur Oral Res recommends the use of reference management software to ensure that the references are correctly formatted. All authors must be included in this section, in contrast to the in-text citations. Journal abbreviations should be formatted according to the PubMed - NLM Journal Title Abbreviations (http://www.ncbi.nlm.nih.gov/journals). Au-

European Oral Research

Official Publication of Istanbul University Faculty of Dentistry

thors are advised to consult a recent issue of the journal. Use single line spacing for this section. Please do not cite unpublished articles, abstracts, personal communications, non-scientific websites or documents such as pamphlets.

AUTHORS ARE RESPONSIBLE FOR SENDING THE FULL TEXT VERSIONS OF ANY CITED PAPER OR TEXTBOOK AS PER THE REQUEST OF THE EUR ORAL RES EDITORIAL BOARD AND/OR THE REVIEWERS.

AUTHORS ARE RESPONSIBLE FOR SENDING THE PROFESSIONAL ENGLISH TRANSLATION OF ANY NON-ENGLISH PAPER OR TEXTBOOK AS PER THE REQUEST OF THE EUR ORAL RES EDITORIAL BOARD AND/OR THE REVIEWERS.

Journal Article in Print

1. Burrow MF, Tagami J, Negishi T. Early tensile bone strengths of several enamel and dentin bonding systems. J Dent Res 1994; 74: 522-8.

Journal Article Electronic Publication Ahead of Print

2. McKeage K. Tobramycin inhalation powder: a review of its use in the treatment of chronic pseudomonas aeruginosa infection in patients with cystic fibrosis. Drugs 2013; [Epub ahead of print] Available from: http://link.springer.com/article/10.1007%2 Fs40265-013-0141-0

Book

3. Mueller HJ, Freeman D. FT-IR spectrometry in materiolography. 2nd Ed., Ohio: American Society for Metal 1994, p.51-56.

Chapter in a book

4. Alexander RG. Considerations in creating a beautiful smile. In: Romano R, editor. The art of the smile. London: Quintessence Publishing, 2005, p.187-210.
5. Hudson FB, Hawcroft J. Duration of treatment in phenylketonuria. In: Seakins J, Saunders R, editors. Treatment of inborn errors of metabolism. London: Churchill Livingstone, 1973, p.51-56.

Thesis

6. Maden I. Effect Of Nd:YAG Laser Treatment In Addition To Scaling And Root Planning. Doctoral Dissertation, Istanbul University Institute of Health Sciences Periodontology Department, 2009.

Tables, Figures and Legends

Please set table format to custom borders, no vertical lines, no shades, no background colors, 3 pt line for top and bottom borders, 1 pt horizontal row lines, cell alignment center. Tables should be numbered consecutively with Arabic numerals in the order mentioned in the text. All tables must be included in the main body of the article and be placed near their first mention in the text. All tables should be self-explanatory. Please provide full explanation for abbreviations even if they were presented in the main text. Legends should be written in Times New Roman Italic font and be positioned right above the table.

Figures (photographs, graphs, charts, drawings, pictures, etc.) should be numbered consecutively with Arabic numerals in the order of mention in the text. High quality pictures with 300 dpi resolution in JPEG JPG, BMP, TIFF, PNG or PSD file formats are generally acceptable for publication. Drawings and shapes should be in vector format. All figures must be included in the main body of the article and be placed near their first mention in the text. Legends should be written in Times New Roman Italic font and be positioned below the figure.

Figure 1. Panoramic radiograph of the patient taken 6 months after surgery, note irregular borders of the lesion.

Submission checklist

1. "Manuscript Submission Form" signed by the corresponding author.
2. "Copyright Transfer Agreement Form" signed by all authors.
3. "Disclosure of Potential Conflict of Interest Form" signed by all authors.
4. Title page
5. Abstract & Keywords page
6. Main text
7. Tables, figures and their legends should be embedded in the main text and are not to be sent separately.

How to submit ?

Eur Oral Res is only accepting electronic submissions. Manuscripts may be submitted by registering at https://mc04.manuscriptcentral.com/eores

Need assistance ?

Please contact editorial office by sending an e-mail to: dentistryeditor@istanbul.edu.tr or disdergi@istanbul.edu.tr

Postal address: İstanbul Üniversitesi Diş Hekimliği Fakültesi Dergisi İstanbul Üniversitesi Diş Hekimliği Fakültesi Kütüphanesi Dergi Yayın Kurulu Odası 34093 Çapa-Fatih, İSTANBUL/TURKEY

Phone: +90 212 414 20 20 (extension 30348)
Fax: +90 212 414 25 70

What's next ?

If you have successfully submitted your forms and manuscript, please continue reading this document to know about the Eur Oral Res editorial process.

General information

Peer-review evaluation and publishing of articles submitted to Eur Oral Res are managed electronically through the online system via e-mail correspondence. Corresponding authors will be notified by e-mail upon receipt of a new manuscript and will have further information regarding the editorial process. It is the responsibility of the corresponding author to communicate with the other participants of the study about the submission of the manuscript, its content and authorship requirements.

Who makes the decisions ?

Eur Oral Res Editorial Board oversees the manuscript evaluation process. The Editor-in-Chief is the only person who can officially accept a paper.

European Oral Research

Official Publication of Istanbul University Faculty of Dentistry

Initial examination

Editor-in-Chief and editorial assistants check the submission files to confirm the availability of the required documents. Please note that the Manuscript Submission Form, Copyright Transfer Agreement Form and Conflict of Interest Disclosure Form must be included in the original submission. Corresponding authors of incomplete submissions will be notified via e-mail. Editorial process cannot proceed until all relevant documents are signed and submitted electronically.

Careful manuscript preparation is the crucial part of peer-review process. Editorial assistants will evaluate the manuscript to ascertain conformity to the following standards: consistency to journal style, clear and concise writing, proper use of English grammar and spelling, technical quality, correct formatting of references and documentation of ethical conduct. All eligible manuscripts will also be scanned with anti-plagiarism software.

Manuscripts that fail to conform to journal expectations in any of the above mentioned issues will be returned to authors without review. This is a frequent cause of delay in the publication of articles and may even result in immediate rejection. All issues regarding the outline of the manuscript should be resolved before further evaluation. Manuscripts which pass the initial examination are presented to the Editorial Board by the Editor-in-Chief.

Peer-review

Eur Oral Res operates a double-blind peer review system. Identities of the Editorial Board members who perform the initial examination and those of the reviewers who evaluate the manuscript remain unknown to the authors. All manuscripts are treated as privileged information. Editorial Board members and reviewers are instructed to exclude themselves from reviewing any manuscripts that might involve a conflict of interest.

Editorial Board requests the opinion of, at least, two independent expert reviewers. Those who accept the invitation are expected to provide written critical reviews of the submission within 21 days of receipt. If one of the reviewers gives a negative feedback while the other's response is positive, Editor-in Chief or Editorial Board invites a third reviewer. Editor-in-Chief and Editorial Board reserve the right to obtain reports from biostatistics experts of their choosing at any time during the process, who might also suggest corrections in the manuscript.

21 day time limit will apply for the correction of the manuscript, at the end of which the corresponding author must return a revised version of the documents. Changes should be highlighted in red in the revised manuscript to facilitate reading. Authors should also provide itemized, point-by-point responses to reviewers' comments in a separate file. The manuscript will be automatically rejected if no answer has been received from the authors. If authors submit the revised paper after the time limit is reached, it will be treated as a new submission. Revised manuscripts will be re-evaluated by Editor-in-Chief and Editorial Board and will be sent back to reviewers.

Acceptance for publication requires at least, but not limited to, final positive responses from two reviewers. In light of their recommendations, the Editor-in-Chief and the Editorial Board members choose between the following options: "accept submission", "revisions required", "resubmit for review" or "decline submission".

"Accept submission" indicates that the manuscript can be published as is. If there are "revisions required", all major changes in the manuscript must be confirmed by the reviewer who had originally suggested the revisions. In some cases, editorial team may think that your manuscript deserves re-evaluation after substantial changes which cannot be completed within reasonable time limits. Therefore, the editor may encourage authors to re-submit their manuscript by selecting "resubmit for review". Such conditions include, but not limited to, increasing the sample size, performing more statistical tests or correcting multiple errors that impede understanding. Re-submitted manuscripts will be treated as new submissions. On the other hand, if the "decline submission" decision has been reached, your manuscript has been found unsuitable for publication and you cannot submit the same manuscript to this journal.

An e-mail notification that includes the formal letter of approval will be sent to the corresponding author. Rejection e-mail will include reviewers' comments and suggestions. Accepted manuscripts will be forwarded to the publisher.

Table 1. *Concise explanation of the table contents (SD: standard deviation, CTA: cartilage tissue area, NBA: new bone area).*

	Control group (Mean % ± SD %)	First group (Mean % ± SD %)	Second group (Mean % ± SD %)
CTA	21.41 ± 4.2	2.5 ± 2.4	11.42 ± 4.2
NBA	11.48 ± 0.2	21.41 ± 14.22	11.41 ± 4.2

After acceptance

Production department transforms the manuscript files into an article and sends the galley proofs to the corresponding author via e-mail. All authors should carefully check the final PDF proof version of the article for minor punctuation or spelling errors, correct presentation and positioning of the tables, figures and their captions. Corrected page proofs should be returned via e-mail within 7 days of receipt. Major changes such as adding new paragraphs, changing the title or the name order of the authors and modifying visual elements will not be allowed at this stage.

Publication

Articles will normally appear in the order in which they were accepted as publication,

however, Editor-in-Chief and Editorial Board reserve the right to modify this schedule in the presence of critical scientific issues.

To speed up the process, articles will be first published online, followed by the print version of the journal. Both versions will have identical page numbers. Therefore, no change can be made in the article in between the online and in print publication steps.

DOI number

Digital Object Identifier (DOI) number is a unique alphanumeric identifier assigned by a registration agency. Once it is assigned to an article, the DOI will never change, therefore, it is ideal for citing and linking electronic documents. Your article will be assigned a DOI number provided by the CrossRef registration agency, immediately after it is published online.

Changes to authorship

This statement concerns the addition, deletion, or rearrangement of author names in the authorship of accepted manuscripts. Requests to add or remove an author or to rearrange the author names must be sent to the Editor-in-Chief from the corresponding author of the accepted manuscript. This document must include: the reason the name should be added or removed, or the author names rearranged and written confirmation (e-mail, fax, letter) from all authors that they agree on the addition, removal or rearrangement. In the case of addition or removal of authors, written confirmation from the author being added or removed must be included. Requests that are not sent by the corresponding author will be forwarded by the Editor-in-Chief to the corresponding author, who must follow the procedure as described above. Production of the accepted manuscript is suspended until authorship has been agreed. The name and the order of the authors cannot be changed once the article is published online or in print.

Data access and retention

Authors may be asked to provide the raw data of their investigations during the editorial process or after publication of the article. Such materials include, but not limited to, original submission files, unedited versions of the printed and/or digital radiographs, unedited versions of the printed and/or digital photographs, histologic slides, original outputs from clinical and/or experimental diagnostic and/or interventional devices, original data sheets of statistical software and technical data sheets of any substance used in the research project. Authors should retain such materials for a reasonable period of time after the publication of their paper.

Correction, Retraction & Removal

A formal correction will be issued in the journal by the Editor-in-Chief, if only a small portion of otherwise reliable article is flawed in a way that does not severely affect the findings reported in it (such as mistakes in the spelling of a drug, miscalculation of a formula, mismatch between images and their captions or incorrect author list). Online articles will not be corrected directly. An erratum (for publishing error) or a corrigendum (for author error) will be published in the next issue of the journal.

Articles may be retracted by its authors or by the Editor-in-Chief under the advice of the scientific community. If authors are willing to retract an article before it is published (accepted or under review), requests must be sent to the Editor-in-Chief from the corresponding author of the manuscript. This document must include: the reason the article should be retracted and written confirmation (e-mail, fax, letter) from all authors that they agree on the retraction. Reasons for editorial retraction include, but not limited to, unreliable publications as a result of misconduct or honest error, redundant publication, major plagiarism, copyright infringement and unethical research. A formal retraction announcement written by the Editor-in-Chief will be published in the print edition of the journal. For online articles, the PDF pages remain with a watermark on each page to notify it is retracted.

In rare circumstances, an article can be completely removed from the online database. Such conditions include, but not limited to, defamation, infringement of legal rights, court orders and claims in the article that might pose serious health risks. Title and author names will remain in the web page while the text will be replaced by a notification indicating that the article has been removed for legal reasons.

European Oral Research

Official Publication of Istanbul University Faculty of Dentistry

Contents

Original Research Articles

eor.istanbul.edu.tr

Official Publication of Istanbul University Faculty of Dentistry

Eur Oral Res 2021; 55(3): 104-109

Original research

Quality and reliability of web-based information regarding restorative treatment in pediatric patients

Berna Kuter[1], **Alp Abidin Ateşçi**[2], **Ece Eden**[3]

Purpose
The aim of the present study is to assess the quality and reliability of web-based information about restorative treatment in pediatric patients on the internet using different scales.

Materials and Methods
Websites obtained by using keywords about restorative treatment in pediatric patients on Google and Yandex were included in the study. The study was conducted in English on a total of 440 websites. Websites were evaluated using the quality criteria for consumer health information (DISCERN toolkit), Journal of American Medical Association (JAMA) benchmarks, and Health on the Net Code of Conduct Certification (HONCode).

Results
The mean DISCERN points of the websites were moderate. Among the evaluated websites, the quality of the knowledge in 20% of the websites was low. The rate of websites with a score below 40 was 37.5%. None of them has reached an excellent score. No websites met all JAMA criteria. There was no HONCode Certificate on any website.

Conclusion
This study showed that the quality of the web-based information about restorative treatment in pediatric patients was generally inadequate and scientifically imperfect.

Keywords: *Internet, DISCERN, JAMA, HONCode, quality of web information*

Introduction

The internet is a resource of knowledge about healthcare for both professionals and patients (1). Information technology has begun to modify the traditional medical approach from treatment towards prevention. World Health Organization declared that 71% of internet users used the internet to obtain information about health topics (2). However, there is no regulation about the content of the health issues posted, and everybody could post them online. Formerly, a person in need of dental treatment was informed by the dentist (3-5). However, nowadays, most of the patients inform themselves through the internet, even before going to the dentist.

Several different dental treatment options and dental materials are available for each case. Dentist's experience could influence the choice of treatment and materials (6). Wuollet *et al*. (7) stated that there were differences in the preferred materials among dentists. The different factors such as materials available, working time, tooth prognosis, material strength, co-operation, experience, and aesthetics affect dental treatment preference. Patient co-operation plays an important role in the choice of the treatment

ORCID IDs of the authors: B.K. 0000-0002-1234-8237; A.A.A. 0000-0001-6346-3801; E.E. 0000-0001-8427-0427

[1]*Department of Paediatric Dentistry, Faculty of Dentistry, Izmir Democracy University, Izmir, Turkey*

[2]*Pedodontics, Private Clinic, Turkey*

[3]*Department of Paediatric Dentistry, Faculty of Dentistry, Ege University, Turkey*

Corresponding Author: Berna Kuter

E-mail: berna.kuter@idu.edu.tr

Received: 17 October 2020
Revised: 12 January 2021
Accepted: 26 January 2021

DOI: 10.26650/eor.2021812053

How to cite: *Kuter B, Atesci AA, Eden E. Quality and reliability of web-based information regarding restorative treatment in pediatric patients. Eur Oral Res 2021; 55(3): 104-109.*

and materials for the restoration of extensive primary tooth decay. However, parents generally have no idea on such factors and they try to decide which treatment is the best for their children based on what they read online. Thus, the accuracy, standard, and trustworthiness of the information on the internet are crucial (8). It was stated that many health sites included captious, inappropriate, and inaccurate information (9,10). Several validation tools were developed that can assess various properties of internet sites to help to choose quality websites on health-related information. DISCERN Toolkit, JAMA Benchmarks and HONCode are some of those (11-13).

Availability of internet and easy access makes it an important tool to inform health-related issues of the population. Therefore, the purpose of this study is to assess the quality and reliability of web-based information in English websites on restorative treatment of children using different scales. The null hypothesis tested in this study is that no difference could be found among websites in terms of quality.

Materials and Methods

Data gathering

Internet search was carried out using Google and Yandex search engines. While determining the keywords, ideas of parents experiencing dental caries in their children's primary teeth, patients attending dental treatment for their children, dentists and people who has no idea about the subject were used. They were asked to suggest keywords or phrases for reaching information on treatment of deciduous teeth. Collected words and phrases were tested on several search engines before being included in the study. Then, 11 keywords were identified by excluding keywords and phrases which referred to internet sites that are not related with the subject. Keywords selected were all phrases and listed as follows: "deciduous teeth treatment(s)", "primary teeth treatment(s)", "milk teeth treatment(s)", "baby teeth treatment", "deciduous teeth restorations", "primary teeth restorations", "milk teeth restorations" and "baby teeth restorations", "primary teeth fillings", "milk teeth fillings" and "baby teeth fillings". Internet search was conducted by one pediatric dentist. The websites were evaluated between 25/03/2020 and 08/04/2020 and number of pages from Google and Yandex search engines on each keyword are presented in Table 1. The search was planned in English language on a total of 440 websites. Each keyword was searched on each search engine and the first 40 websites were assessed.

Inclusion and exclusioion criteria

Dental health centers websites, medical information websites, professional organization websites and hospital websites were included the study. The duplicate websites, links to research studies, advertisements, discussion groups, videos and images were excluded from the evaluation.

The evaluation of websites

Websites were evaluated with the DISCERN toolkit, JAMA benchmarks, and HONCode.

DISCERN toolkit: The websites were analyzed with the DISCERN toolkit (11). DISCERN tool kit includes 3 parts and 16 questions which is scored from 1 to 5. Part 1 includes 8 questions that evaluate the relevance of the publications while part 2 composed of 7 questions evaluating the quality of the information. The last part contains a question which evaluates the general quality of the website. According to the DISCERN toolkit, the total average scores of websites were divided into 5 groups as dental health-care center, informative, organizational and hospital (Table 2). The evaluator read all the information about primary teeth treatments and rated each website according to the DISCERN toolkit. Obtained data were calculated as mean score, percentages and ranges.

JAMA benchmarks: This tool was published for property standards for websites data on health by Silbergin, Lundberg, and Musacchio (12). Four main characteristics as, authorship, attribution, disclosure and currency were used as the criteria of JAMA.

Health on the net code of conduct (HONCode): This certification is maintained by an independent institution (Health on

***Table 1**. Keywords about restorative treatment in pediatric patients and approximate number of webpages obtained.*

Keywords	Google	Yandex
deciduous teeth treatments	4,390,000 pages	212,000 pages
primary teeth treatments	125,000,000 pages	1,000,000 pages
milk teeth treatments	62,500,000 pages	374,000 pages
baby teeth treatments	190,000,000 pages	1,000,000 pages
deciduous teeth restorations	683,000 pages	336,000 pages
primary teeth restorations	2,920,000 pages	79,000 pages
milk teeth restorations	7,790,000 pages	783,000 pages
baby teeth restorations	14,500,000 pages	633,000 pages
primary teeth fillings	3,320,000 pages	740,000 pages
milk teeth fillings	1,110,000 pages	140,000 pages
baby teeth fillings	3,710,000 pages	1,000,000 pages

***Table 2.** The total average DISCERN scores for different types of websites.*

DISCERN Score (16-75)	Total	Dental Health Center	Informative	Organizational	Hospital
Very Poor (16-26)	3	3	0	0	0
Poor (27-38)	15	12	1	2	1
Fair (39-50)	16	11	3	1	1
Good (51-62)	6	2	4	0	0
Excellent (63-80)	0	0	0	0	0

the net, Lausanne, Switzerland) (13). The credibility and the property of the web data were stated on the website contained on eight standards. These are complementarity, privacy, authoritativeness, attribution, justifiability, disclosure, transparency and variations of advertisements.

Statistical analysis

The collected data from all groups were imported to Statistical Package for Social Sciences (SPSS) for Windows software, version 16.0 (SPSS Inc., Chicago, IL, USA). The frequency of each variable was calculated by descriptive statistics. All variables were analyzed for normality using the Shapiro–Wilk test. The distribution was found to be skewed. As the distribution of the data did not meet the requirements for normality and homogeneity of variances assumptions, continuous variables that belong to the websites's Discern scores were analysed by the nonparametric Kruskal-Wallis by ranks and Mann-Whitney U tests for multiple and pairwise comparisons, respectively,. The chi-square test was used to compare the categorical demographic variables among the JAMA groups. The confidence interval was set to 95% and $p < 0.05$ was considered statistically significant.

Results

A total of 440 websites were identified and duplicate web pages, advertisements, and research articles were eliminated before evaluation. The websites considered for evaluation were dental health centres' websites (57.5 %), medical information websites (25.0 %), professional organization websites (10.0 %) and hospital websites (7.5 %). 60 % of the analysed websites were originated from USA. Canada was the second country with 12.5 %, and Australia and India were in the third with 5.0 %. These countries were followed by England, Lithuania, Malta, Russia, Sri Lanka, Ukraine and Vietnam (2.5 %).

Discern Results: The DISCERN points of the websites was fair (average 40.15) (range: 23-58), and findings are presented in Table 2. The quality of the data in 20% of the websites were low (scores of 1 and 2). The percentage of websites, with a total score of 40 and above is 62.5% and the rate of websites with a score below 40 is 37.5%. No website has reached excellent score (63-80). Date of information and sources generally were not mentioned in the websites, in addition, duration, and limits of restorative treatment of children were not clear (Figure 1). References were not supplied. The scores of questions about aim, alternative treatments and benefits of treatments were presented in Table 3.

According to Kruskall-Walls test, at least one statistically significant difference between websites was found ($p = 0.034$). Then each pair of the websites were compared by Mann Whitney U Test at 0.01 significant level. Since significant level was getting higher, 0.01 significant level was selected for each pair of the website comparisons. The scores of dental health-centre websites were higher than information websites ($p = 0.005$). However, dental health centre websites' scores were not statistically higher than organization websites ($p = 0.494$), and hospital websites ($p = 0.315$). The scores of the information websites were not statistically significantly different from the hospital websites ($p = 0.499$), and organization websites ($p = 0.077$). The scores of hospital and organization websites were not statistically significantly different ($p = 0.593$).

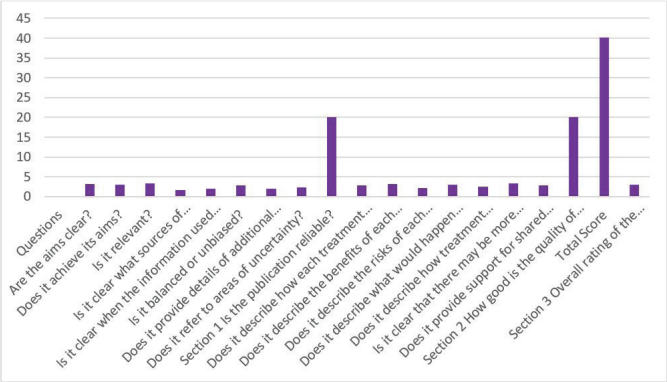

Figure 1. *The Average of Questions of DISCERN Score.*

Table 3. *The Average and Sections DISCERN Score.*

		Dental Health Center	Informative	Organizational	Hospital
Average DISCERN Score	40,15	38,2	47,6	39	37
Section 1 (items1-8)	20,1	18,6	25,3	19,3	20
Section 2 (items 9-15)	20	19,7	22,2	19,6	17
Section 3 (16. item)	2,9	2,8	3,3	3	3

JAMA Results: No websites met all JAMA criteria. 30.0 % of websites only met author criterion. 12.5 % of them met reference criterion, 40.0 % had currency criterion. No websites met disclosure criterion. 30.0 % of websites displayed an author, 12.5% of websites referenced their information, none of the websites mentioned about disclosure, and 40.00 % of websites displayed a date of publication.

Dental health-centre websites had high authorship JAMA points. However, there was no statistically significant difference between dental health-centre websites and the other kinds of webpages according to the authorship JAMA points. This is presented in Table 4 ($X^2=2.632$ df=1, $p = 0.052$).

Dental health-centre websites obtained higher points (17.5%) than the other websites in the currency JAMA criterion (Table 4). There was statistically significant difference between dental health-centre websites and the other kinds of webpages according to the currency JAMA points ($X^2=7.882$ df=1, $p = 0.049$). There was no statistically significant difference between the scores of the websites according to the attribution JAMA criterion. ($X^2=2.159$ df=1, $p = 0.540$). Organization websites had significantly lower points than the dental health-center and information websites in the currency JAMA criterion. This was presented in Figure 2 ($X^2=7.882$ df=1, $p = 0.049$).

When the JAMA criteria scores were analysed in relation to the country of origin, the websites of Ukraine, England, Malta, Vietnamese, Lithuania did not meet any JAMA criteria (Figure 3). The websites of Russia met only currency JAMA criterion.

HonCode Results: There was no website with HonCode Cer-

***Table 4**. JAMA Criteria Distribution and Comparison Between Type of Websites (%) Chi-Square Test: * p < .05 significant difference between the groups (type of websites).*

	Frequency	Type of web site	Frequency	p
Author	30.0	Dental Health Center	17.5	0.452
		Information	10.0	
		Organization	0.0	
		Hospital	2.5	
Attribution	12.5	Dental Health Center	5.0	0.540
		Information	5.0	
		Organization	2.5	
		Hospital	0.0	
Disclosure	0.0	Dental Health Center	0.0	1.00
		Information	0.0	
		Organization	0.0	
		Hospital	0.0	
Currency	40.0	Dental Health Center	17.5	0.049
		Information	15.0	
		Organization	7.5	
		Hospital	0.0	

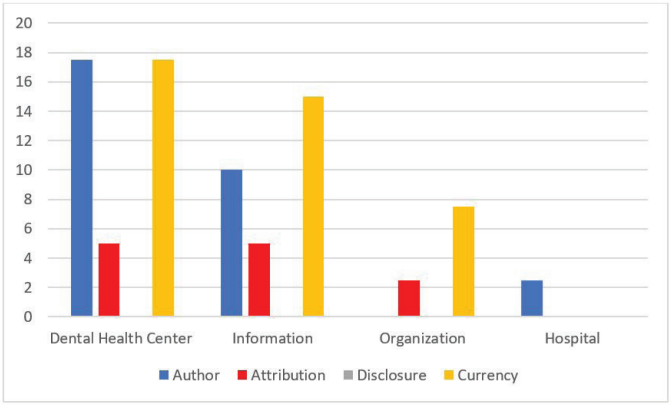

***Figure 2.** JAMA Criteria Scores of Websites (%).*

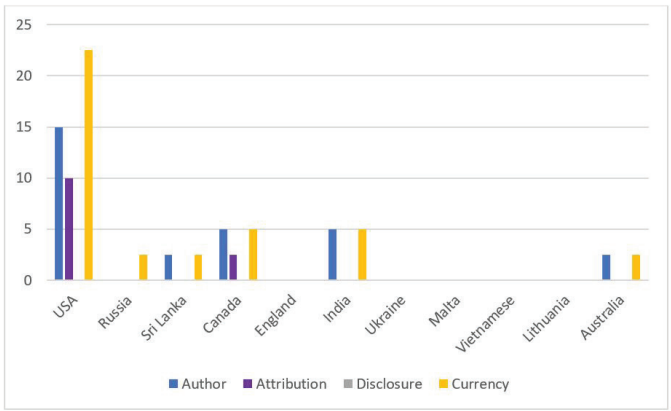

***Figure 3.** JAMA Criteria Scores of Countries (%).*

tificate.

Discussion

This study evaluated the quality of provided information related to the restorative treatment of children on internet. The findings demonstrated that the information was likely to be inadequate or incorrect. Although internet users often prefer to look at the first page, as suggested, the first available 40 websites were evaluated in two search engines for each keyword (14). Internet provides very broad information, and it is not easy to distinguish the proper informative results for the parents who are seeking help from internet. As patients' demand on learning more about the treatment possibilities from internet increased, the type of correspondence between physicians and patients changed (15). All healthcare workers started to show presence on the internet.

A previous study, evaluating the quality of oral hygiene training, reported a moderate score for quality of oral health statement available on the web (16). Similarly, the information on dental trauma on the internet was found to be limited in quantity and quality (17). It is important to guide patients and guide people on dental treatment possibilities. It was stated that dentists could provide patients with appropriate information by referring patients to approved websites, for example on the thumb sucking habit (18). When the latter was evaluated the reliability of websites on the thumb sucking habit by the DISCERN tool, the highest points obtained from 36 websites was 55 out of 80, and the lowest points was 16 out of 80. Baybek and Tuncer (19) evaluated the quality of information on webpages on orthognathic surgery in Turkey using the DISCERN toolkit and stated that the quality of information on orthognathic surgery on the internet was low. It was noted that higher quality information was provided by public institutions that are not concerned about profit. Stinson *et al.* (8) stated that high-quality internet health information was present at an appropriate reading level for youth with juvenile idiopathic arthritis and their parents. The average DISCERN scores of websites were generally poor or fair in this study. Only two dental health centers and four information websites scored good. 38 of the first 40 websites were private dental health-centre websites, 2 websites were public organizations. Private clinics had higher DISCERN scores than the others. However, both public and private websites did not score excellent in this study.

None of the websites examined includes all criteria as regard JAMA benchmarks. These results were similar to the other recent studies (20,21). Dental health-center websites had a higher score than the others. This could be explained by higher currency scores. The part of disclosure scores was insufficient on all websites and public websites did not score both attribution and disclosure JAMA scores. Furthermore, author JAMA score was low in public websites.

HON is a non-governmental organization (13). It promotes a code of conduct for websites providing health information. The applicant is a direct indicator of the quality of information. However, it was shown that none of the evaluated websites had a HONCode Certificate.

The uncontrollable nature of the internet makes healthcare professionals vary in advising their patients to read more from online sources (22,23). One of the main reasons for this may be an increase in websites concerning special

implementation. These private practice websites often advertise rather than inform, and this could lead to questions in patients' minds. Thus, there is a gap that should be filled by non-profit educational institutions which publish websites with sufficient and clear information on dental health.

Based on our findings, internet information about dental restorative treatment for children is limited. The web-based information was deficient both for the reliability of the publications and the quality. English websites were searched, and it could be a limitation for this study. However, English is widely used, and we may conclude that this first research using three different methods to assess the quality of data on the webpages about restorative treatment in pediatric patients showed that the information on the topic is scarce.

Conclusion

This study showed that the quality of the web-based information about restorative treatment in pediatric patients is generally inadequate, limited, scientifically imperfect and insufficient. There is a need for a qualified web page to guide parents who are interested in dental treatment for their children.

Türkçe Özet: *Pediatrik hastalarda restoratif tedavi ile ilgili web tabanli bilgilerin kalitesi ve güvenilirliği. Amaç: İnternet, diş hekimleri ve hastalar için kolayca ulaşılabilir bir diş sağlığı bilgi sağlayıcısıdır. Araştırmanın amacı, internette pediatrik hastalarda restoratif tedavi ile ilgili web tabanlı bilgilerin kalitesini ve güvenilirliğini farklı ölçekler kullanarak değerlendirmekti. Gereç-Yöntem: Çocuk hastalarda restoratif tedavi ile ilgili anahtar kelimelerle Google ve Yandex arama motorları kullanılarak internet üzerinden çocukların restoratif tedavisi üzerine araştırma yapıldı. Çalışma, toplam 440 web sitesinde İngilizce olarak yapıldı. Web siteleri, DISCERN (tüketici sağlık bilgileri için kalite kriterleri), JAMA (Amerikan Tıp Derneği Dergisi) ve HONCode (İnternet Sağlık Kuralları Sertifikasyonu) kalite kriterleriyle değerlendirildi. Bulgular: Web sitelerinin ortalama DISCERN puanları orta düzeydeydi. Değerlendirilen web siteleri arasında, web sitelerinin % 20'sinin bilgi kalitesi düşüktü. Puanı 40'ın altında olan web sitelerinin oranı % 37,5'ti. Hiçbiri mükemmel bir puana ulaşmadı. Tüm JAMA kriterlerini karşılayan web sitesi yoktu. Hiçbir web sitesinde HONCode Sertifikası yoktu. Sonuç: Bu çalışma çocukların restoratif tedavileri ile ilgili internetteki bilgilerin kalitesini farklı ölçeklerle değerlendiren ilk araştırmadır. Bu çalışma, pediatrik hastalarda restoratif tedaviye ilişkin web tabanlı bilgilerin kalitesinin genellikle yetersiz ve bilimsel olarak eksik olduğunu göstermiştir. Ebeveynler, çocuklarının restoratf tedavisine ilişkin web sitelerindeki bilgilerin yanlışlıkları ve kısıtlılıkları konusunda uyarılmalıdır. Anahtar Kelimeler: Internet, DISCERN, JAMA, HONCode, web bilgilerinin kalitesi.*

Ethics Committee Approval: Not required.

Informed Consent: Not required.

Peer-review: Externally peer-reviewed.

Author contributions: BK, EE participated in designing the study. BK, EE participated in generating the data for the study. BK, AAA participated in gathering the data for the study. BK, AAA, EE participated in the analysis of the data. BK wrote the majority of the original draft of the paper. BK participated in writing the paper. AAA has had access to all of the raw data of the study. BK and EE have reviewed the pertinent raw data on which the results and conclusions of this study are based. BK, AAA, EE have approved the final version of this paper. BK guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: The authors declared no conflict of interest.

Financial Disclosure: The authors declared that they have received no financial support.

References

1. Anderson JG, Rainey MR, Eysenbach G. The impact of CyberHealthcare on the physician-patient relationship. Journal of Medical Systems 2003;27:67–84. [CrossRef]
2. Andreassen HK, Bujnowska-Fedak MM, Chronaki CE, Dumitru RC, Pudule I, Santana S, Voss H, Wynn R. European citizens' use of E-health services: A study of seven countries. BMC Public Health 2007;7:53-60. [CrossRef]
3. Eysenbach G, Powell J, Kuss O, Sa ER. Empirical studies assessing the quality of health information for consumers on the World Wide Web: a systematic review. JAMA 2002;287:2691–700. [CrossRef]
4. Nghiem AZ, Mahmoud Y, Som R. Evaluating the quality of internet information for breast cancer. Breast 2016;25:34–7. [CrossRef]
5. Livas C, Delli K, Ren Y. Quality evaluation of the available Internet information regarding pain during orthodontic treatment. Angle Orthod 2013;83:500–6. [CrossRef]
6. Widstrom E, Linden J, Tiira H, Seppala TT, Ekqvist M. Treatment provided in the public dental service in Finland in 2009. Community Dental Health 2015;32:60-4.
7. Wuollet E, Tseveenjav B, Furuholm J, Waltimo-Sirén J, Valen H, Mulic A, Ansteinsson V, Uhlen MM. Restorative material choices for extensive carious lesions and hypomineralisation defects in children: a questionnaire survey among Finnish dentists. Eur J Paediatr Dent. 2020;21:29-34.
8. Stinson JN1, Tucker L, Huber A, Harris H, Lin C, Cohen L, Gill N, Lukas-Bretzler J, Proulx L, Prowten D. Surfing for juvenile idiopathic arthritis: perspectives on quality and content of information on the Internet. J Rheumatol 2009;36:1755-62. [CrossRef]
9. Martins EN, Morse LS. Evaluation of internet websites about retinopathy of prematurity patient education. Br J Ophthalmol 2005;89:565–8. [CrossRef]
10. Lau L, Hargrave DR, Bartels U, Esquembre C, Boufett E. Childhood brain tumor information on the Internet in the Chinese language. Childs Nerv Syst 2006;22:346–51. [CrossRef]
11. Charnock D, Shepperd S, Needham G, Gann R. DISCERN: an instrument for judging the quality of written consumer health information on treatment choices. J Epidemiol Community Health. 1999;53:105–11. [CrossRef]
12. Silberg, W. M., Lundberg, G. D., & Musacchio, R. A. Assessing, controlling, and assuring the quality of medical information on the Internet: Caveant lector et viewor e let the reader and viewer beware. JAMA 1997;277: 1244–5. [CrossRef]
13. Baujard, V, Boyer, C, Geissbuhler, A. Evolution of health web certification through the HONcode experience. Studies in Health Technology and Informatics 2011;169: 53–7.
14. van Deursen AJ, van Dijk JA. Using the Internet: skill related problems in users' online behavior. Interacting with Computers 2009;21:393–402. [CrossRef]
15. Palmer NG, Yacyshyn JR, Northcott HC, Nebbe B, Flores-Mir C, Major PW. Canadian orthodontist Internet user profile. Angle Orthod 2006;76: 92-7.
16. Verhoef WA, Livas C, Delli K, Ren Y. Assessing the standards of online oral hygiene instructions for patients with fixed orthodontic appliances. JADA 2015;146:310–7. [CrossRef]
17. Ghazaleh SA, Hassona Y, Hattar S. Dental trauma in social media—Analysis of Facebook content and public engagement. Dental Traumatology 2018;34:394–400. [CrossRef]
18. Shital KDP, Bargale S, Pandya P, Bhatt K, Barad N, Shah N, Venkataraghavan K, Ramesh K. Evaluation of Health on the Net seal label and DISCERN as content quality indicators for patients seeking information about thumb sucking habit. J Pharm Bioallied Sci. 2015;7:481–5. [CrossRef]

19. Bavbek NC, Tuncer BB. Information on the Internet Regarding Orthognathic Surgery in Turkey: Is It an Adequate Guide for Potential Patients? Turkish J Orthod 2017;30: 78-83.
20. Passos K. K, Leonel A. C, Bonan P. R, Castro J. F, Pontual M. L., Ramos-Perez F. M & Perez, D. E. Quality of information about oral cancer in Brazilian Portuguese available on Google, Youtube, and Instagram. Medicina Oral, Patologia Oral, Cirugia Bucal 2020;25:346–52. [CrossRef]
21. Leira Castelo-Baz P, Pérez-Sayáns, M., Blanco, J, & Lorenzo-Pouso, A. I. Available patient-centered Internet information on peri-implantitis. Can our patients understand it? Clinical Oral Investigations 2019;23:1569–74. [CrossRef]
22. Aldairy T, Laverick S, McIntyre GT. Orthognathic surgery: is patient information on the internet valid? Eur J Orthod 2012;34:466-9.
23. de Boer MJ, Versteegen GJ, van Wijhe M. Patients' use of the Internet for pain-related medical information. Patient Educ Couns 2007;68:86-97. [CrossRef]

eor.istanbul.edu.tr

Official Publication of Istanbul University Faculty of Dentistry

Eur Oral Res 2021; 55(3): 110-115

Original research

Orthodontic treatment need and occlusal traits in the early mixed dentition among 8-9-year old Saudi children

Guna Shekhar Madiraju[1]
Sarah Ahmed Alabd-rab Alnabi[2],
Anfal Saeed Almarzooq[2]

Purpose
The present study aimed to assess the prevalence of occlusal traits and to evaluate the orthodontic treatment need among children aged 8-9 years in Al Ahsa, Eastern region of Saudi Arabia.

