ISSN 1852-4834 on line version
versión electrónica

ACTA ODONTOLOGICA LATINOAMERICANA

Vol. 37 Nº 2 2024

aol

ACTA ODONTOLÓGICA LATINOAMERICANA
An International Journal of Applied and Basic Dental Research

EDITORIAL POLICY

Although AOL will accept original papers from around the world, the principal aim of this journal is to be an instrument of communication for and among Latin American investigators in the field of dental research and closely related areas.
Particular interest will be devoted to original articles dealing with basic, clinic and epidemiological research in biological areas or those connected with dental materials and/or special techniques.
Clinical papers will be published as long as their content is original and not restricted to the presentation of single cases or series.
Bibliographic reviews on subjects of special interest will only be published by special request of the journal.
Short communications which fall within the scope of the journal may also be submitted.
Submission of a paper to the journal will be taken to imply that it presents original unpublished work, not under consideration for publication elsewhere. To this end, the authors will state their agreement with the editorial policy. The papers cannot later be published elsewhere without the express consent of the editors.
To favour international diffusion of the journal, articles will be published in English with an abstract in Spanish or Portuguese.
Acta Odontológica Latinoamericana may use Internet programs and tools to detect plagiarism, self-plagiarism, duplication and fragmentation.
Regarding the ethics of the publication process, Acta Odontológica Latinoamericana complies with the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals, International Committee of Medical Journal Editors. (https://www.icmje.org/icmje-recommendations.pdf)
The editors of Acta Odontologica Latinoamericana have no commercial interest, nor do they endorse or vouch for commercial products or diagnostic and therapeutic procedures mentioned in the publications.
Articles accepted for publication will be the property of Acta Odontológica Latinoamericana and express the opinion of the authors.
Acta Odontológica Latinoamericana is an open access publication. The content of the publication is licensed under a Creative Commons Attribution-Noncommercial 4.0 International License (CC BY-NC 4.0)

Peer review process
Acta Odontologica Latinoamericana uses double-blind review, which means that both the reviewer and author identities are concealed from the reviewers, and vice versa, throughout the review process.
The review process will be carried out by two reviewers selected by the editorial board, from specialists in each field. The reviewers will undertake the review process in order to achieve the highest possible standard of scientific content. Confidentiality, impartiality and objectivity will be maintained during the entire peer review process. If discrepancies arise, a third reviewer will be invited to participate. The reviewers will have a period of 4 weeks to review the manuscript and based on their report, the Editorial Committee may request modifications and decide on acceptance or rejection of the manuscript. The revised version of manuscripts with a recommendation of major modifications, will be sent back to the reviewers for final evaluation.
The manuscripts accepted for publication will be submitted to idiomatic evaluation and revision, edition and layout. The authors will proofread their manuscript and must approve the final version prior to publication.

Policies on Conflict of Interest
All authors must state any financial or other conflict of interest that could be interpreted as a bias in the results or interpretation of the results reported in their manuscript.

Human and Animal Rights, and Informed Consent
All investigators should ensure that the planning, conduct, and reporting of human research are in accordance with the Helsinki Declaration as revised in 2013. The authors must state in their manuscript that they have obtained informed consent of the people involved and that the project has been approved by an institutional ethics committee
Studies involving laboratory animals must comply with the "Guide for the Care and Use of Laboratory Animals", National Academy of Sciences. Washington DC and have the approval of an institutional committee

Publisher
Producción Gráfica: Panorama gráfica & diseño
e-mail: panoramagyd@gmail.com

Acta Odontológica Latinoamericana is the official publication of the Argentine Division of the International Association for Dental Research.

Revista de edición argentina inscripta en el Registro Nacional de la Propiedad Intelectual bajo el N° 284335.

Acta Odontológica Latinoamericana: an international journal of applied and basic dental research. - Vol. 1, no. 1 (1984) - Buenos Aires

Cuatrimestral, 1984-1986; irregular, 1987-1993, semestral, 1996-2008, cuatrimestral, 2009-
Artículos en inglés, sumarios en inglés y castellano o portugués.
Variante de titulo: AOL.
Titulo clave abreviado: Acta Odontol. Latinoam.
Directores: Romulo Luis Cabrini (1984-2015); Maria E. Itoiz (2015-2018); Maria E. Itoiz y Ricardo Macchi (2018-2022); Ricardo Macchi y Sandra J. Renou (2022-
Indizada en **MEDLINE/ PubMed**: Vol. 1, n° 1 (1984) - ; **SciELO**: Vol 22 (2009)-
Se encuentra incorporada a **Latindex** (categoria 1, directorio y catálogo), **Núcleo Básico de Revistas Científicas Argentinas** (2007-) por Resolución n° 1071/07 CONICET, Scopus: (1984-1986, 1990, 1993-1994, 1996-2016) (August 2023-) y PubMed Central (PMC) (August 2021-).
Registrada en: The Serials Directory, Ulrich's Periodicals Directory y SCImago Journal.
Direccion electronica: http://www.actaodontologicalat.com/
ISSN 1852-4834 versión electrónica

Este número se terminó de editar el mes de Septiembre de 2024

CONTENTS

Acta Odontológica Latinoamericana
From volume 27 (2014) AOL is published in digital format with the *Open Journal System* (OJS). The journal is Open Access. This new modality does not imply an increase in the publication fees.

Editorial Board

Contact us

Cátedra de Anatomía Patológica, Facultad de Odontología, Universidad de Buenos Aires.
M.T. de Alvear 2142 (C1122AAH) Buenos Aires, Argentina.
http://www.actaodontologicalat.com/contacto.html
actaodontologicalat@gmail.com

https://doi.org/10.54589/aol.37/2/99

Effect of two oxygen-inhibiting agents on the surface microhardness of giomer restorative materials

Diana J De La Cruz, Melvin Cisneros-del Águila

Universidad Científica del Sur, Lima, Lima, Perú

ABSTRACT

Giomers are bioactive hybrid restorative materials consisting of composite resin and glass ionomer filler pre-reacted on the surface, which maintain acceptable clinical qualities over time. One of the main factors that explains this is the surface hardness that is achieved by inhibiting the oxygen layer. ***Aim:*** *To compare the effect of blue and conventional Mylar strips used as oxygen inhibiting agents on the surface microhardness of giomer restorative materials.* ***Materials and Method:*** *A total 96 giomer specimens were prepared in disc-shaped molds 2 mm tall x 5 mm in diameter (ISO 4049: 2019-05). The specimens were grouped according to type of giomer: Beautifil II (BII) or Beautifil II LS (BIILS), and according to the type of Mylar strip: conventional, blue, or control group without strip. They were subsequently subjected to the Knoop (KHN) microhardness test. The database was analyzed with Stata SE v18 statistical software, and two-way ANOVA was performed.* ***Results:*** *Interaction was found between the type of giomer and Mylar strip (p=0.039). Significant differences were found between surface microhardness values according to the type of giomer (0.001) and the type of Mylar strip (0.001). Beautifil II LS presented significant differences between conventional Mylar strip vs. without Mylar strip (43.58 ± 1.65 vs. 40.44 ± 2.12) and between blue Mylar strip and without Mylar strip (44.69 ± 1.75 vs. 40.44 ± 2.12). In the Bonferroni Post hoc test, a significant difference was found between Conventional Mylar Strip and without Mylar Strip (p=0.001) and Blue Mylar Strip and without Mylar Strip (p=0.001).* ***Conclusion:*** *The use of blue and conventional Mylar strips inhibits the oxygen layer on the Beautifil II and Beautifil II LS giomers, endowing them with high values of surface microhardness.*

Keywords: *dental materials - glass ionomer cements - hardness*

Efecto de dos agentes inhibidores de oxígeno sobre la microdureza superficial de materiales restauradores giomeros

RESUMEN

Los giomeros son materiales restauradores híbridos bioactivos, constituidos por resina compuesta y relleno de ionómero de vidrio pre-reaccionado en superficie, mantienen cualidades clínicas aceptables en el tiempo; un factor principal para ello es la dureza superficial que se logra inhibiendo la capa de oxígeno. ***Objetivo:*** *Comparar el efecto entre la Tira Mylar azul y convencional al ser utilizados como agentes inhibidores de oxígeno sobre la microdureza superficial de los materiales restauradores Giomeros.* ***Material y Método:*** *Se confeccionaron un total de 96 muestras de giomeros en una matriz en forma de disco, de 2 x 5 mm de diámetro (ISO 4049: 2019-05). Se agruparon las muestras según el tipo de giomero: Beautifil II (BII) y Beautifil II LS (BIILS), y según el tipo de tira mylar: convencional, azul y el grupo control sin tira. Posteriormente fueron sometidas a prueba de microdureza Knoop (KHN). La base de datos fue analizada con el software estadístico Stata SE v18, se realizó la prueba ANOVA de dos factores.* ***Resultados:*** *Se encontró interacción entre el tipo de giomero y de tira mylar (p=0.039), y también diferencias significativas entre los valores de microdureza superficial según el tipo de giomero (0.001) y según el tipo de tira mylar (0.001). Beautifil II LS presentó diferencias significativas entre Tira Mylar Convencional vs sin Tira Mylar (43.58 ± 1.65 vs 40.44 ± 2.12) y entre Tira Mylar Azul y sin Tira Mylar (44.69 ± 1.75 vs 40.44 ± 2.12). En la prueba Post hoc de Bonferroni se encontró diferencia significativa entre Tira Mylar Convencional y sin Tira Mylar (p=0.001) y Tira Mylar Azul y sin Tira Mylar (p=0.001).* ***Conclusión:*** *El uso de las tiras mylar azul y convencional inhiben la capa de oxígeno sobre los giomeros Beautifil II y Beautifil II LS, confiriéndoles valores altos de microdureza superficial.*

Palabras clave: *materiales dentales - cementos de ionómero vítreo - dureza*

To cite:
De La Cruz DJ, Cisneros-del Águila M. Effect of two oxygen-inhibiting agents on the surface microhardness of giomer restorative materials. Acta Odontol Latinoam. 2024 Aug 30;37(2):99-104. https://doi.org/10.54589/aol.37/2/99

Corresponding Author:
Diana Jimena De la Cruz Llanos
diana.de.la.cruz.l@upch.pe

Received: November 2023.
Accepted: June 2024.

INTRODUCTION

Giomers are hybrid restorative materials with bioactive features: they can release and recharge fluoride from the glass ionomer, and have the physical, mechanical and optical properties of resin[1]. Giomers are a special class of dental composites[2] because they contain pre-reacted glass-ionomer (PRG) filler particles in a resin matrix[3]. The PRG filler is formed by an acid-base reaction between polyalkenoic acid and particles of fluoro-boro-aluminosilicate glass, in presence of water, before being added to the resin. S-PRG filler is surface pre-reacted glass-ionomer. Its particles have a three-layered structure[4], consisting of a glass core enveloped by a stable glass-ionomer hydrogel[3], in turn surrounded by the reformed phase or modified surface layer which provides structural protection for the hydrogel[4].

Giomers have fluoride release and recharge properties, and minimize the onset of caries by providing constant remineralization[5]. They are employed as sealants for pits and fissures, liner or base materials, varnish for exposed hypersensitive areas, and for pediatric use[2]. Beautifil II can be used on occlusal and proximal surfaces of posterior teeth, and in patients with high caries index[2, 5]. A longitudinal study showed that after thirteen years, giomers maintained acceptable clinical qualities[4,6]. The giomer Beautifil II LS is a system based on Beautifil II, with low volumetric contraction, chameleon effect, easy manipulation and sustained fluoride release and recharge[2]. According to Alinda[7], Beautifil II is a better alternative than Fuji IX glass ionomer cement, because it has is significantly higher resistance to compression and superior characteristics in terms of surface morphology.

The main factor that affects the hardness of a dental composite is the polymerization process. Since polymerization occurs in open air, oxygen is able to bond to the free radicals, forming peroxi radicals and thereby disrupting or retarding the process[8-9]. As a result, a gelatinous layer is formed: the oxygen inhibition layer (OIL), which contains unreacted resin monomers and oligomers[10] The OIL affects dental composite prognosis negatively because it reduces surface hardness, and fosters microleaks, plaque formation and secondary caries [9].

In order to minimize OIL formation as much as possible, the use of Mylar strips is recommended during photopolymerization to displace the oxygen present on the surface[10]. Mylar strips are easy to use for proximal and buccolingual cavities and provide very smooth surfaces[11-12]. Blue Mylar strips are known to provide higher surface hardness than white strips because when monochromatic light passes through a clear object of the same color, the intensity of the light increases, providing greater polymerization and hardness in composite resins[13]. Hardness also depends on the distance of the light source because it decreases as the distance from the light source increases. Greatest hardness is achieved with the light source at a distance of 00 mm[14]. Blue Mylar strips are characterized by their color system, which helps control perfect fit to the gingival base of the cavity[15].

Using blue and conventional Mylar strips to inhibit OIL formation increases the surface hardness of restorative materials[13,16]. Surface hardness is a major factor for increasing resistance to masticatory forces, thereby preventing the risk of restoration breakage, which would affect its clinical success[9,17]. Sánchez-Sánchez et al.[16] report that there is no consensus regarding the best technique for eliminating the oxygen inhibition layer. The aim of this study is therefore to compare the effects of blue and conventional Mylar strips used as oxygen inhibiting agents on the surface microhardness of giomer restorative materials.

MATERIALS AND METHOD

This *in vitro* study was approved by the Ethics Committee at Universidad Científica del Sur, Lima, Perú (No. 810-2021-POS53). It employed the giomers Beautifil II (Shofu Inc., Lima, Perú) color A3, Beautifil II LS (Shofu Inc., Houston, USA) color A3; clear Mylar strips (Maquira, Lima, Perú) and Mylar Blue View VariStrip (Garrison Dental, Spring Lake, USA).

Sample size

Sample size was calculated by means of a pilot study and by using the formula for comparing means for independent groups with confidence level 0.95, statistical power 0.80, and precision 3.5. The result indicated 16 specimens for each of the six groups (combining giomer types, Mylar strip types and controls). A total 96 specimens were prepared.

Grouping

Groups were as follows: 1) BII-Blue Mylar Strip: Beautifil II light-cured with blue Mylar strip, 2) BII-Conventional Mylar Strip: Beautifil II light-cured with conventional Mylar strip, 3) BII-Control: Beautifil II light-cured without Mylar strip, 4) BIILS-Blue Mylar Strip: Beautifil IILS light-cured with blue Mylar strip, 5) BIILS-Conventional Mylar Strip: Beautifil IILS light-cured with conventional Mylar strip, 6) BIILS-Control: Beautifil II LS light-cured without Mylar strip.

Procedure

Specimens were prepared following ISO standard 4049: 2019-05 – Dentistry: polymer-based restorative materials. A stainless-steel mold was used to prepare 96 disk-shaped specimens 2 mm thick and 5 mm in diameter. The giomer was inserted in the mold in a single increment using a 442-443DDES spatula. Then the Mylar strip (conventional or blue, according to experimental group) was applied, and the mold was pressed with a glass slide to remove excess material and homogenize and level the surface. For control group specimens, pressure was applied using only the glass slide.

When all specimen surfaces had been leveled, photopolymerization was performed according to the manufacturer's recommendations, using a LED light-curing unit (Optilight Color, Gnatus, Lima, Perú), and applying the same time (10 seconds) and power (≥ 1000 mW/cm2) to all groups. The distance between the Mylar strip and the light source was 00 mm in all groups. All specimens were stored in distilled water in an electric muffle for 24 hours at 37 °C13. Then, the specimens were placed on a glass plate covered in red wax and pressed with a clamp to achieve a surface parallel to the floor.

Knoop hardness (KHN) Measurement

KHN was measured with a microhardness tester (HV-1000, LG Digital, Korea) with a load of 100 grams-force at 15 seconds dwell time. Before testing, all specimens were polished with 800, 1000, 1500, 2000 and 2500 grit silicon carbide paper[1]. Readings were taken on the surface of each specimen at 3 equidistant indentation points[13]. The results were averaged independently and reported as KHN values.

Statistical analysis

Data were analyzed using the statistical software Stata 18.0 (Stata Corporation, College Station, TX, USA), and reported as measurements of central tendency and dispersion. The assumptions of normality and homogeneity of variances were evaluated by the Shapiro-Wilk test and Bartlett's test, respectively. Two-way analysis of variance (ANOVA) and post-hoc Bonferroni's pairwise multiple comparison test were applied to determine significant differences. All statistical tests were performed at a confidence level of 0.95 and significance level 0.05.

RESULTS

Two-way analysis of variance (ANOVA) showed interaction between giomer type and Mylar strip type (p=0.039), and significant differences between surface microhardness values (KHN) according to type of giomer (p=0.001) and type of Mylar strip employed (p=0.001) (Table 1).

According to the Mylar strip type, surface microhardness (KHN) was significantly higher in the giomer Beautifil II than in the giomer Beautifil II LS. According to giomer type used, surface microhardness (KHN) in the Beautifil II LS group,

Table 1. Two-way ANOVA for surface microhardness (KHN) according to Giomer type and Mylar strip type

Number of observations = 96			R- squared = 0.9579		
Root MSE = 1.99592			Adjusted R-squared = 0.9556		
Source	SS	df	MS	F	P value
Model	8159.4083	5	1631.8817	409.64	0.001
Giomer type	7971.615	1	7971.615	2001.07	0.001*
Mylar strip type	160.88271	2	80.441354	20.19	0.001*
Giomer type # Mylar strip type	26.910625	2	13.455313	3.38	0.039*
Residues	358.53125	90	3.9836806		
Total	8517.9396	95	89.662522		

*p<0.05 significant, two-way ANOVA. SS: Sum of squares. DF: Degrees of freedom. MS: Mean squares. F: Statistical F. #: Interaction between Giomer type and Mylar strip type.

there were statistically significant differences between conventional Mylar strip and without Mylar strip (43.58 ± 1.65 vs 40.44 ± 2.12) and between blue Mylar strip and without Mylar strip (44.69 ± 1.75 vs 40.44 ± 2.12), while no significant differences were found between blue and conventional Mylar strips (44.69 ± 1.75 vs 43.58 ± 1.75); and in the Beautifil II group there were no significant differences (Table 2). Post-hoc Bonferroni's pairwise multiple comparison test was performed to determine significant differences. Because the comparison was pairwise, it found that there was a significant difference in surface microhardness according to Mylar strip type, that were, between Blue Mylar Strip and Without Mylar Strip (p= 0.001), and between Conventional Mylar Strip and Without Strip Mylar (p= 0.001). There was also a significant difference in surface microhardness according to giomer type (p= 0.001) (Table 3).

Table 2. Mean, standard deviation and confidence intervals for surface microhardness (KHN) according to Giomer type and Mylar strip type

	Mylar strip type		
Giomer type	**Blue Mylar strip Mean (± SD)**	**Conventional Mylar strip Mean (± SD)**	**Without Mylar strip Mean (± SD)**
Beautifil II	61.61 ± 2.38 Ba CI 95% (60.35; 62.88)	61.81 ± 1.81 Ba CI 95% (60.84; 62.77)	59.96 ± 2.17 Ba CI 95% (58.81; 61.12)
Beautifil II LS	44.69 ± 1.75 Ab CI 95% (43.76; 45.62)	43.58 ± 1.65 Ab CI 95% (42.69; 44.46)	40.44 ± 2.12 Aa CI 95% (39.32; 41.57)

X: Medium. SD: Standard deviation. CI: Confidence interval. Two-way ANOVA. Consider each response variable: A, B, a, b separately; capital letters "A" and "B" indicate comparisons within each column (Mylar Strip Type), while lowercase letters "a" and "b" indicate comparisons within each row (Giomer Type). The different letters differ significantly from each other, that is, for the columns: Blue Mylar Strip (BII vs BIILS: p = 0.001); Conventional Mylar Strip (BII vs BIILS: p = 0.001); Without Mylar strip (BII vs BIILS: p = 0.001); and for the rows: Beautifil IILS (BMS vs WMS: p = 0.001) and (CMS vs WMS: p = 0.001).

Tabla 3. Post-hoc Bonferroni's pairwise multiple comparison test for surface microhardness (KHN) according to Giomer type and Mylar strip type

Comparison of surface microhardness	
Mylar strip type	**P value**
Conventional Mylar strip vs Blue Mylar strip	1.000
Without Mylar strip vs Blue Mylar strip	0.001*
Without Mylar strip vs Conventional Mylar strip	0.001*
Giomer type	**P value**
Beautifil II vs Beautifil II LS	0.001*

*p<0.05 significant, Bonferroni Post-hoc Test.

DISCUSSION

In the long term, the oxygen inhibition layer produces a negative effect on the giomer surface by reducing hardness, as a result of which the useful life of the restorative material decreases[9]. Mylar strips displace oxygen from the surface, providing a smooth area, whose hardness depends on the type of Mylar strip used, with blue strips providing better outcomes[13]. The aim of the current study was to identify any differences in giomer surface hardness, thereby providing important information to clinicians regarding oxygen inhibition elements.

A review of the literature revealed studies showing that the oxygen inhibition layer is closely related to composite hardness[9]. Surface hardness represents the material's mechanical resistance to plastic deformation[18] and may be affected by the formation of an oxygen-inhibited layer that fosters the development of plaque, secondary caries and microleaks[9].

The current study showed that using a different Mylar strip color had a positive effect of on the oxygen inhibition layer, and in turn on hardness. Because this was an *in vitro* experimental study, it has certain limitations such as not exactly replicating the clinical conditions in the oral environment, therefore the results may be controversial. Moreover, the results cannot be generalized because only one type of oxygen inhibition agent was used: blue Mylar strip. Once the grouping variables were established: type of giomer and type of Mylar strip, the Two-way ANOVA was applied, there was interaction between Mylar strip type and giomer type. Then we proceeded to evaluate the standardized residuals of the Anova mathematical model, finding that these residuals do meet the assumptions of normality (p=0.120) and homogeneity of variances (p=0.406). In both groups, microhardness was lowest without Mylar strips. Thus, it is recommendable to employ

either of the Mylar strip types – conventional or blue – to achieve better restorations.

The results would be more relevant if a larger sample had been used or if a longitudinal study had been performed. Another important point to consider is polymerization time. If all the giomer specimens had been polymerized simultaneously, the ongoing curing reaction would have been the same for all of them, instead of being shorter for the last specimens prepared[19].

Mousavinasab et al.[13] evaluated the effect of the photopolymerization distance and the color of the transparent Mylar strips on the surface hardness of composite resins. They concluded that blue Mylar strips provided greater hardness than clear strips when the light source was at a distance of 0 mm, and that hardness decreased as distance from the light source increased. In this regard, the current study found the highest value with blue Mylar strips in the giomer Beautifil II LS group, in agreement with the aforementioned study, with the light source at 0 mm. This may be because when a monochromatic light passes through a clear object of the same color (in this case, blue, the intensity of the light increases, causing an increase in polymerization and hardness in the restorative materials.

In the current study, the distance from the light source to the Mylar strip was 00 mm, which may have generated a positive effect on the microhardness values of both giomers. These results contradict Al-Zain [20], who found better behavior of two resin-based composites when the light source was placed at 2 and 8 mm from the conventional Mylar strip than when it was placed at 00 mm.

Gonulol et al.[21] report that the composition of the giomer Beautifil II does not include urethane dimethacrylate (UDMA), which is more hydrophobic than bisphenol A-glycidyl methacrylate (Bis-GMA) and Triethylene glycol dimethacrylate (TEGDMA). Restorative materials with UDMA matrices such as the giomer Beautifil II LS therefore have less water absorption, and in turn, less filler dissolution, thereby increasing surface hardness. This may explain why, in the current study, in which all samples were submerged in distilled water and placed in a muffle at 37 °C for 24 hours to replicate oral conditions, microhardness was higher in the giomer Beautifil II LS group, with both blue and the conventional Mylar strips. Nevertheless, the highest microhardness values were found for the Beautifil II group with conventional Mylar strip, which may contradict the aforementioned. It would therefore be interesting to conduct the same study on a larger sample size.

Sánchez-Sánchez et al.[16] used conventional Mylar strips to eliminate the oxygen inhibition layer on a Coltene brand resin composite before photopolymerization and achieved better stability in the composite. For greater credibility, they suggest further studies. All the aforementioned studies recommend the use of Mylar strips before photopolymerization because of the improvement observed in composite surface hardness. The current study also found favorable results with the use of conventional and blue Mylar strips, in agreement with the study by Barkatin[22] evaluating the degree of conversion of two composites, one methacrylate-based and another silorane-based, using blue and conventional Mylar strips, and achieving positive effects in both materials, which were greatest when conventional Mylar was used on silorane.

Inhibition of the oxygen layer increases giomer surface microhardness, ensuring better treatment outcome as a result of the improved dimensional stability and resistance to wear of restorations. It is therefore considered to be a contribution to clinical application. Moreover, it should be considered that the color of the Mylar strip may foster greater surface microhardness of the giomer restorative materials evaluated. This provides a basis for results to be extrapolated to longitudinal *in vivo* studies.

CONCLUSION

The study concludes that blue and conventional Mylar strips inhibit the oxygen layer on the giomer restorative composites Beautifil II and Beautifil II LS, providing higher surface microhardness. The best results were found in the giomer Beautifil II LS, microhardness differed significantly between blue Mylar and without Mylar strip, and between conventional Mylar and without Mylar strips. The microhardness of giomer Beautifil II LS was higher when blue Mylar strip was used, possibly due to the light passing through the Mylar strip better because they were both the same color.

ACKNOWLEDGEMENTS
The authors thank Dr. Melvin Cisneros, Academic Department of Stomatology, School of Stomatology, Universidad Científica del Sur, for contributing to acquiring the materials, participating in drafting, and critically reviewing the project.

DECLARATION OF CONFLICTING INTERESTS
The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

FUNDING
None.

REFERENCES

1. Kaya MS, Bakkal M, Durmus A, Durmus Z. Structural and mechanical properties of a giomer-based bulk fill restorative in different curing conditions. J Appl Oral Sci [Internet]. 2018;26(0). https://doi.org/10.1590/1678-7757-2016-0662
2. Rusnac ME, Gasparik C, Irimie AI, Grecu AG, Mesaroş AŞ, Dudea D. Giomers in dentistry - at the boundary between dental composites and glass-ionomers. Med Pharm Rep. 2019 Apr;92(2):123–8. https://doi.org/10.15386/mpr-1169
3. Garoushi S, Vallittu PK, Lassila L. Characterization of fluoride releasing restorative dental materials. Dent Mater J. 2018;37(2):293–300. https://doi.org/10.4012/dmj.2017-161
4. Zou J, Du Q, Ge L, Wang J, Wang X, Li Y, et al. Expert consensus on early childhood caries management. Int J Oral Sci. 2022 Jul 14;14(1):1–14. https://doi.org/10.1038/s41368-022-00186-0
5. Salcedo RM, Valverde AP. Giomeros en Odontopediatría. Revisión narrativa. Odontología Sanmarquina. 2020 Nov 13;23(4):445–9. https://doi.org/10.15381/os.v23i4.19105
6. Gordan VV, Blaser PK, Watson RE, Mjör IA, McEdward DL, Sensi LG, et al. A clinical evaluation of a giomer restorative system containing surface prereacted glass ionomer filler: results from a 13-year recall examination. J Am Dent Assoc. 2014 Oct;145(10):1036–43. https://doi.org/10.14219/jada.2014.57
7. Alinda SD, Margono A, Putranto AW, Maharti ID, Amalina R, Rahmi SF. The Comparison of Biofilm Formation, Mechanical and Chemical Properties between Glass Ionomer Cement and Giomer. TODENTJ. 2021 Jul 12;15(1):274–83. https://doi.org/10.2174/1874210602115010274
8. Ueta H, Tsujimoto A, Barkmeier WW, Oouchi H, Sai K, Takamizawa T, et al. Influence of an oxygen-inhibited layer on enamel bonding of dental adhesive systems: surface free-energy perspectives. Eur J Oral Sci. 2016 Feb;124(1):82–8. https://doi.org/10.1111/eos.12231
9. Zakiyah D, Effendy R, Prasetyo E. The effect of glycerin on the surface hardness and roughness of nanofill composite. Conservative Dentistry Journal. 2019 Dec 5;8:104. https://doi.org/10.20473/cdj.v8i2.2018.104-111
10. Robertson L, Phaneuf M, Haimeur A, Pesun I, França R. Degree of Conversion and Oxygen-Inhibited Layer Effect of Three Dental Adhesives. Dent J (Basel). 2016 Oct 27;4(4). https://doi.org/10.3390/dj4040037
11. Sherwood IA, Rathakrishnan M, Savadamaoothi KS, Bhargavi P, Vignesh Kumar V. Modified putty index matrix technique with mylar strip and a new clasification for selecting the type of matrix inanterior proximal/incisal composite restorations. Clin Case Rep. 2017; 5(7): 1141 - 6. https://doi.org/10.1002/ccr3.1006
12. Sismanoglu S, Gumustas B, Yildirim-Bilmez Z. Effect of polishing Systems on fluoride Elease and surface roughness of different restorative materials. Odovtos Int J Dent Sc. 2020; 22(1): 81 - 92. https://doi.org/10.15517/ijds.2020.39151
13. Mousavinasab SM, Barekatain M, Sadeghi E, Nourbakhshian F, Davoudi A. Evaluation of light curing distance and mylar strips color on surface hardness of two different dental composite resins. Open Dent J. 2014;8:144–7. https://doi.org/10.2174/1874210601408010144
14. Borges A, Pitta-Lopes J, Portugal J. Influência do tempo de exposição e distância à luz na capacidade de fotopolimerização de compósitos. Revista Portuguesa de Estomatologia, Medicina Dentária e Cirurgia Maxilofacial. 2015 Jul 1;56(3):166–72. https://doi.org/10.1016/j.rpemd.2015.07.001
15. Borisova-Papancheva T, Panov V, Georgieva S. Видове матрични системи, използвани в денталната медицина. Varna Medical Forum. 2017 Feb 15;6(1):92–102. https://doi.org/10.14748/vmf.v6i1.1922
16. Sánchez-Sánchez J, Rodríguez-Cervantes K, Armas A, García I, Oñate-Negrete H. Técnicas diferentes para eliminar la capa de resina inhibida por oxígeno, en un composite nanohíbrido sometido a desgaste abrasivo. Dominio de las Ciencias. 2018 Apr 30;4:20. https://doi.org/10.23857/dc.v4i2.776
17. Kazak M, Koymen S, Yurdan R, Tekdemir K, Dönmez N. Effect of thermal aging procedure on the microhardness and surface roughness of fluoride ion containing materials. Annals of Medical Research. 2020 Jan 1;27:888. https://doi.org/10.5455/annalsmedres.2019.12.831
18. Labonte D, Lenz AK, Oyen ML. On the relationship between indentation hardness and modulus, and the damage resistance of biological materials. Acta Biomaterialia. 2017 Jul 15;57:373–83. https://doi.org/10.1016/j.actbio.2017.05.034
19. Al-Ahdal K, Ilie N, Silikas N, Watts DC. Polymerization kinetics and impact of post polymerization on the Degree of Conversion of bulk-fill resin-composite at clinically relevant depth. Dental Materials. 2015 Oct 1;31(10):1207–13. https://doi.org/10.1016/j.dental.2015.07.004
20. Al-Zain A, Marghalani H. Influence of Light-curing Distances on Microflexural Strength of Two Resin-based Composites. Operative Dentistry. 2020 May 1;45(3):297–305. https://doi.org/10.2341/19-001-L
21. Gonulol N, Ozer S, Tunc E. Water Sorption, Solubility, and Color Stability of Giomer Restoratives. Journal of Esthetic and Restorative Dentistry. 2014 Sep 1;27. https://doi.org/10.1111/jerd.12119
22. Barekatain M, Mousavinasab SM, Azarbaijani S, Ranjbaran FS, Dadashi MZ. Degree of Conversion of Metacrylate- and Silorane-based Dental Composites at Various Depths and Using Different Matrix Bands. J Mash Dent Sch 2018; 42(2): 133-40. https://doi.org/10.22038/JMDS.2018.10887

https://doi.org/10.54589/aol.37/2/105

Changes in masseter muscle structure, membrane lipid peroxidation and Ca-ATPase activity as effects of different local anesthetics

Carolina de la Cal, Daniel E Di Croce, Delia Takara

Universidad de Buenos Aires. Facultad de Odontología, Cátedra de Biofísica y Bioestadística. Buenos Aires, Argentina.

ABSTRACT

Local anesthetics (LA) can cause undesired effects such as sustained contraction of skeletal muscles as a result of structural and functional changes. Proper skeletal muscle function is controlled by intracellular Ca^{2+} concentration and efficient energy (ATP) production, which is closely related to cell ultrastructure. ***Aim:*** *The aim of this study was to identify the structural and functional changes caused by LAs.* ***Materials and Method:*** *Male Wistar rats weighing 200 to 250g were used (n:49). They were divided into seven groups. One group was not anesthetized or treated (Control). The other six groups underwent intramuscular (IM) anesthesia with xylazine 2% (0.05 ml) and ketamine 50 mg/ml (0.1 ml/100g rat weight), and one of the following was applied to the masseter muscle (MM): no further treatment (Anesthetic Control group, CA); 0.1ml physiological saline solution (group SF); Carrageenin (group Carr) 1% as positive control group; prilocaine (group Pri), mepivacaine (group Mepi); or articaine (group Arti) 0.3M, IM. The animals were euthanized by cervical dislocation one hour after treatment. The effects of the different anesthetics on the MM were evaluated histologically and by electronic microscopy (EM). Ca-ATPase and membrane lipid peroxidation (LPX) were evaluated in muscle homogenates under the same conditions as those used to prepare the histological sections.* ***Results:*** *In general, structural damage and increased muscle contraction were observed in tissues treated with anesthetics. The most extreme values of Ca-ATPase activity and LPX were observed in the positive control group (carrageenin). Results were analyzed by one-way ANOVA for multiple comparisons and Tukey's test ($p < 0.05$).* ***Conclusions:*** *The results suggest that in the short term, local anesthetics affect the muscle function and are associated to structural changes.*

Keywords: *local anesthetics - masseter muscle - electronic microscopy - Ca-ATPase - membrane lipid peroxidation - carrageenin.*

Cambios estructurales del músculo masetero, lipoperoxidación de membrana y actividad de la Ca-ATPasa por efecto de distintos anestésicos locales.

RESUMEN

Los anestésicos locales (AL), pueden causar efectos no deseados como la contracción sostenida de los músculos esqueléticos como consecuencia de cambios estructurales y funcionales. Como es sabido, la función adecuada del músculo esquelético está controlada por la concentración de Ca^{2+} intracelular y por la producción eficiente de energía (ATP), íntimamente relacionado con la ultraestructura celular. ***Objetivo:*** *El propósito de este trabajo fue relacionar los cambios estructurales y funcionales provocados por los AL.* ***Materiales y Método:*** *se utilizaron ratas Wistar macho de 200 a 250g de peso (n:49), bajo anestesia intramuscular (IM) de xilazina 2% (0,05 ml) y ketamina 50 mg/ml (0,1 ml/100g peso de rata) se les aplicó en el músculo masetero (MM): 0,1ml de solución fisiológica (grupo SF) o Carragenina (grupo Carr) 1% como grupo control positivo o Prilocaína (grupo Pri), Mepivacaína (grupo Mepi) y articaína (grupo Arti) 0,3M, IM. Un grupo no recibió tratamiento, grupo Control (C). Se realizó la eutanasia por dislocación cervical a la hora post tratamiento. Se evaluó histológicamente y mediante microscopía electrónica (ME) los efectos de los distintos anestésicos a la hora post-inyección en músculo masetero. Además, se evaluó la actividad Ca-ATPásica y la lipoperoxidación de membrana (LPX) en homogenatos de músculo con las mismas condiciones que el utilizado para realizar los cortes histológicos.* ***Resultados:*** *En general se observaron daños estructurales en los tejidos tratados con anestésicos y aumento de la contracción muscular. Los valores extremos de la actividad Ca-ATPásica y la LPX se observaron en el control positivo (carragenina). Los resultados obtenidos se analizaron por comparación múltiple ANOVA de 1 via y test de Tukey ($p < 0,05$).* ***Conclusiones:*** *los resultados sugieren que los AL, a corto plazo, afectarían la función muscular asociados a los cambios estructurales.*

Palabras Clave: *anestésicos locales - músculo masetero - microscopía electrónica - Ca-ATPasa - lipoperoxidación de membrana - carragenina.*

To cite:
de la Cal C, Di Croce DE, Takara D. Changes in masseter muscle structure, membrane lipid peroxidation and Ca-ATPase activity as effects of different local anesthetics. Acta Odontol Latinoam. 2024 Aug 30;37(2):105-113. https://doi.org/10.54589/aol.37/2/105

Corresponding Author:
Carolina de la Cal
carolina.delacal@odontologia.uba.ar

Received: December 2023
Accepted: June 2024

INTRODUCTION

Local anesthetics (LA) have been used since 1905 to block pain temporarily and reversibly in specific parts of the body. LAs block the transmission of the nerve impulse in any part of the system, causing loss of sensitivity, which recovers completely when the effect wears off[1]. However, LAs may cause an undesired effect involving sustained contraction of skeletal muscles involved in mastication. This condition is called "trismus"[2, 3].

In skeletal muscle, the sarcoplasmic reticulum (SR) is the main regulator of calcium storage, release and reuptake. Glycolysis and mitochondria are responsible for cell production of the ATP required for cell metabolism[4,5].

Proper skeletal muscle function is controlled by intracellular Ca^{2+} concentration and efficient energy (ATP) production, which depend on: (a) the release and reuptake of Ca^{2+} by the sarcoplasmic reticulum (SR) during excitation-contraction (EC) coupling, which controls sarcomere contraction and relaxation; (b) Ca^{2+} uptake in the mitochondrial matrix, which stimulates aerobic ATP production; and (c) entry of Ca^{2+} from the extracellular space by activation of store-operated Ca^{2+} entry (SOCE), an important mechanism for preventing muscle fatigue[6-10].

Calcium uptake into the mitochondria increases ATP production by stimulating oxidative phosphorylation and mitochondrial ATP production, as well as production and/or detoxification of reactive oxygen and nitrogen species (ROS/RNS). Mitochondria in turn modulate the release and reuptake of calcium by the SR. This close spatial Ca^{2+}/ATP/ROS/RNS communication between the SR and mitochondria is facilitated by the structural attachment between mitochondria and the calcium release unit (CRU)[4,11]. While mitochondrial uptake of Ca^{2+} after release from the SR in the muscle can stimulate ATP production by excitation-metabolism coupling, Ca^{2+} signaling is also markedly influenced by mitochondrial function. Firstly, mitochondrial ATP production is used to drive both crossbridge cycling during muscle contraction and that mediated by SR Ca^{2+}-ATPase and elimination of myoplasmic Ca^{2+} during relaxation. Indeed, up to 80% of the ATP consumed during muscle contraction is used to drive elimination of Ca^{2+} by the SR during contractile relaxation[7]. The impact of mitochondrial ATP production is particularly important during activity when glycolytic reserves become depleted[8].