Materials and Methods
A descriptive cross-sectional study was conducted among 282 Saudi children who were randomly selected from those visiting dental outpatient clinics at the College of dentistry in King Faisal University, AlAhsa. All the children were evaluated using the DHC and AC components of Index of Orthodontic Treatment Need (IOTN). Descriptive statistics, chisquare test and Fisher's Exact test were used for data analysis with statistical significance set at $p < 0.05$.

Results
The most prevalent malocclusal trait was crowding (39.7%) followed by increased overjet (28.4%). About 30.9% and 17% of the children were in definite need for orthodontic treatment according to DHC and AC of IOTN, respectively. There was no statistical difference in the distribution of DHC (p=0.116) and AC (p=0.177) scores between the gender.

Conclusion
This study demonstrated high percentage of malocclusal traits and orthodontic treatment need in the mixed dentition period among 8-9-year-old children in the Eastern region. Emphasis should be placed on early orthodontic screening and treatment in the mixed dentition stage of dental development.

Keywords: *Children, index of orthodontic treatment need, malocclusion, mixed dentition, prevalence*

Introduction

Tooth position and occlusion in permanent dentition is determined by the transition of discrepancies from deciduous dentition to permanent dentition through the mixed dentition period. Hence early detection of various occlusal traits in the mixed dentition that influence the development of malocclusion of the permanent dentition is essential. Several orthodontic indices are available in the literature for the assessment of malocclusions such as the Index of orthodontic treatment need (IOTN), peer assessment rating index, Index of complexity, outcome and need (ICON), and Index for preventive and interceptive orthodontic treatment needs (IPION) (1-4). Among these IOTN has been the most widely used index to assess the orthodontic treatment needs in children. It consists of two separate components: Dental Health Component (DHC) and Aesthetic Component (AC). The DHC is the objective component and includes five grades of treatment need, which records the severity of the malocclusion using specific occlusal traits. The IOTN-AC is the subjective component

ORCID IDs of the authors: G.S.M. 0000-0001-9443-646X; S.A.A.A. 0000-0003-3035-2157; A.S.A. 0000-0003-0354-4532

[1]Department of Preventive Dental Sciences, Faculty of Dentistry, King Faisal University, Al Ahsa, Saudi Arabia

[2]General Dentist, College of Dentistry, King Faisal University, Al Ahsa, Saudi Arabia

Corresponding Author: Guna Shekhar Madiraju

E-mail: drvkrdr@gmail.com

Received: 7 December 2020
Revised: 2 January 2021
Accepted: 3 February 2021

DOI: 10.26650/eor.2021836877

How to cite: *Madiraju GS, Alnabi SAA, Almarzooq AS. Orthodontic treatment need and occlusal traits in the early mixed dentition among 8-9-year old Saudi children. Eur Oral Res 2021; 55(3): 110-115.*

and consists of a 10-point scale illustrated by a series of intraoral frontal colour photographs showing different levels of dental attractiveness. It measures the patient's perception of presenting malocclusion and treatment needs through visual assessment (1).

Several observational studies pertaining to occlusal traits of the mixed dentition have confirmed that the occlusal features vary among different populations and ethnic groups (5-9). Most studies conducted on the prevalence of malocclusal traits and orthodontic treatment need in Saudi Arabia were limited to Northern, Central, Western and Southern regions (10-13). To our knowledge, no such data in the literature seems to be available in the population of Eastern region of Saudi Arabia. So, this study was carried out to evaluate the distribution of occlusal traits and orthodontic treatment need in early mixed dentition of 8-9-year-old children of Al Ahsa, in the Eastern region of Saudi Arabia. The null hypothesis tested in this study is that no difference could be found in the distribution of DHC and AC scores between the gender.

Materials and Methods

Ethical statement

The present study was approved by the Research Ethics Committee of the King Faisal University (Registration number: KFU-REC/2020-11-24). This study was designed according to the guidelines of Helsinki declaration and the informed consent was obtained from all study participants.

Sample size estimation

No previous study on the prevalence of orthodontic treatment need in mixed dentition in Eastern Saudi Arabia is available, and so the sample size was estimated at 50% prevalence with 5% precision and a confidence level of 90%. The minimum number of subjects needed for this study was 270. Convenience sampling technique was used to enroll the study sample. Random dates were chosen, and all children aged 8-9 years presenting at the clinics on the day of the study were invited to participate in the study. Child age at the last birthday was considered as the child age at time of examination.

Study design

This descriptive cross-sectional study included children aged 8-9 years, seeking routine dental care in the dental outpatient clinics at college of dentistry in King Faisal University, AlAhsa, during the period extended from January to October 2020. The present study is the first in a series of investigations analysing the prevalence of occlusal traits and orthodontic treatment need in mixed dentition. Participation in the study was voluntary, and no child was refused a consultation. Study sample included Saudi nationality children, of both genders, aged 8-9 years with presence of erupted first permanent molars in the early mixed dentition, and with a written informed consent signed by their parents or guardians. The exclusion criteria were subjects who were non-cooperative, not able to be guided to have a stable occlusion or having incomplete data collection, had developmental anomalies, systemic diseases, or craniofacial anomalies and those with history of previous orthodontic treatment. A total of 305 children were invited to participate in the study. After applying exclusion criteria, 23 children were excluded, and the final sample consisted of 282 children.

Data collection

Clinical examinations were conducted under strict infection control protocol as outlined by the Centers for Disease Control and Prevention were used (14). Each subject was examined using disposable surgical gown and gloves, a face mask, face shield, disposable examination kit and a sterile periodontal probe. The malocclusal traits in the dental examination was predefined and included crowding, anterior spacing, molar relationship, overjet, overbite, open bite and crossbite. Overjet was recorded as normal (0–3mm), increased (>3.5mm), and was measured by periodontal probe from labial surface of the most protruded lower incisor to labial surface of the most protrude upper incisor. Overbite was measured based on the amount of overlap of lower incisors by the upper incisors in vertical dimension. If the overbite was one-third, it was recorded as normal and more than two-thirds were considered a deep bite. If an overbite is zero, it would be edge-to-edge; in the case of no contact between the upper and the lower anterior teeth, it would be considered an open bite. The sagittal molar relationship was recorded according to Angle in habitual inter-cuspidation position. Any deviations from Angle class I relation of half cusp widths or more were recorded as class II or III molar relation. Transverse anomalies included anterior crossbite (upper incisors occluded lingual to lower incisors) and posterior crossbite (two or more lower posterior teeth occluded buccal to their opposing teeth). The children who presented one or more of the following indications were registered as malocclusion: increased overjet (>3.5mm), Angle class III, anterior crossbite, deep overbite (>2/3 overlap), open bite, posterior crossbite, anterior or posterior crowding (>2 mm), and anterior spacing (>4 mm) (15). The highest numerical values of the malocclusion severity were recorded.

The DHC component of the IOTN has been grouped into five grades, where Grades 1-2 represent no or little need for treatment; Grade 3-borderline or moderate need for treatment; and Grades 4-5 represent severe/extreme need for treatment. The IOTN-AC is composed of ten photographs representing different severity and attractiveness levels of anterior malocclusion. It has 10 grades; grades 1-4 represent no/slight need, grades 5-7 represent borderline/moderate need, and grades 8-10 represent severe/extreme need for orthodontic treatment (16). The subjects observed their teeth in the mirror and compared them with one of the ten levels of AC.

Observers' agreement

Two experienced and calibrated examiners, working blind to each other's findings, conducted the examination and recorded the scores. 10% of the cases were randomly selected and re-examined after one-week interval. The intra-examiner and inter-examiner agreements evaluated using the

weighted kappa statistic were found to be 0.87 and 0.89, respectively.

Statistical analysis

The data was entered in Microsoft excel spreadsheet and subjected to statistical analysis by using the Statistical Package for the Social Sciences (IBM SPSS v.20 software for Windows IBM Corp, Armonk, NY, USA). Descriptive statistics and frequencies were ascertained. The chi-square test and fisher's exact test were used to detect any statistical differences between the gender. Chi-square and Fisher's exact test (where necessary) were used to compare the frequencies of occlusal traits. The confidence interval was set to 95% and *p* values of less than 0.05 were considered statistically significant.

Results

The final study sample included 282 children (146 males and 136 females) with a mean age of 9.08±0.32 years (Mean±SD). Gender distribution of the IOTN-DHC grades revealed that majority of cases (43.3%) were in the no/slight need of treatment category followed by 30.9% of the cases that required severe/extreme need for orthodontic treatment, and moderate/borderline need (25.8%) respectively (Table 1). According to the IOTN-AC grades, only 17% of the cases fell in the severe/extreme need of treatment. Most cases were in the no/slight need of treatment (53.9%) followed by moderate/borderline need category (29.1%) (Table 2). There were no statistically significant gender differences in both the DHC (p=0.116) and AC grades (p=0.177).

The prevalence of individual malocclusal traits in the study sample is shown in (Table 3**)**. Crowding was the most frequent trait (39.7%) followed by increased overjet (28.4%) and overbite (16.3%). Angle Class I molar relation was observed in 75.2% followed by 23.4% in Angle class II and 1.4% in class III molar relation, respectively (p=0.256) **(**Table 4**)**. Statistically significant difference was only seen between the genders in the frequency of crowding (p=0.020). Despite females showing more tendency to increased overjet in comparison to males, the difference was not significant (p=0.123).

Table 1. *Frequency of the dental health component of the IOTN by gender.*

IOTN DHC (Grades)	Gender		Total	p-value
	Male, N (%)	Female, N (%)		
No/Little need (1-2)	68 (46.6%)	54 (39.7%)	122 (43.3%)	0.116
Borderline need (3)	41 (28.1%)	32 (23.5%)	73 (25.8%)	
Definite need (4-5)	37 (25.3%)	50 (36.8%)	87 (30.9%)	
Total	146 (51.8%)	136 (48.2%)	282 (100.0%)	

Table 2. *Frequency of the aesthetic component of the IOTN by gender.*

IOTN AC (Grades)	Gender		Total	p-value
	Male, N (%)	Female, N (%)		
No/Little need (1-4)	82 (56.2%)	70 (51.5%)	152 (53.9%)	0.177
Borderline need (5-7)	45 (30.8%)	37 (27.2%)	82 (29.1%)	
Definite need (8-10)	19 (13.0%)	29 (21.3%)	48 (17.0%)	
Total	146 (51.8%)	136 (48.2%)	282 (100.0%)	

Table 3. *Distribution of occlusal traits among the study sample. Percentage values of variables in the table include those registered only as malocclusion. achi-square test; bFisher's Exact test *Gender difference was present only for crowding (p<0.05).*

	Total n (%)	Male n (%)	Female n (%)	p-value
Crowding (>2mm)	112 (39.7%)	48 (32.9%)	64 (47.1%)	0.020[a*]
Overjet (>3.5mm)	80 (28.4%)	34 (23.3%)	46 (33.8%)	0.123[b]
Overbite (>2/3rd overlap)	46 (16.3%)	25 (17.1%)	21 (15.4%)	0.736[b]
Anterior crossbite	40 (14.2%)	20 (13.7%)	20 (14.7%)	0.865[b]
Anterior spacing (>4mm)	20 (7.1%)	8 (5.5%)	12 (8.8%)	0.354[b]
Posterior crossbite	17 (6.0%)	9 (6.2%)	8 (5.9%)	1.000[b]
Anterior open bite	12 (4.3%)	5 (3.4%)	7 (5.1%)	0.736[a]

Table 4. *Distribution of Angle's molar relation among the study sample. *pearson chi-square test.*

Gender	Class I n (%)	Class II n (%)	Class III n (%)	p-value*
Male (n=146)	115 (78.8%)	30 (20.5%)	1 (0.7%)	0.256
Female (n=136)	97 (71.3%)	36 (26.5%)	3 (2.2%)	
Total (n=282)	212 (75.2%)	66 (23.4%)	4 (1.4%)	

Discussion

Children in the age group of 8-9 years are in early mixed dentition stage during which early intervention is possible through preventive and/or interceptive orthodontic procedures which could reduce the complexity and cost of the orthodontic treatment done at later stages. Index for preventive and Interceptive Orthodontic Needs (IPION) has been developed and tested to determine the treatment needs

specifically in mixed dentition. However, studies found that it has limitations, as it lacks sensitivity, related to choosing cases specific for preventive or interceptive orthodontics (17). IOTN was used in the present study as the validity and reliability of this index has been verified in previous studies (18-20).

In our study, with regards to IOTN-DHC, about 30.9% of children have been found to require definite orthodontic treatment need which was higher than that reported in previous studies conducted in Saudi Arabia (19,20). Another study by Alharbi (12) had reported a higher definite orthodontic treatment need compared to that seen in the present study. However, results of these studies cannot be compared to our study as they were conducted in a different and/or wider range of age group than in our study. To our knowledge, this is the first study conducted in early mixed dentition of Saudi children and as such comparison of results from the present study with those of similar population group was limited.

Similar to our finding, Steinmassil *et al.* (7) reported that 30.6% of 8-10-year-old Austrain children were in definite orthodontic treatment need. Studies conducted in similar age group from other countries showed comparatively lower rates of definite need of orthodontic treatment (9,21). Nur Yilmaz *et al.* (8) had reported that 45.9% of 7-8-year-old and 56.9% of 9-10-year-old Turkish children showed definite orthodontic treatment need which was higher than that reported in our study.

The IOTN-AC score of severe/extreme treatment need (17%) seen in this study was higher than that reported in Turkish children from a similar age group but was much lower than that found in a previous study on Saudi children by Alharbi (42.94%) (8,12), although the age of the examined children was different. In the present study, 53.9% of children did not think they needed orthodontic treatment as they rated themselves being in IOTN-AC categories 1-4, despite a substantial score (56.7%) in the IOTN-DHC grade 3 and 4 categories. This was found to be much lower than that seen in Turkish study (84.5%) conducted on subjects in similar age group (8). Recent studies on older children from other regions of Saudi Arabia found that 17.9% of children, did not express any desire for orthodontic treatment (12). The differences in these results could be attributed to the fact that the AC component is based on self-perception of subjects, whereas the DHC includes objective analysis of occlusal characteristics of the dentition. Moreover, variable perception of attractiveness between the patients and clinicians, and those among different cultures or population groups has been ascribed to high discrepancies in treatment needs between the DHC and AC of IOTN. Furthermore, variations in study criteria, design and sample selection in different studies might contribute to conflicting results (22).

There were no statistically significant differences seen in the present study between the gender and orthodontic treatment needs which corroborates with most studies in the literature. Despite not being a precise indicator of self-evaluation, IOTN-AC can be used to reflect the subjective self-perceived treatment need (23). Furthermore, as school children may or may not be aware of their clinical malocclusion, studies on this population group may not reflect the subjective perception of aesthetics.

Most studies in the literature explored the incidence of malocclusal traits, whilst the present study focussed on traits that contributed to the severity of the case. The most prevalent malocclusal trait recorded in the present study was crowding (39.7%) followed by increased overjet (28.4%), which is similar to that seen in previous studies on different age groups in Saudi Arabia (12,19), and in other middle east countries (22,24). Our study presented a slightly higher value of increased overjet (28.4%) when compared to other studies in the literature which had reported prevalence rates ranging between 7.1% to 21.8% in Saudi Arabia (10,19,20). Contrary to this, elevated rates of 55.12% and 44.6% for increased overjet have been reported in studies on Romanian and Nigerian children respectively (21,25). Alajlan *et al.* (20) in their study on Saudi children found deepbite (16.2%) to be the most commonly occurring occlusal trait, which is almost similar to that seen in our study (15.4%). However, other authors had reported a lower rate of 8.6% of deepbite in 8-9-year-old male Saudi children (10). Whilst anterior crossbite was seen in 14.2% of our sample, most studies in other regions of Saudi Arabia had showed prevalence values ranging from 2.80%-11.76% (11,12). On the contrary, studies by Fatani *et al.* (9) and Rapeepattana *et al.* (26) had reported higher values of 22.3% and 18.98% respectively (9,26).

Majority of authors had reported anterior spacing to have occurred in the range of 16.1% - 27.2% (19,26). This is in contrast to our study where only 7.1% of the sample was found to have anterior spacing to be considered as malocclusion. Anterior open bite (4.3%) and posterior crossbite (6%) were the least common traits recorded in this study which corroborates with the finding of other studies (10-12,19). In the present study, Angle class I molar relation was the most frequent finding (75.2%), followed by class II (23.4%) and only a small percentage of class III molar relation (1.4%). Another recent study conducted in 7-12-year-old children of northern Saudi Arabia reported almost similar results including class I (70.4%) and Class II (21.3%) except that Class III molar relation (8.3%) showed higher values than in our study (20). Other studies in children of similar age reported values of 78.7% (9) and 82.2% (25) for Angle class I molar relation, and between 12.9% (25) and 35.9% (21) for class II molar relationship. The frequency for Angle class III molar relationship in our study (1.4%) was lower than most studies reported in the literature and varies between 2.5% and 7.7% (7,21,25). No Statistically significant differences between the gender and recorded malocclusal traits was found, except that the females showed tendency to more crowding (p=0.020) in the present study. Variations in the results of the prevalence of malocclusion traits could be related to differences in ethnicity, criteria of study design, sample size and age groups under investigation. In addition, the role of genetic, environmental, and socio-behavioral factors in the development of malocclusion should be further investigated in Saudi Population.

One limitation was that the present study was an institution-based one conducted at a single centre, in a specific age group on a limited sample size which could affect the generalizability of the findings. During the transition of mixed dentition to permanent dentition, transient malocclusions might be spontaneously corrected, depending on the aetiology and severity. Therefore, the final orthodontic treatment needs in permanent dentition might vary. Early

orthodontic screening and treatment during mixed dentition period might reduce the need for complex treatment at later stages and could pose a huge challenge with regard to implementation and management of resources, especially in Saudi Arabia, owing to its public health system funding for orthodontic treatment.

Conclusion

The present study demonstrated that a high percentage of the 8-9-year-old children were in need of orthodontic treatment need. Crowding and increased overjet were the most common occlusal traits defining the DHC component. No difference between the genders was found with regard to DHC and AC scores. Further research to assess the prevalence of malocclusion in the mixed dentition and need for early orthodontic treatment using larger samples is recommended. Additionally, emphasis should be placed on establishing effective policies to prevent the occurrence of malocclusion.

Türkçe Özet: *8-9 yaş aralığında bulunan Suudi çocuklarda erken karma dişlenme dönemindeki oklüzal özellikler ve ortodontik tedavi ihtiyacı Amaç: Doğu vilayetindeki Suudi çocukların oklüzal özellikleri ile ortodontik tedavi ihtiyacına ilişkin epidemiyolojik veriler yetersizdir. Bu çalışma Suudi Arabistan'ın Doğu bölgesinde bulunan AlAhsa'da yaşayan ve yaş aralığı 8-9 olan çocukların oklüzal özelliklerinin prevalansını ve ortodontik tedavi ihtiyacını değerlendirmeyi amaçlamaktadır. Bireyler ve yöntem: AlAhsa, Kral Faysal Üniversitesi Diş Hekimliği Fakültesi diş polikliniklerini ziyaret eden hastalardan rastgele seçilen 282 Suudi çocuk hasta arasında tanımlayıcı kesitsel bir çalışma yürütülmüştür. Tüm çocuklar Ortodontik Tedavi İhtiyacı İndeksi 'nin (IOTN), DHC ve AC bileşenleri kullanılarak değerlendirilmiştir. Veri analizi için tanımlayıcı istatistikler, ki kare testi ve Fisher's Exact testi kullanılmış ve istatistiksel anlamlılık p ≤0.05 olarak değerlendirilmiştir. Bulgular: En sık görülen malokluzyon özellikleri çapraşıklık (% 39.7) ve ardından artmış overjet (% 28.4) olarak tespit edilmiştir. Çocukların yaklaşık % 30.9'u ve % 17'sinde IOTN'nin sırasıyla DHC ve AC'sine göre ortodontik tedaviye kesin olarak ihtiyaç duyulduğu tespit edilmiştir. Cinsiyetler arasındaki DHC (p = 0.116) ve AC (p = 0.177) puanlarının dağılımında istatistiksel olarak anlamlı bir fark bulunmamıştır. Sonuç: Bu çalışmada, Doğu bölgesinde yaşayan ve yaş aralığı 8-9 olan çocukların, karma dişlenme döneminde malokluzyona sahip oldukları ve ortodontik tedaviye yüksek oranda ihtiyaç duydukları tespit edilmiştir. Diş gelişiminin karma dişlenme döneminde, erken ortodontik tarama ve tedaviye önem verilmesi gerektiği vurgulanmıştır. Anahtar Kelimeler: Çocuklar, ortodontik tedavi ihtiyaç indeksi, maloklüzyon, karışık dişlenme, prevalans*

Ethics Committee Approval: The study was approved by Research Ethics Committee of the King Faisal University (Registration number: KFU-REC/2020-11-24).

Informed Consent: The informed consents were provided by all the participants.

Peer-review: Externally peer-reviewed.

Author contributions: GSM and SAA participated in designing the study. GSM and SAA participated in generating and gathering the data for the study. SAA and ASA participated in the analysis of the data. GSM wrote the majority of the original draft of the paper. SAA and ASA participated in writing the paper. GSM, SAA, ASA have had access to all of the raw data of the study. GSM has reviewed the pertinent raw data on which the results and conclusions of this study are based. GSM, SAA, ASA have approved the final version of this paper. GSM guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: The authors declared no conflict of interest.

Financial Disclosure: The authors declared that they have received no financial support.

References

1. Brook PH, Shaw WC. The development of an index of orthodontic treatment priority. Eur J Orthod 1989;11: 309-20. [CrossRef]
2. Richmond S, Shaw W, O'brien K, Buchanan I, Jones R, Stephens C, et al. The development of the PAR index (Peer Assessment Rating): reliability and validity. Eur J Orthod 1992; 14: 125-39. [CrossRef]
3. Daniels C, Richmond S. The development of the index of complexity, outcome and need (ICON) J Orthod 2000; 27: 149-62.
4. Coetzee CE. Development of an index for preventive and interceptive orthodontic needs (IPION) [MSc dissertation]. South Africa: University of Pretoria; 1999. URI: http://hdl.handle.net/2263/25984.
5. Akbari M, Lankarani KB, Honarvar B, Tabrizi R, Mirhadi H, Moosazadeh M. Prevalence of malocclusion among Iranian children: A systematic review and meta-analysis. Dent Res J (Isfahan) 2016; 13: 387-95. [CrossRef]
6. Mugonzibwa EA, Eskeli R, Kuijpers-Jagtman AM, LaineAlava MT, van't Hof MA. Occlusal characteristics during different emergence stages of the permanent dentition in Tanzanian Bantu and Finnish children. Eur J Orthod 2004; 26: 251-60. [CrossRef]
7. Steinmassl O, Steinmassl PA, Schwarz A, Crismani A. Orthodontic Treatment Need of Austrian Schoolchildren in the Mixed Dentition Stage. Swiss Dent J 2017; 127: 122-28.
8. Nur Yilmaz RB, Oktay I, Ilhan D, Fişekçioğlu E, Özdemir F. Normative and subjective need for orthodontic treatment within different age groups in a population in Turkey. Niger J Clin Pract 2017; 20: 1632-8.
9. Rapeepattana S, Thearmontree A, Suntornlohanakul S. The prevalence of orthodontic treatment need and malocclusion problems in 8–9-year-old schoolchildren: A study in the south of Thailand. APOS Trends Orthod 2019; 9: 99-104. [CrossRef]
10. Alsughier ZA. Prevalence of malocclusal traits among 6–9-year-old male schoolchildren in Rass, Saudi Arabia. J Int Oral Health 2019; 11: 384-7. [CrossRef]
11. Albakri FM, Ingle N, Assery MK. Prevalence of malocclusion among male school children in Riyadh city. Open Access Maced J Med Sci 2018; 6: 1296-9. [CrossRef]
12. Alharbi F. The prevalence of malocclusion traits in Saudi Arabia 2015–2019: An epidemiological cross sectional study. J Int Oral Health 2020; 12: 129-34. [CrossRef]
13. Mohammed Almalky N, Mohammad Elattar H. Prevalence of different types of malocclusion among school children in Makkah Governorate of Saudi Arabia. Int J Dent Oral Sci 2018; 5: 645-8.
14. Recommended infection-control practices for dentistry, 1993 Centers for Disease Control and Prevention. MMWR Recomm Rep 1993; 42(RR-8):1–12.
15. Yu X, Zhang H, Sun L, Pan J, Liu Y, Chen L. Prevalence of malocclusion and occlusal traits in the early mixed dentition in Shanghai, China. Peer J 2019; 7: e6630.
16. Burden DJ, Pine CM, Burnside G. Modified IOTN: an orthodontic treatment need index for use in oral health surveys. Community Dent Oral Epidemiol 2001; 29: 220-5. [CrossRef]
17. Sandoval VP, Ceballos CM, Acevedo AC, Jans MA. Orofacial characteristics in relation to the need of orthodontic treatments in children. Int J Odontostomat 2010; 4:59-64. [CrossRef]
18. Baeshen H. The Prevalence of Major Types of Occlusal Anomalies among Saudi Middle School Students. J Contemp Dent Pract 2017; 18: 142-6. [CrossRef]
19. Alhummayani FM, Taibah SM. Orthodontic treatment needs in Saudi young adults and manpower requirements. Saudi Med J 2018; 39: 822-8. [CrossRef]

20. Alajlan SS, Alsaleh MK, Alshammari AF, Alharbi SM, Alshammari AK, Alshammari RR. The prevalence of malocclusion and orthodontic treatment need of school children in Northern Saudi Arabia. J Orthod Sci 2019; 8:10. [CrossRef]
21. Mohamed M Alizae, Ariffin WFM, Rosli TI, Mahyuddin Alida. The feasibility of Index of Orthodontic Treatment Need (IOTN) in labial segment malocclusion among 8-10 years old. Arch Orofac Sci 2014; 9: 76-84.
22. Abu-Fanas A, Hashim R, Al-Ali S. Orthodontic treatment needs among 9-12 years old children in the Emirate of Ajman, United Arab Emirates. J Adv Oral Res 2015; 6: 39-42. [CrossRef]
23. Grzywacz I. The value of the aesthetic component of the Index of Orthodontic Treatment Need in the assessment of subjective orthodontic treatment need. Eur J Orthod 2003; 25: 57–63. [CrossRef]
24. Hammad M, Awad M. Orthodontic treatment need in Egyptian schoolchildren. Pediatr Dent Journal 2013; 21: 39-43. [CrossRef]
25. daCosta OO, Aikins EA, Isiekwe GI, Adediran VE. Malocclusion and early orthodontic treatment requirements in the mixed dentitions of a population of Nigerian children. J Orthod Sci 2016; 5: 81-6. [CrossRef]
26. Fatani NH, Hammam MB, Oraif H, Taher S, Taju W, Bukhari O. Prevalence of Malocclusion among Schoolchildren in Makkah, Saudi Arabia. Open Access Maced J Med Sci 2019; 7: 856-81. [CrossRef]

eor.istanbul.edu.tr

Official Publication of Istanbul University Faculty of Dentistry

Eur Oral Res 2021; 55(3): 116-123

Original research

Evaluation of flexural properties and dynamic mechanical analysis of glass fiber-reinforced polyamide resin

Senem Ünver[1], **Arzu Zeynep Yıldırım**[1]

Purpose
The aim of this study was to evaluate flexural strength, elastic modulus and dynamic mechanical analysis (DMA) of heat-polymerized polymethyl methacrylate resin, polyamide resin and glass fiber-reinforced polyamide resin.

Materials and Methods
Three groups were determined according to denture base materials as polymethyl methacrylate resin (H), polyamide resin (P) and glass fiber reinforced polyamide resin (R). Sixteen specimens for each denture base material were prepared with dimensions of 64x10x3.3 mm for three-point bending test. Two specimens for each denture base material were prepared with dimensions of 30x10x3 mm for DMA. Polymethyl methacrylate and polyamide specimens were prepared according to the manufacturer's recommendations. The silane was applied to glass fibers (4.5 mm length) 2% by weight of the polyamide resin, they were placed in polyamide resin cartilages and injected to the mold. The thermal aging procedure was applied to half of specimens of each material (n=8). Flexural strength and elastic modulus of the specimens were determined by three-point bending test at a speed of 5 mm/min. DMA was performed to 1 specimen from each group to evaluate viscoelastic properties. Data were analyzed with one-way ANOVA, Tukey and Paired t tests.

Results
A statistically significant difference was found in flexural strength and elastic modulus values of denture base materials (p=0.00). The highest flexural strength and elastic modulus values were observed in polymethyl methacrylate group. There was no significant difference between polyamide and glass-fiber reinforced polyamide groups (p=0.497). No significant difference was determined in all three-denture base materials before and after aging procedure.

Conclusion
The reinforcement with glass-fibers did not affect the flexural strength and elastic modulus of polyamide resin.

Keywords: *Polyamide resin, flexural strength, DMA, strengthening, glass fiber*

Introduction

Polymethyl methacrylate (PMMA) resins have been widely used in removable prosthetic restorations. They have some advantages such as easy manipulation, aesthetic appearance, low water absorption and solubility, compatibility with oral tissues and polishability (1,2). However, polymerization shrinkage, low impact strength, low fatigue resistance and residual monomer content are significant disadvantages (3,4). Metal substructures, which are used to increase durability of the PMMA, may cause allergenic reactions as a result of corrosion. Also, the metal clasps affect aesthetic appearance (5). Different methods have been developed to overcome these disadvantages such as; adding filling materials to rein-

Presented at: *The 25th International Scientific Congress and Exhibition of İzmir Dental Association, İzmir, Turkey, Nov 2018.*

ORCID IDs of the authors: S.Ü. 0000-0001-5565-1167; A.Z.Y. 0000-0002-9332-8982

[1]*Department of Prosthodontics, Faculty of Dentistry, Gazi University, Ankara, Turkey*

Corresponding Author: Senem Ünver

E-mail: dtsenemuysal@hotmail.com

Received: 31 October 2020
Revised: 4 January 2021
Accepted: 9 February 2021

DOI: 10.26650/eor.2021872015

How to cite: *Unver S, Yildirim AZ. Evaluation of flexural properties and dynamic mechanical analysis of glass fiber-reinforced polyamide resin. Eur Oral Res 2021; 55(3): 116-123.*

force of resins, chemical modifications of polymer with copolymerization and cross-linking of resin materials, producing new materials with different polymerization technics (6-8). Strengthening materials such as glass fiber, aramid fiber, nanodiamond powder, zirconium oxide, aluminum oxide, halloysite nanotubes, metal wires and carbon nanotubes can be added to denture base in order to increase fatigue resistance and fracture resistance of the prosthetic base materials (9-17). However, the most effective reinforcement is yet to be determined (18). The glass fiber-reinforcement have showed promising results in previous studies (9,19,20).

Injection molding technique has been developed to improve the physical properties of denture base resins (21). Injection molding technique decreases polymerization shrinkage and improve dimensional stability of the resins compared to compression-molding technique (22-24). Polyamide resin is one of the materials prepared with this technique and it can be used safely in patients who are allergic to metal or resin monomers (25). Polyamide resin has higher elasticity than PMMA, and it can be preferred in the presence of tissue undercuts which cannot be corrected by surgical operations (26,27). However, the flexibility of denture base may lead to permanent deformation in the prosthesis during mastication and, resorption of underlying bone structure due to the vertical stress that occurs from deformation (17). Nakamura *et al.* (9) stated that flexible resins must be strengthened, and glass fiber can be used to increase flexural strength of polyamide.

The glass fiber, which was developed to reinforce the polyamide resin material, was used in this study. Its purpose was to evaluate flexural strength, elastic modulus and dynamic mechanical analysis of PMMA, polyamide resin and glass fiber-reinforced polyamide resin before and after thermocycling procedure. The first null hypothesis was that the glass fiber-reinforcement would affect the flexural properties of the polyamide resin and the second null hypothesis was that there would not be any difference between flexural strength values of denture base materials before and after thermocycling procedure.

Materials and methods

Material selection

One PMMA (Meliodent; Heraeus-Kulzer GmbH, Wehrheim, Germany) and one polyamide resin (Deflex; Nuxen SRL, Buenos Aires, Argentina) were selected. E-glass fibers (PA2(D); Şişecam, İstanbul, Turkey) were added to reinforce of polyamide resin. Three groups were determined according to denture base materials as PMMA (H), polyamide resin (P) and glass fiber reinforced polyamide resin (R).

Fabrication of specimens

Forty-eight flexural strength test specimens were prepared in accordance with ISO 20795-1:2013 with dimensions of 64x10x3.3 mm (16 specimens for each denture base material) (28). For dynamic mechanical analysis, 2 specimens for each denture base material were prepared with dimensions of 30x10x3 mm. For dimensions standardization, metal molds were fabricated. Wax specimens were produced by using metal molds, they were embedded in the cast molds and removed. Separating agent was applied on the cast molds and the molds were left to dry. For PMMA specimens, the powder and liquid (35 gr: 14 ml, powder: liquid) were mixed with a spatula and then the resin was inserted in the cast molds. The cast molds were placed in boiling water and the heat source were switched off for 15 min. Then the specimens were polymerized in boiling water for 20 min according to the manufacturer's recommendation (29). The cast molds were allowed to cool at room temperature. For the polyamide resin specimens, the polyamide resins in the cartridges were heated to a temperature of 280°C for 15 minutes and were injected to the cast molds with a 6-bar for 30 seconds. E-glass fibers with a diameter of 11 µ, which were cut into length of 4.5 mm by manufacturer, were selected. For each specimen, glass fibers were 2% by weight of the polyamide resin. The E-glass fibers were weighed and the silane (Ultradent Silane; Ultradent Products Inc., South Jordan, USA) was applied to the E-glass fibers. The silanized E-glass fibers were placed in the polyamide resin cartridges and mixed thoroughly. The cartridges were heated to a temperature of 280°C for 15 minutes and the polyamide resins were injected to the cast molds with a bar injection pressure for 30 seconds. All of the specimens were straightened by using a diamond burr and were smoothed by using 600-grit silicon carbide paper. After polishing procedure, the dimensions of specimens were measured with digital micrometer. The specimens were stored in water at 37°C for 24 hours before thermocycling procedure. The artificial aging procedure was applied to half of specimens of each material and 2 subgroups were determined. (n=8).

Thermocycling procedure

The artificial aging procedure was performed on half of specimens of each material with the thermal cycling device (SD Mechatronik Thermocycler; SD Mechatronik GMBH, Feldkirchen-Westerham, Germany) for 5000 cycles from 5°C to 50°C temperatures with 60 seconds waiting time.

Flexural strength test

The specimens were placed in a universal test machine (Lloyd LRX; Lloyd Instruments Ltd., Fareham, Hampshire, UK) with a 50 mm interface and subjected to three-point bending test at a speed of 5 mm/min until fracture or maximum deflection occurred to determine of the flexural strength (MPa) and the elastic modulus (GPa) of the specimens. The flexural strength was calculated using the formula $3FI/2bh^2$, where F is the maximum fracture load (N), I is the distance between the supports (mm), b is the width of the specimen (mm), h is the height of the specimen (mm). The elastic modulus (GPa) was calculated according to the formula $F1_1I^3/4bh^3d$, where F1 is the load at a point in the straight-line portion of the load/deflection graph (N), I is the distance between the supports (mm), b is the width of the specimen (mm), h is the height of the specimen (mm), d is the deflection (mm) at load F1.

Scanning electron microscope (SEM) examination

One specimen of glass fiber reinforced polyamide was fractured in liquid nitrogen and the fractured surface was stud-

ied under a scanning electron microscope (SEM) (JEOL JSM 6060LV, Noran Instruments, Japan) after gold sputtering to describe glass fiber distribution and fiber-resin connection.

Dynamic Mechanical Analysis

The dynamical mechanical analysis (DMA) was performed to evaluate viscoelastic properties of PMMA, polyamide resin and glass fiber reinforced polyamide resin materials. The temperature range of DMA (Q800; TA Instruments, New Castle, USA) was from 20°C to 200°C with 5°C/min heat rate.

Statistical analysis

The mean flexural strength and elastic modulus values were calculated by using SPSS statistical software package program (SPSS version 24.0 software; SPSS, Chicago, IL, USA). Shapiro-Wilk test was performed to evaluate the normally distribution of the data and the data showed a normal distribution. Parametric tests were used. Data were analyzed by one-way ANOVA test to study the difference among the denture base materials ($P<0.05$). Tukey test was applied for pairwise comparisons of the groups and paired t test was used to decide the effect of the thermocycling procedure on denture base materials. Level of significance was set at $P<0.05$.

Results

The mean values and standard deviations of flexural strength of denture base materials before and after aging procedure are listed in Table 1. Both before and after thermocycling procedure, the flexural strength values of H groups were significantly higher than P and R groups (P=0.000) (Table 2), however it was observed that there was no significant difference between P and R groups. The mean values and standard deviations of elastic modulus of denture base materials are shown in Table 3. The comparison of elastic modulus values of the denture materials was revealed that there was a significant difference between groups (P=0.000) (Table 4) and the H group had higher values than P and R groups.

The paired t test, which used to evaluate the effect of artificial aging on flexural strength of denture base materials, revealed that there was no significant difference between denture materials before and after thermocycling (Group H, P=0.049; Group P, P=0.554; Group R, P=0.922). Similarly, the aging procedure did not effect of elastic modulus of denture base materials (Group H, P=0.549; Group P, P=0.267; Group R, P=0.321). During the flexural test, all of the PMMA specimens on H groups were fractured. Besides, none of the specimens of P and R groups were fractured and during the test but they detached from the supporting clamps. Therefore, flexural yield strength values of P and R groups were considered as flexural strength.

SEM images of the fractured surface of glass fiber reinforced polyamide specimen are shown in Figure 1 and Figure 2. The glass fibers were not distributed uniformly in polyamide resin and some fibers were bunched together in some areas (Figure 1). Cohesive type failure was detected between glass fiber and polyamide, and the hole of detached fiber was seen at x2000 magnification (Figure 2).

Storage modulus (E') and tan delta of groups before and after aging procedure are presented in Figure 3, Figure 4, Figure 5 and Figure 6 respectively. The glass transition temperatures for the PMMA, polyamide resin and glass fiber-reinforced polyamide resin were found 144.5°C, 134.26°C and 134.22°C respectively (Table 5). After thermocycling procedure, the glass transition temperature decreased for each denture base material. Both before and after thermocycling procedure the form of polyamide resin and glass fiber-reinforced polyamide resin specimens were distorted permanently (Figure 7).

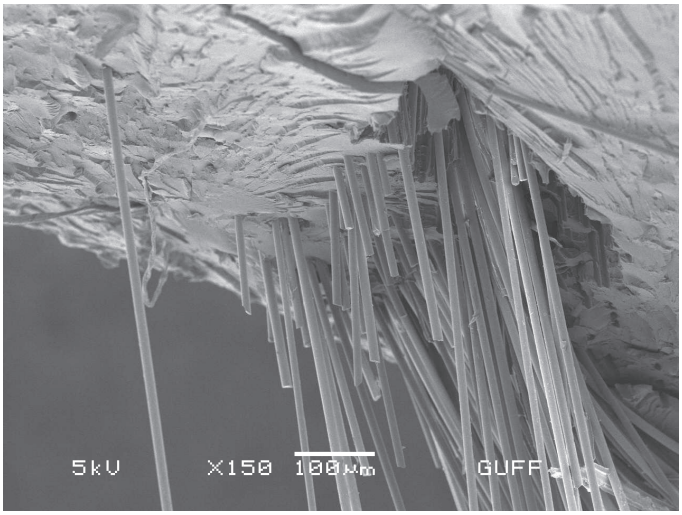

Figure 1. *Fractured surface of glass fiber reinforced polyamide resin specimen under SEM (x150 magnification).*

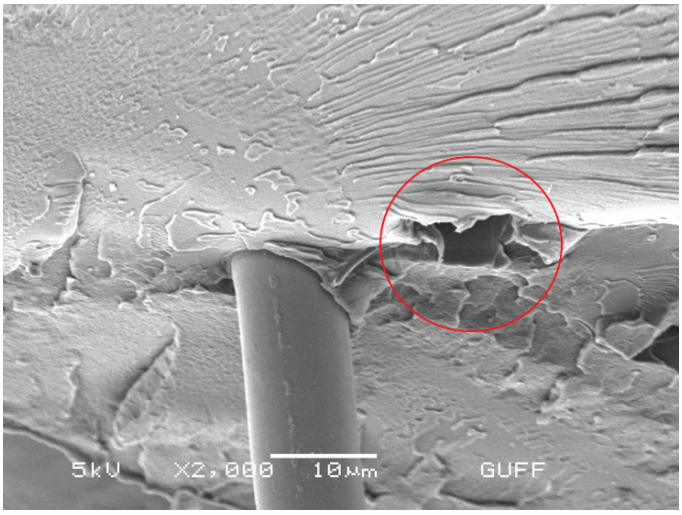

Figure 2. *Fractured surface of glass fiber reinforced polyamide resin specimen under SEM (x2000 magnification).*

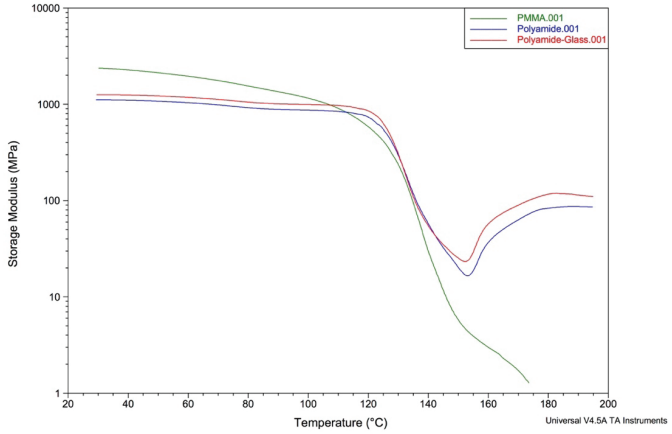

Figure 3. *Storage modulus of the groups before aging procedure.*

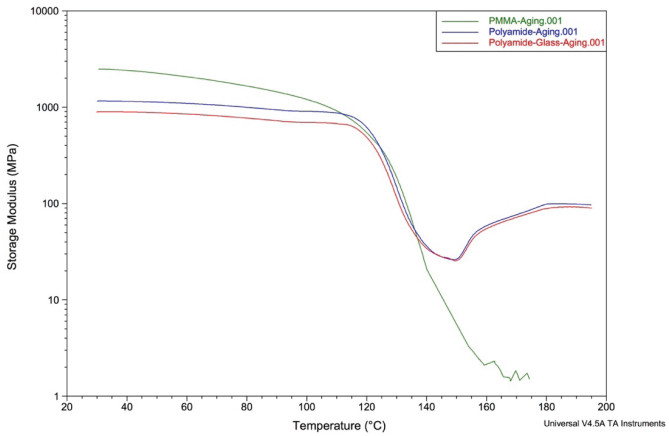

Figure 4. *Storage modulus of the groups after aging procedure.*

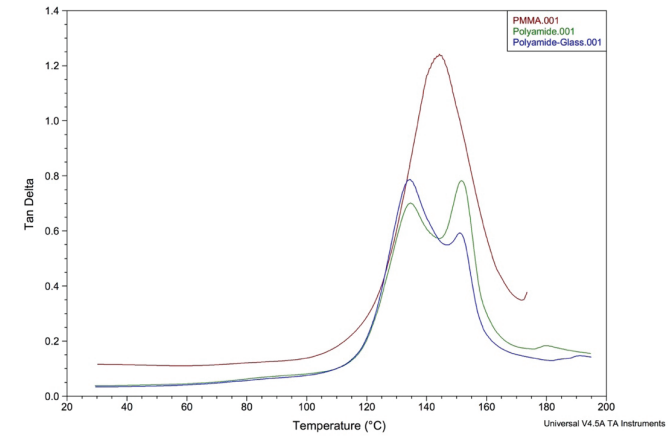

Figure 5. *Tan delta of groups before aging procedure.*

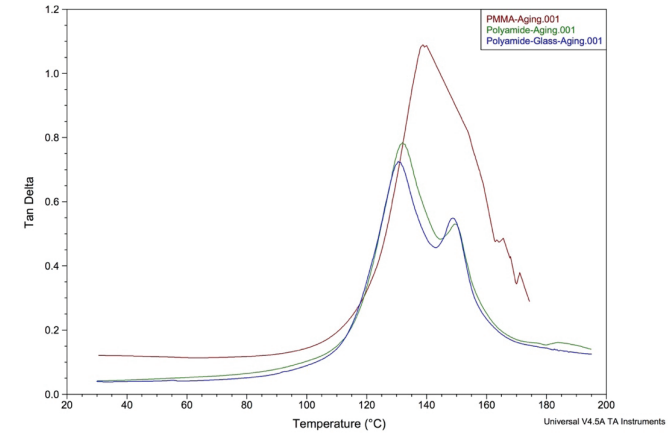

Figure 6. *Tan delta of groups after aging procedure.*

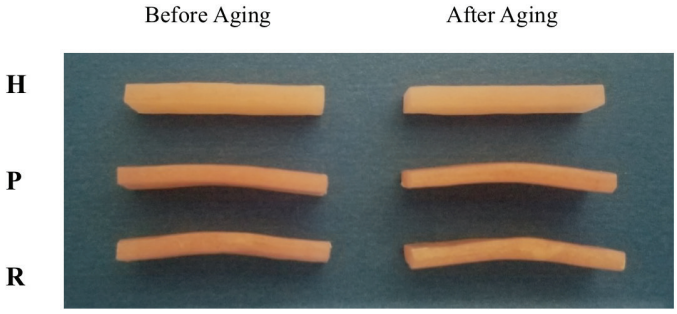

Figure 7. *The specimens after DMA.*

Table 1. *Flexural strength values of the groups (MPa).*

Group		Before Aging Mean ±SD		After Aging Mean ±SD	[c]p
H		119.85 ±15.65		118.9 ±31.05	0.949***
P		80.13 ±12.74		75.3 ±15.53	0.554***
R		72.16 ±13.12		71.73 ±14.8	0.922***
	[a]p	0.000*		0.000*	
Pairwise Comparisons; [b]p	**H-P**	39.72**	**H-P**	43.6**	
	H-R	47.69**	**H-R**	47.17**	
	P-R	7.97	**P-R**	3.56	

(SD: Standard deviation, H: Heat-polymerized polymethyl methacrylate, P: Polyamide, R: Glass fiber reinforced polyamide. [a]One way ANOVA Test*p<0.001, [b]Tukey Test **p<0.05, [c]Paired t Test***p<0.05).

Table 2. *One-way ANOVA analysis of flexural strength values of the groups before and after aging.*

	Sum of Squares	df	Mean Square	F	Sig
Before aging					
Between Groups	10441.958	2	5220.979	27.03	0.000
Within Groups	4056.301	21	193.157		
Total	14498.258	23			
After aging					
Between Groups	11036.133	2	5518.067	11.621	0.000
Within Groups	9971.596	21	474.838		
Total	21007.729	23			

Table 3. *Elastic modulus values of the groups (GPa).*

Group		Before Aging Mean ±SD		After Aging Mean ±SD	[c]p
H		5.79 ±0.64		6.12 ±1.27	0.549***
P		3.03 ±0.69		2.54 ±0.64	0.266***
R		2.61 ±0.63		2.38 ±0.68	0.319***
	[a]p	0.000*		0.000*	
Pairwise Comparisons; [b]p	**H-P**	2.76**	**H-P**	3.58**	
	H-R	3.18**	**H-R**	3.74**	
	P-R	4.21	**P-R**	0.16	

(SD: Standard deviation, H: Heat-polymerized polymethyl methacrylate, P: Polyamide, R: Glass fiber reinforced polyamide. [a]One way ANOVA Test*p<0.001, [b]Tukey Test **p<0.05, [c]Paired t Test***p<0.05).

Table 4. *One-way ANOVA analysis of elastic modulus values of the groups before and after aging.*

	Sum of Squares	df	Mean Square	F	Sig
Before aging					
Between Groups	47.656	2	23.828	55.745	0.000
Within Groups	8.976	21	0.427		
Total	56.632	23			
After aging					
Between Groups	71.471	2	35.735	43.068	0.000
Within Groups	17.424	21	0.83		
Total	88.895	23			

Table 5. *The glass transition temperatures of denture base materials before and after thermocycling procedure.*

Group	Before aging	After aging
H	144.53°C	138.40°C
P	134.26°C	132.26°C
R	134.22°C	130.79°C

(H: Heat-polymerized polymethyl methacrylate, P: Polyamide, R: Glass fiber reinforced polyamide).

Discussion

This in vitro study demonstrated that the PMMA had higher flexural strength and elastic modulus values than the polyamide resin and glass fiber-reinforced polyamide resin. PMMA has been the most commonly used material for removable prosthodontics and polyamide is an alternative. The glass fiber, which were used in current study, has been developed for the reinforcement of polyamide material. The study was planned to determine the effect of this glass fiber on flexural properties of polyamide but, at the same time, the reinforced polyamide was compared with PMMA to decide whether it can replace the PMMA.The reinforcement of polyamide resin decreased the flexural strength and elastic modulus values but there was no statistically significant difference between them. So, these results were rejected the first null hypotheses of this study that the reinforcement would affect the flexural properties of polyamide resin material. Besides, the results confirmed the second null hypothesis that no significant difference would occur between flexural properties of denture base materials before and after thermocycling procedure.

Denture base materials are subjected to various forces such as compression and shear during mastication. Mechanical properties of denture base resins are often evaluated with flexural strength, modulus of elasticity and impact strength (17,30-33). These tests have been approved to imitate the natural forces acting on the prosthesis in oral environment (34). Flexural strength is the ability of a material to oppose the deformation under load and represents the highest stress encountered within the material at fracture moment (35). The denture base must have suitable rigidity to distribute the forces over the dental arch equally. Also, the flexibility of material is important for energy absorption in case of dropping the denture (8). According to ISO 20795-1:2013, flexural strength of denture base materials should be no less than 65 MPa and flexural modulus no less than 2 GPa (28). In the present study, both flexural strength and elastic modulus of each denture base materials were consistent with the specified ISO standards.

Even though PMMA is the most preferred denture base material, flexible resins were introduced by manufacturers as an alternative for constructing complete and partial removable dentures (36). Polyamide resin is usually indicated in patients who have allergy to methyl methacrylate monomer and retention problems due to certain degree of undercuts (25,27). The major connector of the removable denture should have enough rigidity to distribution of the chewing force and low flexural modulus of denture base material is an important disadvantage (6,7). Ucar *et al.* (8) reported that polyamide may be used for an alternative material for construction of complete dentures but not for removable partial dentures.

The denture base materials can be strengthened to prevent fractures which is a common complication. There are some studies that have evaluated the effect of reinforcement of PMMA with different materials, but the those on the reinforcement of polyamide resin are scarce (7,9-17,33). In a previous study, the rigidity of dentures made of polyamide, polyester and conventional heat-polymerized PMMA were compared, and it was concluded that the polyamide, which has low elasticity, needed to be reinforced with metal frames (37). Soygun *et al.* (32) evaluated transverse strength of polyamide, PMMA and reinforced PMMA with different esthetic fibers Polyamide denture base material had higher transverse strength than PMMA and fiber-added groups. Also, no fracture occurred in polyamide specimens. They reported that the polyamide provided better energy absorption due to chemical structure properties. Sasaki *et al.* (17) compared the flexural strength of three different injection-molded thermoplastic denture base resins (polyamide, polyester, polycarbonate) with PMMA They reinforced the denture base materials with using glass fiber-reinforced composite and metal wire. It was stated that the glass fiber-reinforced composite was effective for polyamide and PMMA resins. In the present study, the glass fibers were used for reinforcement of polyamide resin. Glass fiber-reinforced polyamide specimens had lower values than polyamide specimens for both flexural strength and elastic modulus, but the results were not significant. The elastic stiffness of E-glass fibers does not change during heat treatments, but the mechanical strength of the material might be affected by the distribution of fibers inside the matrix (38,39). When the reinforced specimens were prepared, the glass fibers were placed in the polyamide resin cartridges and the resin materials were injected with pressure to the mold. Consequently, the distribution of the fibers in the polyamide were uncontrolled. This result of the present study might be attributed to the non-homogeneous distribution of glass fibers in the material (Figure 1).

Liquid nitrogen was applied to the reinforced polyamide specimen to fracture owing to the fact that none of the reinforced polyamide specimens were fractured during flexural

strength test. The SEM image of the fracture surface of the specimen was shown that the fibers stuck out from polyamide and the fibers showed resistance to fracture (Figure 2). Even though, silan application is an effective method to provide bonding between fiber and polymer matrix, the bonding depends on the mechanical retention caused by polymerization shrinkage of polymer and roughness of fibers (40,41). Therefore, the adhesive type failure could have been observed between fibers and polyamide material.

Thermocycling procedure is an application for testing the long-term clinical use of dental materials and it is based on the temperature changes in the oral environment between 5°C-55°C due to the different types of nutrition (42). After aging procedure, the preservation of physical and mechanical properties of an ideal dental material is important but distance between the polymer chains may extend as a result of heat changes and the material may absorb water (42). So, water immersion causes water molecules to act as plasticizer in the polymer structure and unpolymerized PMMA can be dissolved by cyclic temperature changes (43). Takahashi *et al.* (44). stated that thermocycling significantly decreased flexural strength and elastic modulus of one polyamide (Valplast), but it increased same properties of the other polyamide (Lucitone FRS) This result could be explained with differences in physical properties and composition of different polyamides used in industry. In another study, the effect of thermocycling with different thermal cycles on polyamide and PMMA denture base resins was evaluated and it was concluded that there was no significant difference between flexural strengths of each group (42). In the present study, specimens were subjected to 5000 cycles to imitate the clinical function of 6 months approximately (45). In contrast to the literature, thermocycling procedure did not change the flexural strength and elastic modulus of groups significantly.

DMA, which is commonly studied to describe the mechanical behaviors of polymers and polymer composites, is a technique that oscillating force is applied to a specimen and analyzing the material's response to that force (46). Tg (glass transition temperature) value can be determined by DMA. The Tg represents a major transition for many polymers and physical properties of material change strongly as the material turns from a hard glassy to a rubbery state (47). In the present study, the glass transition temperatures of PMMA, polyamide resin and glass fiber-reinforced polyamide resin were found as 144.53°C, 134.26°C and 134.22°C respectively. The Tg values are high because both the PMMA and the polyamide are amorphous materials (30,48-50). Decrease in the glass transition temperatures of materials after thermocycling shows that the mechanical properties of denture base materials may be affected at mouth temperatures ranging between 5^0C and 55^0C.

The clinical significances of the study are that the polyamide resin must be reinforced, and the temperature changes during clinical usage can be affect the flexural properties and durability of the denture base. There are some limitations in the present study. Only one reinforced material was tested for polyamide resin and only flexural strength, elastic modulus and DMA were tested. The properties of reinforced polyamide with different concentrations of E-glass fibers can be further evaluated. In vitro studies which investigate the different physicomechanical properties of polyamide resins reinforced with different materials would be helpful to provide case-specific solutions.

Conclusion

The PMMA had higher flexural strength and elastic modulus than polyamide resin and glass fiber-reinforced polyamide resin. Using the glass fiber for reinforcement of polyamide resin did not affect flexural strength and elastic modulus of the material. The thermocycling procedure did not change the flexural properties of the denture base materials. According to DMA, polyamide resins are more likely to deform than PMMA resins do.

Türkçe Özet: *Cam Fiber ile Güçlendirilmiş Poliamid Rezinin Bükülme Özelliklerinin ve Dinamik Mekanik Analizinin Değerlendirilmesi. Amaç: Bu çalışmanın amacı, ısı ile polimerize polimetilmetakrilat rezin, poliamid rezin ve cam fiberle güçlendirilmiş poliamid rezin materyallerinin bükülme dayanımı, elastik modülüsü ve dinamik mekanik analizinin (DMA) değerlendirilmesidir. Gereç ve yöntem: Çalışmada, dental kaide materyallerine göre; polimetil metakrilat resin (H), poliamid rezin (P) ve cam fiberle güçlendirilmiş poliamid rezin (R) olmak üzere 3 grup belirlendi. Her bir kaide materyalinden, 3 nokta bükülme testi için 64x10x3,3 mm boyutlarında 16 örnek, dinamik mekanik analiz için 30x10x3 mm boyutlarında 2 örnek hazırlandı. Polimetil metakrilat ve poliamid örnekler firma önerileri doğrultusunda hazırlandı. Poliamid rezinin ağırlığının %2'si kadar 4.5 mm uzunluktaki cam fiberlere silan uygulandı, Cam fiberler poliamid rezin kartilaj içerisine yerleştirildi ve kalıplara enjekte edildi. Her bir materyalin örneklerinin yarısına termal yaşlandırma işlemi uygulandı (n=8). Örneklerin bükülme dayanımı ve elastik modülüsü 5 mm/min hızda üç nokta bükülme testi ile belirlendi. Viskoelastik özellikleri değerlendirmek için her gruptan 1 örneğe DMA yapıldı. Verilerin analizinde one-way ANOVA, Tukey ve Paired t testleri kullanıldı. Bulgular: Protez kaide materyallerinin bükülme dayanımı ve elastik modülüs değerleri arasında istatistiksel olarak anlamlı fark bulundu (P<0.05). En yüksek bükülme dayanımı ve elastik modülüs değerleri polimetilmetakrilat grubunda gözlendi. Poliamid ve cam fiberle güçlendirilmiş poliamid grupları arasında istatistiksel olarak fark tespit edilmedi (P=0.497). Her üç kaide materyalinde yaşlandırma işlemi öncesi ve sonrası değerlerde anlamlı fark görülmedi. Sonuç: Cam fiber poliamid rezinin bükülme dayanımını ve elastik modülüsünü değiştirmedi. Anahtar kelimeler: Poliamid rezin, bükülme dayanımı, dinamik mekanik analiz, güçlendirme, cam fiber.*

Ethics Committee Approval: Not required.

Informed Consent: Not required.

Peer-review: Externally peer-reviewed.

Author contributions: SU, AZYB participated in designing the study. SU participated in generating the data for the study. SU participated in gathering the data for the study. SU participated in the analysis of the data. SU wrote the majority of the original draft of the paper. SU, AZYB participated in writing the paper. SU, AZYB have had access to all of the raw data of the study. SU, AZYB have reviewed the pertinent raw data on which the results and conclusions of this study are based. SU, AZYB have approved the final version of this paper. SU guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: The authors declared no conflict of interest.

Financial Disclosure: This study has been supported by Project of Scientific Investigation of Gazi University (project number: 03/2018-11).

References

1. Uzun G, Hersek N, Tinçer T. Effect of five woven fiber reinforcements on the impact and transverse strength of a denture base resin. J Prosthet Dent 1999;81:616-20. [CrossRef]
2. Köroğlu, A, Özdemir T, Usanmaz A. Comparative study of the mechanical properties of fiber-reinforced denture base resin. J App Polymer Sci 2009;113:716-20. [CrossRef]
3. Ali IL, Yunus N, Abu-Hassan MI. Hardness, Flexural Strength, and Flexural Modulus Comparisons of Three Differently Cured Denture Base Systems. J Prosthodont 2008;17:545-9. [CrossRef]
4. Athar Z, Juszczyk AS, Radford DR, Clark RK. Effect of curing cycles on the mechanical properties of heat cured acrylic resins. Eur J Prosthodont Restor Dent 2009;17:58-60.
5. Kanie T, Arikawa H, Fujii K, Ban S. Flexural properties of denture base polymers reinforced with a glass cloth–urethane polymer composite. Dent Mater 2004;20:709-16. [CrossRef]
6. Doğan OM, Bolayir G, Keskin S, Doğan A, Bek B. The evaluation of some flexural properties of a denture base resin reinforced with various aesthetic fibers. J Mater Sci Mater Med 2008;19:2343-9. [CrossRef]
7. Foo SH, Lindquist TJ, Aquilino SA, Schneider RL, Williamson DL, Boyer DB. Effect of polyaramid fiber reinforcement on the strength of 3 denture base polymethyl methacrylate resins. J Prosthodont 2001;10:148-53. [CrossRef]
8. Uçar Y, Akova T, Aysan I. Mechanical properties of polyamide versus different PMMA denture base materials. J Prosthodont 2012;21:173-6. [CrossRef]
9. Nagakura M, Tanimoto Y, Nishiyama N. Effect of fiber content on flexural properties of glass fiber-reinforced polyamide-6 prepared by injection molding. Dent Mater J 2017;36:415-21. [CrossRef]
10. Al-Harbi FA, Abdel-Halim MS, Gad MM, Fouda SM, Baba NZ, AlRumaih HS, Akhtar S. Effect of Nanodiamond Addition on Flexural Strength, Impact Strength, and Surface Roughness of PMMA Denture Base. J Prosthodont 2019;28:417-25. [CrossRef]
11. He X, Peng J, Peng T, Qian Z. A novel botryoidal aramid fiber reinforcement of a PMMA resin for a restorative biomaterial. Biomater Sci 2017;28:808-16. [CrossRef]
12. Gad MM, Al-Thobity AM, Rahoma A, Abualsaud R, Al-Harbi FA, Akhtar S. Reinforcement of PMMA Denture Base Material with a Mixture of ZrO2 Nanoparticles and Glass Fibers. Int J Dent 2019;2019:1-11.
13. Dhole PI, Shetty R, Huddar D, Sankeshwari B, Chopade S. Reinforcement of Aluminum Oxide Filler on the Flexural Strength of Different Types of Denture Base Resins: An In vitro Study. J Clin Diagn Res 2017;11:101-4. [CrossRef]
14. Abdallah RM. Evaluation of polymethyl methacrylate resin mechanical properties with incorporated halloysite nanotubes. J Adv Prosthodont 2016;8:167-71. [CrossRef]
15. Kumar V, Kumar L, Sehgal K, Datta K, Pal B. A Comparative Evaluation of Effect of Reinforced Autopolymerizing Resin on the Flexural Strength of Repaired Heatpolymerized Denture Base Resin before and after Thermocycling. J Int Soc Prev Community Dent 2017;7:99-106. [CrossRef]
16. Somkuwar S, Mishra SK, Agrawal B, Choure R. Comparison of the flexural strength of polymethyl methacrylate resin reinforced with multiwalled carbon nanotubes and processed by conventional water bath technique and microwave polymerization. J Indian Prosthodont Soc 2017;17:332-9. [CrossRef]
17. Sasaki H, Hamanaka I, Takahashi Y, Kawaguchi T. Effect of reinforcement on the flexural properties of ınjection-molded thermoplastic denture base resins. J Prosthodont 2017;26:302-8. [CrossRef]
18. Takashi T, Gonda T, Mizuno Y, Fujinami Y, Maeda Y. Reinforcement in removable prosthodontics: a literature review. J Oral Rehabil 2017;44:133-43. [CrossRef]
19. Ladha K, Shah D. An In-Vitro Evaluation of the Flexural Strength of Heat-Polymerized Poly (Methyl Methacrylate) Denture Resin Reinforced with Fibers. J Indian Prosthodont Soc 2011;11:215-20. [CrossRef]
20. Gharehchahi J, Aghdaee NA, Kermani N. Evaluation of FRC reinforcing on flexural strength of acrylic resin denture bases. N Y State Dental J 2010;76:40-3.
21. Ganzarolli SM, Mello JAN, Shinkai RS, et al. Internal adaptation and some physical properties of methacrylate-based denture base resins polymerized by different techniques. J Biomed Mater Res Part B: App Biomater 2007;82:169-73. [CrossRef]
22. Anderson GC, Schulte JK, Arnold TG: Dimensional stability of injection and conventional processing of denture base acrylic resin. J Prosthet Dent 1988;60:394-8. [CrossRef]
23. Strohaver RA. Comparison of changes in vertical dimension between compression and injection molded complete dentures. J Prosthet Dent 1989;62:716-8. [CrossRef]
24. Huggett R, Zissis A, Harrison A, et al: Dimensional accuracy and stability of acrylic resin denture bases. J Prosthet Dent 1992;68:634-40. [CrossRef]
25. Stafford GD, Huggett R, MacGregor AR, Graham J. The use of nylon as a denture-base material. J Dent 1986;14:18-22. [CrossRef]
26. Hargreaves AS. Nylon as a denture-base material. Dent Pract Dent Rec 1971;22:122-8.
27. MacGregor AR, Graham J, Stafford GD, Huggett R. Recent experiences with denture polymers. J Dent 1984;12:14657. [CrossRef]
28. International Standards Organization. Dentistry-Base polymers-Part 1: Denture base polymers. ISO 20795-1. International Standards Organization, Geneva, Switzerland; 2013.
29. https://www.kulzer.com/media/webmedia_local/downloads_new/meliodent_1/medliodent_hc/GBA_MelioDent_HC_Regio_INT.pdf
30. Kürkçüoğlu I, Köroğlu A, Özkır SE, Özdemir T. A comparative study of polyamide and PMMA denture base biomaterials: I. Thermal, mechanical, and dynamic mechanical properties. Int J Polym Mater 2012;61:768-77. [CrossRef]
31. Kohli S, Bhaita S. Flexural properties of polyamide versus injectionmolded polymethylmethacrylate denture base materials. Eur J Prosthodont 2013;1:56-60. [CrossRef]
32. Soygun K, Bolayir G, Boztug A. Mechanical and thermal properties of polyamide versus reinforced PMMA denture base materials. J Adv Prosthodont 2013;5:153-60. [CrossRef]
33. Demir H, Gorler O, Dogan A, Ozden S. The assessment of impact properties of a denture base polymer reinforced with various fibers. Int J Acad Res 2017;9:15-9. [CrossRef]
34. McCabe JF, Walls AWG. Applied dental materials. 9th Ed., Copenhagen: Blackwell Munksgaard 2008, p.4-31.
35. Chitchumnong P, Brooks SC, Stafford GD. Comparison of three and four-point flexural strength testing of denture-base polymers. Dent Mater 1989;5:2-5. [CrossRef]
36. Goiato MC, Santos DM, Haddad MF, Pesqueira AA. Effect of accelerated aging on the microhardness and color stability of flexible resins for dentures. Braz Oral Res 2010;24:1149. [CrossRef]
37. Wadachi J, Sato M, Igarashi Y. Evaluation of the rigidity of dentures made of injection-molded materials. Dent Mater J 2013;32:508-11. [CrossRef]
38. Feih S, Manatpon K, Mathys Z, Gibson AG, Mouritz AP. Strength degradation of glass fibers at high temperatures J Mater Sci 2009;44:392-400.
39. Doğan OM, Bolayir G, Keskin S, Doğan A, Bek B, Boztuğ A. The effect of esthetic fibers on impact resistance of a conventional heat-cured denture base resin. Dent Mater J 2007;26:232-9. [CrossRef]
40. Karacaer O, Polat TN, Tezvergil A, Lassila LV, Vallittu PK. The effect of length and concentration of glass fibers on the mechanical properties of an injection-and a compression molded denture base polymer. J Prosthet Dent 2003;90:385-93. [CrossRef]
41. Kanie T, Fujii K, Arikawa H, Inoue K. Flexural properties and impact strength of denture base polymer reinforced with woven glass fibers. Dent Mater 2000;16:150-8. [CrossRef]

42. Aydoğan Ayaz E, Bağış B, Turgut S. Effects of thermal cycling on surface roughness, hardness and flexural strength of polymethylmethacrylate and polyamide denture base resins. J Appl Biomater Funct Mater 2015;13:280-6.
43. Göhring TN, Gallo L, Lüthy H. Effect of water storage, thermocycling, the incorporation and site of placement of glass-fibers on the flexural strength of veneering composite. Dent Mater 2005;21:761-72. [CrossRef]
44. Takahashi Y, Hamanaka I, Shimizu H. Effect of thermal shock on mechanical properties of injection-molded thermoplastic denture base resins. Acta Odontol Scand 2012;70:297-302. [CrossRef]
45. Xie C, Han Y, Zhao XY, Wang ZY, He HM. Microtensile bond strength of one- and two-step self-etching adhesives on sclerotic dentin: the effects of thermocycling. Oper Dent 2010;35:547-55. [CrossRef]
46. Menard KP. Dynamic Mechanical Analysis: A Practical Introduction. 2nd Ed., Boca Raton: CRC Press 1999, p.1-14.
47. Menard KP. Dynamic Mechanical Analysis Basics: Part 2 Thermoplastic Transitions and Properties: Application note. Perkin Elmer Inc 2007.
48. Vojdani M, Giti R. Polyamide as a denture base material: A literature review. J Dent Shiraz Univ Med Sci 2015;16:1-9.
49. http://www.dl.com.tr/downloads/msds/deflex/DEFLEX_MSDS.pdf.
50. https://patentimages.storage.googleapis.com/15/c7/2a/9ba9e36e51977c/US5266655.pdf.

eor.istanbul.edu.tr

Official Publication of Istanbul University Faculty of Dentistry

Eur Oral Res 2021; 55(3): 124-132

Original research

Perceptions of dental students towards online education during the COVID-19 pandemic

Ayca Sarıalioglu Gungor[1], **Yesim Sesen Uslu**[2], **Nazmiye Donmez**[1]

Purpose
This study evaluated the usage habits, attitudes, and perceptions of undergraduate dental students toward distance (online) learning and identified variables related to those attitudes.

Materials and Methods
The study included 1,605 undergraduate dental students who participated voluntarily. The data collection tool consisted of a distance learning attitude scale, a questionnaire on personal information, and open-ended questions. The perceptions of dental students to distance education according to the year and type of dental school they attended were evaluated.

Results
Most students expressed that distance learning in dental courses was not as effective as traditional face-to-face education (59.1%, n=949). While students studying at state universities had a more negative view of distance education, the satisfaction scores of the first-year students were found to be significantly lower than the other students ($p < 0.05$).

Conclusion
Dental students were generally unhappy with the interruption of traditional education caused by COVID-19 and having to continue their education online. However, under the circumstances, they saw it as an advantage allowing them to continue their education and avoid a complete suspension.

Keywords: *COVID-19, survey, distance education, online education, undergraduate student*

Introduction

Distance education is a teaching method transmitted via certain centers and depends on individuals' self-learning goals, educational content, and tools specially designed for learners in myriad environments. Physically, it is a planned form of teaching in which students do not need to be in a specific place; students and teachers keep in touch synchronously or asynchronously via communication technologies with the help of the internet environment (1). Synchronic or asynchronous technologies for online education include websites, podcasts, mobile applications, blogs, discussion boards, internet forums, interactive online tutorials, video conference technology, and virtual learning management systems (2). In distance education, each student learns at their own pace with quick and easy access to the materials. It affords the opportunity to acquire an education and eliminates many expenses, such as transportation and accommodations at a learning institution (1). Another advantage of distance learning is that it can provide students with "easier and more effective access to a wider variety and greater quantity of information" (3). The jus-

ORCID IDs of the authors: A.S.G. 0000-0002-8779-2949; Y.Ş.U. 0000-0001-9601-7410; N.D. 0000-0002-5101-6155

[1]*Department of Restorative Dentistry, Faculty of Dentistry, Bezmialem Vakif University, Istanbul, Turkey*

[2]*Department of Restorative Dentistry, Faculty of Dentistry, Istanbul Okan University, Istanbul, Turkey*

Corresponding Author: Ayca Sarıalioglu Gungor

E-mail: aycagungor83@hotmail.com

Received: 27 January 2021
Revised: 7 March 2021
Accepted: 24 March 2021

DOI: 10.26650/eor.2021869185

How to cite: *Sarialioglu Gungor A, Sesen Uslu Y, Donmez N. Perceptions of dental students towards online education during the COVID-19 pandemic. Eur Oral Res 2021; 55(3): 124-132.*

tification presented by Mattheos *et al*. (4) that information technologies (e-learning, distance learning, simulations, computer-based assessment) are compatible with dental education is that dental technology incorporates hardware and software applications. Currently, information technologies are being used in patient management software, digital X-ray, and so forth in dental practice (5).

Generation Z is classified as the people who were born in the mid-1990s and growing up in the early 2000s. They are mainly characterized by their addiction to computers and other technologies in general. Today's dental school students, part of the Z generation, have been growing up with the Internet, cell phones, laptops, iPads, tablets, and other electronic devices, which became part of their daily lives (6). Online learning is the education they expect to receive, but this does not mean that they are expected to receive it through mobile devices and distance education (7). It is important to understand their perspectives, attitudes, and opinions on distance education.

When the World Health Organization (WHO) declaring COVID-19 as a pandemic in March 2020, the situation was carefully monitored in our country. In Turkey, the first COVID-19 case was identified on March 11, 2020; consequently, primary, secondary, and higher education were suspended on March 16, 2020. Turkey's Council of Higher Education (CoHE) announced on March 23, 2020, that online education would be at the forefront of education due to the pandemic. This extraordinary experience led individuals unable to take advantage of the unexpected upheaval in the education system to seek alternative forms of education. Many teachers and students had to adapt to this system, called the "education system of the future," which they had not experienced before.

As far as we know, while there are many studies in the literature on distance education, there is a shortage of information about the effectiveness of distance education in dentistry. Therefore, this cross-sectional study aimed to evaluate the effectiveness of distance education from dental students' perspectives to emphasize the areas of weaknesses and strengths within the dental schools of Turkey. It was presumed that this information might be helpful to advance distance (online) education for undergraduates across the whole country. The null hypothesis was that online distance education is perceived as effective as the traditional methods of learning in dental schools.

Materials and Methods

Ethical statement

This project has been reviewed and approved by the Ethical Committee of Bezmialem Vakif University (06/108) on May 5, 2020.

Sample size estimation and post-hoc power

The minimum number of participants required was determined by an a priori power analysis using the software package, GPower 3.1. In order to detect an effect size of Cohen's d = 0.5 with 95% power (alpha = 0.05, two-tailed), GPower suggests we would need 210 participants in an independent samples t-test. However, 1,605 students who answered the web-based survey completely during the survey application period were included to increase the reliability of the findings. The post hoc power analysis revealed the statistical power for this study was 1.00 (%100).

Study population

Currently, there are 65 dental schools at 13 private and 52 state universities in Turkey. However, at the time of the survey, only 33 dental schools continued their learning activities with distance education. An invitation was sent to the associate deans for student affairs of the dental schools. Of the 33 dental schools, 13 (8 state universities, 5 foundation universities) agreed to participate in the survey. In Turkey, the dental education curriculum starts with theoretical education and continues with a clinical program in the third year's spring semester until graduation. As determined by the CoHE, practical classroom instruction was suspended, and the recommendation to teach theoretical lessons using digital platforms in the format of distance learning was promptly followed by dental schools during the spring semester. The survey was launched at the time we experienced the first peak period of the pandemic in Turkey. Undergraduate students in dental schools had completed their theoretical courses with distance (online) education activities during the spring semester. Between May 10 and 20, 2020, questionnaires were distributed to all first, second, third, fourth-, and fifth-year undergraduate students in dental schools who had access to their dental theoretical training from home.