In skeletal muscle, the increase in intracellular Ca^{2+} returns to its physiological values due to the activity of SR Ca^{2+}-ATPase, which catalyzes the transport of two Ca^{2+} ions from the cytosol to the lumen of the SR per mole of hydrolyzed ATP, using the magnesium cation as cosubstrate[12-15]. Our group has published several studies on preparations of different rabbit masticatory muscles in which the composition of muscle fibers was determined and related to the distribution of different isoform, enzymatic activity and calcium transport[16-19]. These mechanisms are also altered by different injurious factors such as the action of LAs[20-25]. In addition, in striated muscle, LAs cause the appearance of ROS which may interact with lipids[7].

The current study analyzes at the effect of different LAs on changes in skeletal muscle fiber ultrastructure, SR Ca^{2+}-ATPase activity, and antioxidant response in rat masseter muscle.

MATERIALS AND METHOD

Animals

This study used 49 healthy male Wistar rats weighing 200 to 250g. They were given "ad libitum" access to balanced feed and water and were housed in 32x45x24cm galvanized wire cages containing not more than five animals each, at temperature 21 ºC to 24 ºC; humidity 52% to 56%; and 12/12 h light/dark cycles. The experimental protocol was approved by the Ethics Committee at the School of Dentistry (005/2016 CICUAL-ODONTO-FOUBA Buenos Aires, Argentina), and follows the National Health Institutes Guidelines for the care and use of laboratory animals.

The rats were divided into seven groups of seven animals. One group received no treatment and served as Control (C). The rest received intramuscular (IM) anesthesia in the back leg with xylazine 2% (0.05 ml) and ketamine 50 mg/ml (0.1 ml/100g rat weight) plus one of the following: no further treatment (Anesthesia Control group, CA); or masseter muscle (MM) injection with: 0.1ml physiological saline solution (group SF); carrageenin 1% (group Carr, positive control group)[26]; prilocaine (group Pri), mepivacaine (group Mepi), or articaine (group Arti) 0.3M, IM. The rats were euthanized by cervical dislocation one hour after treatment. Subsequently, the masseter muscle was dissected and processed for evaluation.

Reagents

All reagents were acquired from Sigma Chemicals (St. Luis, Mo., USA). All solvents were analytical grade.

Electronic microscopy

MM specimens were fixed in a solution of glutaraldehyde 2.5% in phosphate buffer 0.1M pH 7.4 for 4hs at 4ºC. Then they were washed twice with phosphate buffer 0.1M, first for 15 minutes and then overnight. The specimens were sent to the Lanais-Mie, Institute of Cell Biology and Neurosciences, run by CONICET, where the tissues continued to be processed. They were fixed for a second time with osmium tetroxide 1% in the same buffer for 60 minutes at 4ºC, rinsed twice for 15 minutes with bidistilled water, and then dehydrated in ascending alcohols (50º, 70º, 96º, 100º) with two 15-minute changes each followed by two 10-minute changes of acetone. Specimens were embedded in "Durcupan" epoxy resin, which was polymerized at 60ºC for 72 hours, and then cut into semi-fine 0.50 µm sections using an ultramicrotome (Reichert Jung Ultracut E) with a glass knife. Sections were mounted on slides and stained with toluidine blue for observation under optical microscope to determine the direction of the section of muscle fibers, and then observe them by electronic microscopy.

For observation by transmission electron microscopy (TEM), ultrafine 70-90 nm sections were cut using the same ultramicrotome. Sections were mounted on a copper grid, contrasted with uranyl acetate and lead citrate (Reynolds' method) and observed under a Zeiss 109 transmission electron microscope. Digital photographs were taken with a Gatan W10000 camera.

To provide information on contractile activity, the fibers on the sections were measured transversely, and the number and size of mitochondria were evaluated because, as mentioned above, they are closely related to contractile activity due to the structural attachment with the calcium release unit (CRU). Mitochondrial size was calculated by the point-counting stereological technique[27] on TEM micrographs taken at magnification 12,000X after superimposing an orthogonal matrix of points 0.50 µm apart on the electronic micrographs. The ratio between the number of points within the mitochondrial outlines and the total number of points covering the whole image was used to calculate the relative area of the fiber occupied by mitochondria. The same set of micrographs was used to determine number of mitochondria per field, and muscle contraction by measuring the distance between Z lines. Sections with any of the following structural alterations were classified as damaged: swollen mitochondria, interrupted Z line, vacuoles, disorganized CRU.

Determination of membrane lipid peroxidation

LAs in striated muscle may cause the appearance of ROS that can interact with lipids. Therefore, a homogenate of MM was prepared in which to determine membrane lipid peroxidation (LPX) by means of the reaction between thiobarbituric acid (TBA) and the aldehyde products derived from the rupture of polyunsaturated fatty acids (thiobarbituric acid reactive substances or TBARS) (µmol MDA/mg Prot) and assess the effects of ROS production[28].

Briefly, the MMs were homogenized in 320 ml of Tris buffer 0.1 N, pH 7.4. Twenty ml were separated to determine proteins, and the rest was centrifuged at 1500 g for 20 min at 4°C. Then, 250 ml of the supernatants were transferred to a tube containing 250 ml trichloroacetic acid. The mixture was centrifuged at 3000 g for 15 min at room temperature. The supernatants were mixed with 250 ml of TBA, boiled for 15 min, and cooled on ice for 5 min to stop the reaction. The resulting reactive TBA species-stained pink (TBARS) were determined in a spectrophotometer (Beckman DU 520) at 540 nm. The acid did not produce color when tested without addition of the sample, showing the absence of a direct reaction to the TBA. The calibration curve was prepared using malondialdehyde (MDA), and each point on the curve was subjected to the same treatment as the muscles. The TBARS were calculated as micromoles of MDA per milligram of protein[29].

Determination of Ca-ATPase activity

SR Ca-ATPase enzymatic activity was determined to corroborate that the pump was not inhibited, preventing muscle relaxation in groups C, CA, SF and Carr, since our team had not analyzed this in any previous study[30]. To do so, sealed rat MM SR membrane vesicles were obtained, with capacity to accumulate calcium, following Champeil et al.[31].

For all the procedures, protein concentration was determined using the technique described by Lowry et al.[32].

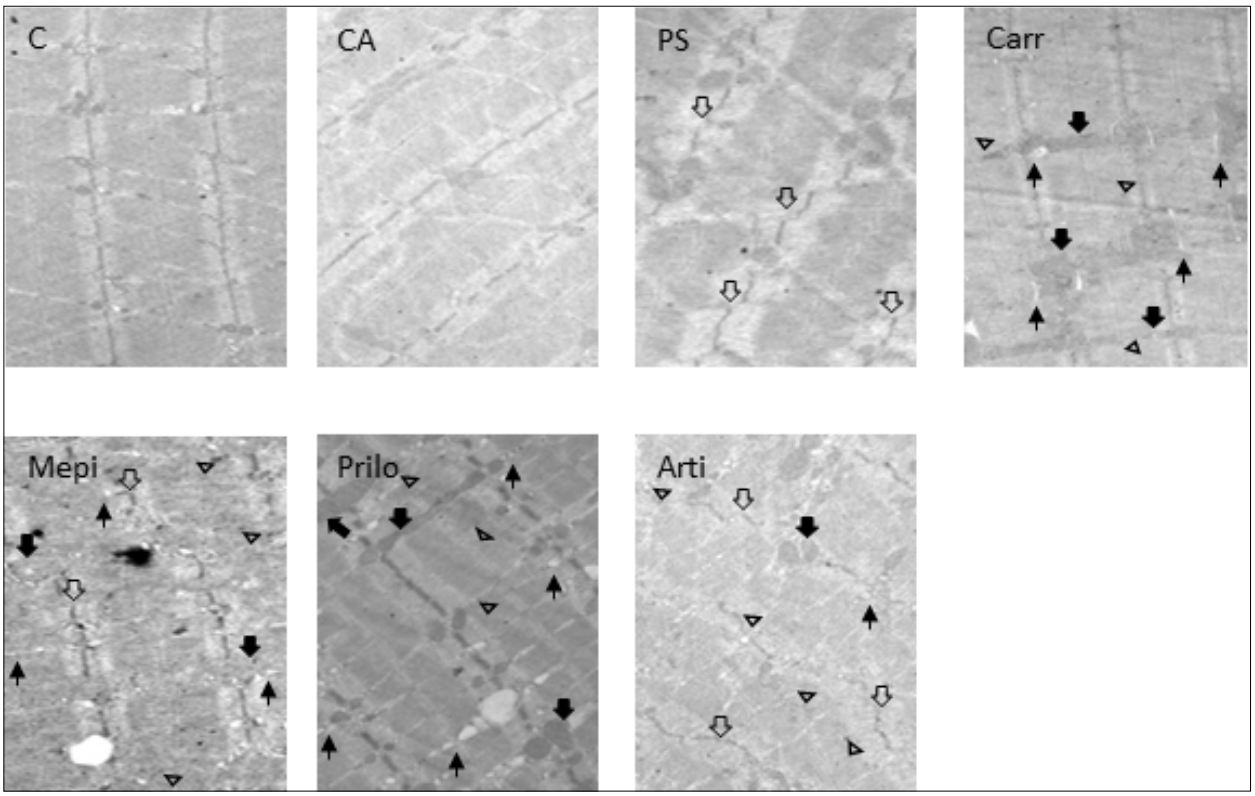

Fig. 1: Electron micrograph of masseter muscle (X12,000) C: Control. CA: Control Anesthetic SF: Physiological saline solution. These three longitudinal sections show that muscle structure is preserved. There is only a partial misalignment of microfibrils in muscles treated with PS (unfilled arrows). Carr: carrageenin. There are many calcium release units (CRU) (small arrows) near the mitochondria (filled arrows) showing clear signs of structural damage/swelling. There are also intermyofibrillar glycogen granules (unfilled arrowheads) near the longitudinal part of the sarcoplasmic reticulum. Mepi: Mepivacaine. Prilo: Prilocaine. Arti: Articaine. The three figures mainly show an increase in size and disorganization of structures, misalignment of myofibrils, and mitochondria with signs of damage/swelling.

The results were analyzed by one-way ANOVA multiple comparison and Tukey's multiple comparisons test, considering $p<0.05$.

RESULTS

Electronic microscopy images of MM showed that in groups C, CA, and SF, which were not treated with drugs, the structures were preserved. At ultrastructure level, partial misalignment of microfibrils (Z lines) was detected in groups SF, Mepi and Arti. In the groups treated with the different LAs, extensive areas with morphological anomalies (such as vacuoles) were observed, among which muscles in group Arti were more damaged than the others (Fig. 1). Muscle contraction values were significantly lower in all groups compared to control ($p<0.001$), with group Carr (positive control) having the greatest contraction (1.39 µm ± 0.03 µm) (Fig. 2).

Mitochondrial count was significantly lower in group Arti than the other groups, in which number of mitochondria was preserved (Fig. 3). Mitochondrial area represents mitochondrial volume and is related to structural damage. Group Prilo had the largest mitochondria (2.04 µm ± 0.42µm), compatible with mitochondrial inflammation, with significant difference ($p<0.001$) with respect to the other groups. In group Mepi, mitochondrial size was similar to that in the control group (C:1.10±0.33; Mepi:1.19±0.23), while the rest of the groups had smaller mitochondria (CA:0.61±0.30; SF:0.56±0.13; Arti:0.36±0.11; Carr:0.39±0.14) (Fig. 4).

Ca-ATPase activity (µmol Pi/ mg protein per hour) differed among groups, with much lower activity in the positive control group (Carr) (C:489.67±11.07; CA:443.47±18.35; PS:417.79±12.13; Carr:294.55±9.70) (Fig. 5).

LPX was significantly higher in the positive control group Carr ($9.85x10^{-3}$ ± $2.12x10^{-4}$) than in the other groups. LPX was also higher in group Prilo

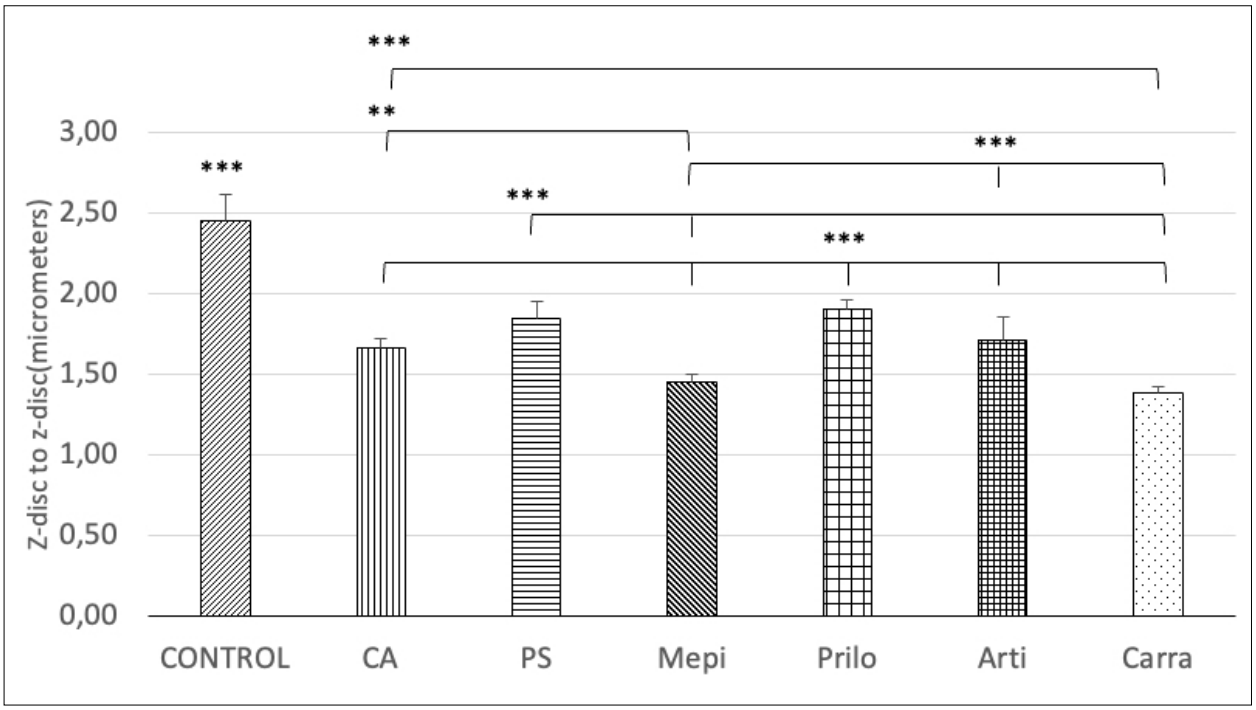

Fig. 2: ***Muscle contraction.*** *Expressed as distance in µm between Z lines. There is a significant reduction (p< 0.001) in all groups compared to control (2.45±0.16), with group Carr showing the greatest contraction (1.39 ± 0.03).*

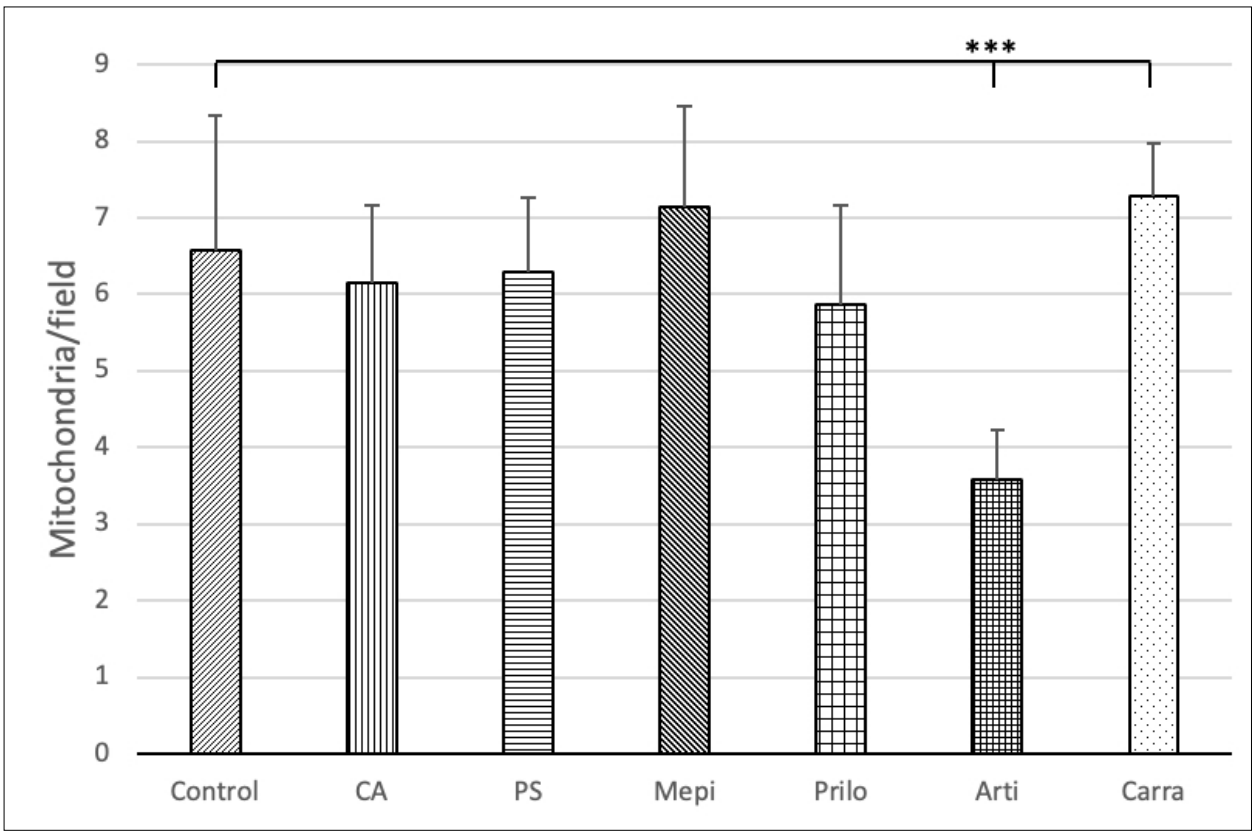

Fig. 3: ***Number of mitochondria.*** *The number of mitochondria per field is lower in group Arti (p=0.002) than in groups C, Carr y Mepi. No difference is observed with the other groups.*

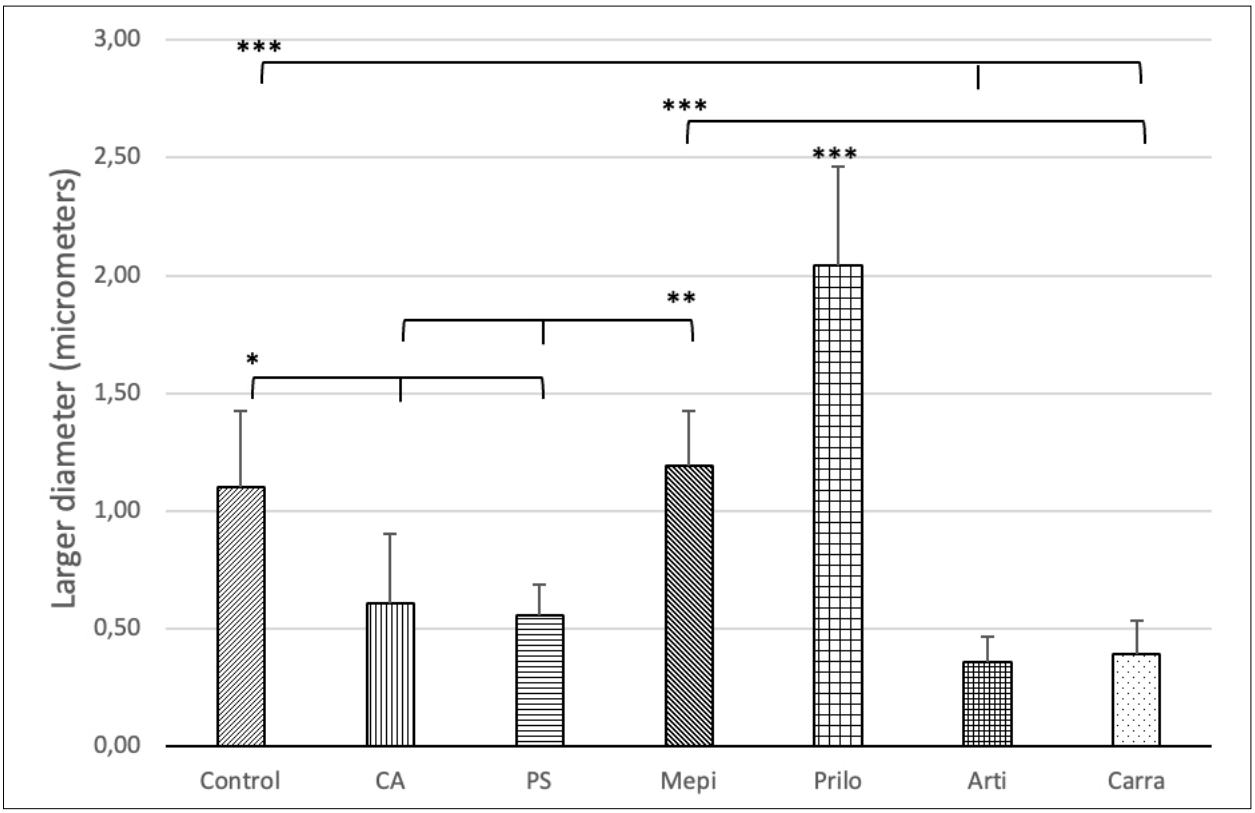

Fig. 4: ***Mitochondrial size.*** *Group Prilo has the largest mitochondria (2.04 µm ± 0.42µm) (p<0.001) compared to the other groups (C:1.10±0.33; CA:0.61±0.30; SF:0.56±0.13; Mepi:1.19±0.23; Arti:0.36±0.11; Carr:0.39±0.14).*

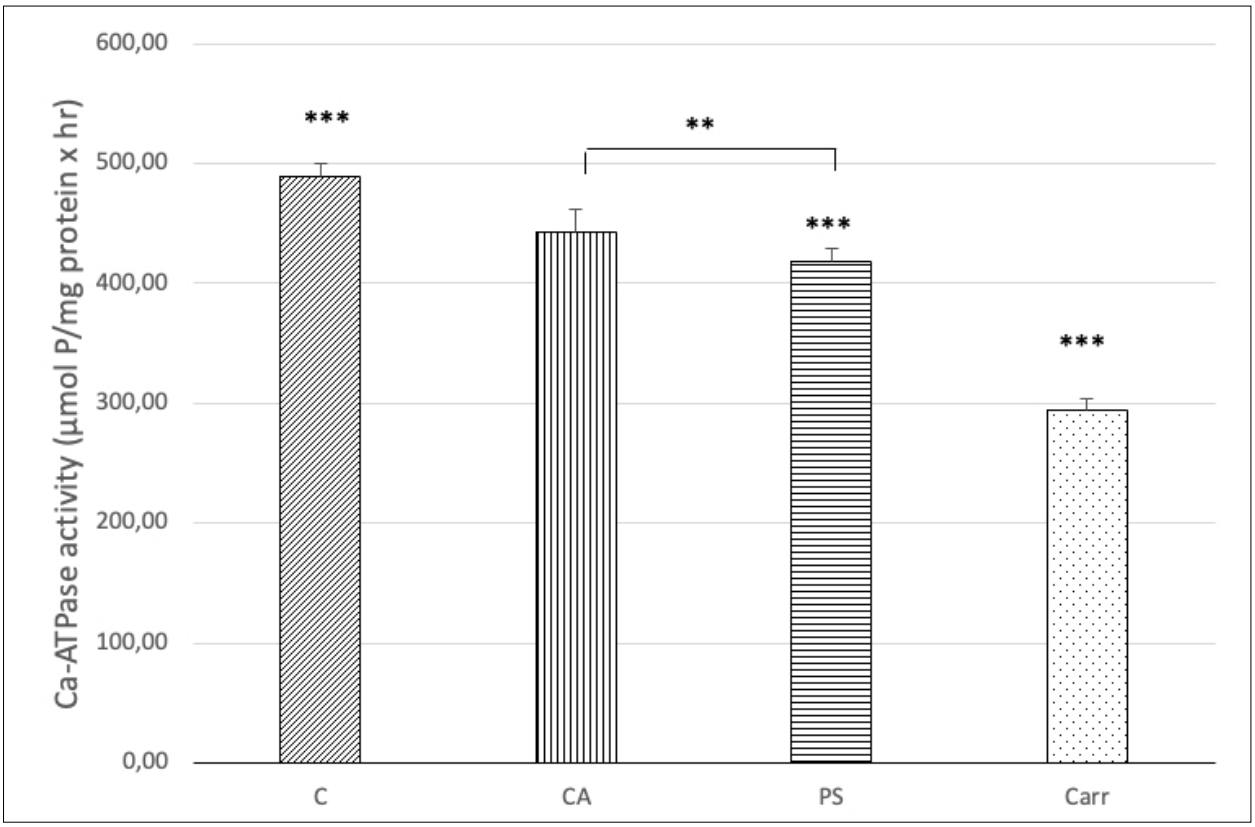

Fig. 5: ***Ca-ATPase activity.*** *There is less activity in all groups than in the control group, with group Carr (positive control) having the lowest enzymatic activity.*

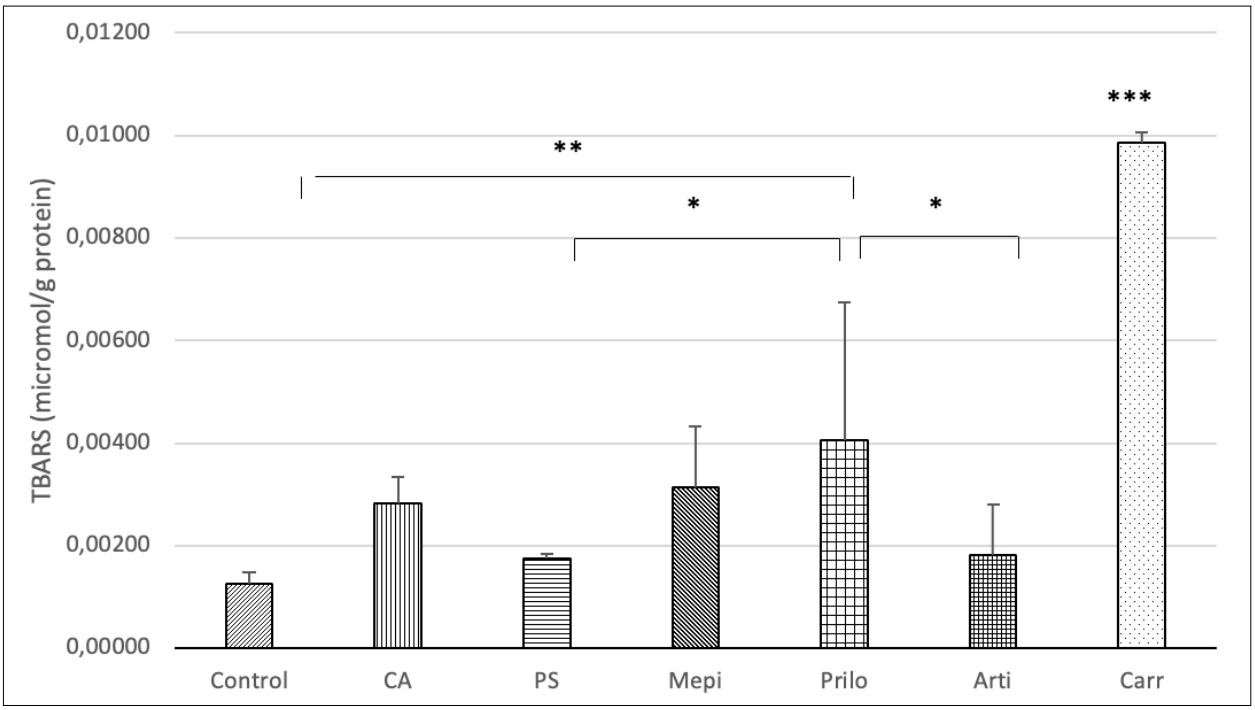

Fig. 6: ***Membrane lipid peroxidation.*** *Group Carr has a highly significant increase in membrane lipid peroxidation compared to the other groups. Group Prilo differs significantly from groups C, SF and Arti.*

($4.05x10^{-3} \pm 2.73x10^{-3}$) than in groups C, SF and Arti ($1.26x10^{-3} \pm 2.30x10^{-4}$; $1.75x10^{-3} \pm 7.78x10^{-5}$; $1.81x10^{-3} \pm 1.02x10^{-3}$) (Fig. 6).

DISCUSSION

All groups treated with LA presented morphological anomalies such as lack of continuity of the muscle tissue. Histological assessment suggests that all the treatments generate muscle contraction, but that in addition, the study LAs, as from the time they are applied, produce different structural changes in the MM, as reported by Gitman et al. (2019)[33]. Our results regarding mitochondrial size agree with several authors who suggest that the increase or reduction in size may be associated to mechanisms of mitochondrial fusion or fission[34,35] as a result of the stress produced in the environment, with the purpose of regulating mitochondrial morphology and function. Other authors attribute the increase in mitochondrial size to an inflammatory response[36].

The Ca-ATPase pump activity values in skeletal muscle found in groups C, CA, SF and Carr were consistent with the results published previously by our group in animals treated with LA, corroborating the interaction of these treatments in pump activity[22,25,37]. The increase in TBARS was used as an indicator of the appearance of ROS. Groups Mepi and Prilo have similar LPX values. It is worth highlighting that the micrograph of group Arti is similar to that of the control group, possibly as a result of destruction of muscle ultrastructures, which may prevent activation of the lipid peroxidation process. Similar effects have been described in salivary glands treated with ionizing radiation[29]. Qaisar et al. (2019) suggests that the increase in oxidative stress is closely related to the malfunction of the Ca-ATPase pump[38]. This is consistent with our results, where the increase in TBARS might be associated with reduction in pump activity.

It is important to consider that the differences observed among the study anesthetics could be owed to their chemical structure. They are all amide groups, with articaine having the greatest molar mass, in addition to containing sulfur and one additional ester group in its composition[39]. These structural features may be related to the extent of the damage recorded in the micrographs.

All of this suggests that in the short term, LAs cause structural changes that affect muscle function. Further medium- and long- term studies are needed on the evolution of muscle structure response such as recovery or increase in the muscle

destruction observed. With regard to the dental anesthetic cartridge, the absence of adrenaline in our determinations may modify the responses obtained in this study.

ACKNOWLEDGMENTS
The authors are greatly indebted to Ricardo Horacio Orzuza for technical assistance.

CONFLICT OF INTEREST
The authors declare no conflicts of interest concerning the publication of this article.

FUNDING
This study was supported by grant from the University of Buenos Aires UBACyT 20020150100119BA.

REFERENCES

1. Bonet R. Anestésicos Locales. Offarm.2011;30(5):42-47.
2. Ogle OE, Mahjoubi G. Local anesthesia: agents, techniques, and complications. Dent Clin North Am.2012;56(1):133-48. https://doi.org/10.1016/j.cden.2011.08.003
3. Martínez Martínez A, Simancas Escorcia V, Díaz Caballero A. Complicaciones asociadas a la anestesia bucal: diagnóstico y manejo. Cient. Dent.2021;18(2) 97-102.
4. Rossi AE, Boncompagni S, Dirksen RT. Sarcoplasmic reticulum-mitochondrial symbiosis: bidirectional signaling in skeletal muscle. Exerc Sport Sci Rev.2009;37(1):29-35. https://doi.org/10.1097/JES.0b013e3181911fa4
5. Dirksen RT. Sarcoplasmic reticulum-mitochondrial through-space coupling in skeletal muscle. Appl Physiol Nutr Metab.2009;34(3):389-95. https://doi.org/10.1139/H09-044
6. Protasi F, Pietrangelo L, Boncompagni S. Calcium entry units (CEUs): perspectives in skeletal muscle function and disease. J Muscle Res Cell Motil.2021;42(2):233-249. https://doi.org/10.1007/s10974-020-09586-3
7. Michelucci A, Liang C, Protasi F, Dirksen RT. Altered Ca2+ Handling and Oxidative Stress Underlie Mitochondrial Damage and Skeletal Muscle Dysfunction in Aging and Disease. Metabolites.2021;11(7):1-19. https://doi.org/10.3390/metabo11070424
8. Protasi F, Pietrangelo L, Boncompagni S. Improper Remodeling of Organelles Deputed to Ca2+ Handling and Aerobic ATP Production Underlies Muscle Dysfunction in Ageing. Int J Mol Sci.2021;22(12):1-25. https://doi.org/10.3390/ijms22126195
9. Michelucci A, García-Castañeda M, Boncompagni S, Dirksen RT. Role of STIM1/ORAI1-mediated store-operated Ca2+ entry in skeletal muscle physiology and disease. Cell Calcium.2018;76:101-115. https://doi.org/10.1016/j.ceca.2018.10.004
10. Koenig X, Choi RH, Schicker K, Singh DP, Hilber K, Launikonis BS. Mechanistic insights into store-operated Ca2+ entry during excitation-contraction coupling in skeletal muscle. Biochim Biophys Acta Mol Cell Res.2019;1866(7):1239-1248. https://doi.org/10.1016/j.bbamcr.2019.02.014
11. Paolini C, Quarta M, Wei-LaPierre L, Michelucci A, Nori A, Reggiani C, Dirksen RT, Protasi F. Oxidative stress, mitochondrial damage, and cores in muscle from calsequestrin-1 knockout mice. Skelet Muscle.2015; 5:1-17. https://doi.org/10.1186/s13395-015-0035-9
12. De Meis L. The sarcoplasmic reticulum. Transport and energy transduction. Ed. John Wiley and Sons, New York.1981.
13. Inesi G. Mechanism of calcium transport. Annu Rev Physiol.1985; 47:573-601. https://doi.org/10.1146/annurev.ph.47.030185.003041
14. Jørgensen MM, Bross P, Gregersen N. Protein quality control in the endoplasmic reticulum. APMIS Suppl.2003;109:86-91.
15. Lodish H, Berk A, Matsudaira P, Kaiser CA, Krieger M, Scott MP, Ziúrsky SL y Darnell. Biología celular y molecular. Ed. Médica Panamericana.2005, 5° Ed.
16. Sánchez GA, Takara D, Toma AF, Alonso GL. Characteristics of the sarcoplasmic reticulum Ca2+-dependent ATPase from masticatory muscles. J Dent Res.2004;83(7):557-61. https://doi.org/10.1177/154405910408300709
17. Di Croce DE, Trinks PW, de la Cal C, Takara D, Sanchez GA. Expresión de la proteína bomba de calcio de retículo sarcoplásmico en el músculo masetero.. Rev. Fac. Odontol, Univ. Buenos Aires 2012; 27(63):24-31.
18. Sánchez GA, Di Croce DE, Casadoumecq AC, Richard SB, Takara D. Characterization of the sarcoplasmic reticulum Ca-ATPase from rabbit temporalis muscle. Arch Oral Biol.2012;57(10):1429-37. https://doi.org/10.1016/j.archoralbio.2012.08.005
19. de la Cal C, Trinks GG, Corti S, Sánchez GA. Differential Effect of Articaine on Sarcoendoplasmic Reticulum Calcium Adenosine Triphosphatase of Medial Pterygoid Muscle. J Oral Facial Pain Headache.2017;31(4):21-28. https://doi.org/10.11607/ofph.1835
20. Takara D, Sánchez GA, Alonso GL. Effect of carticaine on the sarcoplasmic reticulum Ca2+-dependent adenosine triphosphatase. Naunyn Schmiedebergs Arch Pharmacol.2000;362(6):497-503. https://doi.org/10.1007/s002100000259
21. Takara D, Sánchez GA, Toma AF, Bonazzola P, Alonso GL. Effect of carticaine on the sarcoplasmic reticulum Ca2+-adenosine triphosphatase. II. Cations dependence. Naunyn Schmiedebergs Arch Pharmacol.2005;371(5):375-82. https://doi.org/10.1007/s00210-005-1061-7
22. Sánchez GA, Takara D, Alonso GL. Local anesthetics inhibit Ca-ATPaseinmasticatorymuscles.JDentRes.2010;89(4):372-7. https://doi.org/10.1177/0022034510363220
23. Sánchez GA, Casadoumecq AC, Alonso GL, Takara D. Inhibitory effect of lidocaine on the sarcoplasmic reticulum Ca2+-dependent ATPase from temporalis muscle. Acta Odontol Latinoam.2010;23(2):92-8.
24. Sánchez GA, Di Croce DE, Richard SB, Takara D. Effect

of articaine on calcium transport in sarcoplasmic reticulum membranes isolated from medial pterygoid muscle. Acta Odontol Latinoam.2012;25(1):34-9.
25. Sánchez GA, Di Croce DE, de la Cal C, Richard SB, Takara D. Differential mechanism of the effects of ester-type local anesthetics on sarcoplasmic reticulum Ca-ATPase. Naunyn Schmiedebergs Arch Pharmacol.2013;386(12):1061-9. https://doi.org/10.1007/s00210-013-0907-7
26. Albertini R, Villaverde AB, Aimbire F, Salgado MA, Bjordal JM, Alves LP, Munin E, Costa MS. Anti-inflammatory effects of low-level laser therapy (LLLT) with two different red wavelengths (660 nm and 684 nm) in carrageenan-induced rat paw edema. J Photochem Photobiol B.2007;89(1):50-5. https://doi.org/10.1016/j.jphotobiol.2007.08.005
27. Mobley BA, Eisenberg BR. Sizes of components in frog skeletal muscle measured by methods of stereology. J Gen Physiol.1975;66(1):31-45. https://doi.org/10.1085/jgp.66.1.31
28. Buege, J.A., Aust, S.D. Microsomal lipid peroxidation. Methods Enzy-mol. 1978;52:302-310. https://doi.org/10.1016/S0076-6879(78)52032-6
29. de la Cal C, Lomniczi A, Mohn CE, De Laurentiis A, Casal M, Chiarenza A, Paz D, McCann SM, Rettori V, Elverdín JC. Decrease in salivary secretion by radiation mediated by nitric oxide and prostaglandins. Neuroimmunomodulation.2006;13(1):19-27. https://doi.org/10.1159/000093194
30. Baginski ES, Foà PP, Zak B. Microdetermination of inorganic phosphate, phospholipids, and total phosphate in biologic materials. Clin Chem.1967;13(4):326-32. https://doi.org/10.1093/clinchem/13.4.326
31. Champeil P, Guillain F, Vénien C, Gingold MP. Interaction of magnesium and inorganic phosphate with calcium-deprived sarcoplasmic reticulum adenosinetriphosphatase as reflected by organic solvent induced perturbation. Biochemistry.1985;24(1):69-81. https://doi.org/10.1021/bi00322a012
32. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem.1951;193(1):265-75. https://doi.org/10.1016/S0021-9258(19)52451-6
33. Gitman M, Fettiplace MR, Weinberg GL, Neal JM, Barrington MJ. Local Anesthetic Systemic Toxicity: A Narrative Literature Review and Clinical Update on Prevention, Diagnosis, and Management. Plast Reconstr Surg.2019;144(3):783-795. https://doi.org/10.1097/PRS.0000000000005989
34. Andrieux P, Chevillard C, Cunha-Neto E, Nunes JPS. Mitochondria as a Cellular Hub in Infection and Inflammation. Int J Mol Sci. 2021;22(21):11338. https://doi.org/10.3390/ijms222111338
35. Chen X, Ji Y, Liu R, Zhu X, Wang K, Yang X, Liu B, Gao Z, Huang Y, Shen Y, Liu H, Sun H. Mitochondrial dysfunction: roles in skeletal muscle atrophy. J Transl Med. 2023;21(1):503. https://doi.org/10.1186/s12967-023-04369-z
36. Zhao M, Wang Y, Li L, Liu S, Wang C, Yuan Y, Yang G, Chen Y, Cheng J, Lu Y, Liu J. Mitochondrial ROS promote mitochondrial dysfunction and inflammation in ischemic acute kidney injury by disrupting TFAM-mediated mtDNA maintenance. Theranostics. 2021; 11(4):1845-1863. https://doi.org/10.7150/thno.50905
37. Di Croce D, Trinks PW, Grifo MB, Takara D, Sánchez GA. Drug action of benzocaine on the sarcoplasmic reticulum Ca-ATPase from fast-twitch skeletal muscle. Naunyn Schmiedebergs Arch Pharmacol. 2015;388(11):1163-70. https://doi.org/10.1007/s00210-015-1149-7
38. Qaisar R, Bhaskaran S, Ranjit R, Sataranatarajan K, Premkumar P, Huseman K, Van Remmen H. Restoration of SERCA ATPase prevents oxidative stress-related muscle atrophy and weakness. Redox Biol. 2019; 20:68-74. https://doi.org/10.1016/j.redox.2018.09.018
39. Catteral WA, Mackie K.Local Anestethics. Cap 15. Hardman JG, Limbird LE: Goodman y Gilman's. The pharmacological basis of therapeutics. EEUU: McGraw-Hill Companies,1996: 331-347.

https://doi.org/10.54589/aol.37/2/114

Oral manifestations during dengue infection: a systematic review

Gustavo Canales Sermeño[1], Marisel R Valenzuela Ramos[2], Pedro M Dias Monteiro[3], Dario E Medina Castro[4], Nicole K Medina Valera[4]

1. Universidad Nacional "San Luis Gonzaga". Ica, Perú.
2. Universidad Tecnológica de los Andes (UTEA). Abancay, Perú.
3. Universidad César Vallejo (UCV). Lima, Perú.
4. Universidad Nacional de Trujillo (UNT). Trujillo, Perú.