Study design and data collection

An observational, cross-sectional survey design was used to collect information at a given time (8). The data collection tool consisted of three parts: a questionnaire on students' information, a distance education attitude scale, and open-ended questions. Personal information questions were prepared by the researchers based on previous research in the literature and contained 16 questions regarding the respondents' demographic information (gender, type of university, and current year) and mobile technology usage habits (availability of electronic devices, time spent using the internet, etc.) in general (7, 9, 10).

Distance education attitude scale

This scale was developed by Çelik (11) as a five-point, Likert-type scoring instrument consisting of 21 items. The higher the score, the more positive the respondent's attitude is toward distance education. Factor analysis was applied to determine the sub-dimensions of the "attitude scale" within the scope of the Validity-Reliability. Cronbach's alpha coefficients were calculated within the scope of reliability analysis of the scale questions. The statistical significance level (α) was taken as 5% in calculations. Cronbach's Alpha value for "attitude scale questions" was found to be 0.881 (88.1%) (11). Accordingly, the Cronbach's Alpha value of these scale questions exceeding 75% indicates that the reliability of these questions (items) is high. The five optional responses

for each question include: 5 = *strongly agree*, 4 = *agree*, 3 = *undecided*, 2 = *disagree*, and 1 = *strongly disagree*.

Opinions of distance education

This part of the questionnaire consists of three open-ended questions that the researchers of this study generated. While choosing a response was not obligatory, the aim was to gather the students' general views about distance learning (What are the positive aspects of distance education? What are the negative aspects of distance education? What are your suggestions for improvement of distance education?)

Respondents voluntarily consented to take part in the questionnaire and were assured that their answers would be recorded and stored anonymously. They were provided a standardized informed-consent document defining the aim of the study, and the questions were transferred to Google Forms (Google Inc., Mountain View, CA, USA). A web link to the questionnaire was sent by e-mail to full-time lecturers at 23 dental schools to act as contacts, and announcements were disseminated through student communication groups, especially via WhatsApp (WhatsApp Inc., Mountain View, CA, USA) and via student notice boards located in the faculty. The contact people distributed the web link to students willing to participate. A consent form was on the first page of the online questionnaire, which provided prospective participants more detail about the study and obtained their consent to participate. Data collected were stored in a secure database only accessible to the researchers. The data collection process lasted for 10 days. In order to increase participation in the study, announcements were repeated several times during the data collection period. Incomplete questionnaires were excluded to preserve the accuracy of the analysis.

Statistical analysis

Statistical analyzes were performed using Statistical Package for the Social Sciences (SPSS) 22.0 (IBM Corp., Armonk, NY, USA). While continuous variables were presented as median, categorical variables were described in frequencies and percentages. The Shapiro–Wilk normality test was used to determine the normal distribution; as the data were not normally distributed, minus the descriptive statistical methods (mean, standard deviation, frequency), the Kruskal–Wallis test was conducted to compare the parameters between the year of school, and Dunn's test was used to determine the group that caused the difference. The Mann–Whitney U test was used for comparisons between state and private universities. A value of $p < 0.05$ was considered statistically significant. In order to handle any missing data, the direct deletion method was used. All surveys with invalid data were discarded from further analysis, and statistical analysis was conducted on the basis of a complete dataset.

Results

Personal information

The study was conducted with the participation of 1,605 dental students. The demographic data showed a gender distribution of 1,048 females (65.3%) and 557 males (34.7%). Of the participants, 62.5% (n = 1,003) were studying at state universities and 37.5% (n = 602) at private universities; 16.8% (n = 269) were in their first year, 21.6% (n = 346) were in their second, 27.4% (n = 440) were in their third, 20.8% (n = 334) were in their fourth, and 13.5% (n = 216) were in their fifth year. The distribution of demographic characteristics of the students is shown in Table 1.

Distance education attitude scale

Table 2 shows the distribution of students' answers on the distance education attitude scale. Unsurprisingly, the vast majority prefer face-to-face learning over online distance education when the technological conditions at home are at a level adequate to meet distance education standards. The responses of undergraduate dental students on the distance education attitude scale in relation to the type of dental school attended are shown in Figure 1. Statistically significant differences were found between state and private universities on questions 17, 23, 24, 25, 26, 27, 29, 30, 31, 32, 33, 36, and 37 *($p < 0.05$)*.

The responses of undergraduate dental students on the distance education attitude scale according to the year of the dental school are shown in Table 3. There were statistically significant differences in all questions except questions 19, 22, 30, and 31 *($p < 0.05$)*.

Opinions of distance education

Students were asked to specify the positive and negative aspects of distance education and any suggestions for improvement of the system. Table 4 shows representative comments given. Suggestions for improving distance education can be summarized as follows: use videos or live demonstrations instead of PowerPoint slides to present information in a more understandable way; make the lectures interactive and synchronous; limit the duration of the lectures to 30 minutes; and provide training on the system used by the lecturers (each demonstrating a standard lecture).

Discussion

This investigation revealed a negative attitude toward online education among different dental school students during the COVID-19 pandemic in Turkey. According to the results of our study, our null hypothesis was rejected. While before the pandemic, most students (67.2%, n = 1,079) spent 1–3 hours daily on the internet, this period increased to 4–6

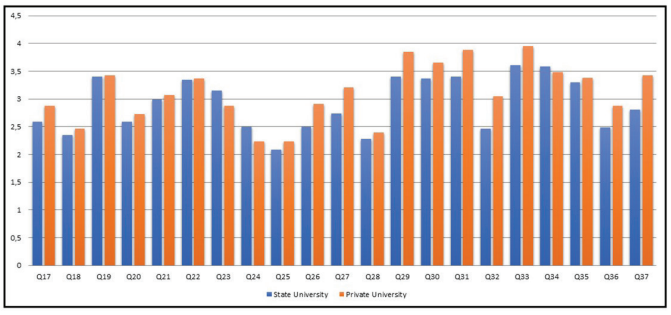

Figure 1. *Responses of undergraduate dental students to distance education attitude scale in relation to the type of dental school attended (Mann Whitney U Test, *p<0.05).*

Table 1. *Personal information of dental students.*

		n	%
Gender (Q1)	Female	1048	65.3
	Male	557	34.7
University (Q2)	State university	1003	62.5
	Private university	602	37.5
Year of the school (Q3)	1st year	269	16.8
	2nd year	346	21.6
	3rd year	440	27.4
	4th year	334	20.8
	5th year	216	13.5
	Total	1605	100
Connection to online courses (Q4)	Mobile phone	510	31.8
	Tablet computer/ Ipad	329	20.5
	Laptop	766	47.7
Internet speed at home (Q5)	1-4 Mbps	196	12.2
	5-8 Mbps	361	22.5
	9-16 Mbps	544	33.9
	16+ Mbps	504	31.4
Number of computers at home (Q6)	None	9	0.6
	1	830	51.7
	2	479	29.8
	3	222	13.8
	4	43	2.7
	5 or more	22	1.4
Do you have a tablet computer at home? (Q7)	Yes	715	44.5
	No	890	55.5
Do you have your own study room? (Q8)	Yes	1197	74.6
	No	408	25.4
Does your mobile phone have an internet package? (Q9)	Yes	1497	93.3
	No	108	6.7
Internet package limit of mobile phone (GB) (Q10)	2 GB	177	11
	4 GB	294	18.3
	6 GB	400	24.9
	10+ GB	734	45.8
Nowadays, how many hours a day do you spend time on the screen? (Q11)	None	20	1.2
	1-3	204	12.7
	4-6	666	41.5
	7-10	539	33.6
	More than 10 hours	176	11
Before this period, how many hours a day did you spend time on the screen? (Q12)	None	148	9.2
	1-3	1079	67.2
	4-6	320	19.9
	7-10	39	2.4
	More than 10 hours	19	1.2

Table 1. *Continue.*

		n	%
Which program does your university use for online (distance) education? (Q13)	Zoom	77	4.8
	Perculus	179	11.2
	Adobe Connect	539	33.6
	Google Meet	147	9.2
	Blackboard Learn	176	11
	Microsoft Teams	270	16.8
	No online course	66	4.1
	Other	151	9.4
How many minutes should online lessons be? (Q14)	20-30	539	33.6
	30-40	622	38.8
	40-50	305	19
	50-60	113	7
	More than 1 hour	26	1.6
Scoring of the tool used to follow online lessons (Q15)	1	127	7.9
	2	76	4.7
	3	92	5.7
	4	118	7.4
	5	196	12.2
	6	189	11.8
	7	308	19.2
	8	245	15.3
	9	105	6.5
	10	149	9.3
The suitability of the environment where the lectures are listened and studied for distance education (Q16)	Not suitable	187	11.6
	Suitable	1418	88.4

hours (41.5%, n=666) during the pandemic. Similarly, a study conducted in Wuhan showed that people spent excessive time on social media during the pandemic (12). The fact that individuals stay home due to social isolation increased internet usage. In this period, more time than expected was spent online; individuals began experiencing excessive anxiety (uneasiness, anger, aggression, etc.) when they were not using the Internet. After school closures, the transfer of education to digital media and confining social life to the home have greatly increased the use of the internet and social media. Clearly, the use of smart devices increased compared to the pre-pandemic period.

Given the rapid spread of COVID-19 and the increase in countries imposing movement restrictions, spending more time in our daily lives at home requires more data use for work and entertainment. This has had a significant impact on the telecom industry. Although there are risks of encountering internet connection problems, there has been an increasing interest in distance education and teleconferencing applications. The demand for software and social media platforms, such as Google Hangouts (Google Inc., Mountain View, CA, USA), WhatsApp video calls, Zoom (Zoom Video Communications, Inc., San Jose, CA, USA), and Microsoft

Table 2. *Distribution of responses to distance education attitude scale.*

	Strongly disagree	Disagree	Undecided	Agree	Strongly agree
I think distance education is a good education model (Q17)	443 (%27.6)	294 (%18.3)	372 (%23.2)	302 (%18.8)	194 (%12.1)
I find distance education more efficient than face-to-face learning (Q18)	650 (%40.5)	299 (%18.6)	250 (%15.6)	190 (%11.8)	216 (%13.5)
I believe that distance education is only an education study that cannot be done in class and / or to support it (Q19)	224 (%14)	232 (%14.5)	282 (%17.6)	398 (%24.8)	469 (%29.2)
Distance education is the education model of the future (Q20)	560 (%34.9)	256 (%16)	263 (%16.4)	252 (%15.7)	274 (%17.1)
In distance education, students learn more independently (compared to face-to-face learning) (Q21)	351 (%21.9)	218 (%13.6)	387 (%24.1)	346 (%21.6)	303 (%18.9)
I prefer online (simultaneous) distance education compared to offline distance education (Q22)	272 (%16.9)	214 (%13.3)	311 (%19.4)	290 (%18.1)	518 (%32.3)
I don't think I learned anything through distance education (Q23)	351 (%21.9)	210 (%13.1)	431 (%26.9)	232 (%14.5)	381 (%23.7)
I find distance education unnecessary (Q24)	577 (%36)	353 (%22)	342 (%21.3)	119 (%7.4)	214 (%13.3)
I work harder in the distance education period than before (Q25)	733 (%45.7)	317 (%19.8)	290 (%18.1)	130 (%8.1)	135 (%8.4)
I can focus on distance education courses (Q26)	449 (%28)	342 (%21.3)	333 (%20.7)	278 (%17.3)	203 (%12.6)
I am satisfied with distance education courses (Q27)	356 (%22.2)	261 (%16.3)	383 (%23.9)	372 (%23.2)	233 (%14.5)
I prefer distance education to face-to-face learning (Q28)	731 (%45.5)	263 (%16.4)	209 (%13)	166 (%10.3)	236 (%14.7)
I have the necessary knowledge infrastructure to follow distance education courses (Q29)	144 (%9)	168 (%10.5)	358 (%22.3)	494 (%30.8)	441 (%27.5)
Whether or not there is an infrastructure for distance education will be an effective criterion in future school preferences (Q30)	178 (%11.1)	173 (%10.8)	372 (%23.2)	473 (%29.5)	409 (%25.5)
Our technological conditions at home are sufficient for conducting distance education activities (Q31)	175 (%10.9)	188 (%11.7)	293 (%18.3)	425 (%26.5)	524 (%32.6)
My university provides all the support we need during the distance education process (Q32)	394 (%24.5)	339 (%21.1)	426 (%26.5)	273 (%17)	173 (%10.8)
I think it is an important advantage that I can receive courses on the computer when necessary (Q33)	121 (%7.5)	144 (%9)	327 (%20.4)	461 (%28.7)	552 (%34.4)
I think a lot is expected from students in the distance education process (Q34)	118 (%7.4)	221 (%13.8)	438 (%27.3)	335 (%20.9)	493 (%30.7)
The success of distance education models depends on the teacher (Q35)	107 (%6.7)	226 (%14.1)	562 (%35)	455 (%28.3)	255 (%15.9)
Instructors can motivate me for distance education courses (Q36)	375 (%23.4)	352 (%21.9)	484 (%30.2)	271 (%16.9)	123 (%7.7)
I think the instructors successfully run the distance education process (Q37)	241 (%15)	266 (%16.6)	481 (%30)	423 (%26.4)	194 (%12.1)

Teams (Microsoft Corporation, Redmond, Washington), has increased accordingly (13-19). Distance education as much as possible continued uninterruptedly in universities in which face-to-face education was interrupted by the pandemic. The top three (and most frequently used) internet communication platforms by dental schools for online education in Turkey were Adobe Connect (Adobe Inc., CA, USA; 33.6%), Microsoft Teams (16.8%), and Perculus (Advancity,

Table 3. *Responses to distance education attitude scale according to the year of the school. Kruskal Wallis Test *p<0.05.*

	1st year	2nd year	3rd year	4th year	5th year	
	Mean±SD (median)	Mean±SD (median)	Mean±SD (median)	Mean±SD (median)	Mean±SD (median)	p
Q17	2.08±1.26 (2)	2.55±1.31 (2)	2.85±1.38 (3)	2.93±1.3 (3)	3±1.4 (3)	0,000*
Q18	1.84±1.25 (1)	2.25±1.42 (2)	2.52±1.48 (2)	2.66±1.45 (2.5)	2.62±1.45 (2)	0,000*
Q19	3.49±1.41 (4)	3.51±1.4 (4)	3.4±1.4 (4)	3.24±1.39 (3)	3.42±1.35 (4)	0.069
Q20	2.23±1.43 (2)	2.41±1.43 (2)	2.78±1.52 (3)	2.91±1.51 (3)	2.83±1.53 (3)	0.000*
Q21	2.79±1.46 (3)	2.9±1.41 (3)	3.09±1.37 (3)	3.24±1.38 (3)	3.02±1.42 (3)	0.001*
Q22	3.2±1.53 (3)	3.43±1.47 (4)	3.35±1.47 (4)	3.32±1.44 (3)	3.47±1.42 (4)	0.276
Q23	3.51±1.39 (4)	3.19±1.44 (3)	2.89±1.45 (3)	2.88±1.46 (3)	2.86±1.36 (3)	0.000*
Q24	2.84±1.49 (3)	2.54±1.4 (2)	2.26±1.32 (2)	2.2±1.3 (2)	2.24±1.32 (2)	0.000*
Q25	1.81±1.22 (1)	2.13±1.35 (2)	2.17±1.31 (2)	2.3±1.31 (2)	2.24±1.26 (2)	0.000*
Q26	2.18±1.3 (2)	2.53±1.36 (2)	2.71±1.4 (3)	2.87±1.33 (3)	3±1.35 (3)	0.000*
Q27	2.26±1.25 (2)	2.8±1.34 (3)	3.05±1.39 (3)	3.22±1.28 (3)	3.18±1.31 (3)	0.000*
Q28	1.83±1.3 (1)	2.2±1.45 (1.5)	2.41±1.52 (2)	2.57±1.5 (2)	2.57±1.54 (2)	0.000*
Q29	3.15±1.36 (3)	3.52±1.3 (4)	3.57±1.21 (4)	3.76±1.1 (4)	3.9±1.13 (4)	0.000*
Q30	3.5±1.31 (4)	3.6±1.25 (4)	3.4±1.29 (4)	3.44±1.27 (4)	3.46±1.28 (4)	0.278
Q31	3.42±1.44 (4)	3.62±1.36 (4)	3.6±1.34 (4)	3.56±1.27 (4)	3.74±1.24 (4)	0.214
Q32	2.39±1.23 (2)	2.61±1.39 (3)	2.76±1.28 (3)	2.73±1.24 (3)	2.93±1.31 (3)	0.000*
Q33	3.41±1.32 (3)	3.61±1.26 (4)	3.78±1.22 (4)	3.93±1.16 (4)	3.96±1.1 (4)	0.000*
Q34	3.8±1.28 (4)	3.75±1.19 (4)	3.49±1.27 (4)	3.47±1.24 (3)	3.07±1.19 (3)	0.000*
Q35	3.12±1.08 (3)	3.27±1.13 (3)	3.43±1.07 (3)	3.4±1.1 (3)	3.33±1.14 (3)	0.002*
Q36	2.17±1.17 (2)	2.45±1.21 (2)	2.76±1.21 (3)	2.84±1.18 (3)	2.94±1.21 (3)	0.000*
Q37	2.67±1.19 (3)	2.95±1.28 (3)	3.15±1.19 (3)	3.13±1.18 (3)	3.29±1.26 (3)	0.000*

Istanbul, Turkey; 11.2%). As an alternative to face-to-face training interrupted by COVID-19, Yuen (20) and Telli (21) reported that many software programs are used in all universities around the world for e-learning, such as Zoom. Mobile learning is a growing trend in dental education, as students prefer smartphones and iPad/computer tablets. The ratio of students connecting to online courses with a cell phone (31.8%) was lower than that for connecting with a laptop (47.7%), although nearly all the students (93.3%) had (mobile) internet data packages. Mobile learning has also been used to access lessons, as there is no computer or laptop at home. Or it may have shifted from having a single computer as a work or learning environment for more than one person at home to mobile learning. The size of the cell phone is much more convenient for students than laptops, but the screen view is considerably reduced. This may have influenced their preference for computers with better image and sound qualities when participating in online courses. Smartphones and laptops were shown to be popular devices in our study and in a study by Khatoon (7). Another study showed strong interest in using the iPad/tablet for future coursework (22).

While coronavirus affects many areas of life, it has caused universities to complete the current year with distance education. Distance education is generally considered the best solution during the current pandemic; however, the lack of internet and computers is one of the most significant problems faced by students (23). In Turkey, roughly 7.5 million students are studying at the undergraduate level in 207 higher education institutions, with 28,941 students enrolled in dental schools (CoHE Information Management System). According to data from the CoHE, 123 of the 207 universities in Turkey have a Distance Education Application and Research Center. While some universities prepared their programs and started distance education on March 23, others are switching to this education system. There is no single method of distance education for universities at present under these extraordinary conditions. Universities use different methods according to available infrastructure and the number of students; some provide simultaneous distance education while others share the course content they have uploaded to the system. However, these methods cover only theoretical lessons. For courses that require practice, the intention is to offer compensation courses in the summer months after the outbreak ends. (24) As dental education requires intensive clinical practice apart from serious theoretical education, this deficiency is causing inevitable anxiety among students (25). In our study, most dental students (45.9%, $n = 737$) expressed the opinion that the distance education model is not an adequate substitute and is not as effective as face-to-face education (59.1%, $n = 949$). The reason students think is possibly due to their views on dental education, which is comprised of both theoretical and practical components. Jordan *et al*. (13) likewise reported that interactive, standard didactic education is more effective than asynchronous online education. However, other studies in

Table 4. *Representative comments by students about positive and negative aspects of distance (online) education.*

Positive Comments	Negative Comments
I can concentrate much better since listening to lessons in the home environment is more comfortable than in the classroom.	My concentration deteriorates when there is a connection problem on the Internet
I don't have to wake up early to attend the courses	When the lessons are explained asynchronous, we cannot ask the sections that we do not understand because we cannot participate actively.
Less waste of time and less tired	If the lesson duration is longer than 30 minutes, my concentration deteriorates because I constantly look at the computer screen
Thanks to the course contents and videos uploaded to the system, I can open the lessons and listen again if something is stuck in my mind	Since some trainers do not have sufficient infrastructure about the system, there may be a waste of time in lecturing.
I find it very successful to plan and implement the Distance education system in a short time.	Only theoretical training can be done with online training. Our clinical training is incomplete

the literature report that dental students have generally positive attitudes toward online learning (26-30). Interpreting these responses as positive feedback would be a natural reaction due to the use of distance education as an emergency solution during the pandemic period. The reason for the difference between the two studies is that dental students require an adequate physical setting and psychomotor skills during their academic years, which cannot be replaced with distance learning, as is currently being conducted during the COVID-19 pandemic (31). Besides, in these studies, the online learning modules were integrated with face-to-face learning, while in the present study, learning was entirely performed via distance learning (i.e., full online). It was previously reported that full online learning leads to a lost sense of reality and learning mostly depends on the dental students' commitment to the courses (32).

Internet difficulties are among the most important problems faced by students thus far in the scope of distance education (33). It has been noted that students' internet quotas are insufficient, as video education requires downloading course materials from time to time. In this process, which became compulsory due to the COVID-19 pandemic, it became crucial to determine whether the infrastructure of each university, the content of the education, the ability of the educators to use this technology, and the students' access to the courses are compatible. Some foundation universities in Turkey have stated that they will contribute hardware or internet access to their students. However, because providing such support for students in public universities with their limited budgets may not be feasible, the perspectives of students in state universities may differ from those studying in private universities. Our findings revealed that dental students at state universities have more negative thoughts about distance education.

Although the technical operation of the system is unproblematic, the necessary interaction required during intensive theoretical lessons may be somewhat restricted. Although most participants (59.1%, n = 949) in our study reported having the technological infrastructure to carry out distance education courses in their homes, more than half (54%) expressed that distance education should be done only when in-class education cannot be provided or to support it. In this study, the preference for online learning was influenced by the year of study. Among students who preferred distance online learning, the percentage of senior students was significantly higher than the young students. The highest rate of negative feedback to the question "I think distance education is a good education model" was from first-year students. However, studies conducted by Sritongthaworn *et al*. (34) and Teo *et al.* (35) reported that younger students tend to adapt to e-learning. The reason may be that younger students have difficulty following and understanding online lessons because they have not yet acquired enough background knowledge in the field.

This study provides valuable data on undergraduate dental students in Turkey. However, there may be differences among the various schools in the country. Therefore, the authors are developing a country-wide study with more participants to examine differences between online and traditional curricula by assessing both undergraduate students' and educators' opinions to achieve more meaningful results. Given the fact that the pandemic may take longer, it is assumed that Augmented Reality/Virtual Reality technology will play a dominant role in the future development of dental education. An educational model can be developed to cover various teaching interests, including digital dental education, Web-based knowledge transfers and digital surface mapping, dental simulator motor skills including IOS, and specialized technologies such as digital radiography.

In this study, only the perspectives of students regarding online education were evaluated. Comparing students' opinions with those of educators will help fill in the gaps of a thorough evaluation of distance education, as it seems likely to continue in the future. Also, this investigation is limited to 23 dental schools and 1605 dental students, a broader sample including more dental schools with a greater number of dental students is desirable.

Conclusion

Within the limitations of this study, it can be concluded that distance (online) education is commonly used by dental schools during the pandemic period, and students' attitudes toward the use of distance learning are not positive.

Türkçe Özet: *Diş hekimliği öğrencilerinin Covid-19 pandemisi sürecinde çevrimiçi eğitim ile öğrenmeye ilişkin algı durumları Amaç: Bu çalışmanın amacı, dişhekimliği lisans öğrencilerinin uzaktan (çevrimiçi) öğrenmeye yönelik kullanım alışkanlıklarını, tutumlarını ve algılarını değerlendirmek ve bu tutumlarla ilgili değişkenleri belirlemektir. Gereç*

ve Yöntem: Çalışmaya gönüllü olarak katılan 1,605 dişhekimliği lisans öğrencisi dahil edildi. Veri toplama aracı olarak uzaktan öğrenme tutum ölçeği, kişisel bilgilerle ilgili bir anket ve açık uçlu sorulardan oluşturuldu. Dişhekimliği öğrencilerinin devam ettikleri yıllara ve dişhekimliği fakültesinin türüne göre uzaktan eğitime yönelik algıları değerlendirildi. Bulgular: Öğrencilerin çoğu tarafından, dişhekimliği derslerinde uzaktan eğitimin geleneksel yüz yüze eğitim kadar etkili olmadığı ifade edildi. (% 59.1, n = 949). Devlet üniversitelerinde okuyan öğrenciler uzaktan eğitime daha olumsuz bakarken, birinci sınıf öğrencilerinin memnuniyet puanları diğer öğrencilere göre anlamlı derecede düşük bulundu (p <0.05). Sonuç: Dişhekimliği öğrencileri, COVİD-19 pandemisine bağlı olarak geleneksel eğitimin kesintiye uğramasından ve eğitimlerine çevrimiçi olarak devam etmek zorunda kalmaktan genel olarak memnun değillerdi. Ancak, bu koşullar altında çevrimiçi eğitimi, eğitimin tamamen askıya alınmadan devam etmesine imkan sağlayan bir avantaj olarak gördüler. Anahtar Kelimeler: COVID-19, anket, uzaktan eğitim, çevrimiçi eğitim, lisans öğrencisi

Ethics Committee Approval: This project has been reviewed and approved by the Ethical Committee of Bezmialem Vakif University (06/108) on May 5, 2020.

Informed Consent: Participants provided informed constent.

Peer-review: Externally peer-reviewed.

Author contributions: ASG participated in designing the study. ASG, YSU, ND participated in generating the data for the study. ASG, YSU participated in gathering the data for the study. ASG, YSU, ND participated in the analysis of the data. ASG wrote the majority of the original draft of the paper. ASG, YSU, ND participated in writing the paper. ASG has had access to all of the raw data of the study. ASG has reviewed the pertinent raw data on which the results and conclusions of this study are based. ASG, YSU, ND have approved the final version of this paper. ASG guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: The authors declared no conflict of interest.

Financial Disclosure: The autzhors declared that they have received no financial support.

References

1. McCutcheon LRM, Alzghari SK, Lee YR, Long WG, Marquez R. Interprofessional education and distance education: A review and appraisal of the current literature. Curr Pharm Teach Learn 2017;9:729-36. [CrossRef]
2. Asarbakhsh M, Sandars J. E-learning: the essential usability perspective. Clin Teach 2013;10:47-50. [CrossRef]
3. Mooney GA, Bligh JG. Information technology in medical education: current and future applications. Postgrad Med J 1997;73:701-4. [CrossRef]
4. Mattheos N, Stefanovic N, Apse P, Attstrom R, Buchanan J, Brown P, *et al.* Potential of information technology in dental education. Eur J Dent Educ 2008;12 Suppl 1:85-92.
5. A. Suner YY, B. Piskin. Mobile learning in dentistry: usage habits, attitudes and perceptions of undergraduate students,. PEERJ 2019;7391.
6. Constantinescu-Dobra A, Maier V. Generation Z and Online Dentistry. An Exploratory Survey on the Romanian Market 2017. p. 291-6.
7. Khatoon B, Hill KB, Walmsley AD. Dental students' uptake of mobile technologies. Br Dent J 2014;216:669-73. [CrossRef]
8. Fraenkel J WN, Hyun H. How to design and evaluate research in education New York: McGraw-Hill Education; 2014.
9. S Deshpande AK, J Chahande. Perceptions of faculty and students regarding use of mobile apps for learning in dentistry: A questionnaire based study Journal of Education Technology in Health Sciences 2016;3:128-30.
10. Rung A, Warnke F, Mattheos N. Investigating the use of smartphones for learning purposes by Australian dental students. JMIR Mhealth Uhealth 2014;2:e20.
11. Çelik A. M-learning attitude scale: validity and reliability analyses. Journal of Research in Education and Teaching 2013;2:172-85.
12. Ni MY, Yang L, Leung CMC, Li N, Yao XI, Wang Y, *et al.* Mental Health, Risk Factors, and Social Media Use During the COVID-19 Epidemic and Cordon Sanitaire Among the Community and Health Professionals in Wuhan, China: Cross-Sectional Survey. JMIR Ment Health 2020;7:e19009.
13. Jordan J, Jalali A, Clarke S, Dyne P, Spector T, Coates W. Asynchronous vs didactic education: it's too early to throw in the towel on tradition. BMC Med Educ 2013;13:105. [CrossRef]
14. Halpin PA, Lockwood MKK. The use of Twitter and Zoom videoconferencing in healthcare professions seminar course benefits students at a commuter college. Adv Physiol Educ 2019;43:246-9. [CrossRef]
15. Martin N, Lazalde OM, Stokes C, Romano D. An evaluation of remote communication versus face-to-face in clinical dental education. Br Dent J 2012;212:277-82. [CrossRef]
16. Poblete P, Nieto E. Does time matter? WhatsApp vs electronic mail for dental education. A pilot study. Eur J Dent Educ 2020;24:121-5. [CrossRef]
17. Das R, Manaktala N, Bhatia T, Agarwal S, Natarajan S, Lewis AJ, *et al*. Efficiency of Mobile Video Sharing Application (WhatsApp(R)) in Live Field Image Transmission for Telepathology. J Med Syst 2020;44:109. [CrossRef]
18. Spallek H, Turner SP, Donate-Bartfield E, Chambers D, McAndrew M, Zarkowski P, *et al*. Social Media in the Dental School Environment, Part A: Benefits, Challenges, and Recommendations for Use. J Dent Educ 2015;79:1140-52. [CrossRef]
19. Alshiekhly U, Arrar R, Barngkgei I, Dashash M. Facebook as a learning environment for teaching medical emergencies in dental practice. Educ Health (Abingdon) 2015;28:176-80. [CrossRef]
20. Yuen J, Xie F. Medical education during the COVID-19 pandemic: perspectives from UK trainees. Postgrad Med J 2020;96:432-3. [CrossRef]
21. Telli Yamamoto G AD. Coronavirüs ve Çevrimiçi (Online) Eğitimin Önlenemeyen Yükselişi. Journal of University Research 2020;3:25-34.
22. Gosper M, Malfroy J, McKenzie J. Students' experiences and expectations of technologies: An Australian study designed to inform planning and development decisions. Australasian Journal of Educational Technology 2013;29:268-82. [CrossRef]
23. Saeed SG, Bain J, Khoo E, Siqueira WL. COVID-19: Finding silver linings for dental education. J Dent Educ 2020.
24. Machado RA, Bonan PRF, Perez D, Martelli JH. COVID-19 pandemic and the impact on dental education: discussing current and future perspectives. Braz Oral Res 2020;34:e083.
25. Deery C. The COVID-19 pandemic: implications for dental education. Evid Based Dent 2020;21:46-7. [CrossRef]
26. Bains M, Reynolds PA, McDonald F, Sherriff M. Effectiveness and acceptability of face-to-face, blended and e-learning: a randomised trial of orthodontic undergraduates. Eur J Dent Educ 2011;15:110-7. [CrossRef]
27. Brumini G, Spalj S, Mavrinac M, Biocina-Lukenda D, Strujic M, Brumini M. Attitudes towards e-learning amongst dental students at the universities in Croatia. Eur J Dent Educ 2014;18:15-23. [CrossRef]
28. Ramlogan S, Raman V, Sweet J. A comparison of two forms of teaching instruction: video vs. live lecture for education in clinical periodontology. Eur J Dent Educ 2014;18:31-8. [CrossRef]
29. Schlenz MA, Schmidt A, Wostmann B, Kramer N, Schulz-Weidner N. Students' and lecturers' perspective on the implementation of online learning in dental education due to SARS-CoV-2 (COVID-19): a cross-sectional study. BMC Med Educ 2020;20:354. [CrossRef]

30. Wang K, Zhang L, Ye L. A nationwide survey of online teaching strategies in dental education in China. J Dent Educ 2021;85:128-34. [CrossRef]
31. Quinn B, Field J, Gorter R, Akota I, Manzanares MC, Paganelli C, *et al*. COVID-19: The immediate response of european academic dental institutions and future implications for dental education. Eur J Dent Educ 2020;24:811-4. [CrossRef]
32. Lo H-C. Utilizing Computer-mediated Communication Tools for Problem-based Learning. Educational Technology & Society 2009;12:205-13.
33. Pontual MLA, do Nascimento EHL, da Cruz Perez DE, Pontual AA, Ramos-Perez FM. Challenges in oral radiology teaching during COVID-19 pandemic. Dentomaxillofac Radiol 2020;49:20200178. [CrossRef]
34. Siritongthaworn S, Krairit D, Dimmitt N, Paul H. The study of e-learning technology implementation: A preliminary investigation of universities in Thailand. Education and Information Technologies 2006;11:137-60. [CrossRef]
35. Teo T, Luan W, Thammetar T, Chattiwat W. Assessing e-learning acceptance by university students in Thailand. Australasian Journal of Educational Technology 2011;27:1356-68. [CrossRef]

eor.istanbul.edu.tr

Official Publication of Istanbul University Faculty of Dentistry

Eur Oral Res 2021; 55(3): 133-138

Original research

The effects of Biofreeze and superficial heat on masticatory myofascial pain syndrome

Deniz Yaman[1], **Cansu Alpaslan**[2], **Oya Kalaycıoğlu**[3]

Purpose
This study aims to assess the influence of superficial heat and Biofreeze on pain, mouth opening (mm), and quality of life in patients with masticatory myofascial pain syndrome (MPS).

Materials and Methods
52 patients with MPS were included in the study. They were randomly divided into two groups. Patients in the Biofreeze group (n = 26) applied 3.5% menthol gel to the masseter and temporal muscles twice a day for seven days, while the other group applied superficial heat. Baseline, 7th, and 21st days of VAS, mouth opening (mm), and Oral Health Impact Profile-14 (OHIP-14) scores of the patients were evaluated statistically.

Results
The mouth opening increased by 4.27 ± 3.80 mm in the Biofreeze group and 2.58 ± 2.16 mm in the superficial heat group. In each group, a significant decrease in VAS and OHIP-14 scores was observed on the 7th day compared to the baseline values ($p<0.001$). There was no statistically significant difference between the two applications on myofascial pain, mouth opening (mm), and OHIP-14 total score variables. The favorable effects of both applications on these parameters were limited to the duration of use.

Conclusion
Biofreeze and superficial heat in MPS were found to increase the quality of life, but the limited effectiveness of these applications underlines the importance of the underlying factors.

Keywords: *Myofascial pain, myofascial pain syndromes, myofascial trigger point pain, menthol, referred pain*

Introduction

Masticatory myofascial pain syndrome (MPS) is characterized by the presence of trigger points that cause local and referred pain in associated structures, such as the teeth and temporomandibular joint (TMJ) (1). Previously, myofascial trigger points were detected in 55.4% of patients who presented to dental clinics for the treatment of chronic head and neck pain, highlighting the wide distribution of MPS (2). Clinically, the restriction of mandibular movements secondary to pain, facial asymmetry, difficulty speaking, vertigo, and tinnitus are common symptoms (3). Several factors, such as sudden muscle loading or chronic injury due to recurrent microtraumas, genetic factors, and stress, contribute to MPS development (4). Treatment strategies aim to relieve pain by inactivating trigger points, relieving local muscle spasms, normalizing the muscle length, and improving functional capacity (5). Local anesthetic/botulinum toxin injections, acupuncture, transcutaneous electrical nerve stimulation (TENS),

ORCID IDs of the authors: D.Y. 0000-0002-1492-6897; C.A. 0000-0003-2092-7842; O.K. 0000-0003-2183-7080

[1]*Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Bolu Abant Izzet Baysal University, Bolu, Turkey*

[2]*Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Gazi University, Ankara, Turkey*

[3]*Department of Biostatistics and Medical Informatics, Faculty of Medicine, Bolu Abant Izzet Baysal University, Bolu, Turkey*

Corresponding Author: Deniz Yaman

E-mail: yamand896@gmail.com

Received: 12 January 2021
Revised: 9 February 2021
Accepted: 24 March 2021

DOI: 10.26650/eor.2021858837

How to cite: *Yaman D, Alpaslan C, Kalaycioglu O. Effect of Biofreeze and superficial heat on masticatory myofascial pain syndrome. Eur Oral Res 2021; 55(3): 133-138.*

ultrasound, superficial heat, massage, cold compression applied to the trigger point, and pharmacological agents are frequently preferred treatment methods (5).

Superficial heat is widely used to increase blood pressure and tissue perfusion as well as to reduce neuropathic pain and joint stiffness (6). It can be applied at home using a variety of methods. This is one of the advantages of superficial heat, which makes it a commonly preferred treatment approach for MPS (6,7). However, the use of alternative agents to relieve pain has become increasingly popular (8,9). Menthol, one of the primary active ingredients in Biofreeze, has been shown to control pain through the stimulation of cold receptors within the opioid or glutamate systems (10). Unlike traditional cold applications, Biofreeze exerts cryotherapeutic effects by blocking sodium channels, which creates a cooling sensation without actively decreasing overall skin temperature, increasing the topical analgesic effects of menthol (11,12). Menthol reduces blood flow and causes vasoconstriction, similar to the outcome observed with ice application (13). However, the isopropyl alcohol and glycerin contained in Biofreeze have also been shown to exert vasodilation effects (14,15). Due to the contradictory nature of these substances, we aimed to investigate the effects of Biofreeze on MPS. To our best knowledge, no previous studies have explored the effects of superficial heat and Biofreeze on the outcomes of pain, mouth opening (measured in mm), and quality of life among patients with MPS. Therefore, we have tested the null hypothesis that no statistically significant difference would be found between Biofreeze and superficial heat in terms of inhibiting the pain associated with MPS by preventing the formation of trigger points. This study also aimed to determine the short and long-term effects of these two applications when used in patients with MPS.

Materials and Methods

Ethical statement

The research was approved by the Local Ethics Committee (No:2015/118), and all participants signed a consent form before the start of the study. The study was carried out compatible with the Declaration of Helsinki.

Sample size estimation

A priori power analysis was performed based on the repeated measures ANOVA with three-time points using the software G*power version 3.1.9.4 (18). A sample size of 52 was found to be adequate to detect a difference in terms of the effect size of f= 0.25 with 80% statistical power at $\alpha = 0.05$.