ABSTRACT

Dengue is a global public health problem, especially in countries with tropical and subtropical climates. ***Aim:*** *To describe the oral manifestations, present during dengue infection.* ***Materials and method:*** *A qualitative systematic review was conducted in OSF Registries. The search was conducted in PubMed, Scielo and Scopus, from June 15 to July 18, 2023, using MeSH term equations with Boolean operators. A total 299 articles were analyzed in three stages, leaving 8 studies for review. RAYYAN was used for selection and tables for study analysis. Studies were assessed under two criteria: metadata, and intraoral and extraoral manifestations of dengue.* ***Results:*** *The review included 8 studies, published in 2012, 2013, 2014, 2019, 2020 and 2021. The studies reported hemorrhagic dengue and type 1 dengue as diagnoses. They recorded presence of hemorrhagic and edematous gums, maculopapular lesions in the mucosa of the lower lip with pain and dysphagia, Pseudomembranous candidiasis, and edematous and erythematous taste buds.* ***Conclusions:*** *Reports of oral manifestations of dengue are deficient, and further research is required to enable correct diagnosis and differentiation from other pathologies.*

Keywords: *dengue - oral manifestations - Aedes aegypti*

Manifestaciones orales durante la infección por dengue: una revisión sistemática

RESUMEN

El dengue es un problema de salud pública mundial en especial cuando se trata de países con climas tropicales y subtropicales. ***Objetivo:*** *Describir las manifestaciones orales presentes durante la infección por dengue.* ***Materiales y Método:*** *Se realizó una revisión sistemática cualitativa registrada en OSF Registries. La búsqueda se realizó en PubMed, Scielo y Scopus; desde el 15 de junio al 18 de julio del 2023, y se empleó ecuaciones de términos MeSH con operadores booleanos. Se obtuvieron 299 artículos analizados en tres etapas, quedando 8 investigaciones para su revisión. Se empleó RAYYAN para la selección y tablas para el análisis de los estudios. Se valoraron los estudios bajo dos criterios: metadatos y manifestaciones intraorales y extraorales por dengue.* ***Resultados:*** *Se revisaron 8 estudios publicados en el 2012, 2013, 2014, 2019, 2020 y 2021. Reportaron como diagnóstico dengue hemorrágico y dengue tipo 1, señalaron la presencia de encías hemorrágicas y edematosas, lesiones maculopapulares en mucosa de labio inferior con dolor y disfagia; asimismo, se registró candidiasis pseudomembranosa, papilas gustativas edematosas y eritematosas.* ***Conclusiones:*** *Los reportes de manifestaciones orales por dengue son deficientes, se requiere de más investigaciones para poder realizar un correcto diagnóstico y diferenciarlo de otras patologías.*

Palabras clave: *dengue - manifestaciones bucales - Aedes aegypti*

To cite:
Canales Sermeño G, Valenzuela Ramos MR, Dias Monteiro PM, Medina Castro DE, Medina Valera NK. Oral manifestations during dengue infection: a systematic review. Acta Odontol Latinoam. 2024 Aug 30;37(2):114-122. https://doi.org/10.54589/aol.37/2/114

Corresponding Author:
Gustavo Canales Sermeño
20186827@unica.edu.pe

Received: December 2023.
Accepted: June 2024.

INTRODUCTION

Dengue is a global public health problem, especially in countries with tropical and subtropical climates, where dengue is endemic. In the last 50 years, the incidence of dengue has multiplied by 30[1].

In Perú, as of week 20 in 2023, 98,760 cases of dengue and 121 deaths from dengue had been reported (93 confirmed and 28 under investigation). These figures have increased since 2017, when as of the same week, 49,031 cases had been reported; and 2022, with 38,887 cases[2].

The departments hardest hit by dengue as of week 20 were Piura, with 28,114; Lima, with 10,431; Ica, with 9,120, and Lambayeque, with 8,550. The departments Arequipa, Moquegua, Huancavelica and Apurimac had not reported dengue cases as of week 20. The most common clinical form of dengue is dengue without warning signs, followed by dengue with warning signs and severe dengue[2].

Dengue is caused by the dengue virus (DenV), which belongs to the family Flaviridae, and has four serotypes (DENV 1-4)[3]. The infection is transmitted by the *Aedesa egypti* mosquito[4], considered to be the main vector[5].

Dengue infection has various presentations, depending on the DenV. It can present as undifferentiated febrile illness or viral syndrome, febrile dengue (which is the classic presentation with fever), dengue hemorrhagic fever and dengue shock syndrome[4,6].

In febrile dengue, symptoms begin with a high fever that lasts 4 to 8 days, severe headache, retroorbital pain, loss of appetite, metallic taste, vomiting, diarrhea, and abdominal pain. There may be rashes on the face, limbs and trunk. Other features include bleeding gums, epistaxis, heavy menstrual periods and gastrointestinal bleeding. Hemorrhagic dengue is characterized by pyrexia, hemorrhagic phenomena, hepatomegaly and features of renal failure. Dengue shock is associated with a rapid pulse, cold and clammy skin, perioral cyanosis and high mortality[4].

The literature reports that oral manifestations are not common in dengue. However, the oral mucosa is affected in 30% of patients with dengue viral infections and more frequently in patients with dengue hemorrhagic fever[7]. Clinically, crusting can be observed on the lips and tongue, and vesicles on the soft palate. Bleeding of the tongue and gums, hemorrhagic plaques, petechiae, purpura, and ecchymosis have also been reported[8].

It is therefore essential for dentists to identify the manifestations of dengue in the oral cavity, as bleeding is often the only early manifestation of the disease. This could help to provide early diagnosis and rapid treatment, thereby avoiding significant complications.

The aim of the current study was to describe the oral manifestations present during dengue infection, based on a literature review.

MATERIALS AND METHOD

Eligibility criteria

This review was registered in OSF Registries[9] and developed to answer the PICO question: What are the oral manifestations that occur during dengue infection?

Studies that were considered for review underwent a selection process according to the inclusion and exclusion criteria established by the authors.

The review included case reports or clinical cases published in any language and any year in the databases used, addressing oral manifestations of dengue, without restrictions due to patient age or systemic status. Any research articles without free access or that did not clearly address the issue were excluded.

Sources of information:

Three databases were used to collect the studies: PubMed, Scielo and Scopus. Articles were collected from June 15 to July 18, 2023.

Search strategy

The search strategy consisted of equations made up of Medical Subject Headings (MeSH NLM) terms and Boolean operators, with the intention of qualifying the information and finding relevant material. All the authors corroborated and approved the strategy and took part in the search. The following formulas were developed and adjusted according to the database: (oral manifestations OR oral implications oral OR clinical oral OR) AND (Dengue OR Breakbone Fever OR Fever, Breakbone OR Classical Dengue Fever OR Classical Dengue Fevers OR Dengue Fever, Classical OR Break-Bone Fever OR Break Bone Fever OR Fever, Break-Bone OR Dengue Fever OR Fever, Dengue OR Classical Dengue OR Classical Dengues OR Dengue, Classical).

The terms were specifically tailored to find studies related to the research objectives.

Study selection process

Three criteria were applied to collect the articles: analysis of the title, the abstract and the full text. The systematic review followed the guidelines established by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020)[10,11].

A total 299 articles were collected from the four databases mentioned above. The articles were uploaded to the RAYYAN web application[12] and any duplicates were identified and deleted from the total number of studies and proceed to the analysis. First, only the titles were analyzed to determine whether the articles fit the previously established inclusion and exclusion criteria. Any articles that did not provide free access were eliminated. Secondly, the abstracts of the selected articles were evaluated, and thirdly, the full texts were analyzed. The articles that passed all three stages were reviewed and analyzed. The entire study selection process is described in a flowchart provided by PRISMA 2020 (Fig. 1).

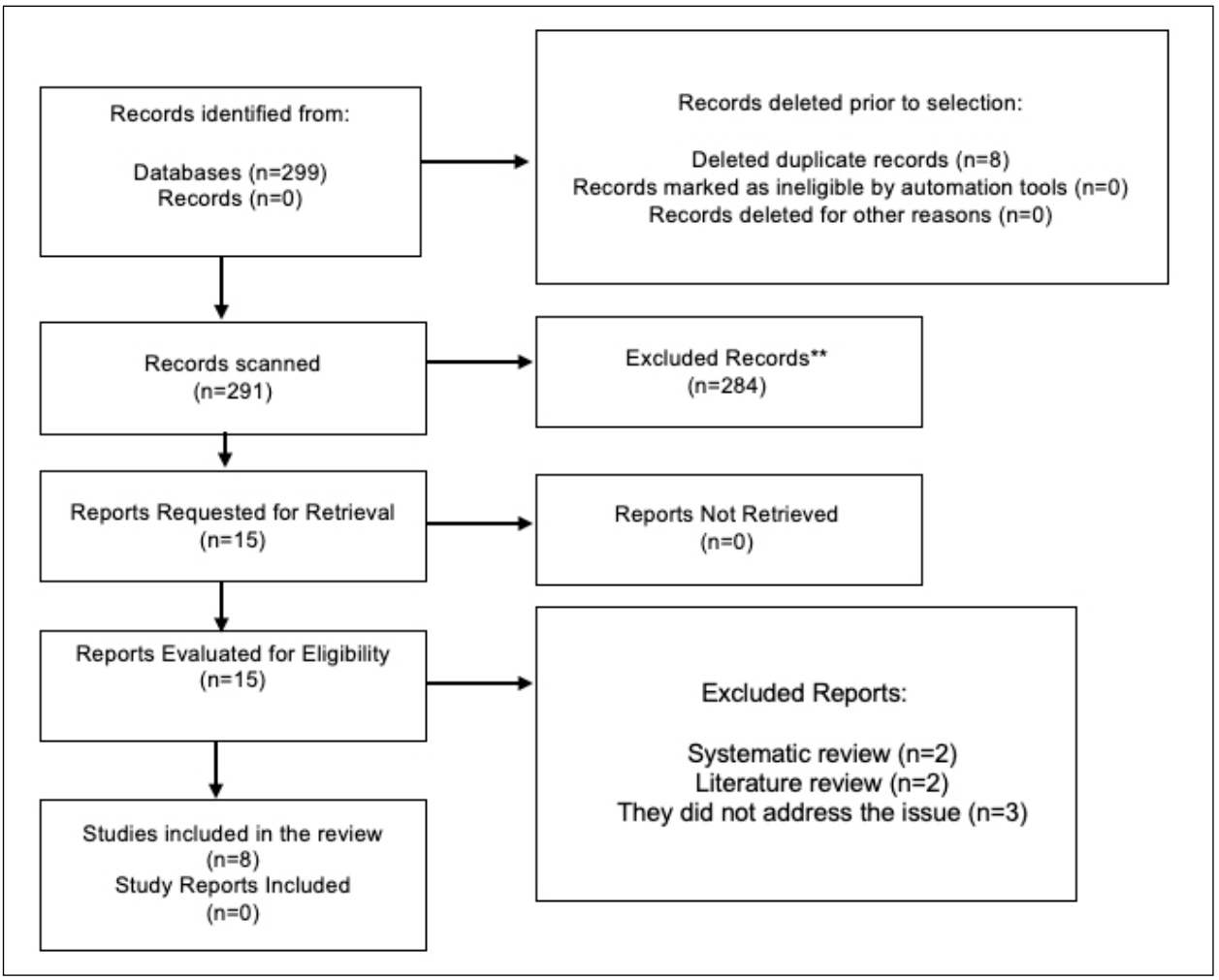

Fig 1: PRISMA-2020 flowchart of the study selection process

Data Extraction Process

The studies were exported from the databases according to the formats they contain, such as PubMed XML, CSV, BibTeX, and then uploaded to the RAYYAN web application[12]. This process was carried out individually, uploading folder by folder. RAYYAN enabled the authors to identify any duplicates automatically.

List of data: The data extracted from the scientific articles were organized in two tables. Table 1 focused on determining the metadata of the studies, taking as indicators database, scientific journal, country, title, authors, type of study and year of publication. Table 2 focused on determining the oral manifestations of dengue, whose indicators were title of the study, population, sex, age, type of dengue, personal

pathological history, extraoral and systemic clinical examination, and intraoral clinical examination.

Bias and Quality Assessment

A limitation to this review was the difficulty in identifying the risk of bias and quality of the included studies, as there is no tool to evaluate case reports or clinical cases.

Meta-analysis

This systematic review was performed only up to the qualitative phase. The type of study of the articles reviewed did not enable the quantitative phase to be performed.

RESULTS

A total 299 scientific articles were collected and uploaded to the Rayyan application for discrimination. The application automatically detected 8 duplicates, which were eliminated from the total collected, leaving 291 studies for analysis. During the first selection phase, studies were selected by reading the title, based on which 284 were eliminated and 15 were accepted, as they met the inclusion criteria. These remaining 15 studies were analyzed in the second phase, which consisted of reading the abstract. Before continuing with the selection phases, free access to these studies was verified, and they were downloaded in PDF format for the next stage. Eight studies qualified for the third and final phase of selection, which consisted of analyzing the full text[6,7,13–18].

Of the 7 articles excluded in the second and third phases, 2 were systematic reviews, 2 were literature reviews, and 3 did not address the topic of the current review. The entire study selection process was organized in a PRISMA 2020 diagram (Fig. 1).

Metadata analysis:

Table 1 describes the main metadata of the articles reviewed. The selected studies were case report

Table 1. Metadata of reviewed articles

Database	Journal	Country	Title	Author(s)	Type of study	Year of publication
Scielo	The South African Dental Association	South Africa	Detrimental orofacial manifestations of dengue and dengue hemorrhagic fever-clinical case series, review of the causes, complications, and vaccine strategies	Dutta SR, Singh P	Case Report	2021
PubMed	Brazilian Journal of Otorhinolaryngology	Brazil	Uncommon oral manifestations of dengue viral infection	Fernandes CIR, etal	Case Report	2020
PubMed	The American journal of tropical medicine and hygiene	Japan	Oral Manifestation Like Forchheimer Spots of Dengue Fever	Yamamoto K	Case Report	2019
PubMed	Quintessence International	Brazil	Severe oral manifestation of dengue viral infection: a rare clinical description	FSC Bridges, et al	Case Report	2014
PubMed	Journal of natural science, biology, and medicine	India	Oral presentation in dengue hemorrhagic fever: A rare entity	Mithra R, et al	Case Report	2013
Scielo	Revista del Nacional (Itauguá)	Paraguay	Granulomatosis with polyangiitis (Wegener's) associated with dengue	Montiel - Jarolín D, et al	Case Report	2013
Scielo	Brazilian Journal of Oral Sciences	Brazil	Unusual yet isolated oral manifestations of persistent thrombocytopenia: a rare case report	Byatnal A, et al	Case Report	2013
PubMed	Oral Surgery, Oral Medicine, Oral Pathology, and Oral Radiology	India	Postextraction bleeding following a fever: a case report	Dubey P, et al	Case Report	2012

studies, of which 5 were indexed to the PubMed database and 3 belonged to Scielo. These studies were published in the following high-impact scientific journals indexed to Scopus: The South African Dental Association[13]; Brazilian Journal of Otorhinolaryngology[6]; The American Journal of Tropical Medicine and Hygiene[14]; Quintessence International[15]; Journal of Natural Science, Biology, and Medicine[7]; Revista del Nacional (Itauguá)[16]; Brazilian Journal of Oral Sciences[17]; and Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology[18]. The countries where the research was conducted were Brazil[6,15,17], India[7,18], Japan[14], South Africa[13] and Paraguay[16]. The studies were published in several years: 1 article in 2012[18], 3 articles in 2013[7,16,17], 1 article in 2014[15], 1 article in 2019[14], 1 article in 2020[6] and 1 article in 2021[13].

Intraoral and extraoral manifestations of dengue:

The studies reviewed reported diagnosed dengue hemorrhagic fever and dengue type 1. Reported oral characteristics or oral manifestations of dengue viral infection included presence of hemorrhagic and edematous gums of the type located in the anterior region of the dental arches, edematous and hemorrhagic labial vermilion, maculopapular lesions in the mucosa of the lower lip with pain and dysphagia. Spontaneous active gingival bleeding was also mentioned[15]. Pseudomembranous candidiasis, edematous and erythematous taste buds, and transient lingual papillitis were reported[6]. Defective post-extraction clot formation with the presence of exudate was described in a dengue patient undergoing dental surgery[18].

Other reports mentioned hemorrhagic plaques surrounded by blue-green pigments, with an irregular surface, on the jugal mucosa and dorsal region of the tongue[7]. Patients also experienced xerostomia, tonsillitis, sacral tongue, pink macules on the hard palate[14], ulcers on the adherent gingiva and jugal mucosa, and blue pigmentations on the posterior mucosa[13,16]. On palpation, edematous, hemorrhagic lesions with rough surfaces bled[17].

These signs and symptoms were present in both male and female patients, with no predilection for either sex. Patient age ranged from 6 to 62 years. Medical histories recorded presence of high fever for 5 days or longer, red spots on the lower appendages, joint pain, blepharoedema, myalgia, cough, subconjunctival hemorrhage, headache, skin rash, bilateral submandibular lymphadenopathy, and respiratory complications, as described in Table 2.

DISCUSSION

Dengue is a viral febrile disease transmitted by the *Aedes aegypti* mosquito, which is common in tropical Southeast Asia[19]. The development of the disease may go unnoticed or generate a severe clinical picture[20]. The World Health Organization (WHO) classified this disease into two groups: severe and non-severe. In Perú, non-severe dengue is more prevalent[21].

Dengue causes various alterations in the oral cavity. Regarding the intraoral and extraoral manifestations, the reviewed studies specified the presence of hemorrhagic and edematous gums, edematous and hemorrhagic labial vermilion, maculopapular lesions in the lip mucosa, cheilophagia and dysphagia. Pseudomembranous candidiasis, edematous and erythematous taste buds, and inflammatory lingual papillitis were also reported. Patients also experienced xerostomia, tonsillitis, sacral tongue, pink macules on the hard palate, ulcers on the adherent gum and jugal mucosa, blue pigmentations on the posterior mucosa. On palpation, the edematous, hemorrhagic lesions with rough surfaces bled. In patients who underwent extractions, the clot was observed to have an infectious process. These results are consistent with those reported by Joob B and Wiwanitkit V, which indicated that oral bleeding was the presentation of dengue hemorrhage[19]. Similarly, Mushtaque RS et al. reported the presence of mild lip swelling in a 32-year-old patient diagnosed with dengue[22]. Thomas EA et al. found small-diameter vesicles on the soft palate, and erythema and crusts on the vermilion and the dorsal part of the tongue[23]. These studies are consistent with the results found in the case reports. However, there is little scientific evidence on dengue and its impact on the oral cavity, even though the disease is endemic in tropical countries.

In dengue case reports published from 2012 to 2021 in PubMed, the evidence collected was limited, as only 8 of the case reports reviewed reported oral manifestations. This number that is too low to increase knowledge in stomatology about this viral infection. Although dengue is endemic in Perú, little evidence of oral manifestations has been reported.

The evidence reviewed shows that the oral manifestations of dengue infection are diverse and

Table 2. Intraoral and extraoral manifestations of dengue

Title	Population, sex and age	Type of dengue fever	Personal pathological history	Extraoral and systemic clinical examination	Intraoral clinical examination
Detrimental orofacial manifestations of dengue and dengue hemorrhagic fever-clinical case series, review of the causes, complications, and vaccine strategies	Male aged 55-65 years (age not specified)	Dengue hemorrhagic fever	Fever for several weeks and body temperature ranging from 110°F to 120°F.	Joint pain from the past few days with a history of fever. On clinical evaluation, petechiae were observed on the upper face and neck. He had an axillary temperature of around 125°F. The respective submandibular lymphadenopathies were obvious.	Raised hemorrhagic plaques on the right posterior buccal mucosa, as well as on the dorsum of the tongue and the floor of the mouth, xerostomia and the tongue were all covered with plaques.
	49-year-old female	Dengue hemorrhagic fever	High fever for more than a few weeks, stomach pain, and occasional bleeding from the nose and gums.	Petechiae all over the body, including the upper and lower appendages, except the palms of the hands and soles of the feet, the lower part of the face, and neck. Axillary temperature 103° F. On palpation, the respective submandibular lymphadenopathy was evident.	Common ulcerative and hemorrhagic ulcers on both sides of the lower jaw, starting from the canine region to the molar. Petechiae were also present on the extraoral inferior aspect and in the intraoral right lower posterior molar and premolar regions. At the intersection of the hard and soft palate and mainly on the hard palate, on both sides, small vessels filled with blood stood out. A blue hemorrhagic strip was observed in the right posterior buccal mucosa strip.
	25-year-old male	Dengue hemorrhagic fever	Headache, orbital pain, hematemesis, and hematochezia.	High fever, muscle pain, and rash.	Petechiae, bleeding gums, ulcer, dry mouth.
Uncommon oral manifestations of dengue viral infection	1 case: Female, 29 years old.	Not specified	Not reported.	High fever, headache, muscle pain, and rash.	Pseudomembranous candidiasis, fungiform papillae that had an edematous and erythematous appearance, characterizing transient lingual papillitis.
Oral Manifestation Like Forchheimer Spots of Dengue Fever	1 case: Male, 6 years old.	Dengue type 1	A 4-day history of fever, cough and blepharedema in 2014.	Bronchial asthma and attention deficit hyperactivity disorder treated with methylphenidate.	Submucosal hemorrhages on the hard palate and pink spots on the soft palate.
Severe oral manifestation of dengue viral infection: a rare clinical description	1 case: Male, 18 years old.	Dengue hemorrhagic fever	Dengue infection	Bilateral subconjunctival hemorrhage, epistaxis, leg skin rash, thrombocytopenic disorder.	Edematous hemorrhagic upper gum in the anterior region, edematous and hemorrhagic upper lip, small maculopapular lesions on the lower lip and mucosa of the left cheek that prevented adequate oral functions and hygiene. Active spontaneous gingival bleeding.

continues on the next page

Table 2. Intraoral and extraoral manifestations of dengue

Title	Population, sex and age	Type of dengue fever	Personal pathological history	Extraoral and systemic clinical examination	Intraoral clinical examination
Oral presentation in dengue hemorrhagic fever: A rare entity	1 case: Middle-aged female.	Dengue hemorrhagic fever	History of fever from 1 week ago and temperature ranged from 102° F to 104° F. The blisters initially began on the left buccal mucosa and then affected the right buccal mucosa, tongue, and the back of the palate. Patient had also complained of red spots on his lower limbs for 3 days. The history revealed that patient had joint pain from the time of the onset of the fever.	Petechiae on upper and lower limbs, face and neck. He had an axillary temperature of approximately 102° F. Bilateral submandibular lymphadenopathies were present.	Raised hemorrhagic plaques on both the right and left buccal mucosa, dorsum of the tongue near the tip. The hemorrhagic plaques were surrounded by blue-green mucosa and the surface of the hemorrhagic plaques was irregular. At the junction of the hard and soft palate, a diffuse area of erosion of 3 × 4 cm was present. The tonsils on the right and left sides were enlarged and swollen. The patient had xerostomia and the tongue appeared to be sore.
Granulomatosis with polyangiitis (Wegener's) associated with dengue	1 case. A 69-year-old male.	Dengue hemorrhagic fever	Bilateral otalgia and mastoiditis 20 days before admission was interpreted as otitis and treated with cephalosporins and imipenem, in addition to myringotomy. A chronic smoker of 10 cigarettes a day for 20 years, he quit 10 years ago.	Bipalpebral edema, pale conjunctivae, nasal passages with blood clots, bilateral free and patent external ear canal.	In the mouth there were ulcers on the lips, tongue and hard palate, some covered with scabs.
Unusual yet isolated oral manifestations of persistent thrombocytopenia: a rare case report	1 case. A 50-year-old female.	Dengue hemorrhagic fever	Patient had had dengue six months earlier, for which she had undergone a blood transfusion, according to previous medical reports.	No significant findings.	Multiple hemorrhagic blisters on the left sublingual mucosa, as well as on the left lateral surface of the tongue and the floor of the mouth. The overlying surface was bluish-black and there was light profuse bleeding on palpation. The right side of the palate and the right posterior buccal mucosa revealed the presence of brown plaques with a rough surface. These lesions bled upon touching.

continues on the next page

Table 2. Intraoral and extraoral manifestations of dengue

Title	Population, sex and age	Type of dengue fever	Personal pathological history	Extraoral and systemic clinical examination	Intraoral clinical examination
Post-extraction bleeding following a fever: a case report	1 case. A 62-year-old male.	Dengue hemorrhagic fever	High fever for 5 days before having the extraction	Facie pale and weak, temperature rose to 100°F, pulse and blood pressure of 94 beats per minute and 130/80 mm Hg. On palpation, the abdomen was found to be tender.	Large clot attached to the exposed alveolus by extraction and a continuous exudation of blood around the periphery of the blood clot formed.

range from swelling in the mucous membranes to bleeding in the gum and lip. It is important to expand the scientific evidence to enable correct differential and definitive diagnosis, since multiple pathologies have similar characteristics.

DECLARATION OF CONFLICTING INTERESTS
The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

FUNDING
None

REFERENCES:

1. Prapty CNBS, Ahmed N, Araf Y, Yang Z, Zhai J, Hosen MJ, et al. Coinfection of COVID-19 and Dengue: A Case Report. Front Med. 2020. https://doi.org/10.3389/fmed.2022.872627
2. National Center for Disease Epidemiology, Prevention and Control. CDC MINSA. 2023. https://www.dge.gob.pe/epipublic/uploads/dengue/dengue_202320_30_121921.pdf
3. Gupta D, Guliani E. Flavonoids: Molecular mechanism behind natural chemoprotective behavior-a mini review. Biointerface Res Appl Chem. 2022;12(5):5983-95. https://doi.org/10.33263/BRIAC125.59835995
4. Roopashri G, Vaishali MR, David MP, Baig M, Navneetham A, Venkataraghavan K. Clinical and oral implications of dengue fever: a review. J Int Oral Health JIOH. 2015;7(2):69-73. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4377157/pdf/JIOH-7-69.pdf
5. Jahan Y, Rahman A. Management of dengue hemorrhagic fever in a secondary level hospital in Bangladesh: A case report. IDCases. 2020; 21:E00880. https://doi.org/10.1016/j.idcr.2020.e00880
6. Fernandes CIR, Perez LE da C, Perez DE da C. Uncommon oral manifestations of dengue viral infection. Braz J Otorhinolaryngol; 86(Suppl 1):3-5. https://doi.org/10.1016/j.bjorl.2016.10.001
7. Mithra R, Baskaran P, Sathyakumar M. Oral presentation in dengue hemorrhagic fever: A rare entity. J Nat Sci Biol Med. 2013;4(1):264-7. https://doi.org/10.4103/0976-9668.107324
8. Chadwick D, Arch B, Wilder-Smith A, Paton N. Distinguishing dengue fever from other infections on the basis of simple clinical and laboratory features: application of logistic regression analysis. J Clin Virol Off Publ Pan Am Soc Clin Virol. 2006; 35(2):147-53. https://doi.org/10.1016/j.jcv.2005.06.002
9. Canales Sermeño G, Valenzuela Ramos MR, Dias Monteiro PM, Medina Castro DE, Medina Valera NK. Case reports on oral manifestations during dengue infection: a systematic review. OSF; 2023. https://doi.org/10.17605/OSF.IO/BTM8Q
10. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:N71. https://doi.org/10.1136/bmj.n71
11. Page MJ, Moher D, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews. BMJ. 2021; 372:N160. https://doi.org/10.1136/bmj.n160
12. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan—a web and mobile app for systematic reviews. Syst Rev. 2016;5(1):210. https://doi.org/10.1186/s13643-016-0384-4
13. Dutta SR, Singh P. Detrimental orofacial manifestations of dengue and dengue hemorrhagic fever-clinical case series, review of the causes, complications, and vaccine strategies. South Afr Dent J. 2021; 76(8):457-64. http://dx.doi.org/10.17159/2519-0105/2021/v76no8a2
14. Yamamoto K. Oral Manifestation Like Forchheimer Spots of Dengue Fever. Am J Trop Med Hyg. 2019;101(4):729. https://doi.org/10.4269/ajtmh.19-0338
15. Pontes FSC, Frances LTM, Carvalho M de V, Fonseca FP, Neto NC, do Nascimento LS, et al. Severe oral manifestation of dengue viral infection: a rare clinical description. Quintessence int berl ger 1985. 2014;45(2):151-6. https://doi.org/10.3290/j.qi.a30992
16. Montiel-Jarolín D, Espínola MA, Arce Kita J, Barrios Velázquez M de los A. Granulomatosis with polyangiitis (Wegener's) associated with dengue.

Rev nac itauguá. 2013;5(2):37-40. http://scielo.iics.una.py/scielo.php?script=sci_arttext&pid=S2072-81742013000200006&lng=en&nrm=iso
17. Byatnal A, Mahajan N, Koppal S, Ravikiran A, Thriveni R, Parvathi Devi MK. Unusual yet isolated oral manifestations of persistent thrombocytopenia: a rare case report. Braz J Oral Sci. 2013;12:233-6. http://revodonto.bvsalud.org/scielo.php?script=sci_arttext&pid=S1677-32252013000400015
18. Dubey P, Kumar S, Bansal V, Kumar KVA, Mowar A, Khare G. Postextraction bleeding following a fever: a case report. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013;115(1):E27-31. https://doi.org/10.1016/j.oooo.2012.03.038
19. Joob B, Wiwanitkit V. Oral manifestations of dengue viral infection. Braz J Otorhinolaryngol. 2017;83(5):605. https://doi.org/10.1016/j.bjorl.2017.02.006
20. Kularatne SA, Dalugama C. Dengue infection: Global importance, immunopathology and management. Clin Med. 2022;22(1):9-13. https://doi.org/10.7861/clinmed.2021-0791
21. Kok BH, Lim HT, Lim CP, Lai NS, Leow CY, Leow CH. Dengue virus infection – a review of pathogenesis, vaccines, diagnosis and therapy. Res Virus. 2022;324:199018. https://doi.org/10.1016/j.virusres.2022.199018
22. Mushtaque RS, Ahmad SM, Mushtaque R, Baloch S. A Curious Case of Dengue Fever: A Case Report of Unorthodox Manifestations. Case Rep Med;2020:1701082. https://doi.org/10.1155/2020/1701082
23. Thomas EA, John M, Kanish B. Mucocutaneous manifestations of dengue fever. Indian J Dermatol. 2010;55(1):79-85. https://doi.org/10.4103/0019-5154.60359

https://doi.org/10.54589/aol.37/2/123

Local application of melatonin associated or not to xenogeneic material, in critical defects of rat calvaria

Karen L Dalla Costa, Juliana Lacerda Dias, Izaura M Crunivel Araújo, Andrei Braga Monteiro, José A Coelho Junior, Rogerio Scipioni Junior, Marcelo Sperandio, Julio C Joly, Daiane C Peruzzo

Faculdade e Centro de Pesquisas Odontológicas São Leopoldo Mandic, Campinas, São Paulo, Brasil.

ABSTRACT

Melatonin (MLT) is a hormone that can stimulate bone formation and inhibit bone resorption, among other functions. ***Aim:*** *To evaluate the effect on new bone formation of MLT applied locally to critical defects created in the calvaria of rats, compared to the effect of Bio-Oss® xenogeneic bone substitute (BO), by analyzing histomorphometry, microtomography and gene expression.* ***Materials and Method:*** *Two critical defects (5.0 mm in diameter) were created in the calvaria of 36 adults male Wistar rats. The rats were divided randomly into two groups: a test group, in which one of the defects was filled with MLT, and the other with MLT with Bio-Oss® (MLTBO), and a control group, in which one of the defects was filled only with the clot (C), and the other with BO. The rats were euthanized 30 days after surgery. Samples of the calvaria containing the critical defects were collected for analysis by histomorphometry, microtomography, and the expression of the genes for type I collagen (COL-I), osteopontin (OPN) and bone morphogenetic protein 2 (BMP-2).* ***Results:*** *A qualitative improvement was observed in bone healing when MLT was used, though there was no statistical difference in the quantification of newly formed bone (p>0.05). Micro-CT showed that bone volume was significantly smaller in absence of BO (p=0.006). Bone trabeculae thickness (p=0.590) and number (p=0.150) were not significantly affected by MLT. Regarding the expression of the genes COL-I, OPN and BMP-2, no significant differences were observed between the MLT, BO and MLTBO groups.* ***Conclusion:*** *Topical application of MLT resulted in a qualitative improvement in bone healing, although it did not affect bone formation quantitatively. In the absence of BO, less bone volume and less bone trabecular thickness were observed.*

Keywords: *calvaria - critical bone defects - bone mineral density - melatonin - bone regeneration.*

To cite:
Dalla Costa KL, Lacerda Dias J, Crunivel Araújo IM, Braga Monteiro A, Coelho Junior JA, Scipioni Junior R, Sperandio M, Joly JC, Peruzzo DC. Local application of melatonin associated or not to xenogeneic material, in critical defects of rat calvaria. Acta Odontol Latinoam. 2024 Aug 30;37(2):123-133. https://doi.org/10.54589/aol.37/2/123

Corresponding Author:
Karen Laurene Dalla Costa
kldallacosta@hotmail.com

Received: February 2024.
Accepted: August 2024.

Aplicação local de melatonina em defeitos em calvárias de ratos associada a material xenógeno

RESUMO

A melatonina (MLT) é um hormônio sintetizado e secretado pela glândula pineal, e que, dentre outras atividades e funções, tem capacidade de estimular a formação e inibir a reabsorção óssea. ***Objetivo:*** *avaliar o efeito da aplicação local do hormônio MLT na neoformação óssea, em defeitos críticos produzidos na calvária de ratos, por meio de análise histomorfométrica, microtomográfica e de biologia molecular, comparando-a com um substituto ósseo de origem xenogênica (Bio-Oss®).* ***Materiais e Método:*** *foram utilizados 36 ratos Wistar machos adultos, nos quais foram produzidos dois defeitos críticos de 5,0 mm de diâmetro cada, que receberam diferentes tratamentos alocados de forma randomizada: no grupo controle, os animais tiveram um dos defeitos preenchidos apenas com coágulo sanguíneo (C) e o outro com substituto ósseo xenógeno (Bio-Oss®, BO); no grupo teste, um dos defeitos foi preenchido apenas com MLT e, o outro, recebeu a associação da MLT ao material sintético xenógeno (MLTBO). Todos os animais foram eutanasiados após 30 dias do pós-operatório. As amostras das calotas contendo os defeitos críticos foram coletadas para análises histomorfométricas, microtomográficas e da expressão gênica de colágeno do tipo I (COL-I), osteopontina (OPN) e proteína óssea morfogenética 2 (BMP-2), por meio de PCR em tempo real.* ***Resultados:*** *Após análise dos dados pode-se observar que não houve diferença estatística na quantificação de neoformação óssea (p>0.05), porém, melhora qualitativa foi observada na cicatrização, quando a MLT foi utilizada. Quanto aos par®metros microtomográficos, foi observado que com ou sem MLT, o volume ósseo foi significativamente menor na ausência de BO. A espessura (p = 0,590) e número (p = 0,150) de trabéculas não foram significativamente afetados pelo uso da MLT. Quanto à expressão gênica de COL-I, OPMN e BMP-2, não foram observadas diferenças significativas entre os grupos MLT, BO e MLTBO.* ***Conclusão:*** *conclui-se que a aplicação tópica de MLT, associada ou não ao BO não afetou quantitativamente a neoformação óssea, porém resultou em uma melhora qualitativa na cicatrização. Adicionalmente, na ausência de BO foi observada menor volume ósseo e menor espessura das trabéculas.*

Keywords: *calvaria - critical bone defects - bone mineral density - melatonin - bone regeneration.*

INTRODUCTION

Melatonin (MLT) or N-acetyl-5-methoxytryptamine is an indoleamine centrally produced by pinealocytes in the pineal gland and released directly into the blood, which acts as a hormone[1]. It also acts on bone formation, promoting osteoblastic differentiation by stimulating the formation of new bone matrix[2-7]. Considering bone metabolism, MLT seems to act directly on osteoclasts through several mechanisms, potentially interfering with their function, thereby inhibiting bone resorption[1,8]. Some studies have shown that topical application of melatonin promotes new bone formation and regeneration in bone defects [9-12].