Patient selection

A total of 52 patients (45 females and 7 males) who were diagnosed with MPS and have no history of occlusal splint usage were included in the study. The patients were divided into 2 groups: the first group received Biofreeze (n=26) while the second one superficial heat treatment (n=26). The inclusion criteria were chronic unilateral pain longer than 3 months and referred pain distributed from myofascial trigger points in the masseter and temporalis muscles to the face, mouth, or TMJ. These inclusion criteria for MPS are consistent with the guidelines of the American Academy of Orofacial Pain and the Research Diagnostic Criteria for TMD (RDC/ TMD) (7). In clinical examination, any tooth or maxillofacial lesions that could be the source of the pain were excluded. Radiographic imaging of the temporomandibular joint was performed to rule out disc displacement, effusion, degenerative disorders. Patients with trigeminal neuralgia, head and neck inflammation, endocrine diseases, and those using oral contraceptive drugs, and those with skin lesions (scar tissue, skin graft) on the treatment area were excluded from the study. The presence of hard, palpable nodules in the masseter and temporalis muscles, indicating the active myofascial trigger points, was the common feature of all patients in this study. By bilateral manual palpation, pain in the muscles was recorded using the visual analog scale (VAS).

Administration of Biofreeze and superficial heat

The patients were divided into two groups randomly. In the Biofreeze group, the patients applied 3.5% menthol gel to the masseter and temporal muscles twice a day for seven days. Patients were informed about the correct application of Biofreeze gel and a marked applicator strip was given to each patient to apply with a standard dose. Patients were told to massage the area with circular movements for about 30 seconds to 1 minute to allow the Biofreeze gel to penetrate under the skin. The remaining half of the patients applied superficial heat to the painful muscle areas twice a day for seven days. In this study, superficial moist heat application was done with a towel soaked in hot water (212^0 F). In this group, each cycle was repeated for 10 minutes and paused for 5 minutes. After three 10-minute heat cycles, with two 5-minute pauses, the superficial heat application took 40 minutes. Since the adipose tissue on the face is not as dense as the other tissues of the body, 5-minute pauses were given to ensure tissue safety against the rapidly rising heat. No application was applied to all patients after the 7^{th} day.

Recording of the clinical data

VAS, mouth opening (mm), and Oral Health Impact Profile-14 (OHIP-14) scores of the patients were compared at baseline, 7^{th}, and 21^{st} days. The unassisted (mandibular) opening without pain and maximum assisted (mandibular) opening of the patients were measured using a millimeter ruler between the edges of the upper and lower middle incisors. The current study assessed the patients' functional limitation, physical pain, psychological discomfort, physical/psychological/social incapacity, and social disadvantage with the OHIP-14 and compare the effects of these two applications at baseline, 7^{th}, and 21^{st} days. The OHIP, originally consisting of 49 questions, uses the World Health Organization (WHO) International Classification of Impairments, Disabilities and Handicaps framework (16). However, OHIP-14 is a shorter, patient-friendly version and consists of 14 questions. The previously validated Turkish translation of the OHIP-14 scale was used in this study. This scale scored between "0" and "4". An increase in the OHIP-14 score indicates the severity of the current problem and a decline in quality of life (17).

Statistical analysis

Data was evaluated by using Statistical Package for Social Science (SPSS Statistics for Windows, Version 25.0 Armonk, NY: IBM Corp, USA). The effects of the two different applications on the unassisted (mandibular) opening without pain and maximum assisted (mandibular) opening, VAS, and OHIP-14 score over time were compared with two-way repeated-measures ANOVA. The differences between the time points for each treatment group were further evaluated using the one-way repeated measures ANOVA followed by Bonferroni post-hoc multiple comparison tests. The confidence interval was set to 95% and p values less than 0.05 were considered statistically significant.

Results

A total of 52 patients with MPS (86.5 % females, 13.5 % males; age range: 18–50 years; mean age: 36.75±10.47) were included in this study. There were no differences between treatment groups in terms of age and gender (Table 1). There were no statistically significant differences between Biofreeze and superficial heat group for all of the four parameters (VAS, OHIP-14, the unassisted (mandibular) opening without pain, and maximum assisted (mandibular) opening) (Table 2). However, the favorable effects of both applications on these four parameters continued only during the usage periods (p<0.001, observed power=1.000).

For each treatment group, a significant difference between the unassisted (mandibular) opening without pain, maximum assisted (mandibular) opening, VAS, and OHIP-14 scores, and usage periods was observed (Table 3).

Table 1. *Comparison of the two groups in terms of age and gender. t-test and Fisher's exact text; sd: standard deviation; p: population correlation coefficient; n: sample size.*

	Total (n=52)	Biofreeze (n=26)	Superficial heat (n=26)	p
Age, mean (±sd)	36.75±10.47	37.35±10.64	36.15±10.47	0.686
Gender, n (%)				
Male	7 (13.5)	24 (92.3)	21 (80.8)	0.419
Female	45 (86.5)	2 (7.7)	5 (19.2)	

On the 7th day, the unassisted (mandibular) opening without pain and maximum assisted (mandibular) opening were statistically significant compared to baseline (p<0.001). On the 21st day, the unassisted (mandibular) opening without pain, and maximum assisted (mandibular) opening significantly reduced compared to the 7th day after treatment, but they were not statistically significant from the baseline

Table 2. *Results of the two-way repeated-measures ANOVA for the unassisted (mandibular) opening without pain, maximum assisted (mandibular) opening, VAS, and OHIP-14 scores. VAS: visual analog scale; df: degrees of freedom; F: F value (the ratio of the model mean square to the error mean square); OHIP-14: The Oral Health Impact Profile-14; [a]Greenhouse-Geisser corrected p-values were used due to the violation of sphericity assumption; Bold p-value indicates statistically significant effect at α=0.05.*

	Type III Sum of Squares	df	Mean Square	F	p[a]	Observed Power
The unassisted (mandibular) opening without pain						
Treatment	1.853	1.000	1.853	0.025	0.875	0.053
Time	380.667	1.363	279.265	63.024	**<0.001**	1.000
Time× Treatment interaction	21.128	1.299	16.262	2.937	0.087	0.320
Maximum assisted (mandibular) opening						
Treatment	3.692	1.000	3.692	0.037	0.849	0.054
Time	251.551	1.496	168.181	34.219	**<0.001**	1.000
Time× Treatment interaction	35.808	1.443	24.821	4.206	0.054	0.563
VAS						
Treatment	20.103	1.000	20.103	1.578	0.221	0.227
Time	328.551	1.186	276.910	238.435	**<0.001**	1.000
Time× Treatment interaction	0.782	1.332	0.587	0.907	0.376	0.244
OHIP-14 Score						
Treatment	1.641	1.000	1.641	0.013	0.912	0.051
Time	9520.192	1.209	7874.080	51.146	**<0.001**	1.000
Time× Treatment interaction	222.936	1.514	147.282	5.024	**0.018**	0.812

Table 3*. Comparison of the long and short-term effects of Biofreeze and Superficial heat groups. VAS: visual analog scale; OHIP-14: The Oral Health Impact Profile-14; n: sample size; mean±SD[a,b]: Same superscript letters indicate a statistically significant difference between the periods based on the Bonferroni post-hoc tests.*

	Biofreeze (n=26)			Superficial heat (n=26)		
	Baseline	**7th day**	**21st day**	**Baseline**	**7th day**	**21st day**
The unassisted (mandibular) opening without pain	31.27±5.59a	35.54±5.49ab	31.65±4.95b	32.15±4.98a	34.73±4.99ab	32.23±4.95b
Maximum assisted (mandibular) opening	35.65±6.73a	39.65±6.61ab	36.35±6.06b	36.31±5.70a	38.04±5.52ab	36.38±5.68b
VAS	6.58±2.04a	3.65±1.65ab	6.62±2.04b	7.31±1.78a	4.19±1.36ab	7.50±1.86b
OHIP-14 Score	39.23±7.80a	30.35±7.40ab	51.50±9.17ab	41.88±9.53a	31.35±8.91ab	48.46±13.32ab

values for each treatment group. After 7 days, the mouth opening increased by 4.27 ± 3.80 mm in the Biofreeze group and 2.58 ± 2.16 mm in the superficial heat group. In each group, a significant reduction in VAS and OHIP-14 scores was observed on the 7th day compared to the baseline values ($p<0.001$). Additionally, the 21st day of VAS and OHIP-14 scores increased significantly compared to the 7th day ($p<0.001$).

Discussion

This study represents the influence of superficial heat and Biofreeze on myofascial pain, mouth opening (measured in mm), and oral health-related quality of life among patients with MPS. The prevalence of myofascial pain was predominantly identified in young women in the present study. Consistent with this result, myofascial pain has been most commonly reported among individuals aged 30–49 years and occurs twice as frequently in women as in men (19,20). Ligament laxity, subluxation, posture disorders, and psychic factors are considered to serve as predisposing factors for the development of MPS (2). However, Brennum *et al.* (21) have postulated that women are more sensitive to pain than men, although no correlation has been found between age and pain perception. Treatment recommendations for MPS range from rest, nonsteroidal anti-inflammatory drugs (NSAIDs), and oral splints to more aggressive and irreversible treatments (22-24). Occlusal splints can change the muscle activity patterns and the positioning of the temporomandibular joint by increasing the vertical dimension of the mouth (25). Hot-cold packs and jaw exercises are considered self-care therapy, and these approaches can reduce parafunctional jaw activities, relieve pain, and improve the range of motion by relaxing the muscles (26-28). Truelove *et al.* (29) stated that oral splint therapy did not provide any advantage over self-care therapy, such as thermal packs, stress reduction, NSAIDs, and jaw relaxation techniques. Therefore, in the present study, we aimed to evaluate the long-term occlusal splint needs of patients who used two basic treatment approaches. Therefore, only patients with MPS who did not use occlusal splints were included in this study.

Heat application, which is preferred for the treatment of myofascial pain, increases metabolism and facilitates circulation by dilating the blood vessels, resulting in increased catabolism, the excretion of lactic acid, and the removal of uric acid and other acidic waste products from muscle cells (30,31). Due to these mechanisms, heat application is thought to reduce fatigue and the signs of aging and produce an analgesic effect on the musculoskeletal system (31). The effects of heat application are not limited to the treatment of neck and back pain, with beneficial effects described for reducing knee pain, temporomandibular joint pain, and delaying exercise-related muscle pain (6,32,33). Chabal *et al.* (34) reported that the analgesic effects of a single one 30-minute thermal application lasted for 2 hours after the application was completed. The principles of thermodynamics suggest that moist heat is more effective than dry heat due to the interactions between molecules (34,35). For this reason, moist heat using a towel soaked in hot water was the preferred method used in our study, with the goal of reducing muscle tension and increasing the size of the mouth opening by providing flexibility to collagen structures, such as tendons, ligaments, joint capsules (6,36,37). A previous study reported that trigger points are inactivated for up to 72 hours by the application of only moist heat, without the need for additional treatment (38). In addition, heat wraps have been found to have more effective analgesic properties than ibuprofen or acetaminophen (39). In patients with acute temporomandibular disorder, the mouth opening was increased by 3.5 to 9 mm following the application of superficial moist heat (6). In our study, an increase of 2.58 ± 2.16 mm was found following superficial heat application.

The use of thermotherapy for the treatment of temporomandibular disorders and neuromuscular disorders has been described in previous studies, but few studies have addressed the amount and or changes in temperature used in the methodology (6,40-43). In these studies, the daily application dose and duration of the heat applications often vary (6,40-43). Previous studies have indicated that pain relief was achieved with a minimum of 20 minutes of heat application (6,40-43). Based on these results, a total of 30 minutes of superficial moist heat, applied once per day, was the preferred approach used in our study, and we found significant improvements in the size of the mouth opening among patients with myofascial pain. However, the patients' acceptance of heat- or cold-based treatments for pain relief may vary. Brandt reported that 60% of participants diagnosed with rheumatoid arthritis (RA) and osteoarthritis (OA) expressed a preference for heat-based treatments for their aching joints, whereas 20% expressed a preference for cold-based applications (44). Cold-based applications exert analgesic effects by increasing the pain threshold and suppressing inflammation (45).

The primary component of the Biofreeze application used in this study is menthol. When menthol is applied to the skin, a cooling effect is mediated through TRPM8 (transient receptor potential cation channel subfamily M [melastatin] member 8), which can prevent inflammatory pain (46,47). TRPM8, also referred to as the menthol receptor, has also been detected in gingiva and incisive papilla (46-48). The wide distribution of menthol receptors throughout oral and craniofacial structures can also result in the topical analgesic effect being mediated through interactions with the dense neural network that is embedded in the dermal-epidermal junction of the skin and mucosa (49,50). It acts as a dose-dependent skin vasodilator, acting through nitric oxide, RhoA/Rho-kinase, and endothelium-derived hyperpolarizing factor (EDHF)-based mechanisms, which can increase cutaneous blood flow (51,52). Increasing attention has been paid to the therapeutic efficacy of menthol for the treatment of neuropathic pain syndromes, which is accompanied by hyperalgesia and allodynia (53). The current study showed an increase in the mouth opening size among patients during the Biofreeze application.

Fibromyalgia (FM) and MPS are the most common types of chronic musculoskeletal pain, reported to affect 80% of the general population (54). Whereas MPS is a regional pain condition that can be managed using conservative interventions, FM is a more complex pain condition, often requiring a multidisciplinary treatment approach, in addition to conservative measures (55,56). The etiology of FB is unknown, whereas MPS has been linked to local injuries and repetitive microtraumas (54). FB is classified as a chronic form of myal-

gia and bilateral, generalized muscle tenderness that lasts at least 3 months (54). In MPS, pain can also persist for more than three months, originating from the trigger point and excessively irritable nodules that respond to palpation (54).

MPS affects the social functioning and physical/psychological health of the affected individual person and results in loss of workdays and the increased need for healthcare (57). Acute MPS is likely to be localized and can heal spontaneously or through the use of simple therapeutic strategies (heat or cold application, physical therapy, dry needling, or injection with a local anesthetic). However, MPS can also occur intermittently due to an underlying structural and environmental stimulus that cannot be corrected (58).

Conclusion

Biofreeze and superficial heat were both found to increase the quality of life, but the limited effectiveness of these applications emphasizes the importance of the underlying structural, postural, and ergonomic factors which should be treated appropriately to control myofascial pain and to prevent recurrence.

Türkçe Özet: *Biofreeze ve yüzeyel ısının mastikatör miyofasiyal ağrı sendromuna (MAS) etkisi. Amaç: Bu çalışma, MAS'lı hastalarda yüzeysel ısı ve Biofreeze'in ağrı, ağız açıklığı (mm) ve yaşam kalitesi üzerindeki etkisini değerlendirmeyi amaçlamaktadır. Gereç ve Yöntem: Çalışmaya dahil edilen MAS'ı olan 52 hasta, yüzeysel ısı ve Biofreeze uygulaması yapılmak üzere rastgele iki gruba ayrıldı. Biofreeze grubundaki hastalar (n=26) günde iki kez olmak üzere yedi gün boyunca masseter ve temporal kaslarına % 3.5 mentol jeli uygularken, diğer grup yüzeyel ısı uyguladı. Hastaların başlangıç, 7. ve 21. gün VAS, ağız açıklığı (mm) ve Ağız Sağlığı Etki Profili-14 (OHIP-14) skorları SPSS ile değerlendirildi. Bulgular: Ağız açıklığı, Biofreeze uygulanan grupta 4,27 ± 3,80 mm, yüzeysel ısı uygulanan grupta 2,58 ± 2,16 mm artmıştır. Her grupta başlangıç değerlerine göre 7. günde VAS ve OHIP-14 skorlarında anlamlı azalma gözlenmiştir (p <0.001). Miyofasiyal ağrı, ağız açıklığı (mm) ve OHIP-14 skorlarında iki uygulama arasında istatistiksel olarak anlamlı fark yoktur (p> 0.05). Her iki uygulamanın bu parametreler üzerindeki olumlu etkileri sadece kullanım süreleri ile sınırlı kalmıştır. Sonuç: Bu çalışmada, Biofreeze ve yüzeysel ısının MPS'deki etkileri analiz edilmiştir. Her iki uygulamanın da yaşam kalitesini artırdığı bulunmuştur, ancak bu uygulamaların sınırlı etkinliği altta yatan faktörlerin düzeltilmesinin önemine dikkat çekmektedir. Anahtar Kelimeler: Miyofasiyal ağrı, miyofasiyal ağrı sendromları, miyofasiyal ağrı tetik noktası, mentol, yansıyan ağrı*

Ethics Committee Approval: Research was approved by the Ethics Committee of the Gazi University (No:2015/118).

Informed Consent: Participants provided informed constent.

Peer-review: Externally peer-reviewed.

Author contributions: DY and CA participated in designing the study. DY and CA participated in generating the data for the study. DY and CA participated in gathering the data for the study. OK participated in the analysis of the data. DY wrote the majority of the original draft of the paper. DY and CA participated in writing the paper. DY and CA have had access to all of the raw data of the study. DY and CA have reviewed the pertinent raw data on which the results and conclusions of this study are based. have DY, CA, OK approved the final version of this paper. DY guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: The authors had no conflict of interest to declare.

Financial Disclosure: The authors declared that they have received no financial support.

References

1. Okada-Ogawa A, Sekine N, Watanabe K, Kohashi R, Asano S, Iwata K, Imamura Y. Change in muscle hardness after trigger point injection and physiotherapy for myofascial pain syndrome. J Oral Sci 2018; 17: 0453.
2. Fricton JR, Kroening R, Haley D, Siegert R. Myofascial pain syndrome of the head and neck: a review of clinical characteristics of 164 patients. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1985; 60: 615-23. [CrossRef]
3. Farina S, Casarotto M, Benelle M, Tinazzi M. A randomized controlled study on the effect of two different treatments (FREMS AND TENS) in myofascial pain syndrome. Eur J Phys Rehabil Med 2004; 40: 293.
4. Velly AM, Gornitsky M, Philippe P. Contributing factors to chronic myofascial pain: case–control study. Pain 2003; 104: 491-9. [CrossRef]
5. Wheeler AH. Myofascial pain disorders. Drugs 2004; 64: 45-62. [CrossRef]
6. Nelson SJ, dos Santos J, Barghi N, Narendran S. Using moist heat to treat acute temporomandibular muscle pain dysfunction. Compendium (Newtown, Pa.) 1991; 12: 808-10.
7. Reiter S, Goldsmith C, Emodi-Perlman A, Friedman-Rubin P, Winocur E. Masticatory muscle disorders diagnostic criteria: the American Academy of Orofacial Pain versus the research diagnostic criteria/temporomandibular disorders (RDC/TMD). J Oral Rehabil 2012; 39: 941-47. [CrossRef]
8. Koppert W, Frötsch K, Huzurudin N, Böswald W, Griessinger N, Weisbach V, et al. The effects of paracetamol and parecoxib on kidney function in elderly patients undergoing orthopedic surgery. Anesth Analg 2006; 103: 1170-6. [CrossRef]
9. McCurdy CR, Scully SS. Analgesic substances derived from natural products (natureceuticals). Life Sci 2005; 78: 476-84.
10. Knowlton WM, Daniels RL, Palkar R, McCoy DD, McKemy DD. Pharmacological blockade of TRPM8 ion channels alters cold and cold pain responses in mice. PloS one 2011; 6: e25894.
11. Hensel H, Zotterman Y. The effect of menthol on the thermoreceptors. Acta Physiol Scand 1951; 24: 27-34. [CrossRef]
12. Gaudioso C, Hao J, Martin-Eauclaire MF, Gabriac M, Delmas P. Menthol pain relief through cumulative inactivation of voltage-gated sodium channels. Pain 2012; 153: 473-84. [CrossRef]
13. Olive JL, Hollis B, Mattson E, Topp R. Vascular conductance is reduced after menthol or cold application. Clin J Sport Med 2010; 20: 372-6.
14. Richter M. The Effects of Biofreeze on Cutaneous Blood Flow in Normal Subjects as Measured by Doppler Ultrasound.
15. Robinson SB, Kesick CM, Kolka MA, Stephenson LA. Topical Nitroglycerin Ointment (2%) Applied to Forearm Skin Increases Skin Blood Flow. USARIEM 2001.
16. Locker D, Jokovic A, Clarke M. Assessing the responsiveness of measures of oral health-related quality of life. Community Dent Oral Epidemiol 2004; 32: 10-8.
17. Balci N, Alkan N, Gurgan CA. Psychometric properties of a Turkish version of the oral health impact profile14. Niger J Clin Pract 2017; 20: 19-24.
18. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G* Power 3.1: Tests for correlation and regression analyses. Behav Res Methods 2009; 41: 1149-60. [CrossRef]
19. Han SC, Harrison P. Myofascial pain syndrome and trigger-point management. Reg Anesth Pain Med 1997; 22: 89-101. [CrossRef]
20. Fricton JR. Clinical care for myofascial pain. Dent Clin North Am 1991; 35: 1-28.
21. Brennum J, Kjeldsen M, Jensen K, Jensen TS. Measurements of human pressure-pain thresholds on fingers and toes. Pain 1989; 38: 211-17.

22. Dionne RA. Pharmacologic treatments for temporomandibular disorders. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1997; 83: 134-42. [CrossRef]
23. Feine JS, Widmer CG, Lund JP. Physical therapy: a critique. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1997; 83: 123-7. [CrossRef]
24. Ekberg E, Vallon D, Nilner M. Occlusal appliance therapy in patients with temporomandibular disorders: a double-blind controlled study in a short-term perspective. Acta Odontol Scand 1998; 56: 122-8. [CrossRef]
25. van Grootel RJ, Buchner R, Wismeijer D, van der Glas HW. Towards an optimal therapy strategy for myogenous TMD, physiotherapy compared with occlusal splint therapy in an RCT with therapy-and-patient-specific treatment durations. BMC Musculoskelet Disord 2017; 18: 1-17.
26. Burgess JA, Sommers EE, Truelove EL, Dworkin SF. Short-term effect of two therapeutic methods on myofascial pain and dysfunction of the masticatory system. J Prosthet Dent 1988; 60: 606-10. [CrossRef]
27. Dworkin SF, Turner JA, Wilson L, Massoth D, Whitney C, Huggins KH, et al. Brief group cognitive-behavioral intervention for temporomandibular disorders. Pain 1994; 59: 175-87. [CrossRef]
28. Dworkin SF, Huggins KH, Wilson L, Mancl L, Turner J, Massoth D, et al. A randomized clinical trial using research diagnostic criteria for temporomandibular disorders-axis II to target clinic cases for a tailored self-care TMD treatment program. J Orofac Pain 2002;16.
29. Truelove E, Huggins KH, Mancl L, Dworkin SF. The efficacy of traditional, low-cost and nonsplint therapies for temporomandibular disorder: a randomized controlled trial. J Am Dent Assoc 2006; 137: 1099-1107.
30. Simons DG, Travel JG, Simons LS. Travell & Simons' myofascial pain and dysfunction: upper half of body. Lippincott williams & wilkins, 1999.
31. Kojima A, Goto K, Morioka S, Naito T, Akema T, Fujiya H, et al. Heat stress facilitates the regeneration of injured skeletal muscle in rats. J Orthop Sci 2007; 12: 74-82. [CrossRef]
32. Petrofsky JS, Laymon MS, Alshammari FS, Lee H. Use of low level of continuous heat as an adjunct to physical therapy improves knee pain recovery and the compliance for home exercise in patients with chronic knee pain: A randomized controlled trial. J Strength Cond Res 2016; 30: 3107-15. [CrossRef]
33. Petrofsky J, Berk L, Bains G, Khowailed IA, Lee H, Laymon M. The efficacy of sustained heat treatment on delayed-onset muscle soreness. Clin *J Sport Med* 2017; 27: 329-37. [CrossRef]
34. Chabal C, Dunbar PJ, Painter I, Young D, Chabal DC. Properties of Thermal Analgesia in a Human Chronic Low Back Pain Model. J Pain Res 2020; 13: 2083. [CrossRef]
35. Poindexter RH, Wright EF, Murchison DF. Comparison of moist and dry heat penetration through orofacial tissues. CRANIO 2002; 20: 28-33. [CrossRef]
36. De Laat A, Stappaerts K, Papy S. Counseling and physical therapy as treatment for myofascial pain of the masticatory system. J Orofac Pain 2003; 17.
37. Nadler SF, Steiner DJ, Erasala GN, Hengehold DA, Abeln SB, Weingand KW. Continuous low-level heatwrap therapy for treating acute nonspecific low back pain. Arch Phys Med Rehabil 2003; 84: 329-34. [CrossRef]
38. Nadler SF, Steiner DJ, Petty SR, Erasala GN, Hengehold DA, Weingand KW. Overnight use of continuous low-level heatwrap therapy for relief of low back pain. Arch Phys Med Rehabil 2003; 84: 335-42. [CrossRef]
39. Nadler SF, Steiner DJ, Erasala GN, Hengehold DA, Hinkle RT, Goodale MB, et al. Continuous low-level heat wrap therapy provides more efficacy than Ibuprofen and acetaminophen for acute low back pain. Spine 2002; 27: 1012-17. [CrossRef]
40. Nelson SJ, Ash Jr M. An evaluation of a moist heating pad for the treatment of TMJ/muscle pain dysfunction. CRANIO 1988; 6: 355-9.
41. de Felício CM, Freitas RL, Bataglion C. The effects of orofacial myofunctional therapy combined with an occlusal splint on signs and symptoms in a man with TMD-hypermobility: case study. IJOM 2007; 33: 21-9.
42. Dıraçoğlu D, Saral İB, Keklik B, Kurt H, Emekli U, Özçakar L, et al. Arthrocentesis versus nonsurgical methods in the treatment of temporomandibular disc displacement without reduction. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009; 108: 3-8. [CrossRef]
43. Nozaki S, Kawai M, Shimoyama R, Futamura N, Matsumura T, Adachi K, et al. Range of motion exercise of temporo-mandibular joint with hot pack increases occlusal force in patients with Duchenne muscular dystrophy. Acta Myol 2010; 29: 392.
44. Brandt KD. The importance of nonpharmacologic approaches in management of osteoarthritis. Am J *Med* 1998; 105: 39-44.
45. Algafly AA, George KP. The effect of cryotherapy on nerve conduction velocity, pain threshold and pain tolerance. Br J *Sports Med* 2007; 41: 365-9.
46. Ferrandiz-Huertas C, Mathivanan S, Wolf CJ, Devesa I, Ferrer-Montiel A. Trafficking of thermoTRP channels. Membranes 2014; 4: 525-564. [CrossRef]
47. Liu B, Fan L, Balakrishna S, Sui A, Morris JB, Jordt SE. TRPM8 is the principal mediator of menthol-induced analgesia of acute and inflammatory pain. PAIN 2013; 154: 2169-77. [CrossRef]
48. Yajima T, Sato T, Hosokawa H, Kondo T, Saito M, Shimauchi H, et al. Distribution of transient receptor potential melastatin-8-containing nerve fibers in rat oral and craniofacial structures. Ann Anat 2015; 201: 1-5. [CrossRef]
49. Finch PM, Drummond PD. Topical treatment in pain medicine: from ancient remedies to modern usage Pain Manag 2015; 5: 359-71.
50. Cliff MA, Green BG. Sensory irritation and coolness produced by menthol: evidence for selective desensitization of irritation. Physiol Behav 1994; 56: 1021-29. [CrossRef]
51. Sun J, Yang T, Wang P, Ma S, Zhu Z, Pu Y, et al. Activation of cold-sensing transient receptor potential melastatin subtype 8 antagonizes vasoconstriction and hypertension through attenuating RhoA/Rho kinase pathway. Hypertension 2014; 63: 1354-63. [CrossRef]
52. Craighead DH, Alexander LM. Topical menthol increases cutaneous blood flow. Microvasc Res 2016; 107: 39-45. [CrossRef]
53. Andersen HH, Olsen RV, Møller HG, Eskelund PW, Gazerani P, Arendt-Nielsen L. A review of topical high-concentration L-menthol as a translational model of cold allodynia and hyperalgesia. Eur J Pain 2014; 18: 315-25. [CrossRef]
54. Bourgaize S, Newton G, Kumbhare D, Srbely J. A comparison of the clinical manifestation and pathophysiology of myofascial pain syndrome and fibromyalgia: implications for differential diagnosis and management. *J* Can Chiropr Assoc 2018; 62: 26.
55. Borg-Stein J, Iaccarino MA. Myofascial pain syndrome treatments. Phys Med Rehabil Clin N Am 2014; 25: 357-74. [CrossRef]
56. Leventhal LJ. Management of fibromyalgia. Ann Intern Med 1999; 131: 850-858. [CrossRef]
57. Sprangers MA, de Regt EB, Andries F, van Agt HM, Bijl RV, de Boer JB, et al. Which chronic conditions are associated with better or poorer quality of life? *J* Clin Epidemiol 2000; 53: 895-907.
58. Gerwin RD. Classification, epidemiology, and natural history of myofascial pain syndrome. Curr *Pain Headache* Rep 2001; 5: 412-20. [CrossRef]

eor.istanbul.edu.tr

Official Publication of Istanbul University Faculty of Dentistry

Eur Oral Res 2021; 55(3): 139-145

Original research

Comparison of efficacy and pain perception using 0.5% Bupivacaine and 2% Lidocaine in periodontal Surgery – A split mouth randomized clinical trial

Shanmukha Srinivas Manikanta Tirumalasetty[1], Dwarakanath Chinniswami Doraisami[2], Konathala SV Ramesh[2], Gautami S Penmetsa[2], NVS Sruthima G[2]

Purpose
To evaluate the effectiveness of bupivacaine and lidocaine local anesthesia on the intra-surgical and post-surgical pain control in patients undergoing periodontal flap surgery.

Materials and Methods
A randomized, single-blind, split-mouth design was employed in patients who are scheduled for periodontal flap surgery for at least two similar sextants with similar anesthetic techniques. Fifty patients (age range 16-65 years, 32 males and 28 females) enrolled in the present study. On one-site, the flap surgery was performed using 2% lidocaine with 1:200000 epinephrine and on the other with 0.5% bupivacaine with 1:200000 epinephrine. Base line clinical parameters, probing pocket depth, clinical attachment level were recorded. Pain during intra operative period, at the time of loss of numbness and for three consecutive days was measured using visual analog scale (VAS).

Results
Significant differences were observed between the two groups in the intra operative pain scores (p=0.0045) and pain scores at the time of loss of numbness (p=0.0005) but not at the 1st, 2nd and 3rd day after the surgery.

Conclusion
Bupivacaine was markedy more effective than the lidocaine. Thus the usage of bupivacaine can be substantiated for periodontal surgeries for the control of pain in the intra operative and immediate post-operative period to increase patients' comfort.

Keywords: *Bupivacaine, Lidocaine, long acting local anesthetics, pain control, periodontal flap surgery*

Introduction

Pain is a highly personalized and unpleasant state accompanying tissue damage as a result of an adequate stimulus. It is a construct including an individual's past experiences, learned responses, and expectations in addition to species specific physiologic responses. Pain can also become a reason for stress and it is mandatory to eliminate this unpleasant feeling (1,2).

Fear of pain has been associated with the dental treatment since ages. Effective control of pain during dental procedures has been one of the most important pre-requisite of painless dentistry. Many dental procedures are often painful, lengthy and can cause major discomfort. In contrast to non-surgical treatments, surgery produces significantly more post-operative discomfort (3). Pain from surgical incision and tissue ma-

ORCID IDs of the authors: G.S.S.M.T. 0000-0002-2194-0435; D.C.D. 0000-0002-0506-5191; S.V.R.K. 0000-0001-7022-0023; S.G.P. 0000-0002-8744-1452; N.V.S.S.G. 0000-0002-7126-5829

[1]Department of Periodontics, GSL Dental College, Rajahmundry, India

[2]Department of Periodontics, Vishnu Dental College, Bhimavaram, India

Corresponding Author: Konathala SV Ramesh

E-mail: ksv006@gmail.com

Received: 19 January 2021
Revised: 5 February 2021
Accepted: 30 April 2021

DOI: 10.26650/eor.2021853507

How to cite: *Tirumalasetty SSM, Chinniswami Doraisami D, Ramesh KSV, Penmetsa GS, Gottumukkala NVSS. Comparison of efficacy and pain perception using 0.5% Bupivacaine and 2% Lidocaine in periodontal Surgery – A split mouth randomized clinical trial. Eur Oral Res 2021; 55(3): 139-145.*

nipulation occurs immediately, but gives way to inflammatory cell recruitment to the injured area over the course of several hours (4). This contributes to sensitization, which results in prolonged episodes of pain. Selection of local anesthesia during surgery will minimize the use of analgesics and provide more patient compliance (5).

Periodontal surgeries are one of the most common surgical dental procedures used for the management of periodontal disease. Periodontal disease is a chronic inflammatory condition that resulrs in an often-painless destruction of tooth supporting tissues (6). On the other hand, periodontal therapy, particularly those requiring extensive surgeries with osseous recontouring or placement of multiple implants, can be significantly painful. Pain from periodontal surgery varies; with mucosal versus osseous procedures having less versus more pain, respectively (4). Hence, effective management of intra and postoperative pain is of utmost importance.

Many strategies have been proposed in medical field to minimize sensitization associated with surgical procedures which includes anoci-association, balanced analgesia and pre-emptive analgesia (6-8). In dentistry, one way to minimize this discomfort to the patient during and after the surgery is the use of pre-operative pain medication using analgesics like ibuprofen, but it can increase intraoperative bleeding (9). An alternative method is the use of long-acting local anesthetics such as bupivacaine to block nociceptive inputs throughout the peri-operative period leading to decreased central sensitization and postoperative pain.

Although, lidocaine is clinically considered to be close to satisfy the requirements of an ideal anesthetic, while performing extensive periodontal surgeries involving extensive areas may require anywhere from 30 to 120 minutes, which may sometimes require an additional reinjection (10). This can lead to intraoperative pain and discomfort and also increased postoperative pain through central sensitization

Bupivacaine was later introduced for this purpose in the perioperative period, as they are comparable in onset and efficacy to lidocaine and has longer duration of action. Long acting anesthetics can attenuate pain for hours after the procedure, when acute pain is most intense thereby making the transition from anesthesia to post-operative pain comfortable. It was hypothesized that bupivacaine would cause effective anesthesia at the time of treatment and also would be effective in controlling postoperative pain over different time intervals following surgery when compared to lidocaine (5). Bupivacaine, an amide local anesthetic solution being lipid soluble, has greater potency with longer action (11).

Most of the research regarding the comparison of bupivacaine and lidocaine for managing intra- and post-operative pain has been carried in the field of oral surgery and endodontics with a sparse literature in periodontal flap surgeries. Therefore, the present study was designed to evaluate the effectiveness and efficacy of bupivacaine and lidocaine on the intra-surgical and post-surgical pain control in patients undergoing periodontal flap surgery. The null hypothesis tested in this study is that there is no difference between bupivacaine and lidocaine in terms of local anesthetic effectiveness and pain control at any time interval.

Materials and methods

Ethical statement

This was a split mouth, randomized, single blind, study which was conducted at Vishnu Dental College. The ethical approval was obtained from institution's ethical committee (VDC/IEC/2012-74) prior to the commencement of the study. All the participants were informed about the procedure and informed consent was obtained before the start of the surgery.

Sample size calculation

Sample size was calculated by using n master 2.0 software (CMS Biostatistics, Vellore, India). The clinically acceptable effect for which non inferiority can be declared is a change of 1 on the visual analogue scale. The true difference is thought to be zero and the expected standard deviation in the population in which the trial is to be conducted is 1.10, alpha level was set at 5% and power was fixed at 80%.

Participants and materials

Fifty particiapants ranging in age range of 16 to 65 years (32 males and 28 females) whom are candidates for periodontal flap surgery in the mandibular posterior region were included in the present study. The exclusion criteria included the patients with known allergy to local anesthetics and patients under any medication which can alter the pain perception (opioids, corticosteroids, NSAIDS, sedatives, anti-anxiety drugs, antidepressants, CNS stimulants) and patients under anti-coagulant therapy, uncontrolled systemic diseases, smokers etc. The materials required for this study included 0.5% Bupivacaine hydrochloride with 1:200000 epinephrine (Marcaine, Cook-Waite company, USA) for the test group and 2% Lidocaine hydrochloride with 1:200000 epinephrine (Xylocaine, Astra zeneca) for the controls, an aspirating syringe with 30-gauge needle (septodont, USA) and a graduated portable aspirator.

Study design

Two sextants in the mandibular posterior region requiring similar periodontal flap surgeries were considered and similar anesthetic techniques and equal amount of anesthesia were administered. The site for choosing either of the anesthesia were randomized using coin flip method and the second periodontal flap surgery was done either on the same day or at least one week after the first surgery.

Data collection

At the time of periodontal surgery, the following parameters were recorded to assess the efficacy of a local anesthetic agent includes: onset of local anesthesia which was determined by the patients' claim and by the loss of sensibility to pricking with periodontal probe (UNC 15 – Hu-Friedy, USA); depth of anesthesia which was assessed by evaluation of pain during surgery using visual analogue scale (VAS); amount of anesthetic solution which was described as the

total volume of anesthetic solution used during surgery. If additional anesthetic solution was needed for re-anesthesia, time at which additional anesthetic solution was administered, volume and anesthetic technique used for re- anesthesia were recorded; Duration of surgery was defined as the time from starting of the incision to the completion of last suture. Osteoplasty; whether it was required or not; Bleeding during surgery variable was defined as all the water and blood collected into a graduated beaker using a portable aspirator. The total amount of blood was calculated by subtracting the volume of water used during the surgery from the total volume of fluid aspirated. Any saliva generated during the procedure was considered negligible since each patient served as his/her own control. Base line clinical parameters were measured before the surgery which includes Plaque index (PI) (Silness and Loe, 1964), gingival index (GI) (Loe and Silness, 1963), Probing pocket depth (PPD), Clinical attachment level (CAL).

Outcome measures

Primary outcome measures considered were the efficacy of bupivacaine over lidocaine and patients' pain perception towards these two anesthetics.

Surgical procedure

Three weeks after initial nonsurgical periodontal therapy (NSPT) which consisted of oral hygiene instructions and full mouth scaling followed by root planing, a periodontal evaluation was performed to confirm the suitability of sites for periodontal surgery. Patients with probing pocket depths higher or equal to 5mm for molars higher than or equal to 6mm for incisors and premolars were scheduled for periodontal flap surgery. All surgeries were performed in the morning hours to provide the patient and investigator sufficient time to evaluate the postoperative discomfort before bedtime and to avoid possible diurnal variation in pain response.

All the surgeries were performed under strict surgical protocol by the same surgeon. Both the sextants in the mandible received inferior alveolar, lingual and long buccal nerve blocks. The same amount of local anesthetic solution (i.e. 1 carpule or 1.8 ml) was deposited for inferior alveolar and lingual nerve block] at each site to achieve surgical anesthesia. An additional carpule was deposited for the long buccal and intra papillary injections. No additional anesthesia was given. If additional anesthetic solution is needed for re-anesthesia (during periodontal surgery), time at which additional anesthetic solution administered, volume and anesthetic technique used for re-anesthesia was recorded. Access flap surgery was performed using crevicular and interdental incisions. A full thickness mucoperiosteal flap was reflected and thorough surgical debridement was done. Direct loop sutures were placed and the area was protected with a non-eugenol dressing. Any discomfort persists after the surgery patients were instructed to take diclofenac sodium (50mg IP).

Postoperative parameters were evaluated using a specially prepared patient handout including: Duration of analgesia - determined by the difference between onset of anesthesia to the ingestion of first rescue analgesic (the patients were requested not to take the analgesic until the pain starts); Recovery from the anesthesia - evaluated by measuring pain at the time of loss of numbness using VAS; Any other significant events related to anesthesia or surgery; Intraoperative and postoperative subjective pain - evaluated by using a questionnaire after the surgery, which consisted of a 100-mm-length VAS, with a 0 anchored by "no pain" and a 100 anchored by "worst pain imaginable". Pain scores were taken immediately after surgery, at the time of loss of numbness (subjects were requested not to take any pain medications until and unless the pain starts and after the surgery for three consecutive days upon waking in the morning; Total number of analgesics used during the postoperative days; Patient preference - Seven days after the completion of the second surgery each patient was asked, "Which one would you prefer for future surgeries?"

Statistical analysis

IBM SPSS V 25.0 (IBM Corp, Armonk, NY, USA) software was used for the data analysis. As the scale variables were not normally distributed non-arametric tests such as Mann- Whitney U test for independent pairwise comparisons and Wilcoxon matched pair test for intragroup comparison were performed. The categorical variables were analyzed using Chi-square test. The confidence level was set to 95% and p values less than 0.05 were considered as statistically significant.

Results

All the fifty patients tolerated the surgical procedure well, except for one patient who had developed swelling post operatively, which was subdued without any further complication. None of the patients developed any other anesthetic related (or) intra and postoperative complications. Periodontal variables: No significant differences were observed between the two sextants, extent of surgery (number of teeth involved), severity of periodontal inflammation (PI, GI) and severity of periodontal involvement (PPD, CAL) (Table1).

There were no major discrepancies between the two local anesthetics in terms of osteoplasty, surgery length, or blood loss volume. Except for the mean onset of numbness, all anaesthetic variables were statistically significant, including lack of sensibility to pricking, the need for additional anaesthesia, the length of anaesthesia, recovery from anaesthesia, and the total number of analgesics used. From a clinical perspective, bupivacaine seemed to produce better results (Table 2 and Table 3).

***Table 1**. Inter-group comparison of periodontal parameters at baseline.*

Variables	LA	Mean	SD	p value
Plaque Index	Bupivacaine	2.36	0.41	0.518
	Lignocaine	2.55	0.97	
Gingival Index	Bupivacaine	2.44	0.37	0.626
	Lignocaine	2.56	0.81	
PPD	Bupivacaine	4.01	0.58	0.4937
	Lignocaine	3.9	0.6	
CAL	Bupivacaine	4.61	0.56	0.8682
	Lignocaine	4.6	0.9	

Table 2. *Comparative evaluation of two groups using non-parametric tests (** p<0.01 inidicates highly statistically significant).*

Variable	LA	Yes	No	p- value
Recovery from anesthesia	Bupivacaine	12	03	0.69
	Lignocaine	01	14	
Osteoplasty	Bupivacaine	10	05	0.0002**
	Lignocaine	11	04	

Table 3. *Comparative evaluation of inter group variations using parametric tests, * p<0.05 statistically significant, ** p<0.01 highly statistically significant.*

Variable	LA	Mean	SD	p- value
Loss of sensibility to pricking (mins)	Bupivacaine	4.96	2.65	0.0202*
	Lignocaine	2.52	1.42	
Additional anesthesia (ml)	Bupivacaine	0.00	0.00	0.016*
	Lignocaine	0.67	0.98	
Duration of surgery	Bupivacaine	119.7	36.46	0.803
	Lignocaine	120.8	29.68	
Volume of blood loss	Bupivacaine	104	38.6	0.9174
	Lignocaine	10.5.67	33.27	
Duration of analgesia (mins)	Bupivacaine	438	72.92	0.00001**
	Lignocaine	164	51.24	
Number of analgesic tablets	Bupivacaine	1.73	1.71	0.0003**
	Lignocaine	4.47	1.25	

Statistically significant differences were observed between the two groups in terms of pain scores immediately after surgery and pain at the time of loss of numbness. No significant differences were observed in pain at first, second and third day after surgery (Figure 1, Table 4).

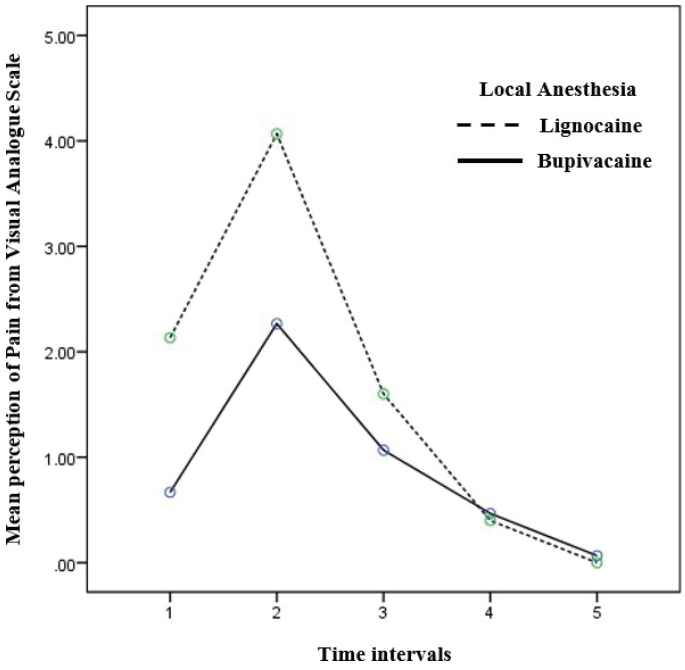

Figure 1. *Comparison of pain scores at five different time intervals.*

Table 4. *Comparison of pain scores at different time intervals, ** p<0.01 highly statistically significant.*

Variables	Groups	Pain score	p-value
Intraoperative pain	Bupivacaine	0.67±0.82	0.0045**
	Lidocaine	2.13±1.46	
Pain at the time of loss of numbness	Bupivacaine	2.27±1.83	0.0005**
	Lidocaine	4.07±1.03	
Day 1	Bupivacaine	1.07±1.33	0.1585
	Lidocaine	1.60±1.84	
Day 2	Bupivacaine	0.47±1.06	0.6187
	Lidocaine	0.40±0.51	
Day 3	Bupivacaine	0.07±0.26	0.7557
	Lidocaine	0.00±0.00	

Discussion

Alleviating the pain is of paramount importance for both patients and dentists. Routine periodontal surgeries are well tolerated by the patients and short acting local anesthetics followed by analgesics are effective in dealing with the pain. However, in surgeries covering large areas of mouth requiring extensive tissue manipulation with osseous recontouring may eventually lead to excessive postoperative pain. Further, the transition between loss of anesthetic effect and onset of pain is the most difficult phase for the patient. Hence, the use of longer acting local anesthetics may be considered in such situations. As reported earlier, periodontal surgeries produce less morbidity and discomfort to the patient (12). Therefore, second surgery was performed one week after the first surgery depending on the patients' comfort. None of the patients reported any discomfort after one week of surgery.

Assessment of pain using pain scales is subjective and highly dependent upon individual experience, therefore in this experimental design the patient served as both the control and test subject as the horizontal VAS was used (13).

The efficacy of bupivacaine as long acting anesthetic has been well substantiated in the literature. The efficacy of 0.5% Bupivacaine HCl was found to be comparable or even more potent than that of 2% Lidocaine HCl. (11,14). According to the available literature, Bupivacaine has a higher efficacy in general dental procedures, especially in extractions. However, no research has been done to date to assess its utility in periodontal surgery.

In the present study, bupivacaine has slightly delayed patient reported onset than that of lidocaine. Onset of complete surgical anesthesia as determined by the loss of pricking with periodontal probe was delayed from 3 to 8 min with Bupivacaine than that of Lidocaine which was statistically significant. This is consistent with previous findings ranging from 6 to 10 min. The delay in the onset can be related to the higher p*Ka* for Bupivacaine which reduces the availability of free base form of anesthetic molecules to diffuse through the nerve membrane (15). In a previous study, loss of sensibility to pricking took an average of 8.1 minutes for bupivacaine, varying from less than 5 to 15 minutes (16).

Bupivacaine is more firmly bound in the nerve membrane (increased protein binding) therefore released more slowly from receptor sites in the sodium channels (15). This study showed that duration of analgesia was 37% higher for Bupivacaine which lasted for up to an average period of 7.3 hrs which was highly significant (p=0.00001*). Similar findings have been reported before (17-19). In a similar study, it was reported that the bupivacaine group was numb for a mean time of 5.9 hrs as compared to 3.9 hrs for the lidocaine group (20). Another one showed that the quadrants received Lidocaine maintained postoperative anesthesia for an average duration of 2.47 hrs while the Bupivacaine had a significantly longer duration of 5.62 hrs (21).

Saline used during the osteoplasty was standardized for all the patients as 20 ml and it was not included at the time of statistical analysis. There were no significant differences in the extent, severity of periodontal involvement, duration of the procedure and surgical trauma (osteoplasty) between the two groups, hence their impact on the intra and postoperative pain was minimized between the groups. Sextants requiring similar anesthetics were selected irrespective of their location because it has been reported that there was no difference in the pain experience and no difference in postoperative pain related to the location of surgery (11,21).

The mean duration of the procedure in this study was 120.26±32.66 min, ranging from 60 to 155 min. This is similar to a study which reported a mean duration of the periodontal flap procedure to be 151.8±52.2 min, ranging from 51 to 255 min. One study reported mean surgical time per quadrant was 60±14.52 min, ranging from 30 to 100 min (22). A clinical trial reported that periodontal surgical procedures required operating times of between 30 to 120 min with a mean duration of 67 min (11).

Bupivacaine has 2.5 times more vasodilating activity when compared to Lidocaine and hence more amount of blood loss can be expected while using Bupivacaine (14). To some degree, these effects may be concentration dependent (23). But in this study, amount of blood loss during the periodontal surgery was similar between the groups accounting for a mean blood loss of 104.00±38.65ml in test group and 105.67±33.27ml in control group; the difference was not statistically significant. This is in contrast with a previous study where bupivacaine group demonstrated more bleeding during periodontal surgeries, but the evaluation of bleeding was done by subjective assessment of the degree of homeostasis achieved (i.e., excellent, limited or poor) which is inaccurate (19). The total blood loss accounted to a combined average of 104.83±35.44ml ranging from 50 to 180ml. This is lesser when compared to a previous study which reported a mean blood loss of 134.4±114.3 ml ranging from 16-592 ml (24). But it is more when compared to other studies which reported a mean blood loss of 59.47±38.2ml ranging from 6.0 to 145.1 ml (25). Both these studies employed variable amount of local anesthetic solutions and used calorimetric methods for measuring blood volume loss, where as in this study measurement was done using a calibrated suction apparatus. The average blood loss per papilla in this study was 7.02 ml for bupivacaine and 7.07 ml for lignocaine, this was similar to another study which reported 10 ml of blood loss per interproximal papilla when a flap was raised or osseous recontouring was performed (24).

Intraoperative pain which was measured immediately after periodontal surgery was greater for lidocaine with statistically significance (p<0.01). This could be due to the shorter duration of action of lidocaine leading to pain and requirement of additional local anesthesia intra-operatively in control group which might have led to postoperative central sensitization. Five patients required an additional 2 ml of Lidocaine each and none of the test sites required additional Bupivacaine.

Recovery from anesthesia was smooth and comfortable with bupivacaine (p<0.0001) due to longer duration of action resulting in suppression of peripheral nociceptive activity in the immediate postoperative period, thereby reduced central sensitization and pain (26). This effect might have reflected on the pain perception immediately after the periodontal surgery, choice of anesthesia for their future surgeries and number of analgesic tablets consumed in test group. However acute pain tends to peak 8 to 10 hours after the procedure (27). Study showed an overall gradual decrease in postoperative pain with the intensity most severe in the immediate postoperative period followed by gradual decrease in the consecutive, first, second and third days after the surgery. But the pain perception was significantly less in the immediate postoperative period in bupivacaine group (p<0.01). This is in agreement with a previous study which showed statistically significant difference in pain perception in the immediate postoperative period over 8 different time periods in first 24 hours between bupivacaine and lidocaine (19). However, this difference was not observed at the first, second and third days post-operatively. One study done in patients undergoing extraction of impacted third molars reported that bupivacaine significantly increased prostaglandin production by stimulating COX-2 gene expression at 48 hours causing increased pain after the local anesthetic effect dissipates (28). But this was not observed in the present study as the intensity of pain gradually decreased over the time period of 72 hours post-operatively. This again demonstrates that the most critical time of pain control is between the loss of anesthesia and the first 24 h after the surgery. Once this critical phase passes out most of the patients often do not require regular dose of analgesics.

In this study, number of analgesic tablets consumed were significantly less (p<0.001) in test group with an average of 1.73±1.71 tablets compared to 4.47±1.25 tablets in control group. Three subjects haven't used even single analgesic tablet and 6 patients required only one analgesic tablet post operatively in bupivacaine group. Similarly, in other studies bupivacaine group took 2.8 postoperative analgesic tablets as compared to 4.3 for the lidocaine group and in other study lidocaine group reported an average of 3.7 tablets versus a significantly smaller amount for the bupivacaine group of 1.6 tablets (19,20).

Being a single blinded study, the patient has no knowledge of what anesthetic is being used. Hence, feedback from the patients can be taken as an accurate appraisal. The patient preference of anesthetic for future surgeries which was recorded 1 week after second surgery for each patient. Out of fifty patients, 39 subjects opted for bupivacaine, 11 subjects preferred lidocaine because of lesser duration of numbness postoperatively and only one patient felt comfortable with lidocaine. These results are in tandem with another study

where bupivacaine was preferred by 14 out of 19 patients and 3 patients preferred lidocaine and one patient did not respond (19).

In terms of adverse events, the toxicity of bupivacaine is less than one-fourth that of lidocaine (14). Bupivacaine is thought to be less safe than other long acting local anaesthetics, especially with regard to cardiac toxicity. Thus, the accentuation of Bupivacaine cardio toxicity must also be considered in patients taking chronic medications that depress cardiac function, such as beta blockers, calcium channel and cardiac glycoside (29,30). A recent meta-analysis reported that there is no evidence could be found to conclude that bupivacaine was less safe than lidocaine (31). No adverse events were reported in this study.

Conclusion

Within the limitations of the present study, it can be stated that 0.5% bupivacaine administered as local anesthetic in periodontal flap surgey is more likely to provide higher intra-operative and immediate post-operative pain control when compared to 2% lidocaine.

Türkçe Özet: *Periodontal cerrahide local anestezik olarak kullanılan %0.5'lik bupivacaine ve 2% lidocaine preparatlarının etkinliğinin ve ağrı algısının karşılaştırılması – Bölünmüş ağız tasarımlı klinik çalışma. Amaç: Periodontal flep cerrahisi uygulanan hastalarda kullanılan bupivacaine ve lidocaine içerikli lokal anestezik maddelerin girişim sırasında ve sonrasında ağrı kontrolü etkinliklerinin karşılaştırılması. Bireyler ve yöntem: Rastgellenmiş, tek kör, bölünmüş ağız tasarımlı bir çalışmada en az iki benzer bölgede periodontal cerrahide benzer anestezi teknikleri uygulanacak bireyler belirlenmiştir. 32 erkek, 28 kadın olmak üzere yaş aralığı 16-65 arasında değişen 50 hasta çalışmaya dahil edilmiştir. Bir bölgede flep cerrahisi sırasında %2'lik lidocaine ve 1:200000 epinefrin diğerinde %0.5'lik bupivacaine ve 1:200000 epinefrin kullanılmıştır. Başlangıçta, temel klinik parametreler, sondalamada cep derinliği, klinik ataşman seviyeleri kayıt edilmiştir. Girişim sırasındaki ağrı, uyuşukluk hissi geçerken hissedilen ağrı ve girişimi takip eden 3 gün içindeki ağrı, vizuel analog skala (VAS) kullanılarak takip edilmiş ve istatistiksel olarak karşılaştırılmıştır. Bulgular: Girişim sırasındaki ağrı skorları bakımından iki grup arasında istatistiksel olarak anlamlı farklılık olduğu belirlenmiştir (p=0.0045). Aynı durum, uyuşukluk hissi geçerken bildirilen ağrı skorları arasında da gözlenmiştir (p=0.0005). Ancak, birinci, ikinci ve üçüncü günler arasında ağrı skoru bakımından bir fark ortaya çıkmamıştır. Sonuç: Bupivacaine içeren preparatın lidocaine içerene göre periodontal cerrahide daha başarılı olduğu öne sürülebilir. Buna bağlı olarak, periodontal flep cerrahisinde girişim sırasındaki ve hemen sonrasındaki ağrı hissini kontrol etmek ve hasta konforunu arttırmak için bupivacaine içeren lokal anesteziklerin kullanımı yararlı olabilir. Anahtar kelimeler: Bupivacaine, lidocaine, uzun etkili lokal anestezik madde, ağrı kontrolü, periodontal flep cerrahisi*

Ethics Committee Approval: The ethical approval was provided by Vishnu Dental College's ethical committee (VDC/IEC/2012-74).

Informed Consent: Participants provided informed consent.

Peer-review: Externally peer-reviewed.

Author contributions: SSMT, DCD, SGP participated in designing the study. SSMT, SVRK participated in generating the data for the study. SSMT participated in gathering the data for the study. SSMT, DCD, SVRK, SGP, NVSSSG participated in the analysis of the data. SSMT wrote the majority of the original draft of the paper. SSMT, DCD, SVRK, NVSSS participated in writing the paper. SSMT, NVSSSG have had access to all of the raw data of the study. SSMT, DCD, SVRK have reviewed the pertinent raw data on which the results and conclusions of this study are based. SSMT, DCD, SVRK, SGP, NVSSSG have approved the final version of this paper. SSMT, DCD, SVRK guarantee that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared that they have received no financial support.

References

1. TateAR, AcsG. Dental postoperative pain management in children. Dent Clin North Am 2002; 46:707–17. [CrossRef]
2. Ozlem Velioglu, Aylin Sipahi, Huseyin Koca, Emre Velioglu. Bupivacaine vs. lidocaine: a comparison of local anesthetic efficacy in impacted third molar surgery. Clin Oral Investig 2020; 24:3539-46. [CrossRef]
3. Matthews DC, McCulloch CAG. Evaluating patient perceptions as short-term outcomes of periodontal treatment: a comparison of surgical and non-surgical therapy. J Periodontol 1993;64:990–7. [CrossRef]
4. Gordon SM, Mischenko AV, Dionne RA. Long-Acting Local Anesthetics and Peri operative Pain Management. Dent Clin North Am 2010;54:611–20. [CrossRef]
5. Coventry J, Griffiths G, Scully C, Tonetti M. Periodontal disease. BMJ 2000; 321:36–9. [CrossRef]
6. Julian Diaz-Abele MD, Mario Luc MD, Alina Dyachenko, Salah Aldekhayel, Antonio Ciampi, Jane Mc Cusker. Lidocaine with epinephrine versus bupivacaine with epinephrine as local anesthetic agents in wide-awake hand surgery: a pilot outcome study of patient's pain perception. J Hand Surg Glob Online 2020;2:1-6. [CrossRef]
7. Katz J. George Washington Crile, anoci-association, and pre-emptive analgesia. Pain 1993;53:243–5. [CrossRef]
8. Kehlet H, Dahl JB. The value of "multimodal" or "balanced analgesia" in postoperative pain treatment. Anesth Analg 1993;77:1048–56. [CrossRef]
9. Woolf CJ, Chong MS. Preemptive analgesia--treating postoperative pain by preventing the establishment of central sensitization. Anesth Analg 1993;77:362–79. [CrossRef]
10. Braganza A, Bissada N, Hatch C, Ficara A. The effect of non-steroidal anti-ınflammatory drugs on bleeding during periodontal surgery. J Periodontol 2005;76:1154–60. [CrossRef]
11. Dal Pra DJ, Strahan JD. A clinical evaluation of the benefits of a course of oral penicillin following periodontal surgery. Aust Dent J 1972;17:219–21. [CrossRef]
12. Pradhan S, Shrestha R, Gorkhali RS. Pain perception after periodontal therapies. J Nepal Soc Perio Oral Implantol 2018;2:56-60. [CrossRef]
13. Scott J, Huskisson EC. Vertical or horizontal visual analogue scales. Ann Rheum Dis 1979;38:560. [CrossRef]
14. Chandrashekhar Pandey, Alexander Speedie, Rahul Jaiswal, Shishir Lanjewar, Yadnesh Dondulkar. Role of bupivacaine in dentistry. Int J Oral Health Med Res 2019; 6:53-55.
15. MoorePA, DunskyJL. Bupivacaine anesthesia—a clinical trial for endodontic therapy. Oral Surg Oral Med Oral Pathol 1983;55:176–9. [CrossRef]
16. McLureHA, RubinAP. Review of local anaesthetic agents. Minerva Anestesiol 2005;71:59–74.
17. Malamed SF. Handbook of Local Anesthesia. Elsevier Health Sciences; 2014.
18. Trieger N, Gillen GH. Bupivacaine anesthesia and post-operative analgesia in oral surgery. Anesth Prog 1979; 26:20–3.
19. Chapman PJ, Macleod AWG. A clinical study of bupivacaine for mandibular anesthesia in oral surgery. Anesth Prog 1985;32:69–72.
20. Bouloux GF, Punnia-Moorthy A. Bupivacaine versus lidocaine for third molar surgery: a double-blind, randomized, crossover study. J Oral Maxillofac Surg 1999;57:510–4. [CrossRef]

21. Sampaio RM, Carnaval TG, Lanfredi CB, Horliana ACRT, Rocha RG, Tortamano IP. Comparison of the anesthetic efficacy between bupivacaine and lidocaine in patients with irreversible pulpitis of mandibular molar. J Endod 2012;38:594–7. [CrossRef]
22. Bellini HT. The time factor in periodontal therapy. A pilot study correlating treatment time and plaque, calculus, pocket depth, and number of teeth. J Periodontal Res 1974; 9:56–61.
23. Aps C, Reynolds F. The effect of concentration on vasoactivity of bupivacaine and lignocaine. Br J Anaesth 1976;48:1171–4. [CrossRef]
24. Baab DA, Ammons Jr WF, Selipsky H. Blood loss during periodontal flap surgery. J Periodontol 1977;48:693–8. [CrossRef]
25. Zigdon H, Levin L, Filatov M, Oettinger-Barak O, Machtei EE. Intraoperative bleeding during open flap debridement and regenerative periodontal surgery. J Periodontol 2012; 83:55–60. [CrossRef]
26. Gordon SM, Brahim JS, Dubner R, McCullagh LM, Sang C, Dionne RA. Attenuation of pain in a randomized trial by suppression of peripheral nociceptive activity in the immediate postoperative period. Anesth Analg 2002; 95:1351–7. [CrossRef]
27. Moore PA. Bupivacaine: a long-lasting local anesthetic for dentistry. Oral Surg Oral Med Oral Pathol 1984;58:369–74. [CrossRef]
28. Gordon SM, Chuang BP, Wang XM, Hamza MA, Rowan JS, Brahim JS, et al. The differential effects of bupivacaine and lidocaine on prostaglandin e2 release, cyclooxygenase gene expression and pain in a clinical pain model. Anesth Analg 2008;106:321–7. [CrossRef]
29. Adsan H, Tulunay M, Onaran O. The effects of verapamil and nimodipine on bupivacaine- induced cardiotoxicity in rats: an in vivo and in vitro study. Anesth Analg 1998;86:818–24. [CrossRef]
30. Roitman K, Sprung J, Wallace M, Matjasko J. Enhancement of bupivacaine cardiotoxicity with cardiac glycosides and beta-adrenergic blockers: a case report. Anesth Analg 1993;76:658–61. [CrossRef]
31. Su N, Wang H, Zhang S, Liao S, Yang S, Huang Y. Efficacy and safety of bupivacaine versus lidocaine in dental treatments: a meta-analysis of randomised controlled trials. Int Dent J 2014;64:34–45. [CrossRef]

eor.istanbul.edu.tr

Official Publication of Istanbul University Faculty of Dentistry

Eur Oral Res 2021; 55(3): 146-151

Original research

The effects of final irrigants on the push-out bond strength of two calcium silicate-based root canal sealers: an *in vitro* study

Davut Celik[1], **Ayse Tuba Ozalp Koca**[2], **Tugba Kosar**[1], **Tamer Tasdemir**[1]

Purpose
The purpose of the present study was to investigate the effect of the different irrigant combinations used in final irrigation on the push-out bond strength of root canal sealers that have different compositions.

Materials and Methods
In total 60 dentinal slices in 1 mm thickness were collected from 15 extracted mandibular premolar teeth; 4 slices from each tooth. 3 canal-like artificial cavities were opened on each dentinal slice. Samples were divided into 4 experimental groups, each of which consisted of 15 samples. In group 1, samples were immersed in 5.25% NaOCl and 17% EDTA solutions respectively; in group 2, immersed in 5.25% NaOCl and 2% CHX solutions respectively; in group 3, immersed in 5.25% NaOCl, 17% EDTA and 2% CHX solutions respectively; and in group 4 immersed in distilled water. After drying with absorbent papers, each cavity in dentinal slice sample was filled with different sealer (Endoseal MTA, Tech Biosealer Endo or AH Plus). Two days later, the push-out bond examination was performed.

Results
AH Plus showed higher push-out bond strength value in two combinations (group 2 and 3) in which final irrigants contained CHX ($p<0.001$). Dentinal push-out bond strengths of root canal sealers from Endoseal MTA and Tech BioSealer Endo were not affected by final irrigant ($p=0.965$).

Conclusion
Using CHX after NaOCl in final irrigant increases push-out strength of epoxy resin-based sealer but, did not create any difference in dentinal push-out bond strength of calcium silicate-based sealers.

Keywords: *Calcium silicate-based sealer, chlorhexidine gluconate, precipitate, push-out bond strength, root canal irrigants*

Introduction

It is widely accepted thaat there is a positive correlation between the outcome of endodontic treatment and the technical quality of the root canal sealing (1, 2). One of the main aims of root canal filling is to seal the prepared canal to prevent the tissue fluids, bacteria and/or bacterial products to enter into it (3). In order to achieve this aim in root canal filling procedure, it is critical that the sealer used with gutta-percha provides an optimum adhesion to root canal walls (4, 5).

Final irrigation of the root canal is performed after root canal shaping in order to reduce the pre-obturation microbial load within the canal system and to minimize future failure (6). Successive administration of sodium hypochlorite (NaOCl) solution and ethylenediaminetetraacetic acid (EDTA) is the most common final irrigation method used all around the world in order to ensure the chemical debridement of root canals and to remove the smear layer take place on dentinal surfaces after the

ORCID IDs of the authors: D.Ç. 0000-0002-1062-0241; A.T.Ö.K. 0000-0001-6920-1300; T.K. 0000-0001-5764-5510; T.T. 0000-0001-8512-5280

[1]*Department of Endodontics, Faculty of Dentistry, Karadeniz Technical University, Trabzon, Turkey*

[2]*Department of Endodontics, Faculty of Dentistry, Bahcesehir University, Istanbul, Turkey*

Corresponding Author: Tamer Tasdemir

E-mail: tamertd72@yahoo.com

Received: 4 January 2021
Revised: 17 April 2021
Accepted: 1 May 2021

DOI: 10.26650/eor.2021922696

How to cite: *Celik D, Ozalp Koca AT, Kosar T, Tasdemir T. The effects of final irrigants on the push-out bond strength of two calcium silicate-based root canal sealers: an in vitro study. Eur Oral Res 2021; 55(3): 146-151.*

instrumentation (7, 8). On the other hand, it was claimed that it is not expected to see a positive therapeutic effect of NaOCl solution while there is EDTA in the canal (9). Besides the high concentrations of NaOCl is toxic and can irritate the periapical tissues (10). Therefore, chlorhexidine gluconate (CHX) was suggested as an alternative irrigant solution for NaOCl (11). Even though CHX has substantivity property along with antimicrobial activity, it does not have the capability of dissolving vital or necrotic tissues (12). In fact, an irrigant with all the desired physiochemical and ideal microbiological properties has yet to be introduced (13, 14). Therefore, different irrigation solutions that have positive effects on antimicrobial properties of NaOCl can be used after NaOCl for effective irrigation (13, 15). However, it is important to be aware of the possibility that irrigation solutions used successively can chemically react with each other. For example, a mixture of NaOCl and CHX produces an orange-brown, hard-to-remove precipitate that colors the walls of the pulpal cavity (16). Previous researches reported that this precipitate contains para-chloroaniline (PCA), a toxin produces methemoglobin and has a potential carcinogenic effect in time (17, 18). However, recent studies that used advanced analysis techniques showed that the precipitate produced by a mixture of these solutions do not contain PCA (6, 19, 20).

There is a limited data on the effect of precipitates that accumulated in canal walls on the push-out bond strength of sealers to the canal walls. Graziele Magro *et al.* (14) reported that the produced precipitates after final irrigation, which used 2% CHX solutions with different formulas, did not change the push-out bond strength of AH Plus, an epoxy-based sealer. Neelakantan *et al.* (21) reported that using EDTA in final irrigation increased the adhesion force of AH Plus sealer to the root dentin significantly when it is compared with a final irrigation that used NaOCl.

Recently, a number of calcium-silicate based root canal sealers that have the positive attributes of bioceramic cement were introduced. Endoseal MTA and Tech Biosealer Endo are the two examples of these type of sealers. In literature, there is limited knowledge about whether final irrigants affects the push-out bond strength of calcium silicate-based sealers to root canal dentin. The purpose of the present study was to evaluate influence of NaOCl+EDTA, NaOCl+CHX or NaOCl+EDTA+CHX final irrigation regimens on the bond-strength of two calcium silicate based (Endoseal MTA and Tech Biosealer Endo) and one epoxy resin based (AH Plus) sealer. The null hypothesis is that the order of the irrigant solutions used in final irrigation does not affect the dentinal push-out bond strength of these root canal sealers.

Materials and Methods

Ethical statement

This study was reviewed and approved by the Research Ethics Committee of Karadeniz Technical University (Protocol Number: 2021/75). The manufacturers and the material contents of root canal sealers tested in this study can be found in Table 1.

Table 1. *The ingredients and manufacturers of the tested materials.*

Sealer	Manufacturer	Composition
Endoseal MTA	Maruchi, Wonju, Korea	Calcium silicates, calcium aluminates, calcium aluminoferrite, calcium sulfates, radiopacifier, thickening agent
Tech BioSealer Endo	Isasan SRL, Revello Porro, Italy	*Powder*: White Portland cement, bismuth oxide, anhydride, sodium fluoride *Liquid*: Alfacaine SP solution (4% articaine + 1/100.000 epinephrine)
AH Plus	Dentsply DeTrey GmbH, Konstanz, Germany	**Epoxy paste:** diepoxy, calcium tungstate, zirconium oxide, aerosol, and dye **Amine paste:** 1-adamantane amine, N.N'dibenzyl-5 oxanonandiamine-1,9, TCD-diamine, calcium tungstate, zirconium oxide, aerosol, and silicone oil

Sample preparation

Samples were prepared by following the technique described by Scelza *et al.* (22). Newly extracted 15 mandibular premolars were chosen. Teeth were stored in 0.02% sodium azide solution at 4°C until the experiment. Soft tissue residuals on teeth were removed by using a scalpel and crowns were removed by using a low-speed diamond disc (Micracut 125; Metkon, Bursa, Turkey) under continuous water wash. The same low-speed diamond disc was used in order to create 4 horizontal cross-sections (1 ± 0.1 mm thick) in each root in coronal to apical direction. 60 root slices were obtained through this protocol. In each root slice, 1 mm thick cylindrical carbide bur was used in order to open three canal-like cavities parallel to the root canal (Figure 1).

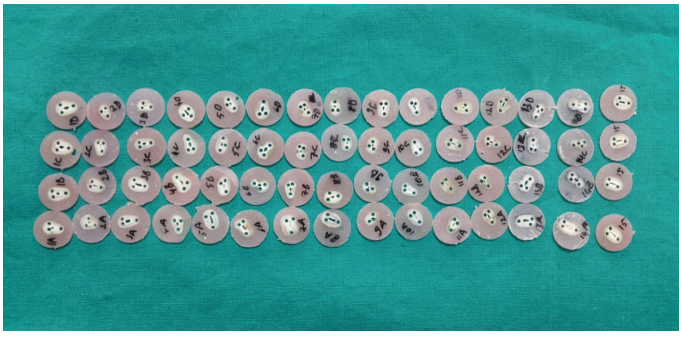

Figure 1. *1.0 mm thick slices were obtained from the roots and three canal-like cavities were opened on each slice.*

The cavities were opened perpendicular to the surface under continuous water wash. The cavities were preserved so as to the distance between the external cement and root canal wall to be 1 mm in minimum. All root slices were divided into 4 different experimental groups according to the final irrigant administration, so each group has 15 slices:

Group-1; the samples were immersed in 5 mL 5.25% NaOCl (Wizard; Rehber Kimya, Istanbul, Turkey) for 15 minutes and then in 5 mL 17% EDTA (Wizard; Rehber Kimya, Istanbul, Turkey) for 3 minutes.

Group-2; the samples were immersed in 5 mL 5.25% NaOCl for 15 minutes, then after drying with absorbent papers, they were immersed in 5 mL 2% CHX (Consepsis; Ultradent, South Jordan, UT, USA) for a minute.

Group-3; the samples were immersed in 5 mL 5.25% NaOCl for 15 minutes and then in 5 mL 17% EDTA for 3 minutes. After drying with the absorbent paper, they were immersed in 5 mL 2% CHX for a minute.

Group-4; the samples were immersed in 5 mL distilled water for 15 minutes.

Then the cavities were dried with absorbent papers and each of the cavities in each of the root slice was randomly filled with one of the chosen sealers: Endoseal MTA (Endoseal; Maruchi, Wonju, Korea), Tech Biosealer Endo (Isasan; Rovello Porro, Italy) or AH Plus (Dentsply DeTrey GmbH, Konstanz, Germany). All of the sealers were mixed and put in cavities according to the protocols provided by the manufacturers. In order to prevent the bubble formation, a weak vibration was applied while placing the sealers into cavities. Lastly, root slices filled with 3 different sealers was incubated at 37° C and 95% relative humidity for 48 hours before push-out analysis in order to harden them completely (Figure 2).

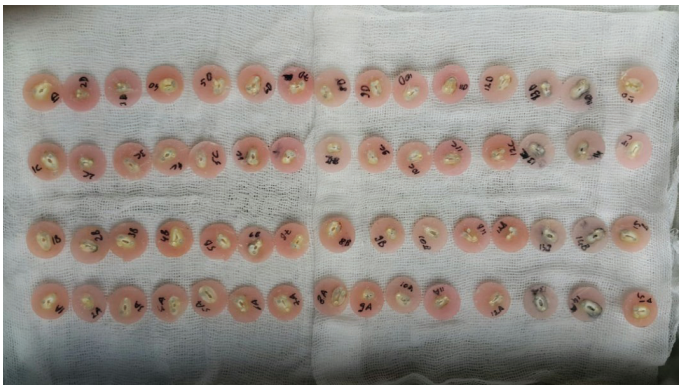

Figure 2. *Each cavity in the dentinal slices was filled with a different sealer.*

Push-out bond strength test

A plunger tip (0.8 mm in diameter) was placed in a way that it was on top of only the test material. The force was always applied in coronal-apical direction. Loading was performed on a universal testing machine (Lloyd Instruments, Foreham, UK) at 0.5 mm/min speed until material dislocation occurred (Figure 3). The load at failure was recorded (in Newtons) and the values were used to calculate the push-out strength in megapascals according to the formula used by Bitter *et al.* (23).

Statistical analysis

The collected data from all groups were imported to Statistical Package for Social Sciences (SPSS) for Windows software, version 17.0 (SPSS Inc., Chicago, IL, USA). The fitness of the data to a normal distribution was analyzed with the Kolmogorov-Smirnov test. The mean, and standard deviation values of the data belong to the independent variables that affect the push-out bond strength (irrigants and canal sealers) were calculated. The mean values of these groups were compared by using one-way ANOVA test ($p<0.05$) and the irrigation protocol values corrected by Bonferroni test with a new threshold level, $p=0.00833$.

Figure 3. *Loading was performed on a universal testing machine at 0.5 mm/min speed until material dislocation occurred.*

Results

The dentinal push-out bond value for each group and the comparison of the groups are summarized in Table 2. AH Plus showed higher push-out bond strength value on CHX groups (Group 2 and 3) than the other two groups ($p<0.001$). The push-out bond strength of Endoseal MTA and Tech BioSealer Endo root canal sealers to dentine were not influenced by the final irrigation protocol ($p=0.965$).