MLT plays a role in reducing cell degeneration by stimulating the production of type I collagen (COL-I), thereby modulating osteoblastic activities[13,14]. MLT can also participate in regulating the inhibition of bone resorption, which occurs through the negative regulation of osteoclasts mediated by inhibition of the RANKL molecule action[8,15].

In dentistry, MLT is a potential adjuvant in dental implant osseointegration[16,12], and used in the treatment of oral cancer[17], as an analgesic, and to induce new bone formation after extraction of third molars[18], as well as to repair damage caused by periodontitis[19-21]. It has also been shown to influence *in vitro* osteoblastic function, improve bone regeneration when administered by mouth daily to ovariectomized mice[22], and increase bone strength in naturally aged mice[23].

Studies using MLT have been conducted with the aim of improving dental implant surfaces, bone substitutes and materials that can accelerate osseointegration[11,14,24,25]. However, there is still little information about the MLT mechanism of action in bone matrix formation, especially when it is administered locally.

The aim of this study was therefore to evaluate the effect of local application of MLT on new bone formation when placed in critical defects created in the calvaria of rats, compared to MLT with a xenogeneic bone substitute (Bio-Oss®), through histomorphometry, microtomography and analysis of COL- I, osteopontin (OPN) and bone morphogenetic protein 2 (BMP-2) gene expression.

MATERIALS AND METHOD

Sample characteristics

Thirty-six healthy male rats (*Rattus norvegicus* – Wistar) were selected, 3 months old, and weighing approximately 300g. The animals were acclimatized for a period of 10 days before the surgical procedures, with water and food *ad libitum*, housed in cages with wood shavings, at controlled temperature (21ºC) and lighting (12/12-hour light-dark cycles), at the vivarium at *Faculdade São Leopoldo Mandic*, after approval by the Ethics Committee (2019/012, SLM. CEUA. F8-00).

Sample size was calculated using the equation for finite population recommended by Zar (2010): n = (z2. dp2) / d2 + z2 (dp2 / N), in which n = finite population; z = confidence interval (α = 0.05 or z = 1.96); d = error (20%); dp = variance (25%). The calculation was: n = (1.962. 252)/202+1.962. (252/16) = 4.37. Adjusted for expected death of animals, 6 animals were allocated for each analysis, totaling 36 rats.

Experimental design

Two circular defects were created in the calvaria of each rat (see Surgical Procedure, below). The animals were randomized into two groups and treated as follows: a) 18 rats were treated with MLT, and had one defect filled with 0.015g of MLT (Active Pharmaceutica, China) - MLT group; and the other defect filled with a combination of 0.025g of xenogeneic bone substitute (Bio-Oss®, Geistlich Biomaterials, Switzerland) and 0.0075g of MLT (Active Pharmaceutica, China) - MLT/BO group; and b) 18 rats had one defect filled with 0.050g of xenogeneic bone substitute (Bio-Oss® Geistlich Biomaterials, Switzerland) - BO group; and the other defect filled only with the blood clot - C group. After the applications, the defects were covered with a single collagen membrane (Bio-Gide®, Geistlich Biomaterials, Switzerland). Six animals from each group were used for histomorphometric analysis, 6 for microtomographic analysis, and 6 for analysis of COL-I, OPN and BMP-2 gene expression (Fig. 1).

Surgical procedures

The animals were weighed and anesthetized according to body weight by intramuscular injection in the outer thigh with a ketamine solution (10 mg/kg/IM) (Francotar®, Virbac do Brasil Industria e Comércio LTDA, Roseira, SP, Brazil) and xylazine hydrochloride (75mg/kg/IM) (Virbaxil® 2%, Virbac do Brasil Industria e Comércio LTDA, Roseira, SP, Brazil), which act as a anesthetic, and as a sedative

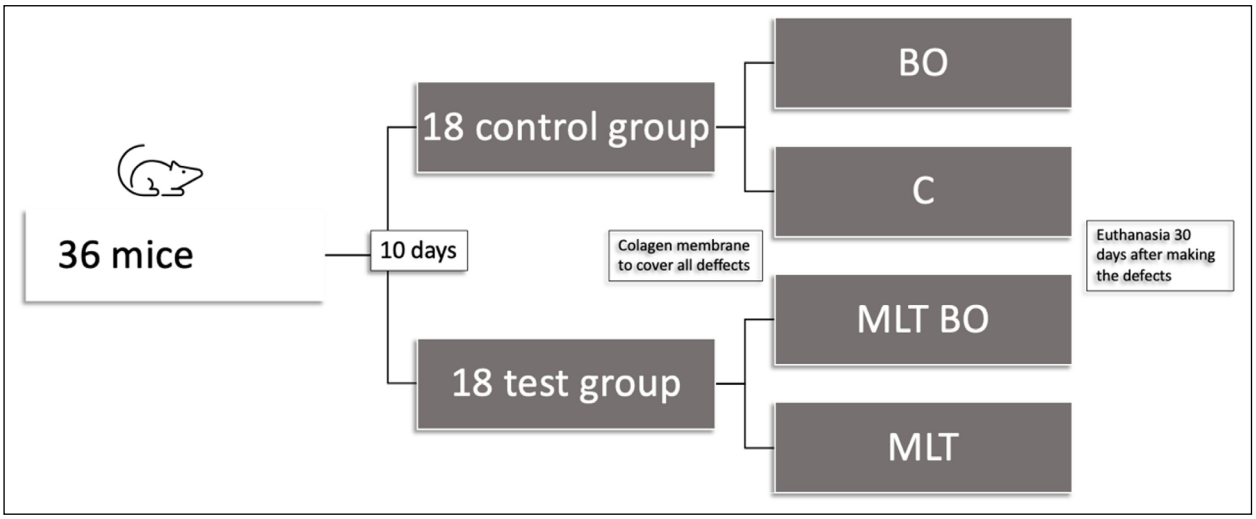

Fig. 1: Flowchart of the study experimental design

and muscle relaxant respectively. After confirming analgesic effectiveness, the animals were marked with their number on the tail, shaved in the calvarial region and disinfected with iodinated alcohol. A straight sagittal incision measuring approximately 1.5 cm was made in the median region of the cranium, followed by wide lateral divulsion and exposure of the calvaria. Two circular defects were created in the calvaria, laterally to the sagittal suture, under constant irrigation with saline, using a 5mm trephine drill (Neodent®, Curitiba, Brasil) with a Neodent® Neosurg Pro implant motor (Neodent®, Curitiba, Brasil) and a SG20 Handpiece (Neodent®, Curitiba, Brasil), at 1200 rpm, taking the necessary care to avoid causing additional injuries (Fig. 2).

The filled critical defects were covered with collagen membrane (Bio-Gide®, Geistlich Biomaterials, Switzerland) and sutured with simple stitches using a 4-0 silk thread (Ethicon® Johnson & Johnson Johson & Johnson do Brasil Comercio de Produtos para Saude Ltda,Brasil). A sterile gauze soaked in 0.9% saline solution was kept over the animals' eyes throughout surgery to prevent the corneas from drying out.

In the immediate postoperative period, the animals were medicated with a single 0.3 mL dose of 5% levofloxacin antibiotic (Isofarma Indústria Farmacêutica Ltda, Precabura, Ceara, Brasil), and 0.3 mL of dipyrone analgesic (Algivet®, Vetnil, Louveira, São Paulo, Brasil) every 12 hours for 3 days.

Thirty days after surgery, all the animals were euthanized with deepening anesthesia using barbiturate [sodium thiopental (71-73-8) 150mg/kg and lidocaine (137-58-6) 10mg/mL]

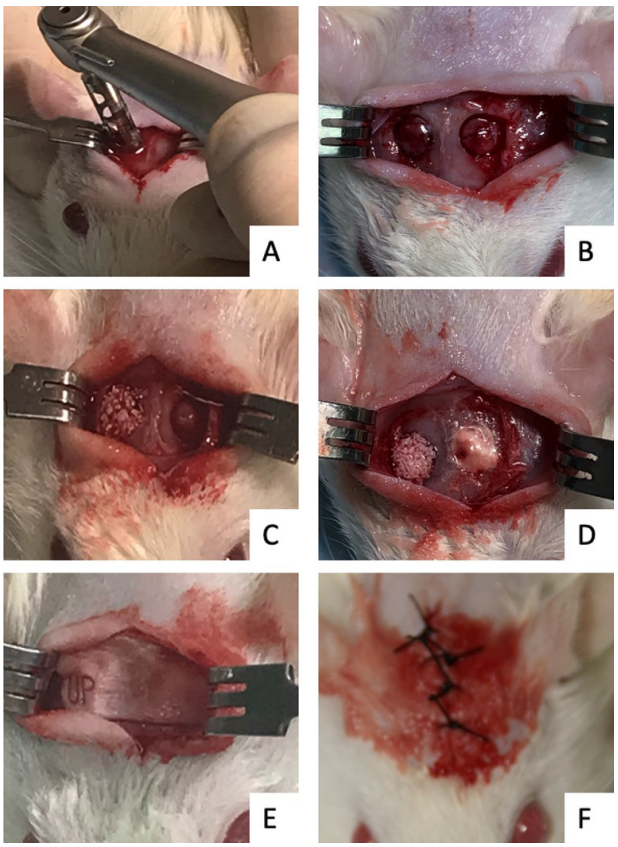

Fig. 2: Surgical sequence of the experiment
A) Creation of defects with a 5mm trephine; B) Cranial perforations; C) Control group, with defects filled with BO (left) and Clot (right); D) Test group, with defects filled with MLT/BO (left) and MLT (right); E) Installation of the membrane covering the defects after insertion of the biomaterials without suture; F) Simple suture with silk thread.

intraperitoneally[26]. After euthanasia, the calvaria was removed using a 2 cm diameter diamond disc with a handpiece, washed with 0.9% saline solution, identified, and stored for subsequent bone analysis.

Bone sample analysis

Bone histomorphometry

For the histological procedures, the calvaria specimens were sectioned into blocks, fixed in a 10% buffered formalin solution (pH 7.4) for 24 hours at room temperature, and then decalcified in a 20% formic acid solution (Merck, Darmstadt, Germany) for 5 days. Once the mineral portion had been removed, the specimens were washed in running tap water, dehydrated with ethyl alcohol, and cleared with alcohol/xylene using a Leica TP 1020 tissue processor (Leica Biosystems, Nussloch, Germany). Then, they were impregnated with liquid paraffin and cut into 4 µm histological sections using a Leica RM2245 microtome (Leica Biosystems, Nussloch, Germany) to prepare slides corresponding to each bone defect. The sections were stained with hematoxylin-eosin (HE), and coverslips were applied using Permount® resin mounting medium (Fisher Scientific, USA).

The histological slides were analyzed by a blinded calibrated researcher (JLD), using a Nikon Eclipse Ci-S microscope (Japan) at 40x objective. The Image J software developed by the US National Institutes of Health (NIH) was used to quantify the area of new bone formation on images in JPEG format, at software default parameters (Fig. 3).

After all areas of new bone formation had been measured on each slide, they were added, to provide a single value in micrometers, which was considered for statistical analysis. Qualitative analysis was performed to identify the following aspects of new bone formation: presence/absence of inflammatory infiltrate, location of new bone formation (close to or distant from residual bone), and presence of mature lamellar bone.

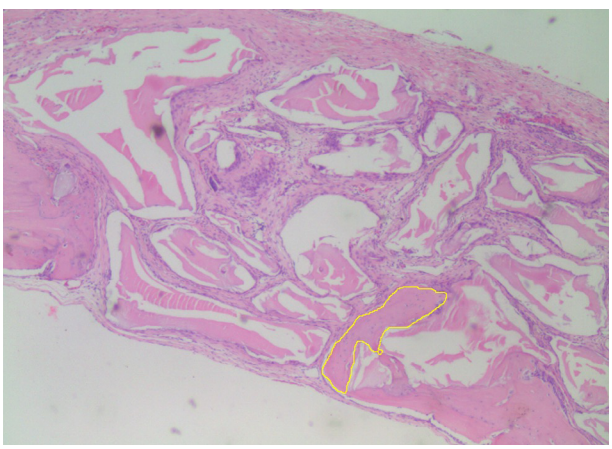

Fig. 3: Photograph of a histological slide analyzed by Image J Software. Measurement of an area of new bone formation highlighted by the yellow line

Microtomography

The calvaria samples were fixed in 10% buffered formaldehyde solution (pH 7.4) and sent for micro-CT analysis to the Brazilian National Nanotechnology Laboratory (LNNano) of the Brazilian Center for Research in Energy and Materials (CNPEM), Campinas, SP. The micro-CT images were acquired using a SkyScan 1272 *micro-CT* scanner (Bruker, Kontich, Belgium) at the following *acquisition* parameters: 11µm voxel size, 360º gantry rotation, 0.4º rotation step, 2 frames and 0.5mm aluminum filter.

Images were reconstructed with NRecon software (Bruker, Kontich, Belgium) at the following standardization parameters: ring artifact correction = 5, smoothing = 0 and beam hardening artifact correction = 0. The reconstructed images were reoriented using the Dataviewer software (Bruker, Kontich, Belgium), according to each defect, considering the convexity of the calvaria, so that each side was analyzed separately by rotating a standard vertical axis (Fig. 4).

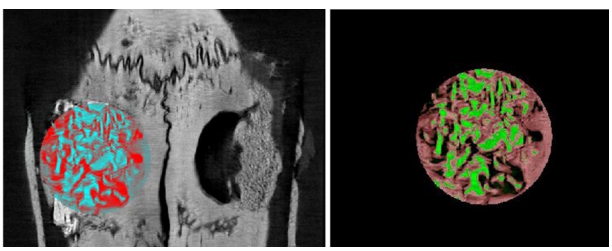

Fig. 4: Microtomographic images – segmentation of the bone defect (defect filled with BO)

The groups of reoriented images were then analyzed in the CTAn software (Bruker, Kontich, Belgium). The region of interest delimited consisted of a disc-shaped volume, 5 mm in diameter, with a height according to the height of each calvaria. Image segmentation was performed automatically, with the exclusion of any shades of gray that stood out (which is characteristic of the BO material). After image segmentation, the parameters of bone quantity (bone volume) and bone quality (thickness and

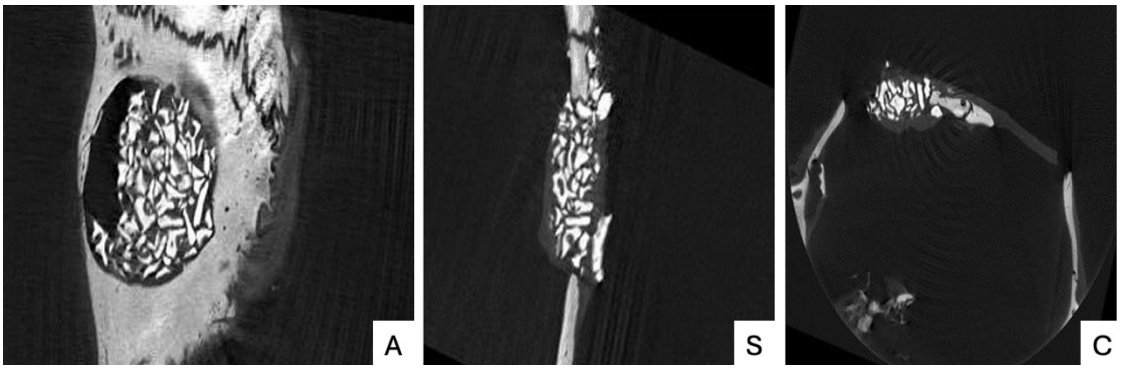

Fig. 5A: Axial (A), sagittal (S) and coronal (C) section - defect filled with BO

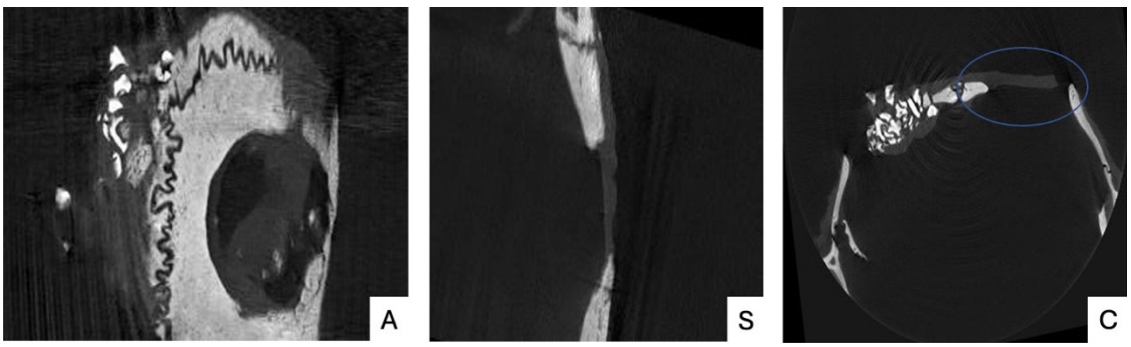

Fig. 5B: Axial (A), sagittal (S) and coronal (C) section - defect filled with MLT

number of trabeculae) were calculated and tabulated for subsequent statistical analysis (Fig. 5A and B).

Analysis of gene expression

Total RNA was extracted using Trizol reagent (Thermo Fisher Scientific, São Paulo, Brazil) according to the manufacturer's instructions. Briefly, the newly formed tissue was collected from each defect, crushed, and homogenized with 1 mL of Trizol. The aqueous and organic phases were separated by adding 0.2 mL of chloroform, followed by centrifugation (12000 g, 15 minutes, 4ºC). RNA was precipitated from the aqueous phase with 0.5 mL of isopropanol (12000 g, 15 minutes, 4 ºC), washed with 75% ethanol and resuspended in water. For reverse transcription PCR (RT-PCR), 1µg RNA samples were treated with 1U DNAse I enzyme. The cDNA synthesis was performed using a RevertAid H Minus First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, São Paulo, Brazil) according to the manufacturer's instructions. Briefly, the following reagents were mixed: 1µg of RNA, 0.5 µg of oligo(dT)18, 1 mM of the dNTP mix, 200U of RevertAid H Minus M-MuLV Transcriptase and 20U of RiboLockRNAse Inhibitor. The reaction ran at 42 °C for 60 minutes, after which it was halted by heating at 70 °C for 5 minutes.

For quantitative PCR (qPCR, also called real-time PCR), primer pairs (oligonucleotides) for OPN, COL-I, BMP2 genes (Table 1) were designed for amplification of their regions of interest, using the Primer Express software (Life Technologies, São Paulo, Brazil). Amplification reactions occurred using 40 ng of cDNA and 0.3 µM of primer pairs, added to Maxima SYBR Green qPCR Master Mix (Thermo Fisher Scientific, São Paulo, Brazil). The reaction conditions were 10 minutes at 95 °C, followed by 40 cycles at 95 °C, 15 seconds, and at 60 °C, 1 minute. The 7500 Fast Real Time PCR System (Life Technologies, São Paulo, Brazil) was used. Expression levels were quantified using

Table 1. Primer sequence.

Oligonucleotide	Sequence
O–N - rat F	5'-AAGCCTGACCCATCTCAGAA-3'
O–N - rat R	3'-ATGGCTTTCATTGGAGTTGC-5'
COL–1 - rat F	AAGCCTGACCCATCTCAGAA
COL–1 - rat R	ATGGCTTTCATTGGAGTTGC
BMP2 – rat F	5'-ACTACCAGAAACGAGTGGGAA-3'
BMP2 – rat '	5'-GCATCTGTTCTCGGAAAAACCT-3'

COL1 = type 1 collagen, OPN = osteopontin, BMP2 = Human Bone Morphogenetic Protein-2, F = forward, R = reverse.

an SDS software (Life Technologies, São Paulo, Brazil), and the relative expression between samples was calculated according to the threshold cycle (Ct) comparison method, based on the formula 2-ΔΔCt. To normalize expression levels, the GAPDH gene was used as a reference gene (internal reaction control).

Statistical analysis

Data referring to histomorphometry, microtomography and biomolecular analysis were tested for normal distribution and homoscedasticity. Then, treatments were compared using t*wo*-way *ANOVA* for *randomized blocks*. Tukey's test was used for histomorphometry and microtomography, Kruskal-Wallis test for biomolecular analysis, and Dunn's test for multiple comparisons. All statistical analyses were conducted at a 5% significance level, using a SPSS 23 software (SPSS INC., Chicago, IL, USA).

RESULTS

Bone histomorphometry

For the sample size of this study and for the measures of dispersion observed within each of the four treatments, no statistically significant differences ($p = 0.869$) were identified in the mean values of new bone formation when the defects in the calvaria were treated with BO and/or MLT (Fig. 6).

For qualitative analysis, the slides were digitized and evaluated using a 40X microscope objective, regarding the following aspects: presence/absence of inflammatory infiltrate, location of new bone formation (close to or distant from the residual bone)

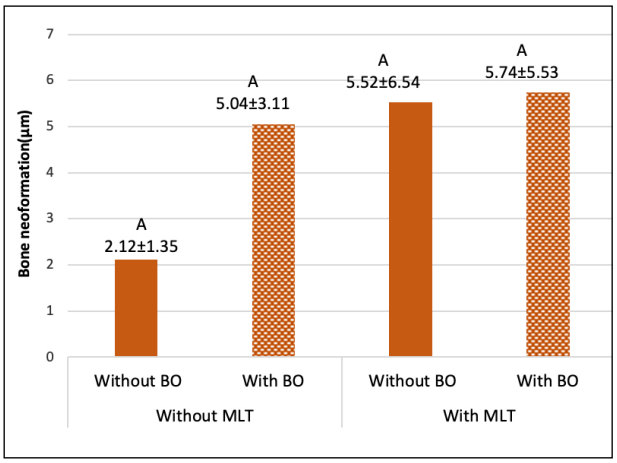

Fig. 6: Bar chart of mean values of new bone formation in defects created in the calvaria of rats, with or without BO and MLT. Legend: Same capital letters indicate that treatments do not differ significantly (ANOVA and Tukey's test, alpha=5%).

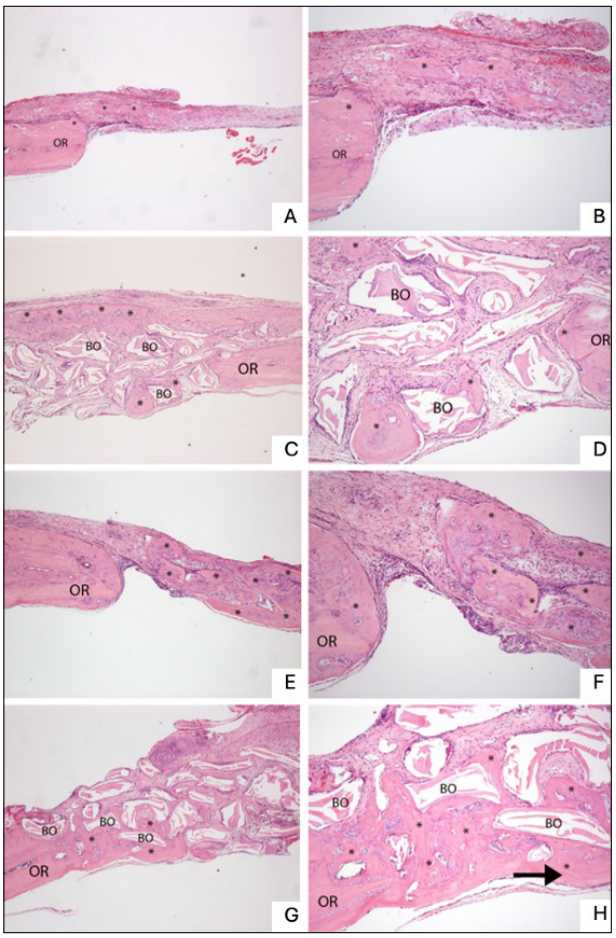

Fig. 7: Photomicrograph of HE-stained histological slides of bone defects

Left column (H&E) original magnification X40; Right column (H&E) original magnification X100. Histological characteristics: A) Sample of bone defect filled with clot (control) showing small areas of newly formed bone (*) mainly in the area adjacent to the residual bone (OR) at the edge of the defect. B) Higher magnification of the previous image showing areas of newly formed bone and absence of significant inflammatory infiltrate. C) Sample of bone defect filled with Bio-Oss® showing areas of newly formed bone (*) in an area adjacent to the residual bone (OR) and permeated with biomaterial particles (BO). D) At higher magnification, biomaterial particles can be seen near the newly formed bone and the absence of significant inflammatory infiltrate. E) Sample of a bone defect filled with MLT, showing a relevant amount of newly formed bone (*) even in areas distant from the residual bone (OR). F) The newly formed bone (*) can be observed in greater detail in absence of significant inflammatory infiltrate. G) Sample of bone defect filled with MLT and Bio-Oss® showing a large amount of newly formed bone (*). H) At higher magnification, extensive new bone formation can be seen amidst the biomaterial particles (BO) and the absence of significant inflammatory infiltrate (H&E original magnification X40). In some areas, the newly formed bone exhibits characteristics of mature lamellar bone (arrow).

and presence of mature lamellar bone, as identified in the histological analysis (Fig. 7).
Histological findings showed that MLT promoted an improvement in bone healing, with greater bone formation in the group with the addition of MLT. In addition, the presence of bone formation distant from the remaining bone and the presence of mature lamellar bone were observed in the MLT group.

Microtomography

For volume data, two-way analysis of variance for randomized blocks demonstrated a statistically significant interaction between the use of BO and MLT ($p = 0.006$). Bone volume was significantly smaller when MLT was used in the absence of BO. It was also verified that when MLT was used, the bone volume was significantly lower in the absence of BO (Fig. 8).

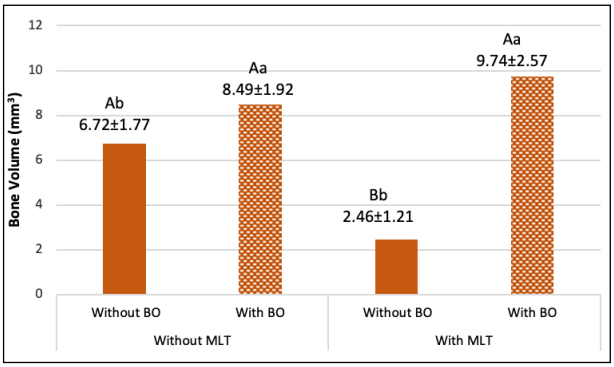

Fig. 8: Bar chart of bone volume in defects created in the calvaria of rats, with or without BO and MLT. Legend: for data on thickness (p = 0.860) and number (p = 0.167) of trabeculae, two-way analysis of variance for randomized blocks indicated that there was no statistically significant interaction between the bone substitute and melatonin.

For thickness ($p = 0.860$) and number ($p = 0.167$) of trabeculae, there was no statistically significant interaction between BO and MLT. The thickness ($p = 0.590$) and number ($p = 0.150$) of trabeculae were also not significantly affected by MLT, either in the presence or the absence of BO. However, the use of BO had a statistically significant influence on the thickness ($p = 0.001$) and number ($p < 0.001$) of trabeculae. Trabecular thickness was significantly smaller in the presence of BO than when BO was absent, whether associated with MLT (Fig. 9). A significantly greater number of trabeculae ($p<0.05$) was observed in the presence of BO, with or without the use of MLT (Fig. 10).

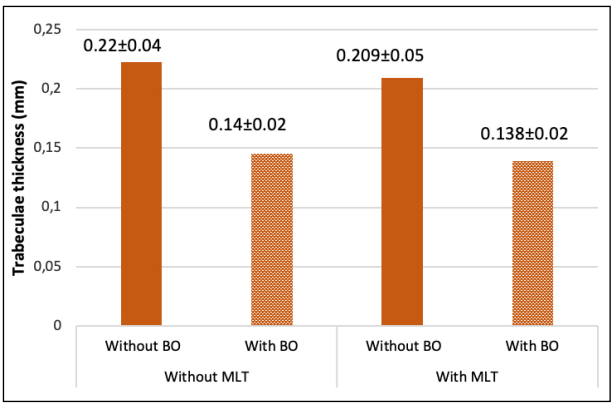

Fig. 9: Bar chart of trabecular thickness in defects created in the calvaria of rats, with or without BO and MLT.

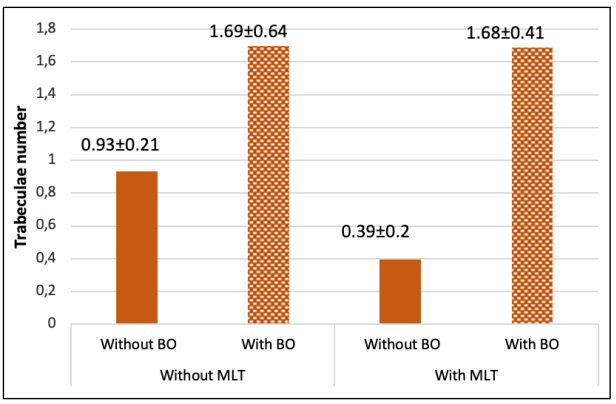

Fig. 10: Bar chart of the number of trabeculae in defects created in the calvaria of rats, with or without BO and MLT.

Quantification of COL-I, OPN and BMP-2 gene expression

The control group showed significantly higher COL-I gene expression than the MLT group ($p=0.0291$). No statistical differences were observed between BO and MLTBO ($p=0.9183$). The control group also showed significantly higher COL-I gene expression than the BO group ($p=0.0374$). There was no significant difference in the comparisons between MLT and BO ($p>0.9999$), MLT and MLTBO ($p=0.7855$), or BO and MLTBO ($p=0.9183$).
Regarding OPN gene expression, there was a tendency for better results in the groups in which MLT was used, though the difference was not statistically significant.
Regarding BMP-2 gene expression, the defects in the control group showed significantly higher gene expression than the defects in the MLTBO group ($p=0.0216$). For the other comparisons, no statistical differences were observed ($p>0.05$).

DISCUSSION

MLT acts on bone tissue by inducing osteoblastic differentiation from mesenchymal cells, and by inhibiting the action of osteoclasts[27-30]. Several studies have suggested positive effects of MLT on periodontal treatment and dental implant osseointegration[12,16,23,24]. However, there is no complete understanding of the MLT action on bone formation and of MLT behavior when associated with biomaterials. The present study demonstrated that the local administration of MLT, alone or associated with BO, induced more qualitative effect than quantitative effect on bone formation, according to the parameters evaluated.

The histomorphometric findings showed that the different treatments did not influence new bone formation differently. Even so, in the histological analysis, the MLT group showed presence of bone formation distant from the remaining bone, and presence of mature lamellar bone, as observed in previous studies[9,11,14,24,31]. Furthermore, in the present study, MLT promoted greater bone formation than the control, but there was no statistical significance, possibly because a single application of MLT may not suffice to trigger a significant quantifiable effect. It could be hypothesized that the combined administration of systemic MLT during the post-surgical period could provide longer MLT action and greater impact on the histological results.

Based on the microtomographic analysis, MLT did not significantly influence parameters of quantity (bone volume) and of quality (bone trabecular thickness and bone trabeculae number). The bone volume inside the defects was significantly smaller when MLT was used in the absence of BO.

Other authors have used Cone Beam Computed Tomography (CBCT) to evaluate the effects of topical MLT application on bone regeneration of isolated vertical defects created with plastic capsules in rat calvaria[11]. The capsules used in the test group were filled with 10 mg of MLT powder[11]. Micro-CT images showed a significant increase in bone volume in the test group, suggesting that MLT induced vertical bone regeneration in isolated defects[11].

Such different results found in the current study in relation to cited study[11] could be due to a difference in the bone defects, since in the present study, the bone defects were truly critical because the bone was completely removed with a trephine drill, whereas in the aforementioned study[11], the defect was demarcated with a trephine, and perforations were made in the cortical bone, but it remained in place, which would increase the bone defect nutrition and accelerate bone formation in 4 weeks.

Regarding other microtomographic parameters analyzed in present study, the trabeculae thickness and number were not significantly affected by MLT. This agrees with another study on MLT, which found no significant differences in bone density measured using cone beam tomography[18]. Said study measured bone density between the dental alveoli before the extraction of third molars in humans, and again 60 days after surgery. The alveoli received either 3 mg of MLT or hydroxymethyl cellulose gel as placebo[18].

Another study evaluated the effect of topical application of MLT on accelerating bone formation in rabbit tibias, compared to xenogeneic grafts and with blood clots in control sites[14]. Radiographic evaluation after 60 days showed lower density in control defects compared to native bone, and greater bone formation in defects that received MLTBO compared to those that received only BO[14]. MLTBO resulted in an increase in formation of cortical bone in length and width in the initial stages (15 and 30 days), while the difference was not significant at the end of 12 weeks[14].

Those findings[14] differ from the outcomes of the present study, in which the application of MLT, alone or associated with BO, did not result in greater bone formation. For trabeculae thickness and number (bone quality parameters), there was no statistically significant interaction between BO and MLT. It is important to consider that the present analysis was performed at only one time point (30 days post-operatively) and used microtomography (a method that increases sensitivity and specificity of the analysis).

In addition to histomorphometry and microtomography, the present study analyzed COL-I, OPN and BMP- 2 gene expression, with the aim of understanding the influence of MLT, whether or not associated with BO, on the reconstruction of critical defects. Concerning bone metabolism, MLT stimulates the synthesis of type I collagen[3]. It also interacts with the cells and extracellular matrix of bone tissue, promoting the union of osteoblastic cells with the organic and mineral phases of the matrix, and controlling the functions of bone cells, such as the OPN gene[7]. MLT also promotes bone tissue repair,

acting concomitantly with the BMP-2 gene[32,33].

COL-I gene expression did not differ statistically between the group in which MLT was added to the xenogeneic bone material (MLT+BO) and the control group, though it was significantly higher in the control group than in the MLT and BO groups. This suggests that the addition of MLT to the biomaterial may increase COL-I protein production by osteoblasts. However, these results should be interpreted with caution, as they could vary depending on the MLT routes of administration (topical or systemic), as well as the MLT ideal dose, since MLT in small concentrations may not be able to change COL-I protein synthesis, while MLT in high doses could cause hyperoxidation and cell damage[29].

In the present study, OPN gene expression did not differ significantly between groups, even though it was higher in the groups in which MLT was applied. According to the literature, OPN protein is secreted by osteoblasts and osteoclasts at the end of the bone mineralization process, and directly or indirectly controls bone mass, bone mineral quantity, and bone orientation[7].

A previous study investigated the role of MLT in bone metabolism using an *in vitro* experimental model based on osteoblast cultures to observe COL-I and OPN gene expression[7]. It demonstrated that MLT upregulated gene expression and the secretion of COL-I and OPN proteins[7]. Those results differ from the present study, probably because the cited study was conducted *in vitro* with MLT in osteoblast cell culture, whereas the present study used MLT in bone tissue, which is a complex structure.

BMP-2 gene expression was significantly lower with MLT associated with BO than in the control group, while no differences were observed with the other groups. According to literature, BMP-2 protein is essential at the beginning of the healing process, and its greatest production occurs during the first days of the reparative process[32].

In the current study, BMP-2 gene expression was similar in the MLT, BO and MLTBO groups. Another study agglutinated MLT and BMP-2 protein in chitosan/hydroxyapatite (HAp) scaffolds, successfully attenuating osteoclastic differentiation induced by BMP-2[33]. The authors therefore recommended chitosan/HAp scaffolds loaded with MLT/BMP-2, which would have dual functions in bone regeneration: increasing bone formation and inhibiting osteoclastic activity[33].

It should be highlighted that it was difficult to compare the present findings with other studies in the literature, since there were major differences in methodology and experimental design. One of these differences is the time at which euthanasia was performed (in the current study, only once, at 30 days). Topical application of MLT might have more significant effects during the initial stages of the proliferative process of bone repair, increasing the production of osteoblasts and thereby inducing greater bone formation, as reported in some studies[9,14,21], though impossible to observe with the method used herein.

A recently published study[34] on calvarial defects in female rats with and without osteoporosis, in which MLT was applied with or without BO, found that in the osteoporosis group, the defects treated with MLT showed higher mineral filling than the other treatments. Such results were not observed in the group of systemically healthy rats. Thus, experimental designs using different species, with different health conditions, as well as different MLT concentrations and routes of administration make it difficult to compare results. Therefore, future research using a larger sample number, with euthanasia at earlier times, and with systemic administration of MLT could provide additional relevant insights regarding new bone formation in critical defects.

CONCLUSION

It can be concluded that the topical application of MLT, whether or not associated with a xenogeneic biomaterial, provided a qualitative improvement in healing, but did not have a quantitative effect on new bone formation. In the absence of the xenogeneic biomaterial used herein, lower bone volume and thinner bone trabeculae were observed.

ACKNOWLEDGMENTS

The authors thank all the members of São Leopoldo Mandic Research Institute for their unwavering support and for providing resources throughout the course of this research.

DECLARATION OF CONFLICTING INTERESTS

The authors declare no potential conflicts of interest regarding this research or the authorship and/or publication of this article.

FUNDING

None.