Regardless of the root canal sealer, the use of CHX in final canal irrigation resulted in higher bond strength than

Table 2. *Push-out bond strength (MPa, means±standard deviations) of root canal sealers in canal-like cavities irrigated with different regimes.*

Sealer	NaOCl + EDTA	NaOCl + CHX	NaOCl + EDTA + CHX	Distilled water	Total
AH Plus	8.12±2.37^{a}	12.87±3.89^{b}	10.54±4.65^{b}	6.68±2.59^{a}	9.55±4.16^{A}
Endoseal MTA	5.98±2.2	6.07±2.25	6.87±3.63	5.22±1.6	6.04±2.53^{B}
Tech Biosealer Endo	4.02±0.96	5.17±2.1	5.19±2.11	4.41±1.39	4.7±1.74**B**

CHX: chlorhexidine; EDTA: ethylenediamine tetra acetic acid; NaOCl: sodium hypochlorite. The different small letters indicate significantly differences between final irrigation groups ($p<0.05$).The different capital letters indicate significantly differences between sealers ($p<0.05$)

the other two groups (p=0.003). No difference occurred between the two CHX combinations, likewise, the push-out bond strengths of the other two groups were also similar to each other (p=0.26).

AH Plus showed stronger dentinal push-out bond strength than bioceramic based root canal sealers independently of the final irrigation solutions (p<0.001).

Discussion

The present findings indicate a significant difference in the performance of the tested materials. AH Plus produced stronger push-out bond-strength to the root dentin than Endoseal MTA and Tech Biosealer Endo. Moreover, the push-out bond strength of AH Plus was affected by final irrigant. AH Plus presented stronger push-out bond strength when NaOCl-CHX used as a final irrigant rather than NaOCl-EDTA or distilled water. The push-out bond strength of Endoseal MTA and Tech Biosealer Endo to the root dentin were not affected by final irrigant. Therefore, the null hypothesis was partially rejected.

Different from the previous studies, there is a methodological aspect of this study to be considered. Standard canal-like holes were opened on dentin slices obtained from extracted human teeth in order to establish a more standardized groundwork. Moreover, sealers and irrigants were compared in the same dental samples which allow for better control on confounding factors such as dental age, sclerosis, micro-stiffness of dentine, canal shape, etc. Thus, this experimental setup overcomes the effects of different dentine sources on study design by allowing for placing three different sealers in the same slice (22, 24, 25). All of these biological-chemical-physical variances of the root dentine can increase sealer retention in the undercut nonprepared areas of natural root canals. Also, all the canal-like cavities were opened as a cylinder-shape with 1mm in diameter in order to maintain a standard root canal anatomy between the groups (25). In addition, the holes were filled only with sealers so that the load is fully applied to this material. Thus, erroneous interpretations of the performance of the gutta-percha are avoided (26). Although this method does not exactly reflect clinical practice is very useful for standardizing examples.

After discovering that the precipitate produced after successive usage of CHX and NaOCl does not contain a carcinogenic PCA, CHX solution, which has a potential to contribute to the antimicrobial activity of NaOCl, can be added to the standard irrigation protocol more easily (6, 19, 20). As it is planned to examine the effects of the precipitates, which emerge upon mixing the two solutions, on the dentinal adhesions of root canal sealers, neutralizing solutions were not used between the successive irrigants in the CHX groups of the present study. Push-out bond strength of calcium silicate-based sealers was not affected by irrigant combinations, while the push-out bond strength of AH Plus sealer to the dentine surprisingly increased compared to NaOCl-EDTA combination in dentinal samples that were immersed in NaOCl and CHX solutions successively. In a previous study, different CHX formulations used in final irrigant caused a higher chemical precipitate and smear layer on radicular dentin compared to irrigation protocol that contains 17% EDTA and 2.5% NaOCl (14). However, these residuals did not change the push-out bond strength of epoxy resin-based sealer (AH Plus) on radicular dentin (14, 27). The reason for this difference between the result of the present study and the results of previous studies may be due to the fact that the push-out bond strength experiments in those studies are performed on the main root canals. AH Plus can chemically bond to dentinal collagen amino groups. In order to establish a good bonding, it is necessary to bring out and more importantly to protect the collagen network (28, 29). From this perspective, amino groups that were dwelled on dentine depending on the irrigation solution can affect the adhesion of resin-based sealer to the canal walls (30).

There is a limited data in the literature about the dentinal push-out bond strength of both calcium silicate-based sealers, Endoseal MTA and Tech Biosealer Endo. In the present study, when final irrigant is not taken into account, Endoseal MTA and Tech Biosealer Endo root canal sealers showed weak dentinal push-out bond strength compared to AH Plus. Similarly, Oliveira *et al.* reported that AH Plus had significantly higher bond strength than both MTA Fillapex and iRoot SP (31). Furthermore, in a recent systematic review and meta-analysis, it has been reported that bioceramic sealers do not perform better than traditional epoxy sealers in terms of dislodgement resistance (32). *Silva et al.* used artificial canals opened in dentinal slices for push-out strength experiment, as the present study, and found that push-out strength of Endoseal MTA is weaker than of AH Plus but stronger than of MTA Fillapex (25). The push-out bond strength of Tech Biosealer Endo did not improve with the irrigation protocols in the present study. These results are in accordance with an earlier report that presented the push-out strength of Tech Biosealer Endo when root canals were irrigated with NaOCl-EDTA or NaOCl-CHX (33).

Conclusion

Within the limitations of this *in vitro* study, using CHX after NaOCl in final irrigation leads to increase in push-out bond strength of epoxy resin-based sealers, whereas it does not affect the dentinal bond strength of calcium silicate-based sealers.

Türkçe Özet: *Final irrigantların iki kalsiyum silikat esaslı kök kanal patının push-out bağlanma dayanımına etkileri: in vitro çalışma. Amaç: Bu çalışmanın amacı, final irrigasyonda kullanılan farklı irrigant kombinasyonlarının, farklı içeriklere sahip kök kanal patlarının push-out bağlanma dayanımları üzerine etkilerini incelemektir. Gereç ve Yöntem: 15 adet alt premolar dişin her birinden 1 mm kalınlığında 4 dilim olacak şekilde, toplam 60 dentin dilimi toplandı. Her dentin dilimi üzerinde 3 adet kanal benzeri yapay kavite açıldı. Örnekler her biri 15 örnekten oluşan 4 deney grubuna ayrıldı. Örnekler grup 1'de sırasıyla %5.25 NaOCl ve %17 EDTA solüsyonlarına; grup 2'de sırasıyla %5.25 NaOCl ve %2 CHX solüsyonlarına; grup 3'te sırasıyla %5.25 NaOCl, %17 EDTA ve %2 CHX solüsyonlarına; ve grup 4'de distile su içine daldırıldılar. Dentin dilimlerindeki her bir yapay kavite emici kâğıtlarla kurutulduktan sonra, farklı kanal patlarıyla (Endoseal MTA, Tech Biosealer Endo veya AH Plus) dolduruldu. İki gün sonra, push-out bağlanma dayanımı testi yapıldı. Bulgular: CHX içeren iki final irrigant kombinasyonunda da (grup 2 ve 3) AH Plus daha yüksek push-out bağlanma dayanımı gösterdi (p <0,001). Endoseal MTA ve Tech Biosealer Endo kök kanal patlarının push-out bağlanma dayanımları final irriganttan etkilenmedi (p=0,965). Sonuç: Final irrigasyonda NaOCl'den sonra CHX kullanılması epoksi rezin bazlı kanal patının push-out bağlanma dayanımını arttırırken, kalsiyum silikat esaslı kanal patlarının*

bağlanma dayanımında herhangi bir fark oluşturmadı. Anahtar kelimeler: Kalsiyum silikat esaslı kanal patı, klorheksidin glukonat, çökelti, push-out bağlanma dayanımı, kök kanal irrigantları.

Ethics Committee Approval: This study was reviewed and approved by the Research Ethics Committee of Karadeniz Technical University (Protocol Number: 2021/75).

Informed Consent: Participants provided informed consent.

Peer-review: Externally peer-reviewed.

Author contributions: DC, TT participated in designing the study. ATOK, TK participated in generating the data for the study. ATOK, TK participated in gathering the data for the study. DC participated in the analysis of the data. DC, TT wrote the majority of the original draft of the paper. DC, TT participated in writing the paper. ATOK has had access to all of the raw data of the study. DC, TT have reviewed the pertinent raw data on which the results and conclusions of this study are based. DC, ATOK, TK, TT have approved the final version of this paper. DC, ATOK, TK, TT guarantee that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: The authors declared no conflict of interest.

Financial Disclosure: The authors declared that they have received no financial support.

References

1. Dugas NN, Lawrence HP, Teplitsky PE, Pharoah MJ, Friedman S. Periapical health and treatment quality assessment of root-filled teeth in two Canadian populations. Int Endod J 2003; 36: 181–92. [CrossRef]
2. Ureyen Kaya B, Kececi AD, Guldas HE, Orhan H. A retrospective radiographic study of coronal-periapical status and root canal filling quality in a selected adult Turkish population. Med Princ Pract 2013; 22: 334–9. [CrossRef]
3. ElAyouti A, Achleithner C, Löst C, Weiger R. Homogeneity and adaptation of a new gutta-percha paste to root canal walls. J Endod 2005; 31: 687–90. [CrossRef]
4. Saleh I, Ruyter I, Haapasalo M, Ørstavik D. The effects of dentine pretreatment on the adhesion of root-canal sealers. Int Endod J 2002; 35: 859–66. [CrossRef]
5. Schwartz RS. Adhesive dentistry and endodontics. Part 2: bonding in the root canal system-the promise and the problems: a review. J Endod 2006; 32: 1125–34. [CrossRef]
6. Thomas JE, Sem DS. An in vitro spectroscopic analysis to determine whether para-chloroaniline is produced from mixing sodium hypochlorite and chlorhexidine. J Endod 2010; 36: 315–7. [CrossRef]
7. Zehnder M, Schmidlin P, Sener B, Waltimo T. Chelation in root canal therapy reconsidered. J Endod. 2005; 31: 817-20. [CrossRef]
8. Haapasalo M, Shen Y, Wang Z, Gao Y. Irrigation in endodontics. Br Dent J 2014; 216: 299-303. [CrossRef]
9. Clarkson RM, Podlich HM, Moule AJ. Influence of ethylenediaminetetraacetic acid on the active chlorine content of sodium hypochlorite solutions when mixed in various proportions. J Endod 2011; 37: 538–43. [CrossRef]
10. Hülsmann M, Rödig T, Nordmeyer S. Complications during root canal irrigation. Endod Topics 2009; 16: 27–63. [CrossRef]
11. Hashem AA, Ghoneim AG, Lutfy RA, Fouda MY. The effect of different irrigating solutions on bond strength of two root canal-filling systems. J Endod 2009; 35: 537–40. [CrossRef]
12. Naenni N, Thoma K, Zehnder M. Soft tissue dissolution capacity of currently used and potential endodontic irrigants. J Endod 2004; 30: 785–7. [CrossRef]
13. Zehnder M. Root canal irrigants. J Endod 2006; 32: 389–98. [CrossRef]
14. Magro MG, Kuga MC, Regina Victorino K, Vázquez-Garcia FA, Aranda-Garcia AJ, Faria-Junior NB, Faria G, Shinohara AL. Evaluation of the interaction between sodium hypochlorite and several formulations containing chlorhexidine and its effect on the radicular dentin - SEM and push-out bond strength analysis. Microsc Res Tech 2014; 77: 17-22. [CrossRef]
15. Kuruvilla JR, Kamath MP. Antimicrobial activity of 2.5% sodium hypochlorite and 0.2% chlorhexidine gluconate separately and combined, as endodontic irrigants. J Endod 1998; 24: 472–6. [CrossRef]
16. Bui T, Baumgartner J, Mitchell J. Evaluation of the interaction between sodium hypochlorite and chlorhexidine gluconate and its effect on root dentin. J Endod 2008; 34: 181–5. [CrossRef]
17. Burkhardt-Holm P, Oulmi Y, Schroeder A, Storch V, Braunbeck T. Toxicity of 4-chloraniline in early life stages of Zebrafish (Danio rerio): II—cytopathology and regeneration of liver and gills after prolonged exposure to waterborne 4 chloraniline. Arch Environ Contam Toxicol 1999; 37: 85–102. [CrossRef]
18. Basrani BR, Manek S, Sodhi RNS, Fillery E, Manzur A. Interaction between sodium hypochlorite and chlorhexidine gluconate. J Endod 2007; 33: 966–9. [CrossRef]
19. Orhan EO, Irmak Ö, Hür D, Yaman BC, Karabucak B. Does Para-chloroaniline really form after mixing sodium hypochlorite and chlorhexidine? J Endod 2016; 42: 455–9.
20. Irmak Ö, Orhan EO, Görgün K, Yaman BC. Nuclear magnetic resonance spectroscopy and infrared spectroscopy analysis of precipitate formed after mixing sodium hypochlorite and QMix 2in1. PLoS One 2018; 13: e0202081.
21. Neelakantan P, Sharma S, Shemesh H, Wesselink PR. Influence of irrigation sequence on the adhesion of root canal sealers to dentin: a fourier transform infrared spectroscopy and push-out bond strength analysis. J Endod 2015; 41: 1108–11. [CrossRef]
22. Scelza MZ, da Silva D, Scelza P, de Noronha F, Barbosa IB, Souza E, De Deus G. Influence of a new push-out test method on the bond strength of three resin-based sealers. Int Endod J 2015; 48: 801–6. [CrossRef]
23. Bitter K, Meyer-Lueckel H, Priehn K, Kanjuparambil JP, Neumann K, Kielbassa AM. Effects of luting agent and thermocycling on bond strengths to root canal dentine. Int Endod J 2006; 39: 809-18. [CrossRef]
24. Silva EJ, Carvalho NK, Zanon M, Senna PM, DE-Deus G, Zuolo ML, Zaia AA. Push-out bond strength of MTA HP, a new high-plasticity calcium silicate-based cement. Braz Oral Res 2016; 30: e84.
25. Silva EJ, Carvalho NK, Prado MC, Zanon M, Senna PM, Souza EM, De-Deus G. Push-out bond strength of injectable Pozzolan-based root canal sealer. J Endod 2016; 42: 1656–9. [CrossRef]
26. Silva EJNL, Carvalho NK, Prado MC, Senna PM, Souza EM, De-Deus G. Bovine teeth can reliably substitute human dentine in an intra-tooth push-out bond strength model? Int Endod J 2019; 52: 1063–9.
27. Magro MG, Kuga MC, Aranda-Garcia AJ, Victorino KR, Chávez-Andrade GM, Faria G, Keine KC, Só MVR. Effectiveness of several solutions to prevent the formation of precipitate due to the interaction between sodium hypochlorite and chlorhexidine and its effect on bond strength of an epoxy-based sealer. Int Endod J 2015; 48: 478–83. [CrossRef]
28. Prati C, Chersoni S, Pashley DH. Effect of removal of surface collagen fibrils on resindentin bonding. Dent Mater 1999; 15: 323–31. [CrossRef]
29. Fisher MA, Berzins DW, Bahcall JK. An in vitro comparison of bond strength of various obturation materials to root canal dentin using a push-out test design. J Endod 2007; 33: 856–8. [CrossRef]
30. Güzel C, Uzunoglu E, Dogan Buzoglu H. Effect of low–surface tension EDTA solutions on the bond strength of resin-based sealer to young and old root canal dentin. J Endod 2018; 44: 485–8. [CrossRef]

31. Oliveira DS, Cardoso ML, Queiroz TF, Silva EJNL, Souza EM, De-Deus G. Suboptimal push-out bond strengths of calcium silicate-based sealers. Int Endod J 2016; 49: 796–801. [CrossRef]
32. Silva EJNL, Canabarro A, Andrade MRTC, Cavalcante DM, Von Stetten O, Fidalgo TKDS, De-Deus G. Dislodgment resistance of bioceramic and epoxy sealers: a systematic review and meta-analysis. J Evid Base Dent Pract 2019; 19: 221-35. [CrossRef]
33. Neelakantan P, Nandagopal M, Shemesh H, Wesselink P. The effect of root dentin conditioning protocols on the push-out bond strength of three calcium silicate sealers. Int J Adhes Adhes 2015; 60: 104–8. [CrossRef]

eor.istanbul.edu.tr

Official Publication of Istanbul University Faculty of Dentistry

Eur Oral Res 2021; 55(3): 152-161

Original research

Evaluation of a collagen-bioaggregate composite scaffold in the repair of sheep pulp tissue

Burak Dayı[1], Deniz Sezlev Bilecen[2,3], Hatice Eröksüz[4], Muhammet Yalçın[5], Vasıf Hasırcı[3,6]

Purpose
This study aimed to compare the effects of the collagen-BioAggregate mixture (CBA-M) and collagen-BioAggregate composite (CBA-C) sponge as a scaffolding material on the reparative dentin formation.

Materials and Methods
CBA-C sponge (10:1 w/w) was obtained and characterized by Scanning Electron Microscopy (SEM) and Mercury Porosimetry. Cytotoxicity of the CBA-C sponge was tested by using the L929 mouse fibroblast cell line. Dental pulp stem cells (DPSCs) were isolated from the pulp tissue of sheep teeth and characterized by flow cytometry for the presence of mesenchymal stem cell marker, CD44. The osteogenic differentiation capability of isolated DPSCs was studied by Alizarin Red staining. The cells were then used to study for the compatibility of CBA-C sponge with cell proliferation and calcium phosphate deposition. The effect of CBA-C sponge and CBA-M on the induction of dentin regeneration was studied in the perforated teeth of sheep for the eight-week period. All the analyses were performed with appropriate statistical hypothesis tests.

Results
CBA-C sponge was found to be biocompatible for DPSCs. The DPSCs seeded on the CBA-C sponge were able to differentiate into the osteoblastic lineage and deposit calcium phosphate crystals *in vitro*. Reparative dentin formation was observed after the second week in the CBA-C sponge applied group. At the end of eight weeks, a complete reparative dentin structure was formed in the CBA-C sponge applied group, whereas necrotic tissue residues were observed in groups treated with the CBA-M.

Conclusion
CBA-C sponge represents a better microenvironment for reparative dentin formation probably due to maintaining DPSCs and allowing their osteogenic differentiation and thus calcium phosphate deposition.

Keywords: *Direct pulp capping, reparative dentin, collagen sponge, BioAggregate, BioAggregate-sponge composite*

Introduction

Vital pulp therapy is defined as a treatment used to perform an indirect pulp application in teeth with deep caries. It can be applied in two ways; by direct pulp capping or pulpotomy in patients with exposed dental pulp tissue (1). Direct pulp capping is a technique in which the pulp protecting agent is directly applied to the exposed pulp tissue. This treatment is used to protect the pulp tissue against further damage, and it allows the regeneration of the dentin pulp complex (2). Dentin pulp complex protection is achieved by performing a single or multilayer pulp capping between the dental tissue and the restorative material. Dentin pulp com-

Presented at: *The characterization data of collagen-BioAggregate scaffold and histopathological findings of fourth and eighth week of collagen-BioAggregate scaffold group of this study was presented as an oral presentation with the title of "The effect of collagen-Bioaggregate scaffold on perforated dental pulp" on the 21th BaSS Congress, May 12-15, 2016, Banja Luka, Bosnia and Herzegovina.*

ORCID IDs of the authors: B.D. 0000-0002-5289-438X; D.S.B. 0000-0002-5708-2276; H.E. 0000-0002-8407-5792; M.Y. 0000-0003-2077-521X; V.H. 0000-0002-3698-8861

[1]Department of Restorative Dentistry, Faculty of Dentistry, Inonu University, Malatya, Turkey

[2]Department of Molecular Biology and Genetics, Faculty of Agriculture and Natural Sciences, Konya Food and Agriculture University, Konya, Turkey

[3]BIOMATEN, METU Center of Excellence in Biomaterials and Tissue Engineering, Ankara, Turkey

[4]Department of Pathology, Faculty of Veterinary Medicine, Firat University, Elazığ, Turkey

[5]Private Practice, Malatya, Turkey

[6]Department of Medical Engineering, Faculty of Engineering, Acibadem University, İstanbul, Turkey

Corresponding Author: Burak Dayı

E-mail: bdayi70@hotmail.com

Received: 10 April 2021
Revised: 4 May 2021
Accepted: 17 May 2021

DOI: 10.26650/eor.2021911441

How to cite: *Dayi B, Sezlev Bilecen D, Eroksuz H, Yalcin M, Hasirci V. Evaluation of a collagen-bioaggregate composite scaffold in the repair of sheep pulp tissue. Eur Oral Res 2021; 55(3): 152-161.*

plex is protected to prevent damages that occur due to the bacterial invasion resulting from operative procedures, the toxicity of the restorative material, and microleakage. Dentin pulp tissue protection is ensured by recovering the vitality of the pulp (3).

Mineral Trioxide Aggregate (MTA) was introduced by Torabinejad and White as the first calcium silicate based material in the 1990s (4). In the following periods newer modifications, such as BioAggregate (in 2006), Biodentine (in 2009), EndoSequence BC RRM (in 2009) and TheraCal LC (patented in 2008) were reported (5,6). BioAggregate is a biocompatible material, and it is safe to use it in the pulp capping process. It is composed of ceramic particles, and its content is similar to the MTA. It can be used in perforation repair and treatment of vital pulp (7).

Tissue engineering is a multidisciplinary field that applies engineering principles to restore, maintain, and develop the normal functions of organs (8). There are three components of tissue engineering; living cells, carriers of cells (scaffolds), and signal molecules such as growth factors (9).

Scaffolds are three-dimensional networks of synthetic or natural polymers that serve as extracellular matrices for cells for a limited duration. They allow cells to migrate and propagate and are used to create tissue mimics in pre-designed forms and structures. Natural polymers have excellent biocompatibility, whereas synthetic polymers possess controlled physico-chemical properties such as dissolution rate, microstructure, and mechanical strength (10). Various synthetic materials do not satisfy the requirements expected of scaffolds in tissue engineering applications, and therefore, animal-derived natural polymers such as collagen and elastin are preferred over the synthetics to create better carriers (11).

Regeneration of pulp tissue is an important alternative for direct pulp capping to more traditional restorative procedures (12). Dental pulp stem cells (DPSCs), which are being used mainly in tooth regeneration studies, are multipotent stem cells that can be isolated from dental tissue for pulp regeneration. They can differentiate into a variety of cell types, including adipocytes, chondrocytes, osteoblasts, and odontoblasts, while exhibiting more substantial odontogenesis characteristics (13). Therefore, it is important to develop new bioactive materials for managing the dental pulp regeneration.

In this study, collagen and BioAggregate materials were mixed and used either directly or in the form of a sponge as a capping material in sheep teeth with perforated pulp tissues. The aim of this study was to compare the effects of collagen-BioAggregate mixture and collagen-BioAggregate composite sponge on reparative dentin formation within an eight-week period. The null hypothesis of this study was that the collagen-BioAggregate mixture and collagen-BioAggregate composite sponge would have no difference in reparative dentin formation during all weeks studied.

Materials and methods

Ethical statement

All the surgical procedures were approved by the Animal Testing Ethics Committee of Firat University, Elazığ, Turkey, with meeting number 2014/9 and decree number 96.

Isolation of DPSCs

Pulps from sheep teeth (n=4) were minced into 1-2 mm pieces with a scalpel under sterile conditions. The pieces were transferred to T25 tissue culture flasks and kept in growth media (DMEM with 4.5 g/L glucose, 10% fetal bovine serum, 1% penicillin-streptomycin, 0.4% amphotericin-B) (5% CO_2, 37^{0}C). The outgrowth of the DPSCs was observed starting from 72 h and visualized under a phase-contrast microscope (Olympus IX70, Leica, USA). The culture was continued until confluency was reached. Cells were harvested by trypsinization, and the cell pellet was passed through a cell strainer (70 µm). The cells were subcultured or frozen in 90% FBS, 10% DMSO solution until use.

Characterization of DPSCs by flow cytometry

The isolated stem cells were analyzed with flow cytometry for the hematopoietic and mesenchymal stem cell markers, CD34 and CD44, respectively (AccuriC6, BD, Germany). Briefly, cells were fixed (PFA,4%) and washed with FACS buffer (PBS, 1:100 BSA, and 1:1000 sodium azide). The cell pellet was resuspended in primary antibody solutions for stem cell markers CD34 and CD44 (100 µL; 1:60 CD34 Rabbit Monoclonal, Abcam, ab81289 and 0.02 µg CD44 Rat Monoclonal, Abcam, ab119335) and incubated for one h at room temperature (14,15). After washing twice with FACS buffer, the cells were incubated with secondary antibodies (10 µg/mL Goat anti-rabbit IgG H+L, Alexa 488, Invitrogen A-11034, and Goat anti-rat IgG H+L, Alexa 647, Invitrogen A-21247) for one h at room temperature. Cell nuclei were stained with Draq5 (1:1000). The cells were washed twice with FACS buffer and resuspended in PBS. Negative controls were unstained cells, isotype control (1:100 Rabbit IgG 488 Isotype control, Abcam ab153686, and 0.02 µg Rat IgG 647 Isotype control, Invitrogen R2a21), and only secondary antibody stained cells.

Osteogenic differentiation of DPSCs and maintenance of osteoblastic cells

Isolated stem cells were subjected to osteogenic differentiation by using OriCell™ Mesenchymal Stem Cell Osteogenic Differentiation Medium (Cyagen, USA) according to the manufacturer's protocol. Briefly, 2x10^4 cells were seeded into six-well plates and incubated for 21 days for differentiation medium containing 10% FBS, 1% Penicillin-Streptomycin, 1% L-Glutamine, 1% β-Glycerophosphate, 0.2% Ascorbate, and 0.01% Dexamethasone. At the end of days 7 and 21, cells were fixed in 4 % PFA for 30 min, washed twice with PBS, and incubated in Alizarin red solution (Cyagen, USA) to stain the calcium deposition. After the osteogenic differentiation process, obtained osteoblasts were maintained in McCoy's 5A medium (Gibco, UK) supplemented with 10% FBS and 1% Penicillin-Streptomycin.

Sample preparation and characterization of collagen-BioAggregate composite sponges

BioAggregate (1.5 mg; DiaDent, Burnaby, BC, Canada) and collagen (15 mg; Sigma-Aldrich, Germany) (10:1, w/w) were mixed and collagen-BioAggregate mixture (CBA-M) sample was prepared.

BioAggregate (1.5 mg; DiaDent, Burnaby, BC, Canada) and collagen (15 mg; Sigma-Aldrich, Germany) were dissolved in acetic acid (0.5%), 51 µL was added to 96 well plates, frozen overnight at -20^{0}C, and lyophilized for 8 h (Labconco, USA) and collagen-BioAggregate composite (CBA-C) sponges (diameter: 1.5 mm, thickness: 1.5 mm) were obtained. Dehydrothermal crosslinking (heating at 150^{o}C for 24 h under vacuum) was applied to the sponges and then kept in a desiccator at room temperature until use. Pure collagen sponges were prepared using the same procedure without the addition of the BioAggregate.

Surface topography of pure collagen and CBA-C sponges were studied using Scanning Electron Microscopy (SEM, Quanta, USA). Their porosity was determined by Mercury Porosimetry (Quantachrome, USA) and ImageJ Analysis Software Programme (NIH, USA).

Determination of cell viability

Cytotoxicity of the sponges was tested using the L929 mouse fibroblast cell line (16, 17). 2x10^4 cells were seeded on 24 well plates and incubated for 24 h for cell attachment. CBA-C sponges were then introduced onto the cells and incubated for 48 h. After 48 h, the medium was removed, and 10% Alamar Blue solution in colorless DMEM (1mL) was added onto the cells and incubated for one h. The optical density was measured at 570 and 595 nm with a microplate reader (SpectraMax M2, Molecular Devices, USA). Cell viability was determined with a calibration curve plotted from percent reductions and corresponding cell numbers (18).

Proliferation of cells on CBA-C sponges

In order to determine whether the CBA-C sponges would represent a compatible environment for cell proliferation, 2x10^4 DPSCs were seeded on composite sponges and subjected to osteogenic differentiation for 21 days in the differentiation medium mentioned in the previous section. In addition, 2x10^4 cells differentiated to osteoblastic cells were seeded on separate sponges and cultured for 21 days in McCoy's 5A maintenance medium. The media were changed every two days. On days 1, 7, 14, and 21, cell numbers on the sponges were determined by Alamar blue cell proliferation assay. DPSCs and osteoblastic cells seeded on tissue culture polystyrene (TCPS) were used as the controls of the experiment. The proliferation assay was performed in triplicate wells for each group.

Calcium phosphate deposition on sponges

To determine the calcium phosphate deposition on sponges, 2x10^4 DPSCs, and DPSCs differentiated to osteoblastic cells were seeded on separate CBA-C sponges. DPSCs seeded on the composite sponges were subjected to the differentiation process for 21 days. Osteoblastic cells seeded on composite sponges, on the other hand, were cultured in the maintenance medium for 21 days. At the end of the culture period, the cells on the sponges were stained with osmium tetraoxide and analyzed with SEM. For osmium tetraoxide staining, cell-seeded sponges were washed with PBS twice and fixed (4% PFA) for 5 min at room temperature. The specimens were then washed with PIPES (piperazine-N, N'-bis (ethanesulfonic acid)) buffer twice and then incubated in 1% osmium tetroxide (OsO4) in PIPES buffer for one h at room temperature. After a second wash with PIPES buffer, the sponges were dehydrated by incubating in a series of ethanol concentrations of 50%, 70%, and 100% at room temperature for 5 min. Specimens were Au-Pd coated under vacuum and examined with SEM (400F Field Emission SEM, USA).

Application and monitoring of the CBA-C sponges and mixture of collagen and BioAggregate in the experimental animals

Randomly selected ten sheeps (Akkaraman sheeps) were deprived of water for six h and fasted for 18 h to prevent nausea and hypersalivation due to the anesthesia. On the day of the experiment, animals were weighed, and their average weight was 42 kg. Doses of the anesthetics were determined according to the average weight of animals.

For the anesthesia, 0.1 mg/kg Alfazyne %2 (Alfasan International B.V., Woerden, The Netherlands) containing XylazineHCl and then 5 mg/kg Ketasol 10% (Richter Pharma AG, Wels, Austria) were applied intramuscularly in semi-membranosus and semi-tendinous regions. This dose of anesthesia did not lead to respiratory depression, and thus intubation was not necessary. Infiltrating local anesthesia was applied by using Jetokain (Adeka, Samsun, Turkey) in order to prevent the pain after the operation.

A portable dental unit system (Dynamic, China) was used for cavity preparation. Diamond carbide bur (number 10, Diatech, USA) was used for the enamel, and tungsten carbide bur (number 10, Diatech, USA) was used for the dentin layer. 1 mm diameter perforations were formed by using the equipments in the pulp tissues of animals under sterile conditions.

The mixture of collagen (15 mg) and BioAggregate (1.5 mg) (CBA-M) was applied to perforated left central and lateral teeth. CBA-C sponges, on the other hand, were cut to fit the perforated area and placed on the right central and lateral teeth. Then, glass ionomer cement (Fuji IX GC, USA) was placed, and the tooth was restored by using the composite filling material (Arabesk, Voco, Germany) and two-step self-etch bonding agent (Clearfil SE Bond, Kuraray Medical, Tokyo, Japan).

Animals were randomly divided into five groups (n: 2) and were decapitated respectively in the first, second, third, fourth, and eighth weeks. Tooth samples were fixed in 10% formaldehyde (Sigma-Aldrich, Germany) and transferred for histological examination.

Fixation of the teeth

Tooth samples in formalin solution were shaken manually (1-2 min) twice a day for ten days to allow better penetration of formalin solution (10%, Sigma Aldrich, Germany) into the teeth. At the end of day 10, teeth were removed from formalin and washed with distilled water for 1-2 min. They were then placed in plastic containers.

Demineralization of teeth

After the fixation of each tooth, ethylenediaminetetraacetic acid (EDTA) based demineralization solution (30 mL; Osteosoft, Merck, Germany) was added to the container and incu-

bated in the dark for five weeks. The solution was refreshed once a week, and each container was gently shaken (1-2 min) twice a day. The demineralization process was finalized at the end of the fifth week when the teeth became extremely flexible.

Preparation of teeth for the histological section

Teeth were placed in embedding cassettes (Isolab Embedding Cassettes, Germany), washed with tap water for several hours, and dehydrated in 70, 80, 96, and finally 99% aqueous ethanol solutions (Sigma-Aldrich, Germany). After dehydration, teeth were incubated in xylene (4 h) in the automatic tissue processor (Leica TP 1020). Teeth were primarily cut into two pieces along their longitudinal axes to obtain sections from pulp tissues and cavities, and then blocked with paraffin.

Ten sections (3µm thick) for each tooth were obtained using a rotary microtome (Leica RM 2125) and placed in the flotation bath (42^0 C, 5-10 s) (Leica HI 1210, Germany). The slides were deparaffinized (68^{0}C for 45 min), immersed in xylene solutions (5 min, 10 times), dried, stained with hematoxylin-eosin and then dehydrated in serial aqueous alcohol solutions followed by drying at 68^{0}C, 10 min. Microscopic imaging and measurements were performed using Olympus BX43 Trinocular attachment Fluorescent Light Microscope and Imaging System with CellSens Standard Software. Inflammation (capillary hyperemia, inflammatory cells and fibrosis) in the pulp was evaluated qualitatively. The amount of reparative dentin was measured in the perforation area from four different points in two tooth sections (Figure 1). Eight measurements were made for each tooth sample.

Statistical analysis

Quantitative data were summarized as mean±standard deviation values. Since the quantitative data did not display a normal distribution with respect to the groups (p<0.05), the non-parametric Kruskal-Wallis H test was utilized for the comparison of the study groups. Significant differences among the groups were determined with the Kruskal-Wallis H test (p<0.05), pairwise comparisons of reparative dentin thickness were performed through the posthoc Bonferroni test (p<0.05). The same time periods of the materials were compared via the Mann Whitney U test. SPSS version 13.0 for Windows (SPSS, Inc., IL, USA) was employed for all analyses. The level of statistical significance was set at $p < 0.05$.

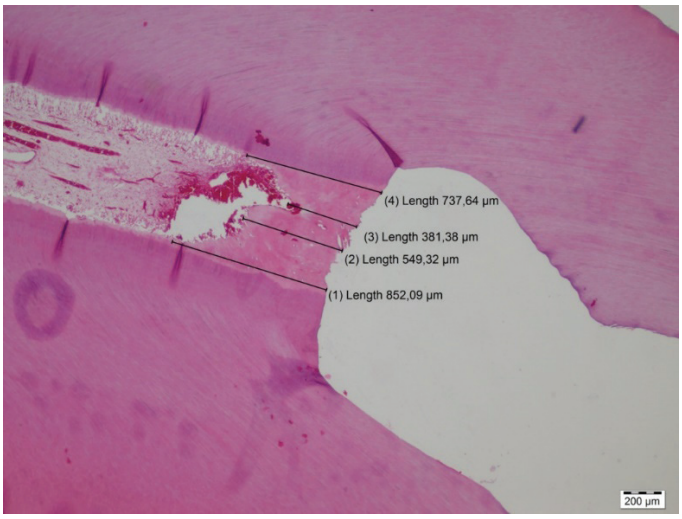

Figure 1. *Quantification of reparative dentin thickness.*

Results

DPSCs isolation and characterization

Stem cells were isolated from sheep dental pulp by using the outgrowth method (Figure 2). The cells were visualized using phase-contrast microscopy and were examined with flow cytometry for their stem cell markers. The cells were found to be negative for hematopoietic stem cell marker CD 34 and positive for the mesenchymal stem cell marker CD 44 (15,19) (Figure 2).

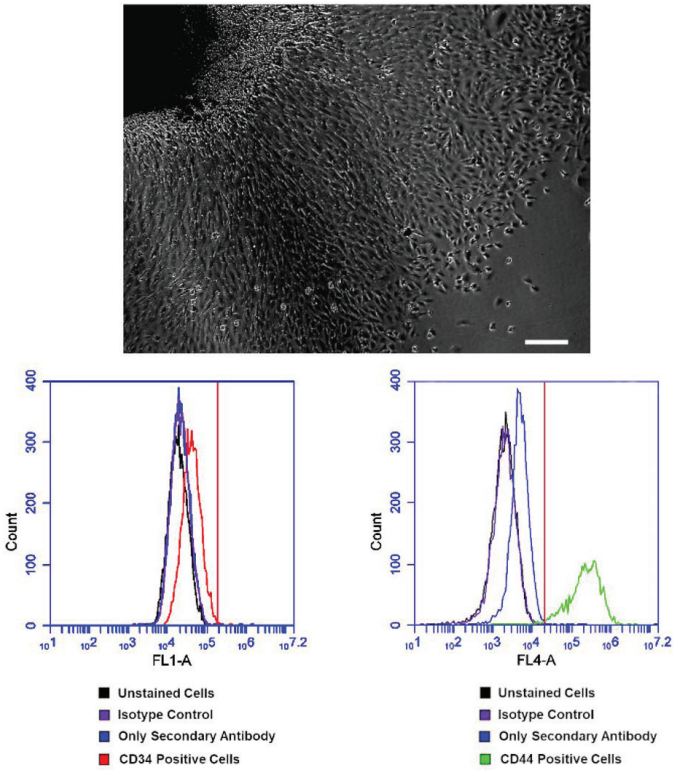

Figure 2. *Outgrowth of DPSCs from minced pulp tissue at the end of week 1 (top). Flow cytometry analysis of isolated DPSCs (bottom). Scale bar: 200 µm.*

Osteogenic differentiation of the isolated cells into an osteoblastic cell lineage

The isolated DPSCs were subjected to osteogenic differentiation for 21 days in an induction medium containing ascorbic acid, dexamethasone, and β-glycerophosphate (20). On days 7 and 21 of osteogenic induction, Alizarin Red staining was performed to visualize the calcium phosphate minerals deposited by the cells as an indicator of osteogenic differentiation (Figure 3). It was observed that induction of 21 days was satisfactory for calcium deposition and thus differentiation.

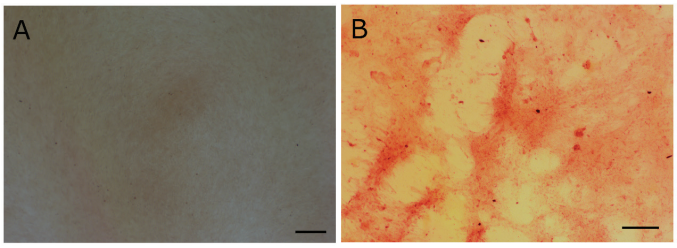

Figure 3. *Alizarin Red staining of DPSCs in osteogenic differentiation. A) 7 days, B) 21 days post osteogenic induction. Scale bar: 200 µm.*

Characterization of pure collagen and CBA-C sponges

The surface characteristics of pure lyophilized collagen and CBA-C sponges were examined with SEM (Figure 4). It was observed that both pure and the composite sponges are highly porous. The addition of BioAggregate did not affect the formation of the highly porous structure of the composite sponges, but the level of porosity was significantly decreased upon BioAggregate addition.

The porosity of the sponges was measured using mercury porosimetry and calculated from the SEM micrographs by using ImageJ software. It was observed that the addition of BioAggregate resulted in a decrease of porosity (reduced from 86% to 59%) when measured with the mercury porosimeter (Table 1). On the other hand, analysis of SEM micrographs by using Image J software indicated that the collagen sponge's porosity was 62% and decreased to 36% upon BioAggregate in a corporation.

Pore size distributions of pure and composite collagen sponges obtained from SEM micrographs are presented in Figure 5. It was observed that the pure collagen sponge has a higher fraction of larger pores than the CBA-C sponge.

Alamar blue cell viability test

In order to study the cell viability directly by Alamar Blue cell viability test, L929 mouse fibroblast cells were seeded on

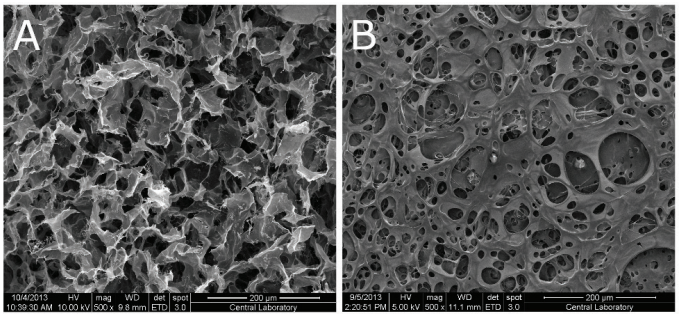

Figure 4. *SEM micrographs of collagen-based sponges. A) Pure collagen, B) Collagen-BioAggregate (10:1, w/w) composite sponge.*

Table 1. *Porosity of the sponges measured with two different methods.*

Sample	Porosity (%)	
	Mercury Porosimetry	SEM
Pure Collagen Sponge	86	62
CBA-C Sponge	59	36

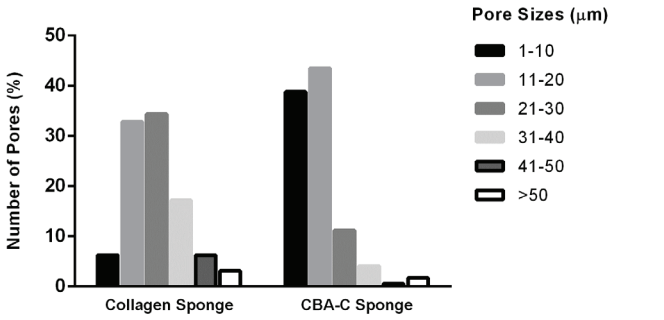

Figure 5. *Pore size distribution of the Collagen and CBA-C sponge as determined by using ImageJ using SEM micrographs.*

TCPS wells, and then pure and composite collagen sponges were placed on top. It was found that the percent viability of cells in the wells where collagen sponges are placed were 70% and 65% with pure collagen and CBA-C sponges, respectively. The viability was less than TCPS control with no sponge (Table 2); however, they are not significantly lower than the 70% limit indicated in ISO10993-5 as the limit of non-cytotoxicity.

Table 2. *Cell viability after 48 h of direct contact with the sponges, *seeded cell density 2x10⁴ cells/well.*

Sample Type	Cell Number*	Viability (%)
TCPS control	204,000±7000	100
Pure Collagen Sponge	142,000±500	70
CBA-C Sponge	133,000±9500	65

Proliferation of DPSCs and osteoblastic cells on CBA-C sponges

The biocompatibility of CBA-C sponges was studied by Alamar Blue cell proliferation assay. For this study, both the DPSCs and osteoblastic cells were seeded on the sponges, and the proliferation of cells on sponges was compared with the cells on TCPS (Figure 6).

It was observed that both stem cells and osteoblastic cells proliferated on composite sponges for 21 days; however, it was also found that the proliferation rates of both

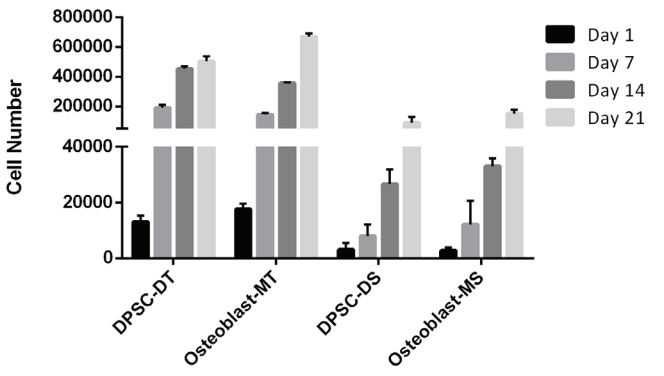

Figure 6. *Proliferation of DPSCs and osteoblastic cells during culture on CBA-C sponge. DPSC-DT: DPSCs in differentiation medium on TCPS; Osteoblasts-MT: Osteoblasts in maintenance medium on TCPS; DPSC-DS: DPSCs in differentiation medium on CBA-C sponge; Osteoblast-MS: Osteoblasts in maintenance medium on CBA-C sponge.*

cell types were lower than the cells on TCPS, probably due to the smaller area of the sponges in comparison to TCPS surface. In addition, at the end of 21 days, the number of cells in the differentiation group (DPSC-DS) was found to be lower than that of the osteoblast maintenance group both on TCPS and on sponges (Osteoblast-MT and Osteoblast-MS; respectively).

Calcium phosphate deposition on CBA-C sponges

Figure 7 shows the SEM micrographs of cells on CBA-C sponges after 21 days of culture. For this study, DPSCs were seeded on the composite sponges and differentiated for 21 days. In addition, osteoblastic cells were seeded on separate composite sponges and cultured in a maintenance medium for 21 days. At the end of 21 days of culture, the sponges were subjected to energy dispersive X-ray analysis (EDX). Deposition of calcium and phosphate elements was observed on CBA-C sponges for both cell types.

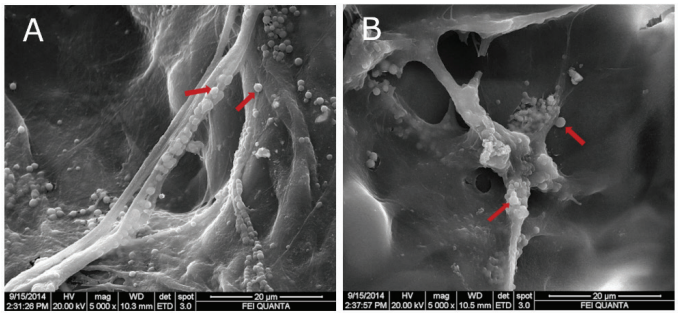

Figure 7. *SEM micrographs of cells on CBA-C sponge after 21 days of culture. A) DPSCs in differentiation medium, B) osteoblastic cells in maintenance medium. Arrows point at the calcium phosphate minerals on the composite structure.*

Histopathological findings of dental samples

Phenotypic characteristics of cells found in reparative dentin tissue in CBA-C sponges and CBA-M applied groups were not different.

Week 1

During the observations at the end of week 1, bleeding was observed in teeth for both CBA-C sponges and CBA-M applied groups. There was no reparative dentin tissue formation in the perforated area.

Week 2

In the CBA-C sponges applied group**,** the reparative dentin tissue formation was started and vascularized fibrous tissue formation was observed in the perforated area. However, hyperemia and porous structured reparative dentin tissue were observed in the pulp tissue in the CBA-M applied group.

Week 3

In the CBA-C sponges applied group, the reparative dentin tissue was formed, but there was a hyperemic porous structure towards the perforated area and in the CBA-M applied group, reparative dentin tissue properly proceeded inside the pulp tissue.

Week 4

At the end of the fourth week, the CBA-C sponges applied group showed less porous reparative dentin tissue than third week, and there were residues of a composite scaffold in the reparative dentin tissue (Figure 8). Except for capillary hyperemia, there was no sign of inflammation in the pulp tissue such as neutrophils, macrophages and lymphocytes infiltrations (Figure 8). In the CBA-M applied group, reparative dentin tissue occupied a large portion of the pulp tissue and necrotic tissues were observed in the perforated area.

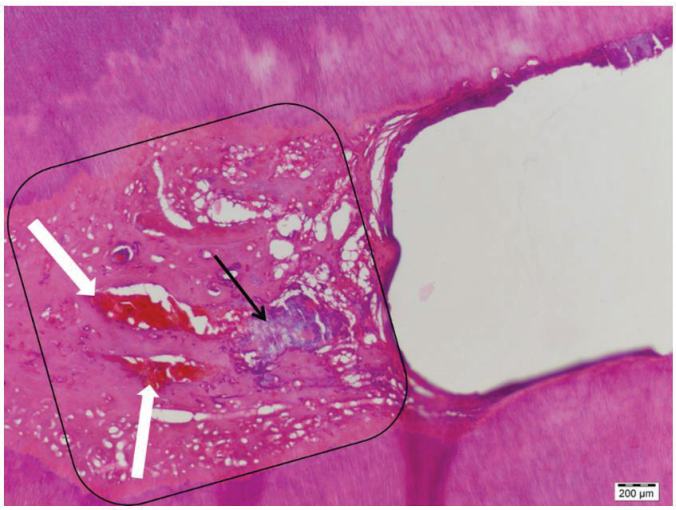

Figure 8. *Light microscopy image of Hematoxylin-Eosin stained tooth sample of CBA-C sponge applied group (week 4). Residual composite sponge (black arrow) and hyperemic areas (white arrows) (4X magnification).*

Week 8

In the CBA-C sponges applied group, complete, homogenous, and compact reparative dentin tissue observed in the perforated area and there were residues of composite sponge in reparative dentin area. The capillary hyperemia was seen in the same area. Integration of the existing dentin tissue with reparative dentin tissue was also observed (Figure 9). In the CBA-M applied group, on the other hand, necrotic tissue residues and hyperemia was observed (Figure 10).

Reparative dentin thicknesses measurements

The mean reparative dentin thicknesses are presented in Table 3. It is observed that reparative dentin tissue formation started on week 2 in both CBA-C sponges and CBA-M applied groups.

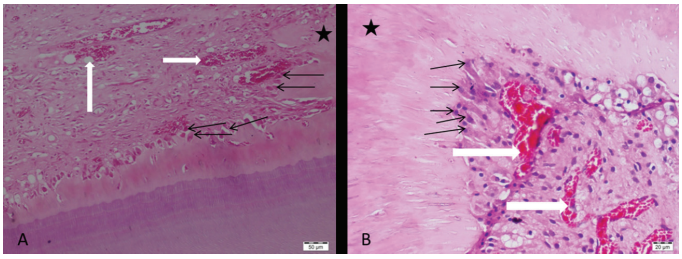

Figure 9. *Light microscopy images of Hematoxylin-Eosin stained tooth sample of CBA-C sponge applied group (week 8). Reparative dentin tissue (black stars), odontoblast-like cells (black arrows), and hyperemic areas (white arrows) (A: 20X magnification, B: 40X magnification).*

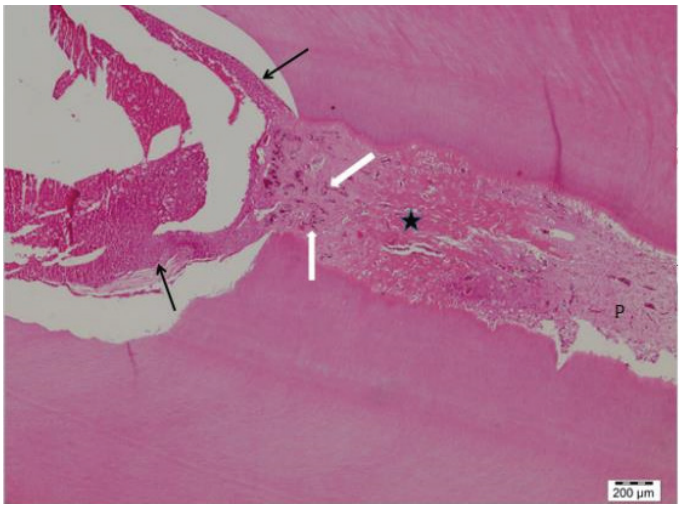

Figure 10. *Light microscopy image of Hematoxylin-Eosin stained tooth sample of CBA-M applied group (week 8). Necrotic tissues (black arrows), hyperemic areas (white arrows), reparative dentin tissue (black stars) and pulp (P) (4X magnification).*

Table 3. *Descriptive values of reparative dentin thicknesses with respect to the time periods. Dentin Tissue Thickness were presented as Mean ± Std Dev. (µm); *: Kruskal Wallis H test; a, b, c, A, B, C: Different superscripts in the rows show a significant difference (Bonferroni test; p<0.05); **: Mann Whitney U test.*

Time periods / Materials	2	3	4	8	*p**
CBA-C (µm)	1339±487[a]	1881±267[b]	1905±552[b]	1951±565[b]	<0.05
CBA-M (µm)	1253±104[A]	2227±393[B]	2759±318[C]	2869±455[C]	<0.05
p**	0.955	<0.001	<0.001	<0.001	

For the CBA-C sponges applied group, the differences were determined between week 2 and weeks 3, 4, 8 (p<0.05). In contrast, the other pairwise comparisons were not significant (p>0.05). Significant differences were found in reparative dentin thicknesses of the CBA-M applied group between the week 2 and weeks 3, 4, 8, and week 3 and weeks 4, 8 (p<0.05); but not between weeks 4 and 8 (p>0.05). As the same time periods of the CBA-C sponges and CBA-M applied groups were compared, significant differences were identified in the comparisons of 3, 4, and 8 weeks (p<0.001). However, a similar comparison was not significant for week 2 of the CBA-C sponges, and CBA-M applied groups (p=0.955).

Discussion

The aim of direct pulp capping is to ensure the continuity of the tooth pulp tissue vitality. This can be achieved by using biocompatible dental materials and induce reparative dentin formation, prevent bacterial infections of the pulp, and ensure the repair of the vital pulp tissue. Recently, studies are carried out on regenerative dentistry practices in pulp capping. Regenerative dentistry focuses on scaffold-based or scaffold-free strategies (21). The use of growth factors and biological molecules with scaffolds as a pulp capping material increases the success of direct pulp capping treatment (22). In this study, collagen and biocompatible BioAggregate materials were either used in the form of composite sponges (CBA-C) as a scaffolding material or as a mixture (CBA-M) and were compared for their effectiveness in the induction of reparative dentin formation.

For this study, DPSCs were used because it is known that DPSCs exhibit mesenchymal stem cell properties and are reported to be multipotent stem cells that can differentiate into osteoblastic cells, including osteoblasts and especially odontoblasts, in addition to other cell types such as adipocytes and neural cells (13, 23, 24). There are still unknowns about the potential and the behavior of the dental pulp progenitor/stem cells (12). The cells isolated from the pulp tissue were, therefore, examined with flow cytometry and found to be positive for mesenchymal stem cell marker CD 44 (15) (Figure 2). After this proof that the isolated cells belong to mesenchymal stem cell lineage, they were then subjected to osteoblastic differentiation. The differentiation towards odontoblasts is similar to osteogenic differentiation, which starts with an increase in ALP expression and is followed by mineralization (20). Within 21 days of induction in the osteogenic differentiation medium, the isolated DPSCs could deposit calcium phosphate minerals, indicating that the cells could differentiate into osteoblastic lineage (Figure 3).

The sponges used in this study were characterized in terms of their porosities, pore size distributions, and cytocompatibilities. The porosity of the sponges was studied both by mercury porosimetry and the surface topography obtained from SEM (Table 1). The lower porosity values obtained from SEM micrographs might be due to the differences in the measurement techniques. In mercury porosimetry, pressure is used, so mercury penetrates deep into the sample (25), while in SEM analysis, only the images of the surface of the sponges are visualized and used for the analysis of porosity. The pore size distribution of the sponges was also analyzed by using the SEM micrographs (Figure 5). It was observed that the CBA-C sponge has a high fraction of smaller pores than the pure collagen sponge. The presence of inorganic material BioAggregate probably physically blocks the pores decreasing the porosity and lowering the average pore size.

The cytocompatibility of CBA-C sponges was shown in the L929 mouse fibroblast cell line by using a direct contact approach (Table 2). The slight reduction in the cell number on both pure collagen and CBA-C sponges, compared to TCPS control, might be either due to the weight applied by the sponges onto the cells or the migration of cells on TCPS into the sponges. In addition, the presence of the sponges on top of the cells may have blocked the diffusion of the culture medium towards the cells underneath the sponge, thus leading to the death of these undernourished cells. Furthermore, the number of live cells in contact with either pure collagen or CBA-C sponges is very similar, indicating that BioAggregate does not have a negative effect on the cytocompatibility of the composite.

The proliferation of DPSCs and osteoblastic cells on the composite sponges were also studied (Figure 6). Both cell types could proliferate on the sponges. The lower number of cells in the differentiation group (DPSC-DS) compared to the osteoblast maintenance groups (Osteoblast-MT and Osteoblast-MS) might be due to the reduced proliferation rate of the stem cells during the differentiation phase (26). The DPSCs seeded on the sponges were able to differentiate to

osteogenic lineage and deposit calcium phosphate minerals (Figure 7) with comparable Ca:P ratios deposited by osteoblastic cells maintained on the sponges for 21 days.

It is known that BioAggregate is not cytotoxic, and it can induce odontoblastic differentiation of tooth pulp stem cells and mineralization under *in vitro* conditions (27-30). In this study, untreated teeth (not used in the experimental groups) of animals were collected, and stem cells were isolated from their pulp tissues. After the differentiation process, osteoblastic cells were seeded on the CBA-C sponges. After 21 days of incubation on the sponges, calcium phosphate deposition was observed on the sponges.

BioAggregate, being a biocompatible cement, can be used in endodontic fillings and repair (31). In a study, the effect of four different materials, including BioAggregate and ProRoot MTA, on odontogenic differentiation of human tooth pulp cells were studied (32). It was found that samples with ProRoot MTA and BioAggregate both presented the ALP activity. ALP is an indicator of early osteoblastic differentiation and has important roles in the mineralization process. However, more mineralized nodules were observed with BioAggregate used samples than with ProRoot MTA samples at the end of the 14th day (32, 33). Another study showed that at the end of the 4 th week MTA, Biodentine and BioAggregate induced adequate reparative dentin formation in rat pulps (34). Similarly, in our study, it was observed that both CBA-C sponges and CBA-M led to osteodentin formation, which showed similar mineralization as the dentin tissue of the tooth 2 weeks post-implementation.

In another study, the biocompatibility of MTA and BioAggregate was evaluated. The materials were produced in line with the producer's instructions, placed in Teflon plates, and implanted in the back of the rats. Rats were sacrificed on the 7th and 30th days, and kidney and liver samples were histologically and morphologically examined. It was found that MTA and BioAggregate caused inflammation in the kidney and liver and that MTA had a higher inflammatory effect than BioAggregate (35). Morais *et al*. and Parirokh *et al*. specified that the inflammatory response of the subcutaneous tissue against MTA reduced after 60 days. However, it was not clearly stated whether there was complete healing compared to the control group (36,37). In our study, no inflammatory response was observed except for capillary hyperemia.

It is known that type I collagen is the main extracellular matrix protein of the pulp and dentin matrix (38). It also has important roles in the adhesion and proliferation of cells (39). Jang et al. investigated the effect of gelatin- and fibrin-based hemostatic hydrogels as a scaffold in regenerative endodontics therapy**.** Gelatin is a collagen based biopolymer protein. And they reported that significantly higher cell viability was observed at the gelatin-based scaffolds group when compared with the fibrin-based scaffolds group (40). We, therefore, used type I collagen as the other component of our sponges.

Kakarla *et al*. (41) used collagen particles impregnated with antibiotics and Pulpotec® cement as a capping material in deciduous (baby) teeth. The histological examinations revealed that inflammation was reduced in both groups on days 7 and 15. At the end of day 30, no inflammation was observed, and the dentin bridge was formed. Furthermore, it was observed that the collagen structure was a better capping material compared to the Pulpotec cement. In another study collagen based scaffolds, which contain polyethyleneimine (PEI) - bonemorphogenic protein-2 (pBMP-2) and/or fibroblast growth factor-2 (pFGF-2) nanoplexes increased cell proliferation, expression levels of BMP-2 and FGF-2, and mineralization when compared to ProRoot- MTA group (42). Similarly, in our study, there were tubule-like structures similar to the osteodentin structure of natural dentin tissue in some samples of CBA-C sponges applied group on week 8. In addition, new tissue formation was observed within some samples of CBA-C sponges applied group on week 8, and this was due to the attachment and penetration of surrounding cells into the sponge, as was also reported in other *in vitro* studies (43).

Dick and Carmichael (44) evaluated the effectiveness of collagen sponge as a capping material and observed that the collagen sponge did not result in a thick dentin bridge formation, but it presented mineralization. In our study at the end of Week 8, the dentin was more compact in the CBA-C sponges applied group, and reparative dentin tissue formation was thicker in the CBA-M applied group. However, necrotic tissue residues seen in the perforation area in the CBA-M applied group may prepare the ground for bacterial infiltration by resorption.

In a clinical study, the effect of dental pulp stem/progenitor cells and collagen sponge on the healing of the human mandible was examined. These cells were isolated from one of the lower third molar teeth of patients. The cells were seeded on the collagen sponge and then placed in the tooth extraction gap. The third molar tooth, which was in the opposite direction, was used as the control sample with no intervention. Dental pulp stem/progenitor cells on the collagen sponges started bone regeneration after three months. According to the radiography results, the cortical bone level was higher in the experimental group compared to the control sample. After one year, a well-organized bone structure was observed in the intervention area, which was shown by radiographic and histological examinations and immunoflourescent microscopy (45). In our study, the histological examinations revealed that after 8 weeks, the perforation area was entirely recovered by the osteodentin structure in both groups; however, in CBA-M, applied group necrotic tissue residues were observed in the cavitation area.

The results of this study show that the collagen-BioAggregate sponges have a porous structure, which is important for the integration and maintenance of cells. The sponges were found to be biocompatible with both DPSCs and osteoblastic cells, and the DPSCs seeded on the sponges can differentiate into the osteoblastic lineage and deposit calcium phosphate mineral. And the histopathological findings of our study showed that collagen-BioAggregate sponges could provide reparative dentin formation more ideally compared to the mixture of collagen-BioAggregate.

Conclusion

CBA-C sponge represents a better microenvironment for reparative dentin formation probably due to maintaining DPSCs and allowing their osteogenic differentiation and thus calcium phosphate deposition. Adding spongy struc-

ture to pulp capping materials can increase success in direct pulp capping treatments.

Türkçe Özet: *Kolajen-bioaggregate kompozit yapı iskelesinin koyun pulpa dokusunun tamirinde değerlendirilmesi. Amaç: Bu çalışmada kolajen-Bio-Aggregate karışımı (CBA-M) ve yapı iskelesi malzemesi olarak kolajen-Bio-Aggregate kompozit (CBA-C) süngerin dentin tamiri üzerindeki etkilerinin karşılaştırılması amaçlanmıştır. Gereç ve Yöntem: Oluşturulan CBA-C sünger (10:1 w/w) Taramalı Elektron Mikroskopu (SEM) ve Civa Porozimetrisi ile karakterize edildi ve CBA-C süngerin L929 fare fibroblast hücre hattı üzerindeki sitotoksik etkisi incelendi. Koyun diş pulpa dokusundan diş pulpa kök hücreleri (DPSCler) izole edildi ve mezenkimal kök hücre belirleyicisi olan CD44 ekspresyonunu incelemek için akış sitometrisi kullanıldı. İzole edilen DPSCler' in osteojenik farklılaşma düzeyi Alizarin kırmızısı ile boyanarak değerlendirildi. CBA-C süngerin biyouyumluluğu, hücre çoğalması ve hücrelerin kalsiyum fosfat biriktirebilmeleri açısından incelendi. CBA-C sünger ve CBA-M' nin dentin rejenerasyonunu indükleyici etkisi perfore edilen koyun diş pulpalarında sekiz haftalık zaman aralığında incelendi ve uygun istatistiksel yöntemlerle analizler değerlendirildi. Bulgular: CBA-C süngerin DPSCler için biyouyumlu olduğu sonucuna varıldı. CBA-C sünger üzerine ekilen DPSCler osteoblastik kökene farklılaştı ve in vitro koşullarda kalsiyum fosfat kristalleri çökelmesi gözlendi. CBA-C sünger uygulanan grupta iki hafta sonra tamir dentini oluşumu gözlendi. Sekiz haftanın sonunda CBA-C sünger uygulanan grupta tamamlanmış tamir dentini oluşumu gözlenirken, CBA-M uygulanan grupta nekrotik doku artıkları gözlendi. Sonuç: CBA-C sünger, DPSCler' in sürekliliğine, osteojenik farklılaşmasına ve dolayısıyla kalsiyum fosfat birikimine izin vermesiyle tamir dentini oluşumu için iyi bir ortam sunmuştur. Anahtar kelimeler: Direkt pulpa kuafajı, tamir dentini, kolajen sünger, BioAggregate, BioAggregate-sünger kompozit.*

Ethics Committee Approval: All the surgical procedures were approved by the Animal Testing Ethics Committee of Firat University, Elazığ, Turkey, with meeting number 2014/9 and decree number 96.

Informed Consent: Not required.

Peer-review: Externally peer-reviewed.

Author contributions: MY, VH participated in designing the study. BD, DSB, HE, MY, VH participated in generating the data for the study. BD, DSB, HE participated in gathering the data for the study. BD, DSB, HE, MY participated in the analysis of the data. BD, DSB wrote the majority of the original draft of the paper. BD, DSB, HE, VH participated in writing the paper. BD, DSB, HE, MY, VH have had access to all of the raw data of the study. BD, DSB, HE, MY, VH have reviewed the pertinent raw data on which the results and conclusions of this study are based. BD, DSB, HE, MY, VH have approved the final version of this paper. BD guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: The authors had no conflict of interest to declare.

Financial Disclosure: This work was funded by the Scientific and Technological Research Council of Turkey (TÜBİTAK), Grant No: 112S584 and by the Research Fund of the Inonu University, Project Number: 2013/80.

Acknowledgement: The authors acknowledge BIOMATEN, the Middle East Technical University Center of Excellence in Biomaterials and Tissue Engineering for the use of the facilities.

References

1. Fuks AB. Vital pulp therapy with new materials for primary teeth: new directions and treatment perspectives. Pediatr Dent 2008;30:211-9. [CrossRef]
2. Dammaschke T. The history of direct pulp capping. J Hist Dent 2008;56:9-23.
3. Briso ALF, Rahal V, Mestrener SR, Dezan Junior E. Biological response of pulps submitted to different capping materials. Braz Oral Res 2006;20:219-25. [CrossRef]
4. Torabinejad M, White DJ, inventors. Tooth filling material and method of use. patent United States Patent & Trademark Office 5,415,547. 1995.
5. Komobayashi T, Zhu Q, Eberhart R, Imai Y. Current status of direct pulp-capping materials for permanent teeth. Dental Mat J 2016;35:1-12. [CrossRef]
6. Suh B, Cannon M, Yin R, Martin D (2008) Polymerizable dental pulp healing, capping, and lining material and method for use. International Patent A61K33/42;A61K33/42 Application number WO2008US5438720080220; Publication number WO2008103712 (A2); Publication date 2008-08-28
7. Park J-W, Hong S-H, Kim J-H, Lee S-J, Shin S-J. X-Ray diffraction analysis of white ProRoot MTA and Diadent BioAggregate. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:155-8. [CrossRef]
8. Scheller E, Krebsbach P, Kohn D. Tissue engineering: state of the art in oral rehabilitation. J Oral Rehabil 2009;36:368-89. [CrossRef]
9. Rosa V, Della Bona A, Cavalcanti BN, Nör JE. Tissue engineering: from research to dental clinics. Dent Mater 2012;28:341-8. [CrossRef]
10. Demarco FF, Conde MCM, Cavalcanti BN, Casagrande L, Sakai VT, Nör JE. Dental pulp tissue engineering. Braz Dent J 2011;22:3-13. [CrossRef]
11. Freyman T, Yannas I, Gibson L. Cellular materials as porous scaffolds for tissue engineering. Prog Mater Sci 2001;46:273-82. [CrossRef]
12. Sloan Alastair J, Waddington Rachel J. Dental pulp stem cells: what, where, how?. Int J Paediatr Dent 2009;19:61-70. [CrossRef]
13. Lan X, Sun Z, Chu C, Boltze J, Li S. Dental pulp stem cells: an attractive alternative for cell therapy in ischemic stroke. Front Neurol 2019;10:824. [CrossRef]
14. Van De Loosdrecht AA, Alhan C, Béné MC, et al. Standardization of flow cytometry in myelodysplastic syndromes: report from the first European LeukemiaNet working conference on flow cytometry in myelodysplastic syndromes. Haematologica 2009;94:1124-34. [CrossRef]
15. Yang M-C, Chi N-H, Chou N-K, et al. The influence of rat mesenchymal stem cell CD44 surface markers on cell growth, fibronectin expression, and cardiomyogenic differentiation on silk fibroin–hyaluronic acid cardiac patches. Biomaterials 2010;31:854-62. [CrossRef]
16. Shiau M-Y, Chiou H-L, Lee Y-L, Kuo T-M, Chang Y-H. Establishment of a consistent L929 bioassay system for TNF-alpha quantitation to evaluate the effect of lipopolysaccharide, phytomitogens and cytodifferentiation agents on cytotoxicity of TNF-alpha secreted by adherent human mononuclear cells. Mediators Inflamm 2001;10:199. [CrossRef]
17. Miranda RB, Fidel SR, Boller MAA. L929 cell response to root perforation repair cements: an in vitro cytotoxicity assay. Braz Dent J 2009;20:22-6. [CrossRef]
18. Al-Nasiry S, Geusens N, Hanssens M, Luyten C, Pijnenborg R. The use of Alamar Blue assay for quantitative analysis of viability, migration and invasion of choriocarcinoma cells. Hum Reprod 2007;22:1304-9. [CrossRef]
19. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci 2000;97:13625-30. [CrossRef]
20. Wang J, Liu B, Gu S, Liang J. Effects of Wnt/β-catenin signalling on proliferation and differentiation of apical papilla stem cells. Cell Prolif 2012;45:121-31. [CrossRef]
21. Dissanayaka WL, Zhang C. Scaffold-based and scaffold-free strategies in dental pulp regeneration. J Endod 2020;46:81–9. [CrossRef]
22. Okamoto M, Matsumoto S, Sugiyama A, et al. Performance of a biodegradable composite with hydroxyapatite as a scaffold in pulp tissue repair. Polymers 2020;12:937. [CrossRef]

23. Komori T. Regulation of osteoblast and odontoblast differentiation by Runx2. J Oral Biosci 2010;52:22-5. [CrossRef]
24. Liu M, Sun Y, Liu Y, Yuan M, Zhang Z, Hu W. Modulation of the differentiation of dental pulp stem cells by different concentrations of β-glycerophosphate. Molecules 2012;17:1219-32. [CrossRef]
25. Moore MJ, Jabbari E, Ritman EL, et al. Quantitative analysis of interconnectivity of porous biodegradable scaffolds with micro-computed tomography. J Biomed Mater Res A 2004;71:258-67. [CrossRef]
26. Cooper G, Hausman R. The cell: a molecular approach. Sinauer Associates, Sunderland, MA;2000.
27. Zhang S, Yang X, Fan M. BioAggregate and iRoot BP Plus optimize the proliferation and mineralization ability of human dental pulp cells. Int Endod J 2013;46:923-9. [CrossRef]
28. Jung JY, Woo SM, Lee BN, Koh JT, Nör J, Hwang YC. Effect of Biodentine and Bioaggregate on odontoblastic differentiation via mitogen-activated protein kinase pathway in human dental pulp cells. Int Endod J 2015;48:177-84. [CrossRef]
29. Yan P, Yuan Z, Jiang H, Peng B, Bian Z. Effect of bioaggregate on differentiation of human periodontal ligament fibroblasts. Int Endod J 2010;43:1116-21. [CrossRef]
30. Yuan Z, Peng B, Jiang H, Bian Z, Yan P. Effect of bioaggregate on mineral-associated gene expression in osteoblast cells. J Endod 2010;36:1145-8. [CrossRef]
31. Saghiri MA, Asatourian A, Garcia-Godoy F, Gutmann JL, Sheibani N. The impact of thermocycling process on the dislodgement force of different endodontic cements BioMed Res Int 2013;2013.
32. Chang S-W, Lee S-Y, Kum K-Y, Kim E-C. Effects of ProRoot MTA, Bioaggregate, and Micromega MTA on odontoblastic differentiation in human dental pulp cells. J Endod 2014;40:113-8. [CrossRef]
33. Cormier C. Markers of bone metabolism. Curr Opin Rheumatol 1995; 7: 243-8. [CrossRef]
34. Kim J, Song YS, Min KS, et al. Evaluation of reparative dentin formation of ProRoot MTA, Biodentine and BioAggregate using micro-CT and immunohistochemistry. Restor Dent Endod 2016;41:29-36. [CrossRef]
35. Khalil W, Eid N. Biocompatibility of BioAggregate and mineral trioxide aggregate on the liver and kidney. Int Endod J 2013;46:730-7. [CrossRef]
36. de Morais CAH, Bernardineli N, Garcia RB, Duarte MA, Guerisoli DM. Evaluation of tissue response to MTA and Portland cement with iodoform. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;102:417-21. [CrossRef]
37. Parirokh M, Mirsoltani B, Raoof M, Tabrizchi H, Haghdoost A. Comparative study of subcutaneous tissue responses to a novel root-end filling material and white and grey mineral trioxide aggregate. Int Endod J 2011;44:283-9. [CrossRef]
38. Ravindran S, Huang C-C, George A. Extracellular matrix of dental pulp stem cells: applications in pulp tissue engineering using somatic MSCs. Front Physiol 2014;4:395. [CrossRef]
39. Wu C-C, Huang S-T, Lin H-C, Tseng T-W, Rao Q-L, Chen M-Y. Expression of osteopontin and type I collagen of hFOB 1.19 cells on sintered fluoridated hydroxyapatite composite bone graft materials. Implant Dent 2010;19:487-97. [CrossRef]
40. Jang JH, Moon JH, Kim SG, Kim SY. Pulp regeneration with hemostatic matrices as a scaffold in an immature tooth minipig model. Sci Rep 2020;10:12536. [CrossRef]
41. Kakarla P, Avula JSS, Mellela GM, Bandi S, Anche S. Dental pulp response to collagen and pulpotec cement as pulpotomy agents in primary dentition: A histological study. J Conserv Dent 2013;16:434. [CrossRef]
42. Chakka LRJ, Vislisel J, Vidal CMP, Biz MT, Salem AK, Cavalcanti BN. Application of BMP-2/FGF-2 gene–activated scaffolds for dental pulp capping. Clin Oral Investig 2020;24:4427-4437. [CrossRef]
43. Doğan A, Munkley A, Thomas S, Moran J. Microscopic evaluation of biocompatibility of osteoblast impregnated human collagen sponges. J Dent Res 1992;71:637.
44. Dick H, Carmichael D. Reconstituted antigen-poor collagen preparations as potential pulp-capping agents. J Endod 1980;6:641-4. [CrossRef]
45. d'Aquino R, De Rosa A, Lanza V, et al. Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells and collagen sponge biocomplexes. Eur Cell Mater 2009;18:75-83. [CrossRef]

55th Volume Index

Subject Index

55th Volume Index

Subject Index

55th Volume Index

Author Index

55th Volume Index

Acknowledgement to Reviewers
September 2020 - September 2021

Editor-in-Chief and Editorial Staff would like to thank following reviewers who have shared their valuable time and expertise to evaluate manuscripts submitted to European Oral Research in 2021.

Ağralı, Ömer Birkan
Aksoy, Umut
Arslan, Ahmet
Aydinbelge, Mustafa
Ayub, Muhammad
Baig, Mirza R.
Balci, Nur
Balik, Ali
Bıyıkoğlu, Basak
Cakici, Fatih
Cheong, Ngeow Wei
Demiriz, Levent
Develi, Tuba
Dogan, Mehmet
Durmuş, Başak
Emes, Yusuf
Eren, Figen
Gad, Mohammed
Geckili, Onur
Genc, Gonca Erkose
Gencel, Burc
Gul, Esma Basak
Hakimeh Siadat
Jaafar Abduo
Kantilieraki, Elena
Kirmali, Omer
Koruyucu, Mine
Kucukekenci, Ahmet Serkan
Kuscu, Ozgur
Nogueira Leal Silva, Emmanuel Joao
Oner Ozdas, Didem
Orhan, Kaan
Oter, Banu
Ozdemir Ozenen, Didem
Pekiner, Filiz
Sari, Fatih
Scribante, Andrea
Singh, Shishir
Sinhoreti, Mario
Sorensen, JA
Sousa-Neto, M. D.
Tekeli, Seda
Telli, Sahure Gonca
Toz Akalin, Tugba
Tuncer, Safa
Ustun, Yakup
Uzun, Ismail
Woestmann, Bernd
Yılmaz, Mustafa


E R