REFERENCES

1. Lee S, Le NH, Kang D. Melatonin alleviates oxidative stress-inhibited osteogenesis of human bone marrow-derived mesenchymal stem cells through AMPK activation. Int J Med Sci. 2018 Jun 23;15(10):1083-1091. https://doi.org/10.7150/ijms.26314
2. Luo C, Yang Q, Liu Y, Zhou S, Jiang J, Reiter RJ, Bhattacharya P, Cui Y, Yang H, Ma H, Yao J, Lawler SE, Zhang X, Fu J, Rozental R, Aly H, Johnson MD, Chiocca EA, Wang X. The multiple protective roles and molecular mechanisms of melatonin and its precursor N-acetylserotonin in targeting brain injury and liver damage and in maintaining bone health. Free Radic Biol Med. 2019 Jan;130:215-233. https://doi.org/10.1016/j.freeradbiomed.2018.10.402
3. Cutando A, Gómez-Moreno G, Arana C, Acuña-Castroviejo D, Reiter RJ. Melatonin: potential functions in the oral cavity. J Periodontol. 2007 Jun;78(6):1094-102. https://doi.org/10.1902/jop.2007.060396
4. Amaral FGD, Cipolla-Neto J. A brief review about melatonin, a pineal hormone. Arch Endocrinol Metab. 2018 Aug;62(4):472-479. https://doi.org/10.20945/2359-3997000000066
5. Roth JA, Kim BG, Lin WL, Cho MI. Melatonin promotes osteoblast differentiation and bone formation. J Biol Chem. 1999 Jul 30;274(31):22041-7. https://doi.org/10.1074/jbc.274.31.22041
6. Suzuki N, Somei M, Seki A, Reiter RJ, Hattori A. Novel bromomelatonin derivatives as potentially effective drugs to treat bone diseases. J Pineal Res. 2008 Oct;45(3):229-34. https://doi.org/10.1111/j.1600-079X.2008.00623.x
7. Dalla-Costa K, Yurtsever F, Penteado J, Martinez EF, Sperandio M, Peruzzo DC. Melatonin has a stimulatory effect on osteoblasts by upregulating COL-1 and OPN expression/secretion. Acta Odontol Latinoam. 2020; 33(2): 125-134. https://doi.org/10.54589/aol.33/2/125
8. Guardia J, Gómez-Moreno G, Ferrera MJ, Cutando A. Evaluation of effects of topic melatonin on implant surface at 5 and 8 weeks in Beagle dogs. Clin Implant Dent Relat Res. 2011 Dec;13(4):262-8. https://doi.org/10.1111/j.1708-8208.2009.00211.x
9. Fernández BE, Díaz E, Fernández C, Núñez P, Díaz B. Ovarian aging: melatonin regulation of the cytometric and endocrine evolutive pattern. Curr Aging Sci. 2013 Feb;6(1):1-7. https://doi.org/10.2174/1874609811306010001
10. Gómez-Moreno G, Aguilar-Salvatierra A, Boquete-Castro A, Guardia J, Piattelli A, Perrotti V, Delgado-Ruiz RA, Calvo-Guirado JL. Outcomes of topical applications of melatonin in implant dentistry: a systematic review. Implant Dent. 2015 Feb;24(1):25-30.. https://doi.org/10.1097/ID.0000000000000186
11. Shino H, Hasuike A, Arai Y, Honda M, Isokawa K, Sato S. Melatonin enhances vertical bone augmentation in rat calvaria secluded spaces. Med Oral Patol Oral Cir Bucal. 2016 Jan 1;21(1):e122-6. https://doi.org/10.4317/medoral.20904
12. Ravi Kiran S, Bammidi N, Kumar AK, Kumar PS, Karnam Y. Evaluation of the Effect of Topical Melatonin Application on Immediately Placed Dental Implants Using Cone Beam Computed Tomography (CBCT). Cureus. 2022 May 23;14(5):e25233. https://doi.org/10.7759/cureus.25233
13. Park KH, Kang JW, Lee EM, Kim JS, Rhee YH, Kim M, Jeong SJ, Park YG, Kim SH. Melatonin promotes osteoblastic differentiation through the BMP/ERK/Wnt signaling pathways. J Pineal Res. 2011 Sep;51(2):187-94. https://doi.org/10.1111/j.1600-079X.2011.00875.x
14. Calvo-Guirado JL, Ramírez-Fernández MP, Gómez-Moreno G, Maté-Sánchez JE, Delgado-Ruiz R, Guardia J, López-Marí L, Barone A, Ortiz-Ruiz AJ, Martínez-González JM, Bravo LA. Melatonin stimulates the growth of new bone around implants in the tibia of rabbits. J Pineal Res. 2010 Nov;49(4):356-63. Retraction in: J Pineal Res. 2021 May;70(4):e12731. https://doi.org/10.1111/j.1600-079X.2010.00801.x
15. Tian Y, Ming J. Melatonin inhibits osteoclastogenesis via RANKL/OPG suppression mediated by Rev-Erbα in osteoblasts. J Cell Mol Med. 2022 Jul;26(14):4032-4047. https://doi.org/10.1111/jcmm.17440
16. Cutando A, Gómez-Moreno G, Arana C, Muñoz F, Lopez-Peña M, Stephenson J, Reiter RJ. Melatonin stimulates osteointegration of dental implants. J Pineal Res. 2008 Sep;45(2):174-9. https://doi.org/10.1111/j.1600-079X.2008.00573.x
17. Farhood B, Goradel NH, Mortezaee K, Khanlarkhani N, Najafi M, Sahebkar A. Melatonin and cancer: From the promotion of genomic stability to use in cancer treatment. J Cell Physiol. 2019 May;234(5):5613-5627. https://doi.org/10.1002/jcp.27391
18. Cobo-Vázquez C, Fernández-Tresguerres I, Ortega-Aranegui R, López-Quiles J. Effects of local melatonin application on post-extraction sockets after third molar surgery. A pilot study. Med Oral Patol Oral Cir Bucal. 2014 Nov 1;19(6):e628-33. https://doi.org/10.4317/medoral.19851
19. Permuy M, López-Peña M, González-Cantalapiedra A, Muñoz F. Melatonin: A Review of Its Potential Functions and Effects on Dental Diseases. Int J Mol Sci. 2017 Apr 19;18(4):865. https://doi.org/10.3390/ijms18040865
20. Purpura, S.; Fernandes, G.V.O.; Oliveira, F.P.; de Castro, F.C. Effects of Melatonin in the Non-Surgical Treatment of Periodontitis: A Systematic Review. Appl. Sci.2022,12,11698. https://doi.org/10.3390/app122211698
21. El-Gammal MY, Salem AS, Anees MM, Tawfik MA. Clinical and Radiographic Evaluation of Immediate Loaded Dental Implants With Local Application of Melatonin: A Preliminary Randomized Controlled Clinical Trial. J Oral Implantol. 2016 Apr;42(2):119-25. https://doi.org/10.1563/aaid-joi-D-14-00277
22. Sharan K, Lewis K, Furukawa T, Yadav VK. Regulation of bone mass through pineal-derived melatonin-MT2 receptor pathway. J Pineal Res. 2017 Sep;63(2):e12423. https://doi.org/10.1111/jpi.12423
23. Igarashi-Migitaka J, Seki A, Ikegame M, Honda M, Sekiguchi T, Mishima H, Shimizu N, Matsubara H, Srivastav AK, Hirayama J, Maruyama Y, Kamijo-Ikemori A, Hirata K, Hattori A, Suzuki N. Oral administration of melatonin contained in drinking water increased bone strength in naturally aged mice. Acta Histochem. 2020 Sep;122(6):151596. https://doi.org/10.1016/j.acthis.2020.151596
24. Salomó-Coll O, Maté-Sánchez de Val JE, Ramírez-Fernández MP, Satorres-Nieto M, Gargallo-Albiol J, Calvo-Guirado JL. Osseoinductive elements for promoting osseointegration around immediate implants: a pilot

study in the foxhound dog. Clin Oral Implants Res. 2016 Dec;27(12):e167-e175. https://doi.org/10.1111/clr.12596
25. Oliveira EA, Dalla-Costa KL, França FM, Kantovitz KR, Peruzzo DC. Influence of melatonin associated with the Bio-Gide® membrane on osteoblast activity: an in vitro Study. Acta Odontol Latinoam. 2022 Sep 30;35(2):90-97. doi: 10.54589/aol.35/2/90. https://doi.org/10.54589/aol.35/2/90
26. Wronski TJ, Dann LM, Horner SL. Time course of vertebral osteopenia in ovariectomized rats. Bone. 1989;10(4):295-301. https://doi.org/10.1016/8756-3282(89)90067-7
27. Reiter RJ, Gultekin F, Manchester LC, Tan DX. Light pollution, melatonin suppression and cancer growth. J Pineal Res. 2006 May;40(4):357-8. https://doi.org/10.1111/j.1600-079X.2006.00325.x
28. Esparza-Guerrero Y, Nava-Valdivia C, Saldaña-Cruz A, Vásquez-Jiménez J, Farias-Cuevas K, Enriquez-Luna A, Gamez-Nava J, Gonzalez-Lopez L, Corona-Sanchez E. El sistema RANK/RANKL/OPG y sus implicaciones clínicas en la osteoporosis. El Residente. 2016; 11 (3): 99-104.
29. Zhou L, Chen X, Yan J, Li M, Liu T, Zhu C, Pan G, Guo Q, Yang H, Pei M, He F. Melatonin at pharmacological concentrations suppresses osteoclastogenesis via the attenuation of intracellular ROS. Osteoporos Int. 2017 Dec;28(12):3325-3337. https://doi.org/10.1007/s00198-017-4127-8
30. Dong P, Gu X, Zhu G, Li M, Ma B, Zi Y. Melatonin Induces Osteoblastic Differentiation of Mesenchymal Stem Cells and Promotes Fracture Healing in a Rat Model of Femoral Fracture via Neuropeptide Y/Neuropeptide Y Receptor Y1 Signaling. Pharmacology. 2018;102(5-6):272-280. https://doi.org/10.1159/000492576
31. Dundar S, Yaman F, Saybak A, Ozupek MF, Toy VE, Gul M, Ozercan IH. Evaluation of Effects of Topical Melatonin Application on Osseointegration of Dental Implant: An Experimental Study. J Oral Implantol. 2016 Oct;42(5):386-389. https://doi.org/10.1563/aaid-joi-D-16-00048
32. Tsuji K, Bandyopadhyay A, Harfe BD, Cox K, Kakar S, Gerstenfeld L, Einhorn T, Tabin CJ, Rosen V. BMP2 activity, although dispensable for bone formation, is required for the initiation of fracture healing. Nat Genet. 2006 Dec;38(12):1424-9. https://doi.org/10.1038/ng1916
33. Jarrar H, Çetin Altındal D, Gümüşderelioğlu M. The inhibitory effect of melatonin on osteoclastogenesis of RAW 264.7 cells in low concentrations of RANKL and MCSF. Turk J Biol. 2020; 44:427-36. https://doi.org/10.3906/biy-2007-85
34. Dalla Costa KL, Abreu LF, Tolomei CB, Eleutério RG, Basting R, Balbinot G, Collares FM, Lopes P, Veiga N, Fernandes GVO, Peruzzo DC. Use of local melatonin with xenogeneic bone graft to treat critical-size bone defects in rats with osteoporosis: a randomized study. J Funct Biomater 2024; 15, 124. https://doi.org/10.3390/jfb15050124

https://doi.org/10.54589/aol.37/2/134

Camouflaged prejudice and the affirmation of skin colour differences: assessment of racism among Brazilian undergraduate dental students

Renata Lamenha-Lins[1], Saul M Paiva[1], Flavio F Mattos[2], Ivana M Prado[1], João L Bastos[3], Junia M Serra-Negra[1]

1. Universidade Federal de Minas Gerais, Faculdade de Odontologia, Departamento de Odontopediatria, Belo Horizonte, Brasil.
2. Universidade Federal de Minas Gerais, Faculdade de Odontologia, Departamento de Saúde Pública, Belo Horizonte, Brasil.
3. Simon Fraser University, Faculty of Health Sciences, Burnaby, Canada.

ABSTRACT

The negative oral health outcomes of disadvantaged racial groups have been well-documented, as racial disparity in oral health persists over time and in different locations[1]. However, it is important to note that skin colour has no biological meaning, and the observed differences can be physiological expressions of social injustice such as racism. ***Aim:*** *The aim of this study was to analyse the association between levels of modern racism (camouflaged prejudice and affirmation of differences) and sociodemographic characteristics of Brazilian dental students.* ***Material and Method:*** *An epidemiological cross-sectional online survey was conducted on 441 Brazilian undergraduate dental students using Google Forms. Participants were recruited via emails and social media, using the snowball technique. The Checklist for Reporting of Survey Studies (CROSS) was followed. The survey used sociodemographic variables, and the Brazilian version of the Modern Racism Scale (B-MRS), which measures the cognitive component of subtle racial attitudes. The scale assesses the central notion of disguised prejudice and has two domains: 'denial of prejudice' and 'affirmation of differences'. Participants' self-declared skin colour was categorized as "white" and "non-white" (black, brown, indigenous, yellow). Univariate analysis and Poisson regression with robust variance were applied.* ***Results:*** *Participants' mean age was 24.1 years (±5.4). Most participants were self-declared as white (54%) and 46% as non-white skin colour. Higher B-MRS overall-scores were observed in male (p=0.008) and non-white (p=0.002) students. B-MRS scores for the domain 'affirmation of differences' (representation of those who believe that whites and non-whites are truly different) were higher among male dental students (PR=1.138; CI 95%: 1.019–1.271) and those from low-income families (PR=1.306; CI 95%: 1.089–1.565). Scores for the domain 'denial of prejudice' (the idea that non-whites use their race to receive legal benefits) were higher among male dental students (PR=1.328; CI 95%: 1.129–1.562).* ***Conclusions:*** *In general, male non-white students had higher modern racism indicators. Male students from low-income families believed that whites and non-whites are truly different, accounting for the affirmation of difference in this sample.*

Keywords: *racism - inequity - health education - discrimination - prejudice - dentistry.*

To cite:
Lamenha-Lins R, Paiva SM, Mattos FF, Prado IM, Bastos JL, Serra-Negra JM. Camouflaged prejudice and the affirmation of skin colour differences: assessment of racism among Brazilian undergraduate dental students. Acta Odontol Latinoam. 2024 Aug 30;37(2):134-143. https://doi.org/10.54589/aol.37/2/134

Corresponding Author:
Junia M Serra-Negra
juniaserranegra@hotmail.com

Received: March 2024.
Accepted: May 2024.

Preconceito camuflado e afirmação das diferenças de cor da pele: avaliação do racismo entre estudantes brasileiros de odontología.

RESUMO

Os impactos negativos da saúde oral dos grupos raciais desfavorecidos têm sido bem documentados, uma vez que a disparidade racial na saúde oral persiste ao longo do tempo e em diferentes locais. No entanto, é importante notar que a cor da pele não tem significado biológico e as diferenças observadas podem ser expressões fisiológicas de injustiça social, como o racismo. ***Objetivo:*** *A teoria do racismo moderno afirma que as atitudes se expressam em formas preconceituosamente camufladas. Associou-se níveis de racismo moderno (afirmação de diferenças) com características sociodemográficas de universitários brasileiros.* ***Material e Método:*** *Uma pesquisa epidemiológica transversal on-line foi realizada com 441 estudantes brasileiros de graduação em odontologia por meio do Google Forms. Os participantes foram recrutados via e-mail e redes sociais, utilizando a técnica bola de neve. Seguiu-se a Lista de Verificação para Relatórios de Estudos de Pesquisa (CROSS). Variáveis sociodemográficas, bem como a versão brasileira da Escala de Racismo Moderno (B-MRS) que mede o componente cognitivo de atitudes raciais sutis foram utilizadas. A escala acessa o preconceito disfarçado, possuindo dois domínios: 'negação do preconceito' e 'afirmação das diferenças'. A cor da pele autodeclarada dos participantes foi categorizada em "branca" e "não-branca" (preta, parda, indígena, amarela). Foram realizadas análises univariada e regressão de Poisson.* ***Resultados:*** *A média de idade foi de 24,1 anos (±5,4). Muitos participantes se autodeclararam brancos (54%) e 46% não-brancos. Maiores escores da B-MRS foram observados em estudantes do sexo masculino (p=0,008) e não-brancos (p=0,002). Os escores da B-MRS para o domínio 'afirmação de diferenças' (representação daqueles que acreditam que brancos e não-brancos são diferentes) foram maiores entre estudantes de odontologia do sexo masculino (RP=1,138; IC 95%: 1,019–1,271) e aqueles de famílias de baixa renda (RP=1,306; IC 95%: 1,089–1,565). Os escores do domínio 'negação do preconceito' (ideia de que os não-brancos usam sua raça para receber benefícios legais) foram maiores entre os participantes do sexo masculino (RP=1,328; IC 95%: 1,129–1,562).* ***Conclusão:*** *Os estudantes homens, não-brancos, apresentavam indicadores de racismo mais elevados. Homens, provenientes de famílias de baixa renda, acreditavam que brancos e não-brancos são verdadeiramente diferentes, o que representa a afirmação da diferença entre o grupo desta amostra.*

Palavras-chave: *racismo - desigualdade - educação em saúde - discriminação - preconceito - odontologia.*

INTRODUCTION

The negative oral health outcomes of disadvantaged racial groups have been well-documented, as racial disparity in oral health persists over time in different locations[1]. However, it is important to note that skin colour has no biological meaning, and the observed differences can be physiological expressions of social injustice such as racism[2]. Understanding how knowledge and power are organised for the benefit of white people is fundamental for addressing racial inequities in oral health[3]. Racial groups are social constructs representing the attributes historically assigned to them by society. Therefore, the critical appraisal of racialised groups requires consideration of social and political contexts that vary across different societies[4].

In Brazil, the myth of racial democracy is widespread in the collective imaginary and intends to mask racism[5,6]. Interraciality and the ideal of harmonious coexistence among racial groups has contributed to the general belief that racial discrimination is not a problem in Brazil[6]. However, white supremacy is consolidated by means of possession of power and unspoken social consensus. The ideology of meritocracy which has become established in Brazilian society intersects with racism to maintain hierarchical racial positions[5].

Moreover, disapproval of explicit racial hostilities driven by contemporary social norms has created more subtle, "modern" expressions of racism, which has spread throughout society and institutions such as dental schools[7,8]. The concept of modern racism emerged as a theoretical construct in the United States after the civil rights movement of the 1950s and 1960s. It originally referred to discrimination against black Americans, mainly due to political opposition to the rights of black people[7-9]. The Modern Racism Scale (MRS) was developed to measure the cognitive component of subtle racial attitudes using a less reactive approach[7].

The MRS was validated in Brazil in 2006 to analyse local racial democracy[10]. The Brazilian version of the Modern Racism Scale (B-MRS) is divided into two domains: 'denial of prejudice' and 'affirmation of differences'. 'Denial of prejudice' captures the idea that racism no longer exists in Brazil but could be used by black people as means to obtain social benefits. 'Affirmation of differences' reflects the idea that black people have characteristics that differentiate them from white people and make them fit only for jobs that require less formal qualification. It focuses on the belief that Blacks and Whites are essentially different[10].

A growing body of evidence has revealed the presence of modern racism in dental schools worldwide. Recent studies have researched the underrepresentation of disadvantaged racial groups in dental education and in the workforce[11], patients' perceptions of racial discrimination in oral healthcare settings[12], and students' experiences of racism at dental schools[13]. Given the evidence of racism in the patient-student-professor-institutional system, it is also necessary to understand how modern racism manifests in dental students. This understanding is key to challenging and dismantling the racist framework within dental education. Such efforts would assist in decolonising the curricula in Brazilian dental schools, enabling the identification and elimination of the roots of racial inequities in oral health[4,5,14,15].

A social psychology study analysing types of prejudice highlighted four profiles: truly prejudiced people, aversive racists, principled conservatives, and modern racists (camouflaged prejudice). Egalitarianism/humanism, social conservatism and economic/political conservatism permeated the prejudiced attitudes of each profile. The prejudice was conservative and demonstrated the effect of ambiguity among Asian university students[16]. It is essential to reflect upon the implications and understand the nature of prejudice[16].

It is crucial to dismantle racial oppression in overwhelmingly white spheres such as dental schools[3], especially in countries such as Brazil, which have limited healthcare system resources. Brazilian-style white supremacy seems to be veiled, operating as a social pact[5]. The denial of racism is a major issue and should be considered as a central point of debate in dental research. This survey was designed to identify modern racism among dental students in Brazil and to identify correlations between B-MRS scores and sociodemographic variables.

MATERIAL AND METHOD

Ethical statement

This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee (Protocol number: #05021018.7.0000.5149 Universidade Federal de Minas Gerais). All participants were informed about

anonymity and data confidentiality and provided informed consent to be part of the study.

Study design and eligibility criteria.
An epidemiological cross-sectional online survey was conducted using Google Forms. The recommendations of the Checklist for Reporting of Survey Studies (CROSS) were followed[17]. The target population was Brazilian dental students enrolled in the country`s public and private higher dental education system, with no restrictions on age, gender, skin colour, ethnicity, or socioeconomic background. Data were collected from November 2021 to July 2022.

Sample selection and recruitment.
Convenience sampling using the snowballing recruitment technique was used to reach the highest possible number of participants from all areas of the country. The researchers presented the survey to professors of public and private dentistry courses throughout the country and invited them to share it with students at their own schools and with other professors in their network. Invitations to participate were extended to students via remote lectures, email and social media, additionally requesting help to contact further eligible subjects. Social media posts and a brief video about the research containing a link to access the questionnaire were distributed through Instagram and WhatsApp. The campaigns were promoted in social media on relevant national and international dates for the struggle against racial discrimination. The first campaign took place on November 20, the Brazilian day of Black Awareness, and the second was launched on March 21, the United Nations International Day for the Elimination of Racial Discrimination. As the sample size expanded, the risk of selection bias was progressively reduced[18]. Descriptive analysis was periodically performed, and data collection stopped when the composition of the sample was balanced according to the distribution of dental students and schools in the country[18,19].

Questionnaire content and data collection
Responses to all survey items were mandatory. Students were asked about their gender identity (female, male, non-binary, prefer not to say)[20], self-declared skin colour (see below), age (in years), and the current semester of the dentistry course in which they were enrolled (first to tenth). Students' age was grouped for data analysis as follows: up to 20 years old, 20 to 35 years old, and over 35 years old. Based on the dental schools represented in the study, two variables were created: regional location of the school (North, Northeast, Midwest, Southeast, South) and administrative type (public, private). Participants' occupation was categorised as either full-time student or student also engaged in paid work unrelated to the course.
Students' self-declared skin colour was classified according to the Brazilian Institute of Geography and Statistics (IBGE) classifications of black, brown, indigenous, white, or yellow. The IBGE criterion was adopted because it is referenced in the enrolment process for Brazilian dental education[21]. Participants' self-declared skin colour was then recategorized as "white" and "non-white" (black, brown, indigenous, yellow) for statistical analysis. This categorisation was adopted to better identify the role of whiteness in the expression of modern racism in Brazilian dental education[5,22–24].
The brown skin colour classification generally refers to a person of mixed skin colour, such as people with indigenous-black, indigenous-white or black-white heritage. Brazilian self-identification of skin colour is a complex topic[22,23]. Deciding 'who is racially who' in Brazil is a longstanding social and political issue[23], primarily because it has been based on phenotypical characteristics. In any case, the structural disposition of racism grants symbolic and material privilege to those considered white, compared to non-white individuals[5].
Data on participants' monthly family income was also collected from the socioeconomic questionnaire administered during enrolment in Brazilian public universities. Participants were grouped into: Class E (up to $235 USD); Class D ($235.01 USD–$703 USD); Class C ($703.01 USD–$1,406 USD); Class B (from $1,406.01 USD–$2,110 USD); and Class A (more than $2,110.01 USD). These groups are based on multiples of the national minimum monthly wage (approximately $235 USD).
The last section of the questionnaire comprised items rated according to the B-MRS[7,10] scale, which assesses the central notion of disguised prejudice[7,8], and was culturally adapted to the Brazilian context using 14 items distributed into two domains: 'denial of prejudice' (9 items) and 'affirmation of differences' (5 items)[10]. All responses were rated on a 7-point Likert-type scale, from 1 = strongly disagree to 7 =

strongly agree. The total score ranged from 14 to 98, with higher scores expressing higher levels of modern racism. The maximum scores are 63 points in the 'denial of prejudice' domain, and 35 points in the 'affirmation of differences' domain.

Data analysis.

Data were analysed using the Statistical Package for the Social Sciences (version 21.0, SPSS Inc., Chicago IL, USA). The total score and domain scores of the B-MRS were the dependent variables. The independent variables comprised participants' gender, self-declared skin colour, age, current semester of the dentistry course, occupation, national region of the dental school of enrolment, school administrative type and family monthly income. Mann–Whitney U and Kruskal–Wallis tests were used to analyse the association between participants' total and domain scores on the B-MRS and their sociodemographic characteristics. Bonferroni and Tukey's HSD post-hoc tests were used to identify the pairwise comparisons in which statistically significant differences occurred. Univariate and multivariate analyses were performed using Poisson regression with robust variance. For the multivariate analysis, variables with a p-value ≤ 0.20 in the univariate (non-adjusted) model were included in the adjusted model, and effect changes were considered in the final model. The level of statistical significance was set at 5% ($p < 0.05$).

RESULTS

A total 485 users accessed the electronic form and 441 (90.9%) participated in the survey. Participants' mean age was 24.1 years (±5.4), ranging from 17 to 53 years. Of the participants, 54.9% *(n*=242) were self-declared white and 45.1% non-white (n=199). More white dental students studied at paid private educational institutions (52.2%). In contrast, most non-white dental students studied at public free of charge dental schools (62.6%).

Table 1 shows the sociodemographic status of participants and provides a univariate analysis of the relationship between these characteristics and their total sum-score on the B-MRS scale. Higher B-MRS sum-score was observed for male (p = 0.008) and non-white (p = 0.002) participants. Assessed by age group, Bonferroni's test revealed higher B-MRS sum-score for students over 35 years old than for those aged 20 to 35 years (p = 0.024). Regarding family income, Bonferroni's test showed that sum-score was significantly higher for students in Class E on the B-MRS scale than for those in Class D (p = 0.002), Class C (p = 0.001) or Class A (p = 0.001). Students enrolled in private dental schools had higher B-MRS sum-score than those from public dental schools (p = 0.001). Bonferroni's test showed higher B-MRS mean sum-score for students from the north than for those from the south of Brazil (p = 0.012). Tukey's HSD test identified that participants enrolled from the seventh to tenth semesters of the course had higher B-MRS mean sum-score than students in the fourth to sixth semesters (p = 0.048). B-MRS in the domains of 'denial of prejudice' and 'affirmation of differences' were higher among male participants (p = 0.018 and p = 0.014 respectively). Non-white dental students had higher sum-score in the 'affirmation of differences' domain (p = 0.037) (Table 2).

Poisson regression analysis with robust variance estimation showed that total B-MRS sum-score was significantly higher among males (Prevalence Ratio [PR] = 1.130; 95% Confidence Interval [CI]: 1.027–1.244), and among students at private dental schools (PR = 1.404; 95% CI: 1.272–1.550). Participants from Class E (PR = 1.321; 95% CI: 1.134–1.538) and Class B (PR = 1.188; 95% CI: 1.008–1.402) had higher B-MRS sum-score than those from Class A (Table 3).

Male participants had higher sum-score in the 'denial of prejudice' domain (PR = 1.126 95% CI 1.018–1.245 p = 0.021) than females. Participants from the family income Class E group had higher sum-score in the 'denial of prejudice' domain than students with higher family incomes (PR = 1.328 95% CI = 1.129–1.562 p = 0.001) (Table 4). Sum-score in the 'affirmation of differences' domain was higher among male students (PR = 1.138 95% CI 1.019–1.271 p = 0.022) than among females. Dental students from income Class E scored higher in the 'affirmation of differences' domain (PR = 1.306 95% CI 1.089–1.565 p = 0.004) than students from higher income families (Table 5).

DISCUSSION

The results suggest an interesting background to the (re)arrangement of power and racism in Brazilian dental education system. Alongside its purpose of measuring subtle individual-level racist beliefs[7,10], the B-MRS has evidenced the pathways used by

Table 1. Descriptive sociodemographic and economic data of Brazilian dental students and univariate analyses between the independent variables and the total score on the Brazilian version of the Modern Racism Scale.

Variables	Dental students	Total score on the B-MRS			
	N (%)	Mean (± SD)	Min – Max	Median	*P*
Sex					
Female	343(78.1)	32.5(15.7)	14 – 98	29.0	**0.008***
Male	96(21.9)	37.2(16.9)	14 – 93	36.0	
Self-declared race					
White	242(54.9)	32(15.7)	14 – 98	29.0	**0.002***
Non-white	199(45.17)	35.8(16.3)	14 – 86	32.0	
Age group					
Up to 20 years old	78(17.7)	33.8(15.1)	14 – 93	31.0	**0.030****
20 to 35 years old	341(77.5)	32.9(15.7)	14 – 98	29.0	
Over 35 years old	21(4.8)	42.4(21.6)	14 – 82	43.0	
Socioeconomic status					
Class E	53(12.5)	42.1(19.4)	14 – 86	41.0	**0.005****
Class D	122(28.8)	32.3(14.2)	14 – 79	30.5	
Class C	116(27.4)	31.7(14.2)	14 – 93	28.5	
Class B	59(13.9)	35.5(19.5)	14 – 98	32.0	
Class A	74(17.5)	31.1(14.2)	14 – 84	28.0	
Administrative type of the HEI					
Public	236(54.3)	30.5(16.1)	14 – 98	25.0	**<0.001***
Private	199(45.7)	37(15.3)	14 – 93	36.0	
Semester of dental course					
1st to 3rd semester	119(27.0)	31(14.7)	14 – 86	31.0	**0.025****
4th to 6th semester	89(20.2)	30.2(15.3)	14 – 84	24.0	
≥7th semester	233(52.8)	35(16.8)	14 – 98	32.0	
Occupation					
Full-time student	297(70.0)	33.1(16)	14 – 93	30.0	0.229*
Studies and works	127(30.0)	34.7(16.2)	14 – 98	34.0	

B-MRS = Brazilian version of the Modern Racism Scale; HEI = Higher Education Institution; N = Number of participants; SD = Standard Deviation; Min = Minimum; Max = Maximum; P = Probability value. *Mann-Whitney test; **Kruskal-Wallis test. Values in bold indicate statistically significant difference.

white people keep prejudice camouflaged in Brazil. The high percentage of white dental students in the study population stands out and shows that dental education in Brazil is still an environment for white students[25]. However, this national scenario is changing. A study from southern Brazil observed a recent increase in non-white students at Brazilian universities[25]. An official national survey also observed an increase in non-whites enrolled at universities[25].

An important finding in this study was the difference in B-MRS sum-score between male and female students. A plausible explanation for the higher scores among males is in the complexity of the systems of oppression present in the intersections of social categories such as skin colour, gender and socioeconomic class[26]. As the socially dominant group, men enjoy the benefits of their position, and the dominant discourse may cause them to express racial discrimination more assertively, based on the theory of the intersectionality of racism and sexism[25-27]. For instance, health inequities linked to skin colour are usually associated with gender inequities, and such intersections leave black women in more unfavourable positions[28].

Table 2. Descriptive sociodemographic and economic data of Brazilian dental students and bivariate analyses between the independent variables and the domains 'denial of prejudice' and 'affirmation of differences' of the Brazilian version of the Modern Racism Scale.

Variables	Dental students	Denial of Prejudice				Affirmation of Differences			
	N (%)	Mean (± SD)	Min – Max	Median	*P*	Mean (± SD)	Min – Max	Median	*P*
Sex									
Female	343(78.1)	21.32(10.84)	9 – 63	19	**0.018***	11.16(6.15)	5 – 35	10	**0.014***
Male	96(21.9)	24.22(11.51)	9 – 60	23.5		12.96(6.69)	5 – 33	12	
Self-declared race									
White	242(54.9)	21(10.61)	9 – 63	18.5	0.069*	11(6.18)	– 35	9	**0.037***
Non-white	199(45.1)	23.1(11.46)	9 – 54	21		12.18(6.41)	– 35	11	
Age group									
Up to 20 years old	78(17.7)	22.08(10.66)	9 – 60	19	0.079**	11.7(5.89)	– 33	11	0.412**
20 to 35 years old	341(77.5)	21.53(10.8)	9 – 63	19		11.37(6.27)	– 35	10	
Over 35 years old	21(4.8)	28.67(14.22)	9 – 52	30		13.76(8.07)	– 30	15	
Socioeconomic status									
Class E	53(12.5)	27.92(13)	9 – 54	25[a]	**0.005****	14.17(8.26)	– 35	12	0.189**
Class D	122(28.8)	21.4(9.86)	9 – 50	19[b]		10.87(5.54)	– 29	11	
Class C	116(27.4)	20.55(9.55)	9 – 60	18[b]		11.15(5.91)	– 33	9	
Class B	59(13.9)	23.25(13.28)	9 – 63	22[ab]		12.20(7.29)	– 35	11	
Class A	74(17.5)	20.5(10.37)	9 – 55	17[b]		10.64(5.05)	– 29	9.5	
Semester of dental course									
1st to 3rd semester	119 (27.0)	21.9(10.34)	9 – 54	19[ab]	**0.040****	11.1(5.85)	– 32	10	0.102**
4th to 6th semester	89(20.2)	19.71(10.56)	9 – 55	16[a]		10.53(6)	– 29	8	
≥7th semester	233(52.8)	22.81(11.48)	9 – 63	21[b]		12.13(6.6)	– 35	11	
Occupation									
Full-time student	297(70.0)	21.67(10.77)	9 – 60	19	0.258*	11.44(6.41)	– 35	10	0.422*
Studies and works	127(30.0)	23.06(11.6)	9 – 63	21		11.65(6.04)	– 35	11	

HEI = Higher Education Institution; N = Number of participants; SD = Standard Deviation; Min = Minimum; Max = Maximum; P = Probability value. *Mann-Whitney test; **Kruskal-Wallis and post-hoc test. Bolded values indicate statistically significant difference. Different letters indicate statistically significant difference.

This reasoning is also consistent with the lower B-MRS sum-score observed among the students with the highest socioeconomic status, particularly compared to those of the lowest socioeconomic group. However, it would be reductionist to assume that this result reflects their real positions of social power. Further qualitative studies might provide greater insight into why participants from the more vulnerable groups, such as Class E, had higher scores in the B-RMS domains. Defence mechanisms that cause the oppressed to become oppressors[7] might be a reasonable explanation, and different study designs could further investigate this possibility[7,8,10]. The presence of internalised racism may explain the higher B-MRS sum-score among participants from a lower economic class. People in vulnerable situations tend to accept their own oppression and internalise the racism perpetuated by the majority in society[29]. Furthermore, there is the social desirability of being accepted in society [30,31].

The B-MRS encompasses items regarding blacks' rights, which require tailored public policies[10]. However, there is well-known opposition from high-income groups in Brazilian society against important affirmative action initiatives in Brazil, such as the Quota Law, which gives preference to racially marginalised students enrolling in public federal universities by reserving 50% of student vacancies for them[26]. Based on

Table 3. Poisson regression of dental students' sociodemographic characteristics associated with the total score on the Brazilian version of the Modern Racism Scale.

Variables	Total score on the B-MRS			
	Unadjusted model	*P*	Adjusted model	*P*
	PR (95% CI)		PR (95% CI)	
Sex				
Male	1.145 (1.032 – 1.270)	0.011	1.130 (1.027 – 1.244)	**0.013**
Female	1		1	
Self-declared race				
Non-white	1.102 (1.009 – 1.205)	0.031	1.048 (0.958 – 1.147)	0.307
White	1		1	
Age group				
Up to 20 years old	0.796 (0.630 – 1.006)	0.056	0.887 (0.717 – 1.098)	0.270
20 to 35 years old	0.775 (0.623 – 0.964)	0.022	0.833 (0.692 – 1.003)	0.054
Over 35 years old	1		1	
Socioeconomic status				
Class E	1.353 (1.152 – 1.588)	<0.001	1.321 (1.134 – 1.538)	**<.0.001**
Class D	1.037 (0.911 – 1.180)	0.583	1.015 (0.897 – 1.149)	0.810
Class C	1.019 (0.893 – 1.162)	0.784	1.050 (0.919 – 1.200)	0.474
Class B	1.139 (0.958 – 1.355)	0.140	1.188 (1.008 – 1.402)	**0.040**
Class A	1		1	
Administrative type of the HEI				
Private	1.214 (1.111 – 1.326)	<0.001	1.404 (1.272 – 1.550)	**<.0.001**
Public	1		1	
Semester of dental course				
1st to 3rd semester	0.944 (0.854 – 1.045)	0.266	0.917 (0.813 – 1.035)	0.161
4th to 6th semester	0.865 (0.766 – 0.977)	0.020	0.952 (0.846 – 1.071)	0.411
≥ 7th semester	1		1	

B-MRS = Brazilian version of the Modern Racism Scale; HEI = Higher Education Institution; PR = Prevalence Ratio; CI = Confidence Interval; P = Probability value. Values in bold indicate statistically significant difference.

the ideology of meritocracy, the Brazilian economic and political élite resist the idea of social minorities occupying spaces they used to inhabit[5,27]. In this regard, the lower sum-score among Class A students might be seen as an attempt to hold onto power.

Our study found higher B-MRS sum-score among students at private than at public dental schools. It may be intuitive to assume that this result is closely linked to the abovementioned Quota Law[27], and the consequent increase of non-white students at public institutions. If so, it is important to devise political initiatives to support opportunities for non-whites to have increased access to private dental schools[30]. In this context, two governmental funding programmes have been created to promote access of students of lower socioeconomic status to private higher education: the Student Loan Fund (FIES) and the University for All program (PROUNI)[30]. These programmes are crucial to increase the opportunities of low-income groups, which consist mainly of non-whites, to have access to higher education. As a result of these initiatives, the racial and socioeconomic profiles of dental students enrolled in Brazilian public and private higher education have become more diverse[27-31], as can be seen from the findings of this study, although they do not enable the researchers to address the social hierarchies among students[30]. Further research is needed to analyse the social dynamics and anti-racist engagement in both private and public Brazilian dental education[15].

The discussion should extend to all Latin America. The 2021 Pan American Health Organization report "Health of Afro-descendant People in Latin America" concluded that people of African descent live

Table 4. Poisson regression of the sociodemographic characteristics of dental students associated with the score on the 'denial of prejudice' domain of the Brazilian version of the Modern Racism Scale.

Variables	Denial of Prejudice			
	Unadjusted model	*P*	Adjusted model	*P*
	PR (95% CI)		PR (95% CI)	
Sex				
Male	1.136 (1.019 – 1.267)	0.022	1.126 (1.018 – 1.245)	**0.021**
Female	1		1	
Self-declared race				
Non-white	1.100 (1.002 – 1.208)	0.046	1.045 (0.950 – 1.149)	0.365
White	1		1	
Age group				
Up to 20 years old	0.770 (0.610 – 0.972)	0.028	0.850 (0.688 – 1.049)	0.130
20 to 35 years old	0.751 (0.607 – 0.930)	0.009	0.814 (0.681 – 0.974)	**0.025**
Over 35 years old	1		1	
Socioeconomic status				
Class E	1.363 (1.151 – 1.614)	<0.001	1.328 (1.129 – 1.562)	**<.0.001**
Class D	1.045 (0.908 – 1.202)	0.542	1.020 (0.889 – 1.171)	0.775
Class C	1.003 (0.870 – 1.157)	0.965	1.030 (0.892 – 1.189)	0688
Class B	1.135 (0.944 – 1.364)	0.177	1.179 (0.988 – 1.406)	0.068
Class A	1		1	
Semester of dental course				
1st to 3rd semester	0.960 (0.863 – 1.068)	0.452	0.919 (0.807 – 1.047)	0.204
4th to 6th semester	0.864 (0.760 – 0.982)	0.025	0.943 (0.834 – 1.067)	0.354
≥7th semester	1		1	

B-MRS = Brazilian version of the Modern Racism Scale; HEI = Higher Education Institution; PR = Prevalence Ratio; CI = Confidence Interval; P = Probability value. Values in bold indicate statistically significant difference.

with a wide range of disadvantages compared to the white population. The disadvantages experienced by people of African descent in Latin America occur in a context of discrimination and racism, often exacerbated by gender inequalities[32]. Perhaps these data justify the higher rates of prejudice related to gender and low income among the university students who participated in our study. Attending university and envisioning a professional career, which other family members may not have been able to do, may have influenced an argumentation stance with camouflaged prejudice among men, compared to women in the study sample.

There are limitations to this study. The snowball sampling method facilitated access to participants, considering the geographical size of Brazil, but limited direct interaction with the researchers. Participants may have had doubts and questions during the survey but could not contact the researchers for clarification. A future qualitative study may be beneficial in this context. Additionally, the desire to be socially acceptable may also have interfered with the responses[31]. Confidentiality was guaranteed to all participants, but some participants may still have felt embarrassed to admit their own prejudice to themselves. It is important to note that white students predominated in the dental schools observed. Brazilian laws have attempted to correct the historical debt owed to black people in the country by encouraging affirmative action in university admissions. It is important to discuss the levels of prejudice within universities[11,12].

Future population-based research with a qualitative or mixed methods approach[10] could expand on the findings of this study. The defence of ethnic minorities needs to be encouraged in various sectors of society around the world. Intervention studies are needed to evaluate anti-racist educational strategies aimed at dental students and this could be a rich research topic to be developed at all dental education institutions.

Table 5. Poisson regression of the sociodemographic characteristics of dental students associated with the score on the 'affirmation of differences' domain of the Brazilian version of the Modern Racism Scale.

Variables	Affirmation of Differences domain on the B-MRS			
	Unadjusted model	*P*	Adjusted model	*P*
	PR (95% CI)		PR (95% CI)	
Sex				
Male	1.161 (1.032 – 1.307)	0.013	1.138 (1.019 – 1.271)	**0.022**
Female	1		1	
Self-declared race				
Non-white	1.107 (1.000 – 1.226)	0.049	1.055 (0.949 – 1.172)	0.321
White	1		1	
Age group				
Up to 20 years old	0.850 (0.649 – 1.112)	0.235	0.964 (0.746 – 1.246)	0.779
20 to 35 years old	0.826 (0.642 – 1.063)	0.137	0.872 (0.690 – 1.101)	0.249
Over 35 years old	1		1	
Socioeconomic status				
Class E	1.332 (1.103 – 1.610)	0.003	1.306 (1.089 – 1.565)	**0.004**
Class D	1.022 (0.888 – 1.176)	0.761	1.006 (0.878 – 1.152)	0.934
Class C	1.048 (0.907 – 1.211)	0.523	1.089 (0.939 – 1.263)	0.260
Class B	1.147 (0.953 – 1.381)	0.146	1.208 (1.013 – 1.440)	**0.036**
Class A	1		1	
Semester of dental course				
1st to 3rd semester	0.915 (0.814 – 1.029)	0.139	0.914 (0.794 – 1.052)	0.208
4th to 6th semester	0.868 (0.757 – 0.995)	0.042	0.967 (0.838 – 1.116)	0.647
≥7th semester	1		1	

B-MRS = Brazilian version of the Modern Racism Scale; HEI = Higher Education Institution; PR = Prevalence Ratio; CI = Confidence Interval; P = Probability value. Values in bold indicate statistically significant difference.

CONCLUSION

This study found higher sum scores of denial prejudice and affirmation of difference among male than female students. Camouflaged prejudice about the difference in skin colour between whites and non-whites was evident among participants with low family income. Despite legal attempts at historical reparations, there is still a lot of inequality in Brazil and throughout Latin America between whites and non-whites, as well as gender inequality. The topic needs to gain more visibility, and global discussions about prejudice should be encouraged.

DECLARATION OF CONFLICTING INTERESTS

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

FUNDING

This study was financed by the Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazilian Ministry of Science, Technology and Innovations, Brazil (Grant CNPq 301973/2022-9).

REFERENCES

1. Bastos JL, Celeste RK, Paradies YC. Racial inequalities in oral health. J Dent Res. 2018;97(8):878-886. https://doi.org/10.1177/0022034518768536
2. Flanagin A, Frey T, Christiansen SL. Updated guidance on the reporting of race and ethnicity in medical and science journals. JAMA. 2021;326(7):621-627. https://doi.org/10.1001/jama.2021.13304
3. Lala R, Gibson BJ, Jamieson LM. The relevance of power in dentistry. JDR Clin Trans Res. 2021;6(4):458-459. https://doi.org/10.1177/2380084421998619

4. Fleming E, Raskin SE, Brody E. From describing disparities to understanding why disparities exist: Anti-racist methods to support dental public health research. J Public Health Dent. 2022;82(1):73-78. https://doi.org/10.1111/jphd.12503
5. Schucman LV, Melo WC. White Supremacy, Brazil Style. NACLA Rep Am. 2022;54(2):1-7. https://doi.org/10.1080/10714839.2022.2084991
6. Brito L. Portraits of black politics and resistance in Brazil. NACLA Rep Am. 2022;54(2):129-131. https://doi.org/10.1080/10714839.2022.2084971
7. McConahay JB, Hardee BB, Batts V. Has racism declined in America? It depends on who is asking and what is asked. J Confl Resolut. 1981;25(4):563-579. https://doi.org/10.1177/002200278102500401
8. McConahay JB. Modern racism and modern discrimination: The effects of race, racial attitudes, and context on simulated hiring decisions. Pers Soc Psychol Bull. 1983;9(4):551-558. https://doi.org/10.1177/0146167283094004
9. Bonilla-Silva E. Racism without racists: Color-blind racism and the perspective of racial inequality in America. 4th Edition. Lanham: Rowman & Littlefield Publishers, Inc.; 2014.
10. Santos WS, Gouveia VV, Navas MS, Pimentel CE, Gusmão EES. Escala de racismo moderno: Adaptação ao contexto brasileiro. Psicol Estud. 2006;11(3):637-645. https://doi.org/10.1590/S1413-73722006000300020
11. Smith SG, Banks PB, Istrate EC, Davis AJ, Johnson KR, West KP. Anti-racism structures in academic dentistry: supporting underrepresented racially/ethnically diverse faculty. J Public Health Dent. 2022;82(1):103-113. https://doi.org/10.1111/jphd.12509
12. Singhal A, Jackson JW. Perceived racial discrimination partially mediates racial-ethnic disparities in dental utilization and oral health. J Public Health Dent. 2022;82(1):63-72. https://doi.org/10.1111/jphd.12515
13. Ahmadifard A, Forouhi S, Waterhouse P, Muirhead V. A student-led qualitative study to explore dental undergraduates' understanding, experiences, and responses to racism in a dental school. J Public Health Dent. 2022;82(1):36-45. https://doi.org/10.1111/jphd.12514
14. Ali K, McColl E, Tredwin C, Hanks S, Coelho C, Witton R. Addressing racial inequalities in dental education: decolonizing the dental curricula. Br Dent J. 2021;230(3): 165-169. https://doi.org/10.1038/s41415-020-2598-z
15. Demopoulos CA, Kohli R, Dhar S, Raju K. Racial and oral health equity in dental school curricula. J Public Health Dent. 2022;82(1):114-122. https://doi.org/10.1111/jphd.12516
16. Son Hing LS, Chung-Yan GA, Hamilton LK, Zanna MP. A two-dimensional model that employs explicit and implicit attitudes to characterize prejudice. J Pers Soc Psychol. 2008;94(6):971-87. https://doi.org/10.1037/0022-3514.94.6.971
17. Sharma A, Duc NTM, Thang TLL, Nam NH, Ng SJ, Abbas KS, et al. A Consensus-Based Checklist for Reporting of Survey Studies (CROSS). J Gen Intern Med. 2021;36(10):3179-3187. https://doi.org/10.1007/s11606-021-06737-1
18. Heckathorn DD. Snowball Versus Respondent-Driven Sampling. Sociol Methodol.2011;41(1):355-366. https://doi.org/10.1111/j.1467-9531.2011.01244.x
19. Morita MC, Uriarte Neto M, Fontanella VRC, Haddad AE. The unplanned and unequal expansion of Dentistry courses in Brazil from 1856 to 2020. Braz Oral Res. 2021;35:e009. https://doi.org/10.1590/1807-3107bor-2021.vol35.0009
20. Tadiri CP, Raparelli V, Abrahamowicz M, Kautzky-Willer A, Kublickiene K, Herrero MT, et al. Methods for prospectively incorporating gender into health sciences research. J Clin Epidemiol. 2021;129:191-197. https://doi.org/10.1016/j.jclinepi.2020.08.018
21. Brazil. Statistical Synopsis of ENEM 2021 [Internet]. National Institute of Educational Studies and Research Anísio Teixeira (INEP); 2022 Jul https://www.gov.br/inep/pt-br/acesso-a-informacao/dados-abertos/sinopses-estatisticas/enem
22. Mendes K. 'I am Indigenous, not pardo': Push for self-declaration in Brazil's census [Internet]. Mongabay News & Inspiration from Nature's Frontline; 2021 Jun. https://news.mongabay.com/2021/06/i-am-indigenous-not-pardo-push-for-self-declaration-in-brazils-census/
23. Costa ES, Schucman LV. Identidades, Identificações e Classificações Raciais no Brasil: O Pardo e as Ações Afirmativas. Estud Pesqui Psicol. 2022;22(2). https://doi.org/10.12957/epp.2022.68631
24. Santos RV, Bastos JL, Kaingang JD, Batista LE. Should there be recommendations on the use of "race" in health publications? An emphatic "yes", especially because of the implications for antiracist practices. Cad Saúde Pública. 2022;38(3):e00021922. https://doi.org/10.1590/0102-311x00021922
25. Fernandez MDS, Pontes AFL, Casarin M, Feijo JDS, Pola NM, Muniz FWMG Factors associated with poor academic performance among undergraduate dental students: A cross-sectional study. J Dent Educ. 2023;87(4):514-522. https://doi.org/10.1002/jdd.13134
26. Collins, P.H. Black Feminist Thought: Knowledge, Consciousness, and the Politics of Empowerment. New York: Routledge; 2009.
27. Crenshaw K. Mapping the Margins: Intersectionality, Identity Politics, and Violence against Women of Color. Stanford Law Rev. 1991;43(6):1241-1299. https://doi.org/10.2307/1229039
28. Uzogara EE. Dark and sick, light and healthy: black women's complexion-based health disparities. Ethn Health. 2019;24(2):125-146. https://doi.org/10.1080/13557858.2017.1315376
29. Sosoo EE, Bernard DL, Neblett EW. The influence of internalized racism on the relationship between discrimination and anxiety. Culture Divers Ethnic Minor Psychol. 2020;26(4):570-580. https://doi.org/10.1037/cdp0000320
30. Reis DB, Santos JR. Wins and Uncertainties After 10 Years of Affirmative Action. NACLA Rep Am. 2022;54(2):203-208. https://doi.org/10.1080/10714839.2022.2084992
31. Mourao P, Junqueira A. Through the Irregular Paths of Inequality: An Analysis of the Evolution of Socioeconomic Inequality in Brazilian States Since 1976. Sustainability. 2021;13(4):2356. https://ideas.repec.org/a/gam/jsusta/v13y2021i4p2356-d503701.html.
32. PAHO. Las personas afrodescendientes de América Latina viven en condiciones muy desiguales que repercuten en su salud y bienestar, según un estudio de la OPS. 2021. Available from: https://www.paho.org/es/noticias/3-12-2021-personas-afrodescendientes-america-latina-viven-condiciones-muy-desiguales-que

https://doi.org/10.54589/aol.37/2/144

Dental caries lesions and impact on quality of life in adolescents living in urban and rural areas. A case study

Carlos Fernández[1,2], Maria C Pagano[1], Pablo A Salgado[1,2], Angela B Argentieri[1,2], Aldo F Squassi[1,2], Noemí E Bordoni[1,2]

1. Universidad de Buenos Aires, Facultad de Odontología, Cátedra de Odontología Preventiva y Comunitaria, Buenos Aires, Argentina
2. Universidad de Buenos Aires, Facultad de Odontología, Instituto de Investigaciones en Salud Pública, Buenos Aires, Argentina

ABSTRACT

The concept of quality of life (QoL) developed from early studies on subjective wellbeing and satisfaction with life, interpreted as resulting from living conditions, and recognized by means of objective indicators. Indicators have been developed and validated to measure the relationship between quality of life and health ***Aim:*** *To determine, during an Educational Social Practice, the association between presence of dental caries lesions and its impact on quality of life of adolescents living in rural and urban areas in Argentina.* ***Materials and Method:*** *This was an observational cross-sectional study in (a) a rural area (Tres Isletas; Chaco Province/Schools No. 601 and 477) and (b) an urban area (Villa Soldati; Buenos Aires City/Nuestra Sra. Fátima School). The sample included 40 students from the urban area (UG) and 30 from the rural area (RG). A calibrated researcher (Kappa 0.80) collected the survey data and performed the clinical examinations. The following were recorded: (a) sociodemographic distribution in terms of age and gender; (b) presence of dental biofilm according to Silness and Löe; caries lesions according to ICDAS II /INTC criteria, and (d) DMFT, total and per component. Quality of life was assessed using the CPQ 11-14r. Statistical processing included calculation of frequency distribution of the variables (X± DS), and chi-square and Mann-Whitney tests to calculate association and comparisons between groups.* ***Results:*** *The sample included 47 girls (67.1%) and 23 boys (32.9%). Distribution according to sex and age did not differ significantly between sites (UG:12.9 years ± 0.5 and RG:11.8 years ±1.1). No significant difference was found between sites for a) plaque biofilm (p=0.759); b) DMFT, total value and per component, or c) individual and grouped ICDAS scores. Percentage analysis of the CPQr 1-14 scores showed significant differences between groups for questionnaire total scores (26.9% ± 2.2 in UG and 4.1% ± 0.8 in RG) and for the different domains.* ***Conclusion:*** *Perceived impact on quality of life caused by dental caries lesions was significantly higher in urban than the rural group, although neither the dental caries process nor the amount of biofilm differed significantly between groups.*

Key words: *quality of life - dental caries - population groups*

To cite:
Fernández C, Pagano MC, Salgado PA, Argentieri AB, Squassi AF, Bordoni NE. Dental caries lesions and impact on quality of life in adolescents living in urban and rural areas. A case study. Acta Odontol Latinoam. 2024 Aug 30;37(2):144-150. https://doi.org/10.54589/aol.37/2/144

Corresponding Author:
Angela Argentieri
abargentieri@gmail.com

Received: April 2024.
Accepted: July 2024.

Lesiones de caries dental e impacto sobre calidad de vida en adolescentes residentes en áreas urbanas y rurales. Estudio de caso

RESUMEN

El concepto de calidad de vida (CV) se desarrolló a partir de estudios tempranos sobre bienestar subjetivo y satisfacción con la vida[3], interpretado como resultante de las condiciones de vida empleando para su reconocimiento. Para medir su relación con la salud se han desarrollado y validado indicadores. ***Objetivo:*** *Determinar la asociación entre presencia de lesiones de caries dental y el impacto que producen sobre la calidad de vida de adolescentes residentes en áreas rural y urbana República Argentina), en un caso de Practica Social Educativa.* ***Materiales y Método:*** *Estudio observacional de corte transversal en (a) área rural (Tres Isletas; Provincia Chaco/escuelas N° 601 y N° 477) y (b) área urbana (Villa Soldati; CABA/ escuela Nuestra Sra. Fátima). La muestra incluyó 40 escolares residentes en área urbana (GU) y 30 residentes en área rural (GR). Un investigador calibrado (Kappa 0.80) realizó el relevamiento de datos y el examen clínico Se registraron (a) distribución sociodemográfica en términos de edad y género; (b) presencia de biofilm dental segùn Silness y Löe; lesiones de caries según criterios ICDAS II /INTC (d) cálculo de CPOD total y por componente. La calidad de vida fue evaluada mediante cuestionario CPQ 11-14r. El procesamiento estadístico incluyó cálculo de distribución de frecuencias de las variables (X± DS) y las pruebas chi cuadrado y Mann-Whitney.para calcular asociacion y comparaciones. entre grupos.* ***Resultados:*** *La muestra incluyó 47 mujeres (67,1%) y 23 hombres (32,9%). La distribución según sexo y edad no mostró diferencias significativas entre las sedes (GU:12,9 años ± 0,5 y GR:11,8 años ±1,1). No se encontraron diferencias significativas entre las sedes al comparar a) biofilm de placa (p=0,759); b) CPOD, valor total y por componentes y c) códigos de ICDAS individuales y agrupados. El análisis porcentual del índice CPQr 1-14 reveló diferencias significativas en el puntaje total (26,9% ± 2.2 en GU y 4,1% ± 0.8 en GR) y en los respectivos dominios del instrumento.* ***Conclusión:*** *La percepción del impacto sobre la calidad de vida generado por las lesiones de caries dental resultó significativamente mayor en los escolares urbanos que en los rurales, aunque ni el proceso de caries dental ni la cantidad de biofilm encontrados en cada grupo, revelaron diferencias significativas.*

Palabras claves: *calidad de vida - caries dental - grupos de población*

INTRODUCTION

The Department of Preventive and Community Dentistry at Buenos Aires University's School of Dentistry (FOUBA) coordinates education, clinical care and research within the different subjects taught. In doing so, it undertakes to generate knowledge, implement that knowledge in real scenarios, and develop competencies that will ensure the quality of care provided and the safety of the population covered. Developing protocols to implement knowledge in real scenarios involves specific steps, which include the identification of problems and potential evidence-based solutions, and the analysis of the local and institutional situations. It is also necessary to know about the association between oral health problems and the impact on quality of life perceived by the social actors involved.

It has been demonstrated that people or communities have a perception of the impact of diseases on quality of life, lifestyle, and even on availability of care[1,2]. Within this framework, models have been developed on care of dental caries lesions interpreted as therapeutic expression of NCCD (non-communicable chronic diseases), even based on knowledge of genetics and epigenetics. These models have oriented diagnosis and therapy approaches.

The concept of quality of life (QoL) developed from early studies on subjective wellbeing and satisfaction with life[3], interpreted as resulting from living conditions, and recognized by means of objective indicators[4]. However, this concept was criticized by Shen and Lai[5], who incorporated cultural, economic and political criteria. Bishop et al.[6], Michalos[7], and Caqueo et al.[8,9] related quality of life to subjective wellbeing and satisfaction with life[10-12], and Cummins[13] recognizes social psychology studies as a theoretical framework[14-21].

The WHO currently interprets quality of life as an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns. It is a multi-dimensional concept influenced by the person's physical health, psychological status, level of independence, social relations, and relationship with the environment, and constitutes a dynamic process ruled by the values and criteria of wellbeing, satisfaction and multidimensionality, and includes subjective/objective contents[22-25].

Indicators have been developed and validated to measure the relationship between quality of life and health. The Child Perception Questionnaire CPQ11-14[26] assesses self-perceived oral health status in early adolescents. It is organized into four domains: oral symptoms (OS), functional limitation (FL), emotional wellbeing (EW) and social wellbeing (SW). The original version consists of 47 multiple choice questions, and based upon it, abbreviated versions containing 16 and 8 items have been developed and duly validated (**ISF-16** and **ISF-8**, respectively)[27-30]. Evidence shows the negative association between the extent of the pathology and quality of life estimated by the OHRQol[31] questionnaire, and that treatment of dental caries lesions improves perceived quality of life in children and adolescents[32]. The aim of this study was to determine the association between dental caries lesions and perceived impact on quality of life in a case of educational social practice conducted in rural and urban areas in Argentina on students in the 6th grade of basic schooling.

MATERIALS AND METHOD

Analysis of the situation in the territorial context: Tres Isletas (Chaco Province, Argentina) and Villa Soldati (Comuna 8, Buenos Aires City, Argentina)

Tres Isletas, population is 16,976 (INDEC, 2010), is a town in the center-north of Chaco Province (Argentina) and is the municipal seat of Maipú Department. It is accessed via the paved Carretera 95 (national) and Carretera 9 (provincial), while the other access roads are unpaved (Ruta Provincial 46 and Ruta Provincial 27). The town is located in the warm tropical zone with dry season, with mean annual temperature 21 °C. Healthcare is available at "Nueva Alianza" Healthcare Center and Hospital "Jorge O. Vázquez".

The district Comuna 8 in Buenos Aires City comprises the neighborhoods Villa Soldati, Villa Riachuelo and Villa Lugano, among which Villa Soldati has the second highest population density, with surface area 8.6 Km^2 and population 46,779 (2010). The district's urban fabric is characterized by fragmentation. It has reasonable road infrastructure, with public transportation, highways and large avenues.

Villa Soldati has two level 1 healthcare centers (CESAC No. 6 and No. 24) dependent on the

programmatic area of Hospital Piñeiro. CESAC 6 provides healthcare activities including clinical medicine, nursing, pharmacy, speech and hearing therapy, general medicine, nutrition, obstetrics, dentistry, pediatrics, psychology, educational psychology, OBGYN, and includes social work. CESAC No. 24 provides, among other specialties, anthropology, nursing, speech and hearing therapy, gynecology, physiotherapy, education, general and/or family medicine, nutrition, obstetrics, dentistry and social work.

Institutional intervention scenarios

The educational social practices in the rural area were conducted at two government-run rural schools in Tres Isletas**,** Maipú Department**,** Chaco Province: (a) School No. 601 for Personalized Special Education (Paraje Lalelay) and (b) School No. 477 (M. M. Güemes) for Basic General Education.

The practices in the urban area were conducted at the school Nuestra Sra. de Fátima (Villa Soldati; Comuna 8, Buenos Aires City), a privately managed, coeducational, extended-shift religious school, which depends on Buenos Aires City's Ministry of Education

The study sites had not participated previously in educational extension programs provided by the Department of Preventive and Community Dentistry run by Buenos Aires University's School of Dentistry (FOUBA).

Sample

The sample consisted of 40 6th graders from Nuestra Sra. de Fátima school (Urban Group: UG) and 30 6th graders from the schools in Tres Isletas (Rural Group: RG).

Diagnosis and statistical analysis

On-site research was conducted by a single researcher calibrated for gathering data (Kappa: 0.80)

Analysis of sociodemographic distribution

Sociodemographic distribution in terms of age and gender was analyzed in both groups. The frequency distribution of the variables in the CPQr 11-14 questionnaire was established, including identification of students' language comprehension level according to reports from local teams of teachers (Argentine Ministry of Education and Sport, 2016).

Analysis of dental plaque biofilm and dental caries lesions

Each child was diagnosed for plaque biofilm according to the de Silness/Loe Index[33], and for dental caries lesions according to the DMFT index. Lesions per tooth were recorded according to the ICDAS[2] and INTC[34] criteria.

Results from the two sites were compared using Mann-Whitney's U test.

Analysis of perceived impact of caries lesions on Quality of Life

Data on quality of life were gathered in compliance with ethical and legal requirements. The questionnaire CPQ 11-14 (abbreviated version 8) validated in Spanish and adjusted for semantic equivalence was applied. No suggestions were provided on how it should be completed.

Perceived impact of dental caries on quality of life was recorded and analyzed for both groups, per domain and per item, applying the independence test (chi-square).

RESULTS

Analysis of the sample

The total sample included 47 girls (67.1%) and 23 boys (32.9%) (Table 1).

Table 1. Distribution of total sample according to zone of residence and gender.

		n	%
GENDER	Male	23	32.9%
	Female	47	67.1%
	TOTAL	70	100.0%
ZONE OF RESIDENCE	BUENOS AIRES CITY	40	57.1%
	TRES ISLETAS	30	42.9%
	TOTAL	70	100.0%

Mean age was 12.9 years ± 0.5 in the urban area and 11.8 years ±1.1 in the rural area. In the urban area, mean age was 13 years ± 0.3 for girls and 12.9 years ± 0.7 for boys. In the rural area, mean age was 11.9 years ± 1.1 for girls and 11.8 years ± 0.9 de for boys (Table 2).

Distribution per sex did not differ significantly between sites.

Table 2. Age according to zone of residence and gender

SAMPLE				AGE		
ZONE OF RESIDENCE	n	GENDER	n	Mean	Median	Standard error
BUENOS AIRES CITY	40	Male	11	12.9	13.0	0.7
		Female	29	13.0	13.0	0.3
TRES ISLETAS	30	Male	12	11.8	11.5	0.9
		Female	18	11.9	11.5	1.1

Analysis of dental plaque biofilm and dental caries lesions

Plaque biofilm values (Silness/Loe Index) did not differ significantly (p=0.759) between sites.

Subjects' caries history (DMFT and components D, M or F) did not differ significantly between sites, as shown by the Z value (Table 3).

Table 3. Comparison of dental caries lesions (DMFT) between sites

STATISTICAL TECHNIQUE	INDICATOR			
	D/DMFT	M/DMFT	F/DMFT	DMFT
Mann-Whitney's U test	565.500	562.000	530.000	585.500
Wilcoxon's W	1030.500	1382.000	1350.000	1050.500
Z	-0.412	-0.866	-1.010	-0.173
Asymptotic Sig. (bilateral)	0.680	0.386	0.312	0.863

No significant difference was found between groups for dental caries lesion values, including ICDAS levels 1 and 2 (Tables 4 and 5).

There were significant differences between sites for perceived impact of dental caries lesions on quality of life, both considering the average CPQ 11-14 scores, which were 26.9% for UG and 4.1% for RG, and per domain.

Mean scores on the scale, recorded per domain and per item, differed significantly (Table 6).

DISCUSSION

Social determinants of health are recognized as factors, conditions or circumstances that influence the level of health in individuals and populations, condition general health, and affect and influence the oral component. They have been conceptualized[35] with translation to health systems[36] by authors who have analyzed the consequences of social determinants on the creation of inequities. Lask and Fosson[37] recognized situations that generated predisposing, precipitating, perpetuating and protective factors, and Frenk[38] posed the existence of basic, structural and immediate context determinants.

Eid et al.[39] demonstrated that: **a)** Egyptian adolescents with DMFT≤3 or DT = 0 had significantly

Table 4. Active dental caries lesions (ICDAS II) at both sites

DENTAL CARIES LESIONS	SITES											
	BUENOS AIRES CITY				TRES ISLETAS				TOTAL			
	Mean	Median	Standard deviation	Standard error	Mean	Median	Standard deviation	Standard error	Mean	Median	Standard deviation	Standard error
ICDAS 1	0.1	0.0	0.6	0.109	0.0	0.0	0.2	0.031	0.1	0.0	0.4	0.047
ICDAS 2	0.8	0.0	1.7	0.310	1.1	0.0	1.8	0.126	0.9	0.0	1.7	0.203
ICDAS 3	3.4	3.0	2.9	0.521	3.0	2.5	3.3	0.521	3.2	3.0	3.1	0.370
ICDAS 4	0.8	0.0	1.9	0.346	0.4	0.0	1.1	0.173	0.6	0.0	1.6	0.191
ICDAS 5	1.1	0.0	1.5	0.273	1.5	1.0	1.8	0.284	1.3	1.0	1.6	0.191
ICDAS 6	1.7	0.0	3.2	0.584	2.0	0.0	3.3	0.521	1.9	0.0	3.2	0.382

Table 5. Results of comparison between active dental caries lesions recorded at the two sites.

Statistical technique	Comparison between active dental caries lesions recorded at the two sites					
	ICDAS 1	ICDAS 2	ICDAS 3	ICDAS 4	ICDAS 5	ICDAS 6
Mann-Whitney's U test	589.000	556.000	528.000	546.500	502.000	562.500
Wilcoxon's W	1054.000	1376.000	993.000	1011.500	1322.000	1382.500
Z	-0.372	-0.626	-0.866	-0.936	-1.243	-0.514
Asymptotic Sig. (bilateral)	0.710	0.531	0.387	0.349	0.214	0.607

Table 6. Correlation between mean scores for total CPQr and domains at the sites.

CPQr 11-14	SITES	N of the sample	Mean	Standard Deviation	Standard Error	p
Oral Symptoms	Buenos Aires City	40	3.23	1.75	0.28	0.001
	Tres Isletas	30	1.30	1.37	0.25	
	Total	70	2.40	1.85	0.22	
Functional Limitation	Buenos Aires City	40	2.38	1.92	0.30	0.001
	Tres Isletas	30	0.13	0.43	0.08	
	Total	70	1.41	1.85	0.22	
Emotional wellebeing	Buenos Aires City	40	1.78	1.85	0.29	0.001
	Tres Isletas	30	0.07	0.25	0.05	
	Total	70	1.04	1.64	0.20	
Social wellbeing	Buenos Aires City	40	1.23	2.01	0.32	0.001
	Tres Isletas	30	0.00	0.00	0.00	
	Total	70	0.70	1.63	0.19	
Total	Buenos Aires City	40	8.60	4.54	0.72	0.001
	Tres Isletas	30	1.30	1.37	0.25	
	Total	70	5.47	5.07	0.61	

lower CPQ scores$_{11\text{-}14}$, with values of $p<0.01$ and $p<0.0001$, respectively; **b)** untreated caries lesions were associated with mothers' lower socioeconomic and educational level, and with less regular dental appointments, determining a significant negative impact on 11- to 14-year-olds' QoL[32].

Cadenas de Llano-Pérula et al.[40] evaluated prevalence of caries and malocclusion in urban and rural areas in Perú and compared them to perceived oral health. They found that caries experience and untreated caries lesions were associated with lower socioeconomic level, lower level of maternal education and less frequent toothbrushing, leading to a significant negative impact on students' QoL.

In a critical review on low income and poor oral health, Singh et al.[41] summarized evidence on associations between individual/family income and oral health and the inequalities related to those variables. Studies in the US, Japan and Brazil have shown associations between unequal income in given areas and poor oral health, even though that evidence is not conclusive due to the differences in context and in dental care.

The findings in the current study support the importance of including, in educational social practice projects or studies on populations, an analytical component including family sociodemographic variables, mapping analysis to identify causes and effects of the problem under study, and adequate prioritization of alternatives for solutions. The findings also support the importance of recognizing the perception of dental caries lesions as one of the factors involved in healthcare and provide a basis for the need to increase relevant learning among young adolescents in rural or marginal urban areas by means of intersectoral association between academia and healthcare services.

CONCLUSIONS

Perceived impact of oral health on quality of life was significantly higher among urban than rural students, although no significant difference was found between groups either in the dental caries process or in quantity of biofilm.

DECLARATION OF CONFLICTING INTERESTS

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

FUNDING

None.

REFERENCES

1. Featherstone JDB, Chaffee BW. The Evidence for Caries Management by Risk Assessment (CAMBRA®). Adv Dent Res. 2018 Feb;29(1):9-14. https://doi.org/10.1177/0022034517736500
2. Pitts NB, Ekstrand KR; ICDAS Foundation. International Caries Detection and Assessment System (ICDAS) and its International Caries Classification and Management System (ICCMS) - methods for staging of the caries process and enabling dentists to manage caries. Community Dent Oral Epidemiol. 2013 Feb;41(1):e41-52. https://doi.org/10.1111/cdoe.12025
3. Smith KW, Avis NE, Assmann SF. Distinguishing between quality of life and health status in quality of life research: a meta-analysis. Qual Life Res. 1999 Aug;8(5):447-59. https// doi.org/10.1023/a:1008928518577
4. Hollandsworth JG Jr. Evaluating the impact of medical treatment on the quality of life: a 5-year update. Soc Sci Med. 1988;26(4):425-34. https //doi.org/10.1016/0277-536(88)90311-5
5. Shen S, Lai Y. Optimally Scaled Quality-of-Life Indicators. Social Indicators Research 1998;44:225–254. https://doi.org/10.1023/A:1006824827723
6. Bishop SL, Walling DP, Dott SG, Folkes CC, Bucy J. Refining quality of life: validating a multidimensional factor measure in the severe mentally ill. Qual Life Res. 1999;8(1-2):151-60. https//doi.org/10.1023/a:1026489331009
7. Michalos AC. Social Indicators Research and Health-Related Quality of Life Research. Social Indicators Research 2004;65:27–72. https://doi.org/10.1023/A:1025592219390
8. Caqueo-Urízar A, Gutiérrez-Maldonado J, Miranda-Castillo C. Quality of life in caregivers of patients with schizophrenia: a literature review. Health Qual Life Outcomes. 2009 Sep 11; 7:84. https//doi.org.ar/10.1186/1477-7525-7-84
9. Caqueo Urízar A, Lemos Giráldez S. Calidad de vida y funcionamiento familiar de pacientes con esquizofrenia en una comunidad latinoamericana. Psicothema.2008;20(4):577-582. Spanish
10. Cummins RA. Objective and Subjective Quality of Life: an Interactive Model. Social Indicators Research. 2000;52:55-72. https://doi.org/10.1023/A:1007027822521
11. Huppert FA, Whittington JE. Evidence for the independence of positive and negative well-being: implications for quality of life assessment. Br J Health Psychol. 2003 Feb;8(Pt 1):107-22. https//doi.org/10.1348/135910703762879246
12. Haas BK. Clarification and integration of similar quality of life concepts. Image J Nurs Sch. 1999;31(3):215-20. https://doi.org/10.1111/j.1547-5069.1999.tb00483.x
13. Cummins RA. Moving from the quality of life concept to a theory. J Intellect Disabil Res. 2005 Oct;49(Pt 10):699-706. https//doi.org/ 10.1111/j.1365-2788.2005.00738.x
14. Puget J. Berenstein I. Psicoanálisis de la Pareja Matrimonial. Buenos Aires, Argentina. Ed. Paidós.1989.
15. Kaës R. El grupo y el sujeto del grupo. Buenos Aires, Argentina. Amorrortu Editores, 1993.
16. Kaës R. Comunicación a la Asociación Uruguaya del Psicoanálisis de las Configuraciones Vinculares 2000 https://www.aappg.org/wp-content/uploads/Revista-Grupo-2014.pdf
17. Lacan J. La instancia de la letra. Siglo XXI, México, 1984.
18. Winnicott D. Realidad y juego. Editorial Gedisa, Barcelona, 1979.
19. Aulagnier P. La violencia de la interpretación. Amorrortu Editores, Buenos Aires., 2004.
20. Laplanche J. Nuevos fundamentos para el psicoanálisis. La seducción originaria. Amorrortu Editores, Buenos Aires, 1987:132-133.
21. Campbell A. The sense of well-being in America. McGraw-Hill, New York. 2001
22. Wu C. The role of perceived discrepancy in satisfaction evaluation. Social Indicators Research.2008;88:423-436. https:doi.org/10.1007/s11205-007-9200-9
23. Solberg EC, Diener E, Wirtz D, Lucas RE, Oishi S. Wanting, having, and satisfaction: examining the role of desire discrepancies in satisfaction with income. J Pers Soc Psychol. 2002 Sep;83(3):725-34. https://doi.org/10.1037//0022-3514.83.3.725
24. Buunk A. Belmonte J. Peiro J. Zurriaga R. Gibbons F. Diferencias individuales en la comparación social: Propiedades de la escala española de orientación hacia la comparación social. Revista Latinoamericana de Psicología. 2005;37:561-579. https://doi.org/10.1016/s0120-0534(14)7004-4
25. Urzúa A. Health related quality of life: Conceptual elements. Revista Médica de Chile. 2010;138:341-348. https://doi.org/10.4067/s0034-98872010000300017
26. Jokovic A, Locker D, Guyatt G. Short forms of the Child Perceptions Questionnaire for 11-14-year-old children (CPQ11-14): development and initial evaluation. Health Qual Life Outcomes. 2006 Jan 19;4:4. https://doi.org/10.1186/1477-7525-4-4
27. Jokovic A, Locker D, Stephens M, Kenny D, Tompson B, Guyatt G. Validity and reliability of a questionnaire for measuring child oral-health-related quality of life. J Dent Res. 2002 Jul;81(7):459-63. https://doi.org/10.1177/154405910208100705
28. Núñez Franz,. Rey Clericus R, Bravo-Cavicchioli D,Jiménez del Río P, Fernández Gonzalez C, Mejía Delgado G, Adaptación y validación al español del cuestionario de percepción infantil CPQ-Esp11-14 en población comunitaria chilena. Rev. Esp. Salud Publica [Internet]. 2015 Dic. https://doi.org/10.4321/s1135-57272015000600006
29. Barbosa TS, Tureli MCM, Gaviao MDV. Validity and reliability of the Child Perceptions Questionnai-res applied in Brazilian children BMC Oral Health. 2009; 9:13. https://doi.org/10.1186/1472-6831-9-13
30. Tadakamadla SK, Mangal G, Quadri MFA, Nayeem M, Tadakamadla J. Psychometric Analyses of the Indian (Hindi) Version of the Child Perception Questionnaire (CPQ11–14). Children 2020, 7, 175. https://doi.org/10.3390/children7100175
31. Tadakamadla SK, Mangal G, Quadri MFA, Nayeem M, Tadakamadla J. Psychometric Analyses of the Indian (Hindi) Version of the Child Perception Questionnaire (CPQ11-14). Children (Basel). 2020 Oct 9;7(10):175. https://doi.org/10.3390/children7100175
32. Pinheiro SAA, Rodrigues HB, Santos JTL, Granja GL, Lussi A, Leal SC, Diniz MB. Association of dental caries morbidity stages with oral health-related quality of life

in children and adolescents. Int J Paediatr Dent. 2020 May;30(3):293-302. https://doi.org/10.1111/ipd.12605
33. Aimée NR, Damé-Teixeira N, Alves LS, et al. Responsiveness of oral health-related quality of life questionnaires to dental caries interventions: Systematic review and meta-analysis. Caries Res. 2019; 53(6): 585-598. https://doi.org/10.1159/000500855
34. Löe H. The Gingival Index, the Plaque Index, and the retención Index. J Periodontol 1967;38: 610-616. https://doi.org/10.1902/jop.1967.38.6_part2.610
35. Bordoni N, Squassi A, Diagnostico e interpretacion de caries dental en Odontologia Preventiva. PRECONC. Buenos Aires Argentina. PALTEX/OPS/OMS, 1999.
36. Lalonde M. A New Perspective on the Health of Canadians. Ottawa, Ontario, Canada: Information Canada. 1974. http://www.phac-aspc.gc.ca/ph-sp/pdf/perspect-eng.pdf
37. Dahlgren G, Whitehead M. Policies and Strategies to Promote Social Equity in Health. Stockholm, Sweden: Institute for Futures Studies 1991. https://www.researchgate.net/publication/26594615
38. Lask B, Fosson AR. Childhood Illness: The Psychosomatic Approach-Children Talking with their Bodies. Wiley, London,1989. https://doi.org/10.1192/s0007125000141170
39. Frenk J, Chen L, Bhutta Z, Cohen J, Crisp N, Evans T et al. Health professionals for a new century: transforming education to strengthen health systems in an interdependent world. Health professionals for a new century: transforming education to strengthen health systems in an interdependent world. Lancet. 2010 Dec 4;376(9756):1923-58. https://doi.org/10.1016/s0140-6736(10)61854-5
40. Eid SA, Khattab NMA, Elheeny AAH. Untreated dental caries prevalence and impact on the quality of life among 11 to14-year-old Egyptian schoolchildren: a cross-sectional study. BMC Oral Health. 2020 Mar 19;20(1):83. https://doi.org/10.1186/s12903-020-01077-8
41. Cadenas de Llano-Pérula M, Ricse E, Fieuws S, Willems G, Orellana-Valvekens MF. Malocclusion, Dental Caries and Oral Health-Related Quality of Life: A Comparison between Adolescent School Children in Urban and Rural Regions in Peru. Int J Environ Res Public Health. 2020 Mar 19;17(6):2038. https://doi.org/10.3390/ijerph17062038
42. Singh A, Peres MA, Watt RG. The Relationship between Income and Oral Health: A Critical Review. J Dent Res. 2019 Jul;98(8):853-860. https// doi.org/10.1177/0022034519849557

https://doi.org/10.54589/aol.37/2/151

Bone regeneration by a bone substitute biomaterial containing hydroxyapatite, chitosan, xanthan and graphene oxide supplemented with conditioned medium from mesenchymal stem cells.

Daniel N Rocha[1,3], David GM López[2], José RM Ferreira[1], Marcelo HP Silva[3], Isabela AAAP Filgueiras[2], Bruno F Alves[2], Bruno M Almeida[2], Tatiana Kotaka[2], Leonardo F Buss[2], José L Magnabosco[2], Marcelo L Teixeira[2], Éric D Mariano[1], Mariáh H Cationi[1], Isadora Bosco[1], Marvin Nascimento[3], Raul Canal[4], José G Neves[5], Antonio C Aloise[2], Elizabeth F Martinez[6], Lexie S Holliday[7], André A Pelegrine[2]

1. Department of Bioengineering, R-Crio Criogenia S.A., Campinas, Brazil
2.Division of Implant Dentistry, Faculdade São Leopoldo Mandic, Campinas, Brazil.
3. Department of Materials Engineering-SE/8, Military Institute of Engineering, Rio de Janeiro, Brazil.
4. ANADEM, Brasília, Brazil.
5. Department of Restorative Dentistry, Dental Materials Area, State University of Campinas, Piracicaba, Brazil.
6. Division of Oral Pathology and Cell Biology, Faculdade São Leopoldo Mandic, Campinas, Brazil.
7. Department of Orthodontics, University of Florida, Gainesville, USA.

ABSTRACT

This study analyzed a recently developed bone substitute biomaterial made of chitosan-xanthan-hydroxyapatite-graphene oxide (CXHAG). The CXHAG particles underwent in vitro structural and morphological characterization, and in vivo testing with or without osteogenic conditioned medium from mesenchymal stem cells. ***Aim:*** *The aim of this study was to determine whether the CXHAG novel biomaterial, supplemented with conditioned medium from mesenchymal stem cells, could be useful for bone regeneration.* ***Materials and Method:*** *For the in vitro study, cells were incubated with 20mg of CXHAG granules for 24 hours and a MTT assay was performed to tests for cytotoxicity. For the in vivo study, critical size calvarial bone defects were created in twenty-five rats. One animal had the defect unfilled (Control Group–CG) and was euthanized after 42 days. Twelve rats received the CXHAG particles (Group 1–G1) and the other twelve received the CXHAG particles supplemented with the conditioned medium (Group 2–G2). All G1/G2 grafts were covered with a CXHAG membrane. G1/G2 animals were euthanized after 14 days (T1) or 42 days (T2). The specimens were processed and histologically evaluated.* ***Results:*** *SEM analysis of the CXHAG particles showed granules of 300-400µm, with a rough irregular surface. They were not cytotoxic to dental pulp stem cells in vitro. The CG specimen showed loose immature connective tissue and no bone formation at the center of the defect. G1 and G2 presented remnant biomaterial particles at both time points, but only G2 had bone formation at the center of the defect.* ***Conclusions:*** *The conditioned medium had a positive effect on bone regeneration in rat calvarial critical size defects when associated with the novel bone substitute biomaterial.*

Keywords: *bone regeneration - stem cells - chitosan - xanthan - graphene - conditioned medium.*

Regeneração óssea por meio de biomaterial substituto ósseo contendo hidroxiapatita, quitosana, xantana e óxido de grafeno suplementado com meio condicionado de células-tronco mesenquimais

RESUMO

Este estudo analisou um biomaterial substituto ósseo recentemente desenvolvido feito de óxido de quitosana-xantana-hidroxiapatita-grafeno (CXHAG). As partículas CXHAG observaram caracterização estrutural e morfológica in vitro. Foi testado in vivo, com ou sem meio condicionado osteogênico de células-tronco mesenquimais. ***Objetivo:*** *O objetivo deste estudo foi determinar se o novo biomaterial CXHAG, suplementado com meio condicionado de células-tronco mesenquimais, poderia ser útil para a regeneração óssea.* ***Materiais e Método:*** *Para o estudo in vitro, as células foram incubadas com 20mg de grânulos de CXHAG por 24 horas e foi realizado ensaio de MTT para verificar a citotoxicidade. Para o estudo in vivo, foram criados defeitos ósseos de tamanho crítico na calvária em vinte e cinco ratos. Um animal teve o defeito não preenchido (Grupo Controle – GC) e foi eutanasiado após 42 dias. Doze ratos receberam as partículas CXHAG (Grupo 1 – G1) e os outros doze receberam as partículas CXHAG suplementadas com o meio condicionado (Grupo 2 – G2). Todos os enxertos G1/G2 foram cobertos com membrana CXHAG. Os animais do G1/G2 foram eutanasiados após 14 dias (T1) ou 42 dias (T2). Os espécimes foram processados e avaliados histologicamente.* ***Resultados:*** *A análise SEM das partículas CXHAG mostrou grânulos de 300-400µm, com superfície áspera e irregular. Eles não foram citotóxicos para células-tronco da polpa dentária in vitro. As amostras CG mostraram tecido conjuntivo imaturo frouxo e nenhuma formação óssea no centro do defeito. G1 e G2 apresentaram partículas remanescentes de biomateriais em ambos os momentos, mas apenas G2 apresentou formação óssea no centro do defeito.* ***Conclusões:*** *O meio condicionado teve repercussões positivas na regeneração óssea em defeitos críticos de calvária de ratos quando associado ao novo biomaterial substituto ósseo.*

Palavras-chave: *regeneração óssea - células tronco - quitosana - xantana - grafeno - meio condicionado.*

To cite:
Rocha DN, López DGM, Ferreira JRM, Silva MHP, Filgueiras IAAAP, Alves BF, Almeida BM, Kotaka T, Buss LF, Magnabosco JL, Teixeira ML, Mariano ED, Cationi MH, Bosco I, Nascimento M, Canal R, Neves JG, Aloise AC, Martinez EF, Holliday LS, Pelegrine AA. Bone regeneration by a bone substitute biomaterial containing hydroxyapatite, chitosan, xanthan and graphene oxide supplemented with conditioned medium from mesenchymal stem cells. Acta Odontol Latinoam. 2024 Aug 30;37(2):151-161. https://doi.org/10.54589/aol.37/2/151

Corresponding Author:
André Antonio Pelegrine
pelegrineandre@gmail.com

Received: March 2024.
Accepted: June 2024.

INTRODUCTION

The management of bone defects remains a major clinical challenge for both orthopedics and maxillofacial surgery. Guided bone regeneration (GBR) is commonly used to treat bone defects. The GBR technique uses a membrane as a barrier to exclude the proliferation of epithelial cells and connective tissue[1]. Because membranes can mimic the extracellular matrix, they serve as a support for cell growth, enabling proliferation and differentiation of specific tissues[2]. The principle of using membranes to select cell groups to populate the wound has been called osteopromotion and is usually used in conjunction with bone grafts[3].

Bone grafts used in GBR procedures can be natural or synthetic. They are classified according to source as autogenous (from the same individual), allogeneic (from another individual of the same species), xenogeneic (from an individual of another species) or alloplastic (synthetic materials)[4]. The association of synthetic ceramic derivatives with materials from natural sources seems to be a feasible alternative, since each type of material can contribute its advantages. Associations of ceramic derivatives and biopolymers such as chitosan/xanthan are gaining attention because in addition to being biocompatible and biodegradable, they interact with bone growth factors and receptor proteins[5]. Hydroxyapatite is a bioceramic that is often used for bone reconstruction purposes due to its striking resemblance to bone in terms of structure and characteristics[6]. Moreover, hydroxyapatite can overcome important issues concerning polymers, such as unfavorable mechanical characteristics regarding insufficient tensile and compressive strength. However, although its low resorption rate is related to the advantage of volume maintenance in bone reconstruction surgery, it also requires longer time for reconstruction[7]. Hydroxyapatite is therefore frequently employed in conjunction with various polymers and crosslinkers[6]. In addition, there are reports that biomaterials coated with graphene oxide can increase regenerative potential[8]. These materials can be combined with different polymers, ceramics and metals and, due to their ability to promote osteogenic differentiation, have been increasingly used to improve the physical, chemical and mechanical properties of biomaterials[9].

Critical-sized bone defects are defined as those that will not heal spontaneously within a patient's lifetime. The standard procedures to treat such defects are vascularized bone autografts, distraction osteogenesis, or tissue engineering. Autografts are considered the biological gold standard in the restoration of lost bone structure, due to their osteoinductive, osteoconductive and osteogenic properties. However, the tissue trauma caused when autogenous tissue is harvested has led to increased use of bone substitutes[10]. Nevertheless, bone substitute biomaterials are inadequate for treating critical bone defects because they are only osteoconductive[11]. Numerous recent articles have tested different methodologies[12-16] for adding osteoinductive and ultimately, osteogenic potential to bone substitutes.

Some studies suggest that mesenchymal stem cells or the conditioned culture medium in which they were grown (which contains proteins and growth factors) can improve bone regeneration associated with bone substitutes[17]. Although the use of mesenchymal stem cells has shown promise[18], it involves a major drawback: the need to introduce living cells into patients. For safety reasons, clinical use could be limited to autogenous applications. Even so, there is a concern that the stem cells might differentiate in unexpected ways or transform into a cancerous state.

Certain cell products can also promote tissue healing (e.g., bone cell proteins and growth factors) and may be useful for the treatment of critical size bone defects. Conditioned medium obtained from stem cell culture has been extensively studied in recent years[16,19-21]. The aim of the current study was to evaluate the use of a recently developed biomaterial composed of chitosan, xanthan, hydroxyapatite, and graphene oxide (CXHAG) in critical size bone defects. To potentially introduce osteoinductive properties to this novel biomaterial, a medium conditioned by stem cells was tested.

MATERIALS AND METHOD

Bone Substitute Biomaterial (chitosan-xanthan-hydroxyapatite-graphene oxide - CXHAG)

Graphene oxide (GO) was produced by liquid-phase exfoliation through the chemical route, using the Rourke et al. method[22]. The production of GO and its subsequent addition to the composite was based on the study by Lopes et al.[23]. First, 5 g of graphite flakes (GRAFLAKE 99550, National

Graphite) were suspended with 4.5 g of sodium nitrate ($NaNO_3$, VETEC) and 169 ml of sulfuric acid (H_2SO_4, HERZOG) under magnetic stirring for 2 h. Then, the mixture was cooled in ice, and 22.5 g of potassium permanganate ($KMnO_4$, VETEC) were slowly added and stirred for 2 h. The mixture was then left to stir for 7 days. The resulting mixture was slowly dispersed into 605 ml of 5 wt% H_2SO_4 for 1 h and stirred for a further 3 h. Hydrogen peroxide (16.5 g, 30 vol) was added to considerable effervescence and stirred for 2 h. The mixture was then further diluted with 500 ml of 3 wt% H_2SO_4/0.5 wt% H_2O_2 and left to stir overnight. After this period, the mixture was centrifuged (Hettich, model 420 R, at 9300 rpm, for 30–60 min, in 4 × 250 ml tubes), discarding the supernatant. This washing/centrifugation procedure was repeated 12 times using 500 ml of 3 wt% H_2SO_4/0.5 wt% H_2O_2 and 5 times using 500 ml of deionized water. Finally, vacuum filtration (EDWARDS, Germany) was applied to eliminate any non-oxidized graphite.

Chitosan-xanthan-hydroxyapatite-graphene oxide (CXHAG) was prepared in aqueous solutions rich in calcium and phosphorus precursors, with the addition of chitosan, xanthan, and graphene oxide[24]. The precursor solution was prepared by mixing, under stirring (magnetic stirrer – Quimis, São Paulo, Brazil), a solution of 2 mol/L lactic acid (Merck, Darmstadt, Germany), 1% (w/v) chitosan (Sigma-Aldrich, Saint Louis, USA), 0.5 mol/L calcium hydroxide (Merck, Darmstadt, Germany), 1% (w/w) graphene oxide in relation to the final hydroxyapatite, and a solution of 0.3 mol/L orthophosphoric acid (Merck, Darmstadt, Germany). After 24 hours of stirring, a solution of 1.2 mol/L potassium hydroxide (Sigma-Aldrich, Saint Louis, USA) and 1% (w/v) xanthan gum (Sigma-Aldrich, Saint Louis, USA) was added to adjust the pH to 12 at a temperature of 60 **°C,** enabling precipitation of the chitosan-xanthan-hydroxyapatite-graphene oxide composite.

To produce CXHAG granules, the powder produced was mixed with a 5% (w/v) pluronic aqueous solution and dried at 70 °C for 12 hours. The resulting composite was then ground using an agate mortar and pestle and transferred to a granulometric sieve with a mesh size between 300 and 400 µm **(**Bronzinox, 100 mesh and stainless-steel frame 5" x 2", São Paulo, Brazil) and sieved.

Membranes were produced following the method previously validated by Souza et al. (2022), which used the same materials (i.e., Chitosan/Xanthan membrane containing hydroxyapatite/Graphene oxide)[25].

Biomaterial Characterization

The crystal structure was investigated by X-ray diffraction, using a diffractometer (Malvern Panalytical, model X'Pert-MPD, Worcestershire, UK) on a Cu-Kα (λ=1.540 Å) tube, operating at 40 mA and 40 kV, and a scanning step of 0.02 seconds at 1 s/step. The HighScore Plus software was employed for qualitative analysis of the X-ray diffraction data.

The functional group was identified by Fourier Transform Infrared Spectroscopy (FTIR Prestige-21, Shimadzu, Columbia, USA) in the wavenumber range of 400-4000 cm^{-1}. Spectra were collected as the result of 32-64 scans with a resolution of 4 cm^{-1}. The analyses were performed at room temperature. For the selected spectra, the ratios of integrated intensities, and the integrated areas of the bands corresponding to O-H groups in the range 3470-3450 cm^{-1}, CO_3^{-2} groups in the range of 1380–1580 cm^{-1} and those due to PO_4^{-3} at 900–1300 cm^{-1} were calculated.

CXHAG particle morphology was investigated by scanning electron microscopy (LEO Electron Microscopy, model Leo 440i, Cambridge, UK), whereas energy dispersive spectroscopy (EDS) was used to qualitatively assess the chemical composition.

Lyophilized Conditioned Medium

Human dental pulp stem cells (catalog number PT-5025) obtained from Lonza (Lonza, Cohasset, USA) were used to prepare the lyophilized culture medium as previously described by Buss et al. (2023)[16]. Briefly, the cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Sigma-Aldrich, Saint Louis, USA) containing 10% fetal bovine serum (FBS) (Sigma-Aldrich, Saint Louis, USA) and 1% antibiotic-antimycotic solution (Thermo Fischer/Life Technologies, Carlsbad, USA) and, after 24 hours, the medium was supplemented with 50 µM ascorbic acid, 10 mM ß-Glycerophosphate and 0.1 µM dexamethasone (Thermo Fischer/Life Technologies, Carlsbad, USA) to induce osteogenic differentiation. After 4 days, the conditioned medium was collected, frozen, and transferred to a lyophilizer (lyophilized at -55 °C under a vacuum of 0.040 m Bar for 48 h).

After lyophilization and sterilization by gamma radiation (Sterigenics, Americana, Brazil), the freeze-dried conditioned medium, in powder form, was dissolved in deionized water (20 mg/mL) and filtered through a 0.2 μm filter. Then, it was drip-associated with 100 mg of the biomaterial in 12-well cell culture plates (Corning, New York, USA) immediately before the surgery.

Cytotoxicity Assay of CXHAG Granules

For this study, dental pulp mesenchymal stem cells (Lonza, catalog number PT-5025, Cohasset, USA) were cultivated in 25 cm^2 cell culture flask using DMEM supplemented with 10% fetal bovine serum (FBS), 1% of antibiotics (100 units/ml penicillin, 100 μg/ml streptomycin) (Sigma-Aldrich, Saint Louis, USA), 2 mM L-glutamine (Sigma-Aldrich, Saint Louis, USA) and 100 μM ascorbic acid (Sigma-Aldrich, Saint Louis, USA). After incubation, at approximately 80% confluency, cells were detached using TrypLETM Express Enzyme (1x) (Gibco/ Life Technologies, Carlsbad, USA) at 37 °C for 3 min, and immediately seeded at a cell density of 1×10^4 cells into a 96-well culture plate, a final volume of 100μL/well. Experimental 20mg of CXHAG granules were also incubated in a 12-well plate. The cytotoxicity assay was performed in quadruplicate (n = 4), in accordance with ISO 10993-5 guidelines. After 24 h of incubation, 100 μL of the medium from the experimental pellets were transferred into a 96-well culture plate, 10 μL WST-1 solutions (Roche, Basel, Switzerland) were added to each well, and the cells were incubated at 37 °C in 5% CO_2 for 4 h. After the reaction period, the specimens were gently shaken for 1 min and the absorbance was measured at 450 nm by a microplate reader (Promega, Glomax E8032, Madison, USA).

The culture medium containing WST-1 without cells was used to set the background threshold, while culture medium containing WST-1 with cells was used as a control. As a cytotoxicity control, 50 μL dimethyl sulfoxide (DMSO, Sigma-Aldrich, Saint Louis, USA) was used with 50 μL culture medium.

For data analysis of cytotoxicity assay, the Jamovi© statistical software (2.3.28.0 version) was utilized. The interaction between independent parameters was assessed using ANOVA repeated measures, followed by Tukey's post hoc test. A p-value of < 0.05 was considered statistically significant.

Experimental Design

Twenty-five eight-week-old male Wistar rats, weighing 300-350g, were used in this study, after approval from the Research Ethics Committee for Animal Experimentation of the Faculdade São Leopoldo Mandic (protocol no. 2020/010, approval date March 26th, 2020). The study was carried out in compliance with the ARRIVE guidelines.

One animal was enrolled in the control group (CG), in which nothing was grafted inside the bone defect, and euthanized after 42 days. The other twenty-four animals were randomly divided into two groups: Group 1 (G1) and Group 2 (G2). In G1 (n=12), bone defects were filled with the recently developed biomaterial and covered by the membrane. In G2 (n=12), bone defects were filled with the recently developed biomaterial combined with the conditioned medium and covered by the membrane. The animals in G1 and G2 were euthanized after 14 days (T1) or 42 days (T2).

Surgical Technique

After anesthesia, trichotomy in the region of the calvaria and subsequent antisepsis of the area, a 15 mm long linear incision was made with a scalpel in the integument covering the skull, followed by total flap detachment. The critical bony defect was made using an 8.0 mm diameter trephine drill (Maximus, Contagem, Brazil), crossing the entire bone thickness of the diploe. The bone fragment was removed, exposing the meninges at the bottom of the defect. Sequentially, the defect was filled with biomaterial according to the determined groups, covered with membranes measuring 10 mm x 10 mm (Fig. 1), and finally the flap was repositioned and sutured. The volume of the bone substitute biomaterial used in this study was determined by the aim to completely fill the bone defect.

After surgery, the animals received intraperitoneal postoperative medication for analgesia (Dipyrone 0.5g/mL, Algivet®-VETNIL, Louveira, Brazil).

Histologic Processing and Analysis

After 14 days or 42 days, according to the euthanasia time, the specimens were harvested and processed for histological evaluation. The calvarias were demineralized in 20% formic acid, dehydrated, and embedded in histological paraffin, to cut sections 4 μm thick in the central region of the defects. The sections were stained with hematoxylin-eosin

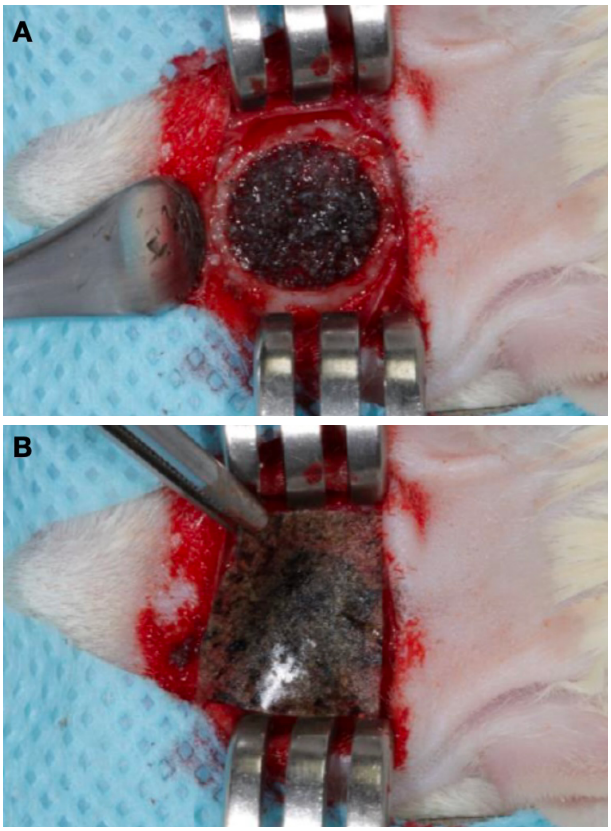

Fig. 1: Critical-size defect used in the study. A) Critical-size defect filled with the novel biomaterial, B) Defect and biomaterial covered with the membrane.

and mounted as photomicrographs on resin slides. Images were captured with a computerized imaging system (AxioVisionrel 4.8, Carl Zeiss, Oberkochen, Germany) coupled to the Axioskop 2 Plus light microscope (Carl Zeiss, Oberkochen, Germany).
Descriptive histological analyses were performed in the center of the defect. For bone formation at the center of the defect, the area of newly formed bone was traced using ImageJ software (National Institutes of Health, Bethesda, USA) on photomicrographs taken at 200x magnification. Photomicrographs were taken under a light microscope using a computerized image analysis system consisting of an Axioskop 2 plus light microscope (Carl Zeiss, Gottingen, Germany) connected to a microcomputer using AxioVision rel. 4.8 image analysis software (Carl Zeiss, Gottingen, Germany). The results were scored in square micrometers and then expressed as a percentage of the total area.

RESULTS

Analysis of the novel bone substitute biomaterial

Fig. 2A presents the XRD analysis of the CXHAG

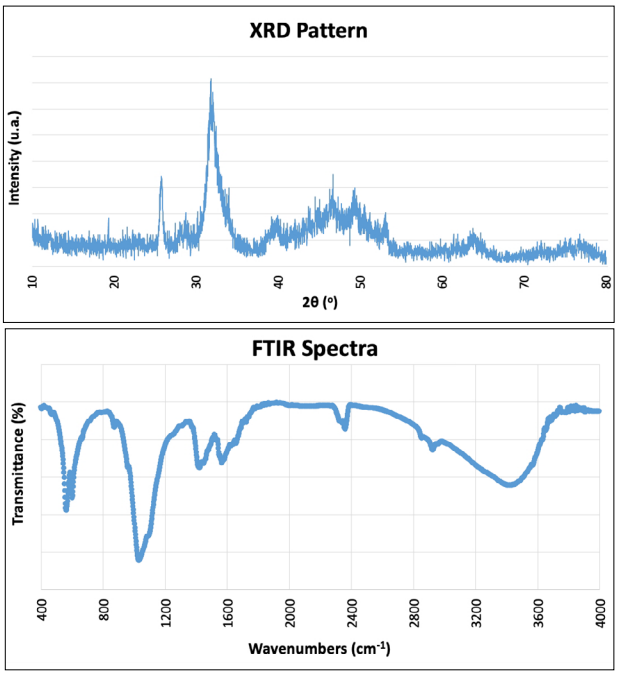

Fig. 2: A) XRD Patterns and B) FTIR Spectra.

granules, revealing patterns indicative of the hydroxyapatite phase, as aligned with JCPDS 09-0432 hydroxyapatite crystallographic record. Fig. 2B displays the spectra for the CXHAG granules, exhibiting bands at approximately 1,095, 1,045, 962, 607 and 577 cm^{-1}, which correspond to phosphate groups. The hydroxyl band at around 3,570 cm^{-1} is not distinctly defined in the granules. Moreover, minor peaks associated with the C-O vibrational bands of carbonate groups are evident within the 1,410-1,490 cm^{-1} range. Additionally, the CXHAG granules exhibit a distinct band for amino groups (C–CH_3), denoting the presence of chitosan, alongside a characteristic band for carboxylic groups (CO_3) from xanthan gum.
Fig. 3 shows that the granulation technology produced irregularly shaped granules with sharp edges. The granule surfaces at the micro level are rough. By measuring the granule dimensions from the SEM images, the value of the experimental granule size was determined to be between 300 and 400 μm.

In vitro assessment

Fig. 4 shows the *in vitro* cytotoxicity assay graph. Through the values for the viability percentage, it was observed that after an exposure of 24 hours to dental pulp mesenchymal stem cells (hDPSCs), no cytotoxicity was observed in the CXHAG group.

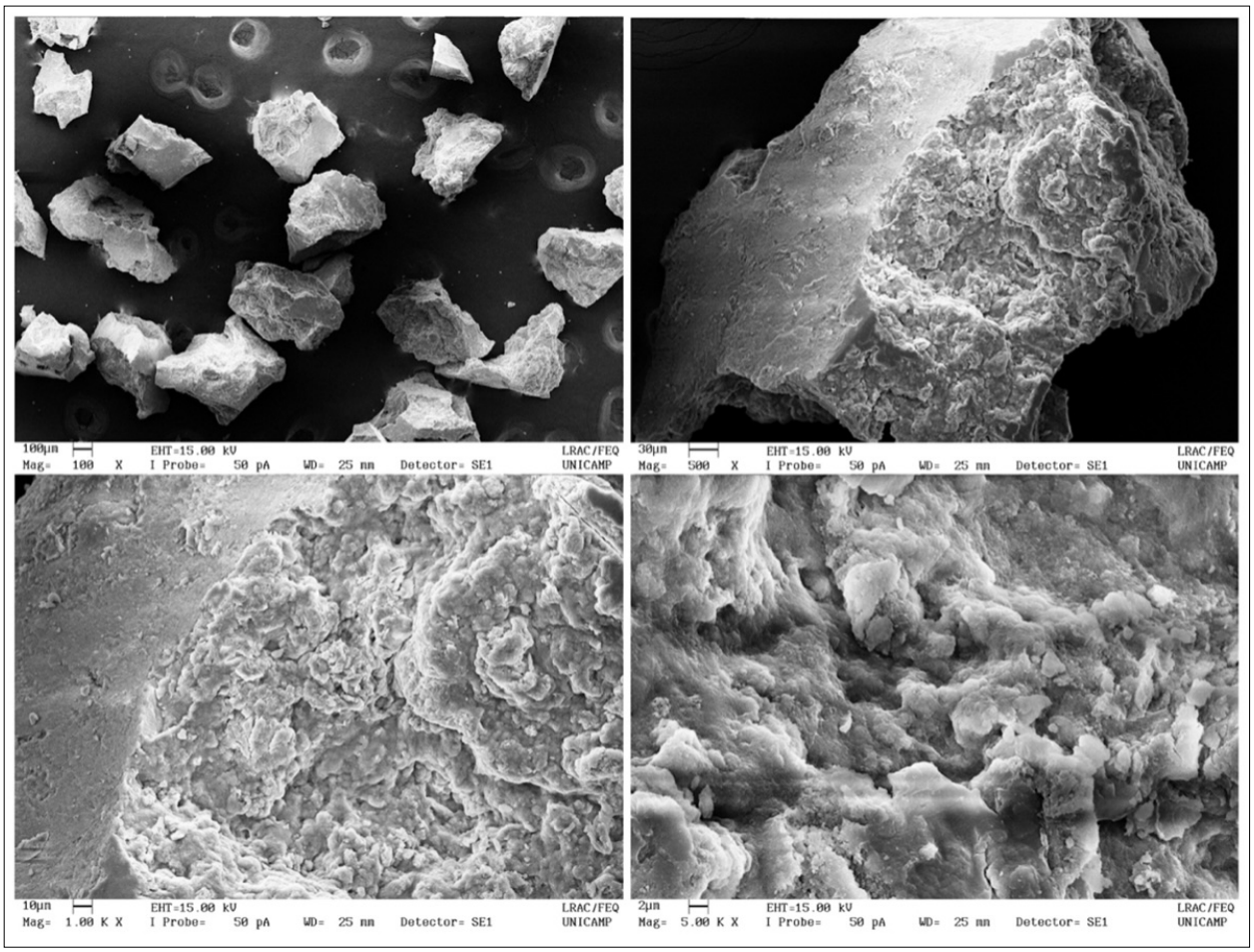

Fig. 3: SEM images of irregularly shaped CXHAG granules with surface microroughness.

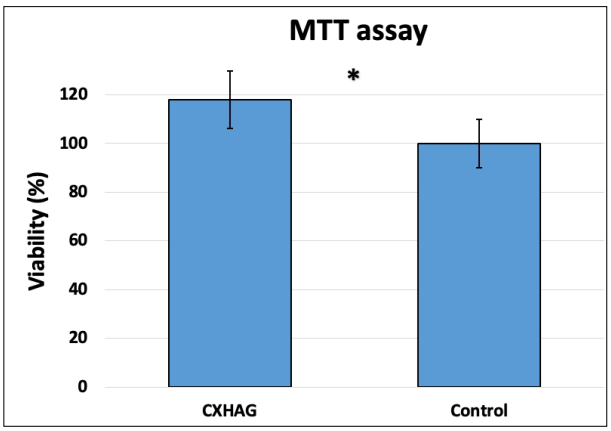

*Fig. 4: Indirect cytotoxicity WST-1 of Hydroxyapatite-Chitosan-Xanthan-Graphene Oxide Composite after 24-hour exposure to hDPSCs. *p<0.05. CXHAG = chitosan-xanthan-hydroxyapatite-graphene oxide.*

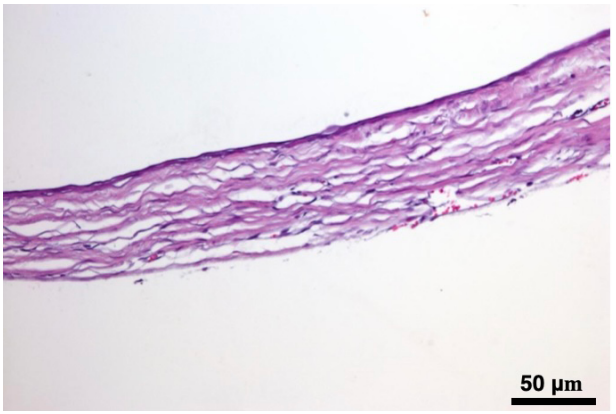

Fig. 5: Photomicrograph of histological section showing no bone formation at the center of the defect in the CG, after 42 days. (Scale bar = 50 µm).

In vivo assessment

Concerning the histologic findings, the CG showed the presence of loose immature connective tissue and no bone formation at the center of the defect (Fig. 5).

G1 presented a remnant of biomaterial particles at both timepoints, always surrounded by loose immature connective tissue. Multinucleated giant cells were observed around some biomaterial particles, especially at 14 days. There was a typical mononuclear inflammatory infiltrate at T1, but it

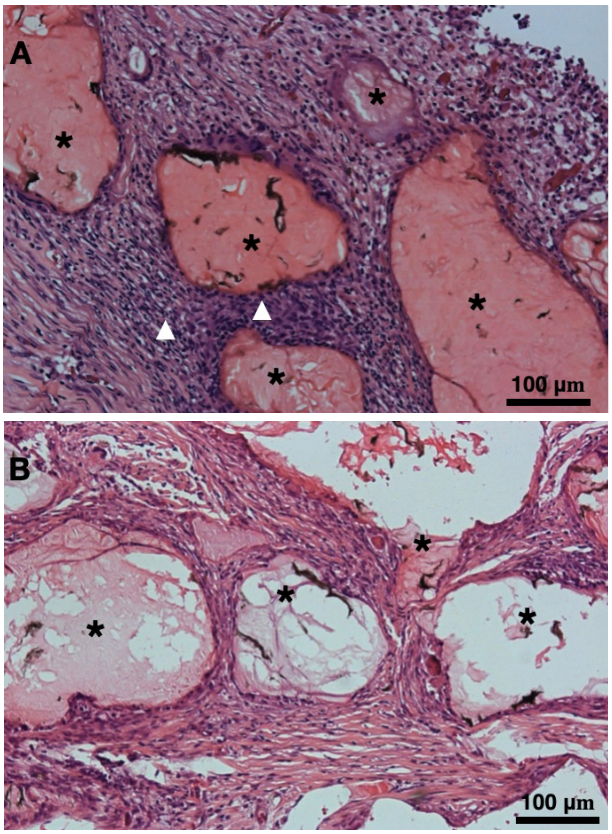

Fig. 6: Photomicrographs of histological sections showing no bone formation at the center of the defect in G1. Remnants of the biomaterial (*) and inflammatory infiltrate (arrowhead). A) 14 days and B) 42 days. (Scale bar = 100 µm).

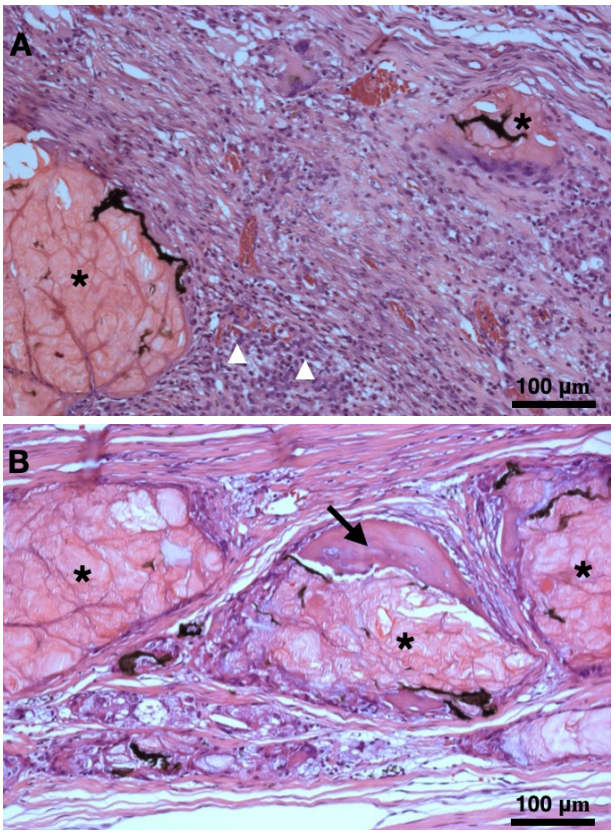

Fig. 7: Photomicrographs of histological sections showing bone formation (arrow) at the center of the defect in G2. Remnants of the biomaterial (*), inflammatory infiltrate (arrowhead), and newly formed bone (arrow). A) 14 days and B) 42 days. (Scale bar = 100 µm).

was attenuated at 42 days (T2) (Fig. 6).

G2 presented a remnant of biomaterial particles at both timepoints, but some bone formation was detected around the biomaterial particles only after 42 days. Of the total area evaluated, the median percentage of newly formed bone was 5.81% (1.95% minimum and 12.25% maximum). Multinucleated giant cells were observed around some biomaterial particles, especially at 14 days. Typically, a mononuclear inflammatory infiltrate was observed on T1. However, at 42 days (T2), there was a decrease in the inflammatory infiltrate (Fig. 7).

DISCUSSION

In the present work, we tested CXHAG granules covered by membranes in rat calvarial critical size defects. We found that bone formation in the center of the critical defect was stimulated only when the granules were supplemented by the conditioned medium from mesenchymal stem cells.

The present study replicated the production of CXHAG membranes previously described by our group[25]. Although the production protocol of these membranes aligns with our earlier publication, this study further investigates our new approach for producing CXHAG granules and their significance in addressing critical bone defect regeneration. The use of a bone substitute biomaterial filling defect and coverage with a membrane is the basis of the guided bone regeneration concept[26] and, therefore, this study adopted it by using CXHAG based biomaterials, either associated or not associated with the conditioned medium derived from mesenchymal stem cell. However, it is important to state that the membranes used in this study were not fixed and, therefore, some micromotion may have occurred, which might have impaired bone regeneration.

The diffraction patterns and FTIR spectra confirmed the chemical composition of the CXHAG granulated biomaterial. Moreover, the sizes of the CXHAG granules produced were predominantly within the 300 to 400 micrometer range, which is

consistent with the current trend in the literature that shows small size granules performing better than large ones[27-28]. A comprehensive examination under varied magnifications revealed distinctive surface topography at the micrometric scale. The irregularity in the granule shape was intended to potentially provide mechanical interlocking and to increase the specific surface area for enhanced cellular interactions. We hypothesized that the design would promote cell adhesion and proliferation. In this regard, this work provided evidence of a higher cellular response in terms of cell viability, which supports this hypothesis, since cell viability percentage was higher in CXHAG than in the control. In this regard, *in vitro* cell cytotoxicity assays utilizing WST play an indispensable role in predicting clinical toxicity. These colorimetric assays provide crucial preliminary data on the potential impacts of a biomaterial on cellular metabolic activity, effectively serving as a proxy for cell health and viability.

In this study, the results of WST-1 assay suggested that CXHAG granules exhibited high cell viability after 24 hours, with significant statistical difference ($p < 0.05$) in comparison to the control cell group. The presence of ions might have contributed to these findings. This result agrees with Souza et al.[25], who showed that hydroxyapatite-chitosan-xanthan-graphene oxide membrane had excellent properties for the cell viability evaluated by the MTT assay. The presence of multinucleated giant cells surrounding some biomaterial particles was expected, as it is a common finding when hydroxyapatite biomaterials are used, especially at early timepoints[29].

The present study provides evidence that the medium conditioned by stem cells obtained from dental pulp stimulated osteogenesis in the center of the defect after 42 days in this experimental model. These findings corroborate our previous study [16], which used the same medium but associated with a bovine hydroxyapatite bone graft, and was also designed with critical size defects and the same 2 timepoints as used in the present research. It is thus feasible to compare the two studies, enabling the understanding that both bone substitute biomaterials (i.e., hydroxyapatite, chitosan, xanthan and graphene oxide versus exclusive bovine hydroxyapatite) can be used as scaffolds for bone tissue engineering purposes. Both the present study and Buss et al.[16] showed no bone formation in the center of the critical sized bone defect with the use of grafts without addition of the conditioned medium, even after 42 days of healing in rat calvaria. These results highlight the inability of osteoconductive biomaterials (e.g., alloplastic or xenogenous bone grafts) to promote bone regeneration in critical areas without the use of tissue engineering/cell therapy approaches.

In the present study, after 14 days, no bone formation was observed in any group, showing that this timepoint is too early for bone regeneration to have occurred, probably due to the size of the critical defect, and consequently, the large distance between the bone walls. However, as bone formation in the center of the defect was only seen after 42 days in G2, and as the presence or absence of the conditioned medium was the only difference between G1 and G2, it can be stated that the medium was the only factor responsible for the difference in osteogenesis between groups. Recent studies have attributed such regenerative effects to the paracrine factors secreted by such cells[30]. Thus, the conditioned medium in which these cells are proliferated has extracellular vesicles and exosomes that might propagate the main regenerative and immunoregulatory characteristics[17,31-32]. The role of the osteogenic conditioned medium in bone formation in the center of the defect is explained by the release of bone regulatory proteins[33]. The presence of exosomes in the osteogenic conditioned culture medium can sensitize cells. However, it is important to note that the level and composition of extracellular vesicles and exosomes in the conditioned medium were not evaluated in this study.

The histological results in the CG (unfilled defect) after 42 days, showing no bone formation, make it clear that the defect used in the present study was a critical size bone defect. For critical size bone defects, the scientific literature shows that the use of tissue engineering concepts can promote earlier bone formation[12-13]. Critical size bone defects enable determination of whether the applied therapies are in fact promoting bone regeneration. It is worth mentioning that the literature considers a defect greater than 6 mm to be critical, since 6 mm is the threshold for spontaneous bone formation, which can occur after 8 weeks[34]. This fact, taken together with the histological results of the CG specimen, makes it clear that the 8mm defect used in the present study really represents a critical bone defect, and therefore

supports the statement that the only variable used in this study (i.e., the conditioned medium in from mesenchymal stem cells) was responsible for the bone formation in the center of the critical bone defect. In this regard, it is important to note that a higher level of bone formation might occur at longer times (e.g., 8 weeks). However, Song et al.[35] showed that there was not complete bone regeneration of 8 mm defects, even after 8 weeks of healing. Moreover, the use of the critical size model healing evaluated at two timepoints enables the evolution of inflammatory infiltrate to be verified over time. In the present study, as in our previous publication[16] in which this same model with xenografts was used, the inflammatory infiltrate decreased between 14 days and 42 days. The trend to have an overrepresentation of the immune/inflammatory processes, with an upregulation of genes associated with leucocyte and T-cell activation at the early healing stages in critical size defects, has been demonstrated by other studies[36-37].

It is important to emphasize that the results of this study cannot be immediately extrapolated to clinical practice, since they derive from an animal model. Therefore, controlled randomized clinical trials are suggested to confirm the regenerating potential of the conditioned medium of mesenchymal stem cells when associated with different scaffolds. Further in vitro studies should be performed to evaluate the effect of the proposed granules on mineralized extracellular matrix as well as on the simulated body fluid[38], and in vivo studies to evaluate the degradation and porosity after bed implantation.

CONCLUSIONS

The biomaterial composed of hydroxyapatite, chitosan, xanthan, and graphene oxide presented irregular granules and showed an improvement in stem cell viability. The conditioned culture medium associated with this biomaterial was able to promote some bone regeneration in the center of critical bone defects in rat calvaria, in contrast to the biomaterial alone.

CONFLICT OF INTEREST

The authors declare no conflicts of interest concerning the publication of this article

FUNDING

This research received funding from the São Paulo Research Foundation–innovative research in small business (FAPESP–PIPE process 2017/08198-5 and process 2018/18890-6) and from Technological Development and Innovative Extension (DT) category 2 of the Brazilian National Council for Scientific and Technological Development (CNPq, grant number 303054/2023-9).

ACKNOWLEDGMENTS

The authors would like to acknowledge Ms. Nadir Freitas for her technical support in histology.

REFERENCES

1. Hasegawa H, Masui S, Ishihata H, Kaneko T, Ishida D, Endo M, Kanno C, Yamazaki M, Kitabatake T, Utsunomiya S, Izumi K, Sasaki K. Evaluation of a Newly Designed Microperforated Pure Titanium Membrane for Guided Bone Regeneration. Int J Oral Maxillofac Implants. 2019; 34:411-422. https://doi.org/10.11607/jomi.6777
2. Cui W, Li X, Xie C, Zhuang H, Zhou S, Weng J. Hydroxyapatite nucleation and growth mechanism on electrospun fibers functionalized with different chemical groups and their combinations. Biomaterials. 2010; 31:4620-4629. https://doi.org/10.1016/j.biomaterials.2010.02.050
3. Caballé-Serrano J, Zhang S, Ferrantino L, Simion M, Chappuis V, Bosshardt DD. Tissue Response to a Porous Collagen Matrix Used for Soft Tissue Augmentation. Materials (Basel). 2019; 12:3721. https://doi.org/10.3390/ma12223721
4. Chavda S, Levin L. Human Studies of Vertical and Horizontal Alveolar Ridge Augmentation Comparing Different Types of Bone Graft Materials: A Systematic Review. J Oral Implantol. 2018; 44:74-84. https://doi.org/10.1563/aaid-joi-D-17-00053
5. Maji K, Dasgupta S, Pramanik K, Bissoyi A. Preparation and characterization of gelatin-chitosan-nanoß-TCP based scaffold for orthopaedic application. Mater. Sci. Eng. C Mater. Biol. Appl. 2018; 86:83-94. https://doi.org/10.1016/j.msec.2018.02.001
6. Soleymani S, Naghib SM. 3D and 4D printing hydroxyapatite-based scaffolds for bone tissue engineering and regeneration. Heliyon. 2023; 9:e19363. https://doi.org/10.1016/j.heliyon.2023.e19363
7. Wach T, Kozakiewicz M. Fast-Versus Slow-Resorbable Calcium Phosphate Bone Substitute Materials-Texture Analysis after 12 Months of Observation. Materials (Basel). 2020; 13:3854. https://doi.org/10.3390/ma13173854
8. Wang F, Saure LM, Shutt F, Lorich F, Rasch F, Nia AS, Feng X, Seekamp A, Kluter T, Naujokat H, Adelung R, Fuchs S. Graphene Oxide Framework Structures and Coatings: Impact on Cell Adhesion and Pre-Vascularization Processes for Bone Grafts. Int J Mol Sci. 2022: 23:3379. https://doi.org/10.3390/ijms23063379
9. Dreanca A, Sarosi C, Parvu AE, Blidaru M, Enacrachi G, Purdoiu R, Nagy A, Sevastre B, Oros NA, Marcus

I, Moldovan M. Systemic and Local Biocompatibility Assessment of Graphene Composite Dental Materials in Experimental Mandibular Bone Defect. Materials (Basel). 2020; 13:2511. https://doi.org/10.3390/ma13112511

10. Sakkas A, Wilde F, Heufelder M, Winter K, Schramm A. Autogenous bone grafts in oral implantology-is it still a "gold standard"? A consecutive review of 279 patients with 456 clinical procedures. Int J Implant Dent. 2017; 3:23. https://doi.org/10.1186/s40729-017-0084-4
11. Yamada M, Egusa H. Current bone substitutes for implant dentistry. J Prosthodont Res. 2018; 62:152-161. https://doi.org/10.1016/j.jpor.2017.08.010
12. de Oliveira E Silva M, Pelegrine AA, Alves Pinheiro da Silva A, Manhães Júnior LR, de Mello E Oliveira R, Gaiba França S, Aloise AC, Ferreira LM. Xenograft enriched with autologous bone marrow in inlay reconstructions: a tomographic and histomorphometric study in rabbit calvaria. Int J Biomater. 2012; 2012:170520. https://doi.org/10.1155/2012/170520
13. Aloise AC, Pelegrine AA, Zimmermann A, de Mello E Oliveira R, Ferreira LM. Repair of critical-size bone defects using bone marrow stem cells or autogenous bone with or without collagen membrane: a histomorphometric study in rabbit calvaria. Int J Oral Maxillofac Implants. 2015; 30:208-215. https://doi.org/10.11607/jomi.4010
14. Lavareda Corrêa SC, Elias de Sousa J, Pasquali PJ, Scavone de Macedo LG, Aloise AC, Teixeira ML, Pelegrine AA. Use of Bone Allograft With or Without Bone Marrow Aspirate Concentrate in Appositional Reconstructions: A Tomographic and Histomorphometric Study. Implant Dent. 2017; 26:915-921. https://doi.org/10.1097/ID.0000000000000669
15. Fontes Martins LC, Sousa Campos de Oliveira AL, Aloise AC, Scavone de Macedo LG, Teixeira ML, Moy PK, Pelegrine AA. Bone marrow aspirate concentrate and platelet-rich fibrin in fresh extraction sockets: A histomorphometric and immunohistochemical study in humans. J Craniomaxillofac Surg. 2021; 49:104-109. https://doi.org/10.1016/j.jcms.2020.12.005
16. Buss LF, de Martin GS, Martinez EF, Filgueiras IAAAP, Magnabosco JL, Alves BF, Almeida BM, Kotaka T, Teixeira ML, Ferreira JRM, da Rocha DN, Canal R, Aloise AC, Holliday LS, Pelegrine AA. Conditioned Media from Human Pulp Stem Cell Cultures Improve Bone Regeneration in Rat Calvarial Critical-Size Defects. J Funct Biomater. 2023; 14:396. https://doi.org/10.3390/jfb14080396.
17. Katagiri W, Watanabe J, Toyama N, Osugi M, Sakaguchi K, Hibi H. Clinical Study of Bone Regeneration by Conditioned Medium From Mesenchymal Stem Cells After Maxillary Sinus Floor Elevation. Implant Dent. 2017; 26:607-612. https://doi.org/10.1097/ID.0000000000000618
18. Kobolak J, Dinnyes A, Memic A, Khademhosseini A, Mobasheri A. Mesenchymal stem cells: Identification, phenotypic characterization, biological properties and potential for regenerative medicine through biomaterial micro-engineering of their niche. Methods. 2016; 99:62-68. https://doi.org/10.1016/j.ymeth.2015.09.016
19. Takeuchi R, Katagiri W, Endo S, Kobayashi T. Exosomes from conditioned media of bone marrow-derived mesenchymal stem cells promote bone regeneration by enhancing angiogenesis. PLoS One. 2019; 14:e0225472. https://doi.org/10.1371/journal.pone.0225472
20. Hiraki T, Kunimatsu R, Nakajima K, Abe T, Yamada S, Rikitake K, Tanimoto K. Stem cell-derived conditioned media from human exfoliated deciduous teeth promote bone regeneration. Oral Dis. 2020; 26:381-390. https://doi.org/10.1111/odi.13244
21. Katagiri W, Takeuchi R, Saito N, Suda D, Kobayashi T. Migration and phenotype switching of macrophages at early-phase of bone-formation by secretomes from bone marrow derived mesenchymal stem cells using rat calvaria bone defect model. J Dent Sci. 2022; 17:421-429. https://doi.org/10.1016/j.jds.2021.08.012
22. Rourke JP, Pandey PA, Moore JJ, Bates M, Kinloch IA, Young RJ, Wilson NR. The real graphene oxide revealed: stripping the oxidative debris from the graphene-like sheets. Angew Chem Int Ed Engl. 2011; 50:3173-3177. https://doi.org/10.1002/anie.201007520
23. Lopes CC, Pinheiro WA, da Rocha DN, Neves JG, Correr AB, Ferreira JR, Barbosa RM, Soares JRF, Santos JL, da Silva MHP. Nanocomposite powders of hydroxyapatite-graphene oxide for biological applications. Ceramics International. 2021; 47:7653-7665. https://doi.org/10.1016/j.ceramint.2020.11.107
24. Navarro da Rocha D, Prado da Silva MH, De Campos JB, Marçal RLSB, Mijares DQ, Coelho PG, Cruz LR. Kinetics of conversion of brushite coatings to hydroxyapatite in alkaline solution. Journal of materials research and technology. 2018; 7:479-486. https://doi.org/10.1016/j.jmrt.2018.02.002
25. Souza AP, Neves JG, da Rocha DN, Lopes CC, Moraes M, Correr-Sobrinho L, Correr AB. Chitosan/Xanthan membrane containing hydroxyapatite/Graphene oxide nanocomposite for guided bone regeneration. Journal of the Mechanical Behavior of Biomedical Materials. 2022; 136:105464. https://doi.org/10.1016/j.jmbbm.2022.105464
26. Retzepi M, Donos N. Guided Bone Regeneration: biological principle and therapeutic applications. Clin Oral Implants Res. 2010; 21:567-576. https://doi.org/10.1111/j.1600-0501.2010.01922.x
27. Leiblein M, Koch E, Winkenbach A, Schaible A, Nau C, Büchner H, Schröder K, Marzi I, Henrich D. Size matters: Effect of granule size of the bone graft substitute (Herafill®) on bone healing using Masquelet's induced membrane in a critical size defect model in the rat's femur. J Biomed Mater Res B Appl Biomater. 2020; 108:1469-1482. https://doi.org/10.1002/jbm.b.34495
28. Klüppel LE, Antonini F, Olate S, Nascimento FF, Albergaria-Barbosa JR, Mazzonetto R. Bone repair is influenced by different particle sizes of anorganic bovine bone matrix: a histologic and radiographic study in vivo. J Craniofac Surg. 2013; 24:1074-1077. https://doi.org/10.1097/SCS.0b013e318286a0a3
29. Barbeck M, Udeabor SE, Lorenz J, Kubesch A, Choukroun J, Sader RA, Kirkpatrick CJ, Ghanaati S. Induction of multinucleated giant cells in response to small sized bovine bone substitute (Bio-Oss™) results in an enhanced early implantation bed vascularization. Ann Maxillofac Surg. 2014; 4:150-157. https://doi.org/10.4103/2231-0746.147106
30. Wang X, Thomsen P. Mesenchymal stem cell-derived small extracellular vesicles and bone regeneration. Basic Clin Pharmacol Toxicol. 2021; 128:18-36. https://doi.org/10.1111/bcpt.13478

31. Cooper LF, Ravindran S, Huang CC, Kang M. A Role for Exosomes in Craniofacial Tissue Engineering and Regeneration. Front Physiol. 2020; 10:1569. https://doi.org/10.3389/fphys.2019.01569
32. Macías I, Alcorta-Sevillano N, Infante A, Rodríguez CI. Cutting Edge Endogenous Promoting and Exogenous Driven Strategies for Bone Regeneration. Int J Mol Sci. 2021; 22:7724. https://doi.org/10.3390/ijms22147724
33. Morrell AE, Brown GN, Robinson ST, Sattler RL, Baik AD, Zhen G, Cao X, Bonewald LF, Jin W, Kam LC, Guo XE. Mechanically induced Ca2+ oscillations in osteocytes release extracellular vesicles and enhance bone formation. Bone Res. 2018; 6:6. https://doi.org/10.1038/s41413-018-0007-x
34. Kim RW, Kim JH, Moon SY. Effect of hydroxyapatite on critical-sized defect. Maxillofac Plast Reconstr Surg. 2016; 38:26. https://doi.org/10.1186/s40902-016-0072-2
35. Song JM, Shin SH, Kim YD, Lee JY, Baek YJ, Yoon SY, Kim HS. Comparative study of chitosan/fibroin-hydroxyapatite and collagen membranes for guided bone regeneration in rat calvarial defects: Micro-computed tomography analysis. Int. J. Oral Sci. 2014; 6:87–93. https://doi.org/10.1038/ijos.2014.16
36. Ivanovski S, Hamlet S, Retzepi M, Wall I, Donos N. Transcriptional profiling of "guided bone regeneration" in a critical-size calvarial defect. Clin Oral Implants Res. 2011; 22:382-389. https://doi.org/10.1111/j.1600-0501.2010.02104.x
37. Calciolari E, Mardas N, Dereka X, Anagnostopoulos AK, Tsangaris GT, Donos N. The effect of experimental osteoporosis on bone regeneration: part 2, proteomics results. Clin Oral Implants Res. 2017; 28:e135-e145. https://doi.org/10.1111/clr.12950
38. Kokubo T, Takadama H. How useful is SBF in predicting in vivo bone bioactivity? Biomaterials. 2006; 27:2907-15. https://doi.org/10.1016/j.biomaterials.2006.01.017

https://doi.org/10.54589/aol.37/2/162

Effect of finishing and polishing systems on surface roughness and color stability of aesthetic restorations exposed to staining solution.

Ana C Ferreira Alvarenga[1], Kamila R Kantovitz[1], Cecília P Turssi[1], Roberta T Basting[1], Waldemir F Vieira-Junior[2], Fabiana MG França[1]

1. Faculdade São Leopoldo Mandic, Campinas, Brasil
2. Universidade Estadual de Campinas, Escola de Odontologia de Piracicaba, Departamento de Dentística Restauradora, Piracicaba, Brasil.

ABSTRACT

The color and the surface roughness of aesthetic restorations are related to the clinical sucess and longevity of these treatments. ***Aim:*** *This study evaluated the influence of finishing and polishing systems, and storage media on the surface roughness and color stability of aesthetic restorative composites.* ***Materials and Method:*** *Cylindrical specimens (n=10) were prepared and treated according to: 1. Type of composite resin (nanofilled- Filtek Z350XT, suprananofilled- Estelite Omega, nanohybrid- Empress Direct); 2. Type of finishing and polishing systems (no polishing, aluminum oxide discs or abrasive rubber polishers); and 3. Type of immersion medium (water or coffee, 3 h/day/30 days). Surface roughness (Ra - μm) and color stability (L, ΔEab, and ΔE00) were evaluated at baseline (after polishing) and final time (after immersion). Data were subjected to Kruskal-Wallis, Mann-Whitney, Wilcoxon, and Student-Newman-Keuls tests (α=0.05).* ***Results:*** *Nanohybrid (p<0.001) and suprananofilled composite resins (p=0.004) showed an increase in Ra after polishing, regardless the finishing and polishing system. After immersion in coffee, the nanofilled composite had the highest roughness values (p=0.032). L values increased for all resins after polishing (p<0.05). Suprananofilled composites had the greatest color stability with the lowest values of ΔEab and ΔE00.* ***Conclusions:*** *Finishing and polishing systems had an impact on the surface roughness and color stability of all aesthetic resins, and their effectiveness depended on the type of composite resin.*

Keywords: *composite resins - surface properties - color - acids*

Efeito de sistemas de acabamento e polimento na rugosidade superficial e estabilidade de cor de restaurações estéticas expostas a solução de manchamento.

RESUMO

A cor e a rugosidade de superfície das restaurações estéticas estão relacionadas ao sucesso clínico e manutenção destes tratamentos ao longo do tempo. ***Objetivo:*** *Este estudo avaliou a influência de sistemas de acabamento e polimento e meios de armazenamento na rugosidade superficial e estabilidade de cor de compósitos restauradores de alta estética.* ***Materiais e Método:*** *Foram preparados espécimes cilíndricos de acordo com os fatores em estudo (n=10): 1. Tipo de resina composta (nanoparticulada- Filtek Z350XT, suprananoparticulada- Estelite Omega, nanohíbrida- Empress Direct); 2. Tipo de sistemas de acabamento e polimento (sem polimento, discos de óxido de alumínio ou borrachas abrasivas); e 3. Tipo de meio de imersão (água ou café, 3 h/dia/30 dias). A rugosidade superficial (Ra - μm) e a estabilidade de cor (L, ΔEab e ΔE00) foram avaliadas no início (pós-polimento) e no final (pós-imersão na solução). Os dados foram submetidos aos testes de Kruskal-Wallis, Mann-Whitney, Wilcoxon e Student-Newman-Keuls (α=0,05).* ***Resultados:*** *As resinas compostas nanohíbrida (p<0,001) e suprananoparticulada (p=0,004) apresentaram aumento em Ra pós-polimento, independentemente do sistema de acabamento e polimento. Em relação ao pós-polimento após imersão em café, a composta nanoparticulada mostrou os maiores valores de rugosidade (p=0,032). Os valores de L aumentaram para todas as resinas pós-polimento (p<0,05). A resina suprananoparticulada apresentou a maior estabilidade de cor com os menores valores de ΔEab e ΔE00.* ***Conclusão:*** *Os sistemas de acabamento e polimento tiveram impacto na rugosidade superficial e estabilidade de cor de todos os compósitos estéticos, e sua eficácia depende do tipo de resina composta.*

Palavras-chave: *resinas compostas - propriedades superficiais - cor - ácidos*

To cite:
Ferreira Alvarenga AC, Kantovitz KR, Turssi CP, Basting RT, Vieira-Junior WF, França FMG. Effect of finishing and polishing systems on surface roughness and color stability of aesthetic restorations exposed to staining solution. Acta Odontol Latinoam. 2024 Aug 30;37(2):162-171. https://doi.org/10.54589/aol.37/2/162

Corresponding Author:
Fabiana Mantovani Gomes França
biagomes@yahoo.com

Received: April 2024.
Accepted: June 2024.

INTRODUCTION

Composite resins consist of an organic polymeric matrix and inorganic fillers bound together by a silane coupling agent[1]. There are several types of composite resins, classified according to filler types and distribution, average filler particle size and polymerization method[2]. Nanocomposites have advantages such as reduced polymerization contraction, improved mechanical properties, enhanced optical behavior, better gloss, improved color stability and reduced wear[3].

To be considered clinically successful, composite resin restorations must have long-lasting and functional qualities, including good marginal adaptation, radiopacity, high wear resistance, ease of application, and resistance to degradation from contact with water and/or other solvents. However, despite improvements in the physical properties of composite resins, they are still subject to degradation in the oral cavity[4].

Composite resins provide acceptable aesthetic results due to their superior optical and mechanical properties[5] and can be used for veneers. Compared to ceramic veneers, composite resin veneers have advantages such as adhesion between the composite resin and the dental substrate, low cost, and shorter clinical manufacturing time. Restoration failure behavior differs between anterior and posterior teeth, and although anterior restorations have fewer secondary caries lesions, they are more often replaced for reasons such as aesthetic appearance and staining[6]. Color stability is thus crucial to aesthetic restorations and has direct impact on the clinical success of restorative procedures[7], so it is important to consider that the conditions to which restorative materials are exposed can influence their chemical degradation.

Another important factor for the success of restorations is surface roughness. The surface roughness of composite resins can be minimized by employing an effective finishing and polishing protocol[8]. A variety of instruments are used for finishing and polishing, including carbide finishing burs, diamond finishing burs, abrasive-impregnated rubber cups and tips, aluminum oxide-coated abrasive discs, abrasive strips, and abrasive pastes. The effectiveness of finishing and polishing systems depends on the substrate, type of abrasive used, time spent with each abrasive, pressure applied, alignment of abrasive surfaces and geometry of abrasive instruments, presence or absence of lubrication, and immediate or delayed timing of polishing after composite resin polymerization[9,10]. Several finishing and polishing systems are available for composite resins. These systems may involve one or several steps and vary significantly in composition, presentation, and the type and hardness of abrasive particles[11].

However, there is limited information in the literature regarding polishing protocols for composite resins with high aesthetic performance. Therefore, the aim of this study was to evaluate the influence of finishing and polishing systems on the surface roughness and color stability of composite resins recommended for high aesthetic performance in anterior teeth, as a result of exposure to chemical challenges. The first null hypothesis was that the finishing and polishing systems would not significantly impact surface roughness of composite resins with high aesthetic performance when they were exposed to coffee. The second null hypothesis was that the finishing and polishing systems would not significantly impact color stability of composite resins with high aesthetic performance when they were exposed to coffee.

MATERIALS AND METHOD

The factors in the study were: 1) Type of composite resin material (Nanofilled, Suprananofilled, Nanohybrid); 2) Type of finishing and polishing systems (no polishing, aluminum oxide discs, abrasive rubber); and 3) Type of immersion medium (water, coffee). The surface roughness (Ra) and color stability (L, ΔEab, ΔE00) performance of the composites were measured at baseline (after polishing) and final time (after immersion).

Sixty cylindrical specimens (n=10 per treatment) of each composite resin (described in Table 1) were manufactured using a rubber mold 2 mm tall and 5 mm in diameter, positioned on a glass plate and polyester strip. The composite resins were inserted in a single increment into the mold, and another polyester strip and glass plate were placed on top for 15 s under a weight of 500 g. The cylinders were photoactivated according to the manufacturer's instructions for 20 s using a LED device at Standard power (Valo, Ultradent, South Jordan, Utah, USA) with a light intensity of 1000 mW/cm^2. The distance between the light source and the specimens was

Table 1. Manufacturer and composition of the composite resins used in this study.

Manufacturer	Composition	
	Matrix	Filler
Filtek Z350 XT 3M (Sumaré, SP, Brazil)	Bis-GMA Bis-EMA UDMA TEGDMA	Nanofilled - Silica: 20 nm (non-agglomerated) - Zirconia: 4-11 nm (unbonded) - Zirconia/silica agglomerates: 4-11 µm - Inorganic particles: 72.5% by weight
Estelite Omega Tokuyama (Encinitas, CA, USA)	Bis-GMA TEGDMA	Suprananofilled - SiO^2-ZrO^2 spherical particles: 200 nm - Inorganic particles: 82% by weight
Empress Direct Ivoclar (Barueri, SP, Brazil)	Bis-GMA UDMA	Nanohybrid - Barium glass, ytterbium trifluoride, mixed oxide, silicon dioxide and copolymer: from 40 nm to 3000 nm (average size = 550 nm). - Inorganic particles: 75-79% by weight

Bis GMA – Bisphenol A glycidyl methacrylate; UDMA - Urethane dimethacrylate; TEGDMA – Triethylene glycol dimethacrylate; Bis-EMA - Bisphenol A glycidyl dimethacrylate ethoxylate.

standardized by the polyester strip. The specimens were placed on cotton moistened with distilled water and stored in a humid environment in a refrigerator. Each specimen was lightly engraved with a number using a high-speed spherical diamond tip (No. 3072, KG Sorensen, Cotia, SP, Brazil) on the surface opposite the testing surface, to ensure that tests were applied and read in a standardized manner on the same face.

For the finishing and polishing procedures, the specimens were divided into three groups, as follows: **1.** Polyester matrix – without finishing and polishing (control group); **2.** Multi-step aluminum oxide discs and diamond paste with polishing felt - medium (40 µm), fine (24 µm), extra fine (8 µm) (SofLex, 3M, Sumaré, SP, Brazil); **3.** Multi-step bullet-shaped abrasive silicone rubber tips impregnated with silicon carbide and aluminum oxide particles, and diamond paste with polishing felt - Coarse/green (40 µm); medium/yellow (30 µm), fine/white (5 µm) (Jiffy, Ultradent, South Jordan, UT, USA), associated with diamond polish - diamond particles in an aqueous base grain size (0.5 µm) (Ultradent, South Jordan, UT, USA).

A base was made of heavy addition silicone paste (Yller, Pelotas, RS, Brazil). Before the silicone was completely set, three test objects were inserted to form recesses to hold the resin blocks. The resin blocks were allowed to fully set within the heavy silicone. All the specimens were polished on this base, with each specimen placed in a recess with the end to be polished facing upwards. Each polishing system was applied to 20 specimens of each resin for 20 s with gentle pressure in the same direction, approximately 25 movements on each specimen at controlled speed of 7,500 rpm[12]. Between the use of each system (felt), the specimen was washed with air and water spray for 10 s with a triple syringe to remove debris. The polishing systems (felts) were used with polishing paste under the same pressure and time conditions. Abrasive rubber tips were discarded after every 3 specimens, while aluminum oxide discs and polishing felt discs with diamond paste were only used once.

The immersion protocol followed that of a previous study[13]. Thirty specimens of each type of composite resin (10 from each of groups 1. 2 and 3) were immersed in either: 1) Distilled water - Chemically pure, free of soluble salts (AM distributor, São Paulo, SP, Brazil) (pH = 6.0); or 2) Coffee made from traditional ground roasted coffee beans (Café Três Corações S.A., Três Corações, MG, Brazil) (pH = 5.39, using MS Tecnopon (Model MPA210, Piracicaba, SP, Brazil). Coffee was prepared by heating water (± 85ºC) for 5 minutes in a microwave (power 100 W), and then pouring it through a plastic strainer lined with a paper filter containing ground coffee beans (water/ground coffee ratio = 700 mL/58 g).

For 30 consecutive days, specimens were immersed in 5 mL of coffee or distilled water at room temperature for 3 hours, and then stored in a humid environment under refrigeration. Fresh distilled water and coffee were used every day.

Average surface roughness (Ra) was evaluated for each specimen at baseline (after polishing) and final time (after immersion in water or coffee),

using a profilometer (Mitutoyo, Suzano, SP, Brazil). Sampling length was 2.4 mm, made up of three 0.8 mm cut-offs taken sequentially in horizontal, vertical and oblique directions at a stylus speed of 0.25 mm/s.

Color was evaluated at baseline (after initial polishing) and final time (after immersion in distilled water or coffee). Before color evaluation, the specimens were washed with distilled water for 60 s and dried with absorbent paper. Color was analyzed with a colorimetry spectrophotometer, CIEl*a*b* system (VITA Easyshade Advance 4.0 - VITA Zahnfabrik, Bad Säckingen, Germany). Briefly, a box with a white background was used to avoid the influence of external light on color measurement. The color was evaluated by three readings on each specimen, with the result being the average of the three values (L*). Regarding the color change parameter (ΔEab), the range to be used to distinguish changes in color values was ΔEab > 1.2. Thus, values greater than 1.2 were considered to be easily observable and clinical changes. This threshold is in line with previous criteria[14], that deem values above 1.2 as not acceptable. In the ΔE00 formula, ΔL* represents the variation of the L* coordinate indicating lightness (black-white axis); ΔC represents the difference in saturation (chromacity); ΔH represents differences in hue; eRT is a function that considers the interaction between chromacity and hue differences in a blue region of the spectrum. The ΔE00 values were calculated sequentially.

$$\Delta E00 = \sqrt{(\Delta L'/kLSL)2 + (\Delta C'/kCSC)2 + (\Delta H'/kHSH)2 + RT(\Delta C'/kCSC)(\Delta H'/(kHSh)}$$

Statistical analysis

As the data did not meet normal distribution and homogeneity of variance, the surface roughness and color (L, ΔEab and ΔE00) of the composite resins subjected to finishing and polishing systems and immersion solutions were analyzed using the Kruskal-Wallis, Mann-Whitney and Wilcoxon tests. Student-Newman-Keuls tests were used for multiple comparisons. Statistical calculations were performed using SPSS 23 (SPSS Inc., Chicago, IL, USA) and BioEstat 5.0 (Fundação Mamirauá, Belém, PA, Brazil) (α=0.05).

RESULTS

Table 2 shows the means and standard deviations of surface roughness (Ra - µm) for the different types of composite resins, according to the finishing and polishing systems and type of immersion medium. For Estelite Omega (suprananofilled, p=0.004) and Empress Direct (nanohybrid, p<0.001), roughness was higher after finishing and polishing than without polishing (using only polyester strip – control group), regardless of the system used. For Filtek

Table 2. Mean (SD) of surface roughness (µm) of the different types of composite resins according to the finishing and polishing system and the immersion medium.

	Experimental Groups	Control (without polishing)	Discs + felts	Rubbers + felts
	Filtek Z350 XT (Nanofilled)			
	Baseline (post-polishing)	0.276 (0.117) **A**	0.313(0.104) **A**	0.339(0.123) **A**
Final	Water (post-immersion)	0.381 (0.221) **a**	0.416(0.153) **a**	0.457(0.242) ***a**
Final	Coffee (post-immersion)	0.425 (0.209) ***a**	0.449(0.118) ***a**	0.385(0.186) ***a**
	Estelite Omega (Suprananofilled)			
	Baseline (post-polishing)	0.306(0.143) **A**	0.416(0.153) **B**	0.464(0.144) **B**
Final	Water (post-immersion)	0.223(0.085) **a**	0.467(0.149) **a**	0.665(0.253) **a**
Final	Coffee (post-immersion)	0.254(0.143) **a**	0.576(0.251) **a**	0.521(0.155) **a**
	Empress Direct (Nanohybrid)			
	Baseline (post-polishing)	0.185 (0.114) **A**	0.317 (0.094) **B**	0.385 (0.093) **B**
Final	Water (post-immersion)	0.139 (0.054) **a**	0.325 (0.137) **a**	0.431 (0.103) **a**
Final	Coffee (post-immersion)	0.237 (0.141) **a**	0.267 (0.090) **a**	0.433 (0.126) **a**

Different capital letters indicate a significant difference between finishing and polishing systems by Kruskal-Wallis and Student-Newman-Keuls tests.
* indicates significant difference in relation to the post-polishing value by Mann-Whitney. Equal lowercase letters indicate no significant difference between specimens immersed in distilled water or coffee by Mann-Whitney.

Z350 XT (nanofilled), there was no statistically significant difference with finishing and polishing or without (using only polyester strip – control group (p=0.269). However, Filtek Z350 XT had the highest roughness values after polishing and immersion in coffee (p=0.032, Table 2). For all other groups, there was no significant difference in the values after finishing and polishing and after immersion (p>0.05).

The L values (lightness) for all composite resins were significantly affected by finishing and polishing systems and by immersion (Table 3). The lowest L value was observed for the control group (without polishing) after immersion in coffee (p<0.001). At baseline, Estelite Omega (suprananofilled) and Empress direct (nanohybrid) had the highest L values when multi-step aluminum discs and felts were used as finishing and polishing system (p<0.001), while for Filtek Z350 XT (nanofilled), there was no significant difference between the finishing and polishing systems (p>0.05).

Table 4 shows that the values of ΔEab (p<0.001) and ΔE00 (p<0.002) were significantly higher after immersion in coffee than in distilled water, regardless of the type of composite resin. Specifically, Empress Direct (nanohybrid) immersed in coffee had the lowest ΔEab (p=0.009) and ΔE00 (p=0.010) values when polished with multi-step aluminum oxide discs + felt disc + paste. Estelite Omega (suprananofilled) (p=0.002) had significantly lower ΔEab and ΔE00 than nanofilled and nanohybrid composite resins, which did not differ significantly from each other. For these two indicators, the color change was significantly greater in the absence of finishing and polishing.

In distilled water, only Estelite Omega (suprananofilled) showed no statistically significant differences for ΔEab (p=0.168) and ΔE00 (p=0.374) values considering the finishing and polishing systems. Filtek Z350 XT (nanofilled) had lower ΔEab (p=0.003) and ΔE00 (p=0.004) when aluminum disc + felts were used. For Empress Direct (nanohybrid), the ΔEab (p=0.005) and ΔE00 (p=0.002) values were significantly higher in the group without polishing than in the group polished with aluminum oxide discs + felt + paste, while the group polished with abrasive rubber had the lowest ΔEab and ΔE00 values.

DISCUSSION

The first null hypothesis – that finishing and polishing systems would not significantly impact the surface roughness of composite resins with high aesthetic performance when they were subjected to a chemical challenge – was rejected. After being treated with finishing and polishing systems, Estelite Omega (suprananofilled) and Empress Direct (nanohybrid) had higher roughness values

Table 3. Means (SD) of color parameters L for composite resins, according to the finishing and polishing system and immersion medium.

	Experimental Groups	Control (without polishing)	Discs + felts	Rubbers + felts
		Filtek Z350 XT (Nanofilled)		
	Baseline (post-polishing)	84.6 (1.6) B	87.9 (1.8) A	86.1 (1.1) A
Final	Water (post-immersion)	81.9 (1.0) *a	84.7 (1.1) *a	84.1 (1.3) *a
Final	Coffee (post-immersion)	70.0 (2.3) *b	75.6 (1.4) *b	73.8 (1.7) *b
		Estelite Omega (Suprananofilled)		
	Baseline (post-polishing)	85.0 (1.9) C	88.9 (1.8) A	86.8 (1.3) B
Final	Water (post-immersion)	84.9 (1.3) a	87.5 (1.5) *a	86.7 (1.4) a
Final	Coffee (post-immersion)	74.0 (0.6) *b	76.8 (1.2) *b	77.3 (1.4) *b
		Empress Direct (Nanohybrid)		
	Baseline (post-polishing)	85.4 (1.4) B	88.1 (1.1) A	86.3 (1.4) B
Final	Water (post-immersion)	82.4 (1.4) *a	85.1 (0.8) *a	83.5 (0.6) *a
Final	Coffee (post-immersion)	68.2 (1.6) *b	73.2 (1.7) *b	70.5 (1.7) *b

Different capital letters indicate an intragroup (in line) significant difference by Kruskal-Wallis and Wilcoxon tests. Different lowercase letters indicate a significant difference between immersion media by Mann-Whitney. * indicates significant difference in relation to the post-polishing value by Mann-Whitney.

Table 4. Means (SD) of ΔEab and ΔE00 for composite resins according to the finishing and polishing system and the immersion medium.

	Experimental Groups	Control (without polishing)	Discs + felts	Rubbers + felts
ΔEab	*Filtek Z350 XT (Nanofilled)*			
	Water (post-immersion)	3.40 (0.74) A**b	2.23 (0.55) B*b	2.94 (0.57) A**b
	Coffee (post-immersion)	16.94 (4.22) A##a	14.34 (3.23) AB#a	13.20 (1.64) B#a
	Estelite Omega (Suprananofilled)			
	Water (post-immersion)	1.99 (0.50) A*b	1.92 (0.87) A*b	1.61 (0.31) A*b
	Coffee (post-immersion)	14.28 (1.13) A#a	14.06 (1.22) A#a	11.93 (1.10) B#a
	Empress Direct (Nanohybrid)			
	Water (post-immersion)	3.75 (1.13) A**b	3.65 (0.77) A**b	2.39 (0.68) B**b
	Coffee (post-immersion)	18.25 (1.64) A##a	15.17 (1.91) B#a	17.31 (1.88) AB##a
ΔE00	*Filtek Z350 XT (Nanofilled)*			
	Water (post-immersion)	2.35 (0.47) A**b	1.64 (0.34) B*b	2.09 (0.36) A**b
	Coffee (post-immersion)	12.15 (2.78) A##a	10.38 (2.36) A#a	9.82 (1.21) A##a
	Estelite Omega (Suprananofilled)			
	Water (post-immersion)	84.9 (1.3) a	87.5 (1.5) *a	86.7 (1.4) a
	Coffee (post-immersion)	74.0 (0.6) *b	76.8 (1.2) *b	77.3 (1.4) *b
	Empress Direct (Nanohybrid)			
	Water (post-immersion)	2.39 (0.60) A**b	2.44 (0.41) A**b	1.68 (0.30) B**b
	Coffee (post-immersion)	12.96 (1.27) A##a	10.75 (1.36) B#a	12.31 (1.39) A###a

Different capital letters indicate significant differences between finishing and polishing systems by Kruskal-Wallis and Student-Newman-Keuls tests ($p<0.05$). Different lowercase letters indicate intragroup significant difference immersed in distilled water or coffee by Kruskal-Wallis and Student-Newman-Keuls tests ($p<0.001$). * indicates significant difference among the type of finishing and polishing immersed in water by Kruskal-Wallis and Wilcoxon tests. ** indicates significant difference among composite resins immersed in water, considering each system separately by Mann-Whitney and Wilcoxon tests. # indicates significant difference among composite resins immersed in coffee, considering each finishing and polishing system separately by Mann-Whitney and Wilcoxon tests.

than when not polished (using only polyester strip – control group), regardless of the type of system used. This agrees with another study reporting that the smoothest surfaces were observed for the polyester strip (control group) [15]. The smooth surface formed by the matrix tends to be rich in the organic matrix and free of any compounds that are inhibited by air[15]. However, the removal of the outermost composite layer by finishing and polishing procedures is necessary to produce a harder, wear-resistant, color-stable restoration[16]. In addition, most aesthetic restorations require the removal of excess material and refinement in anatomy, thus necessitating the use of finishing and polishing systems. However, these treatments can produce variability in the degree of roughness as well as the removal of the resin oxygen-inhibited layer[17].

In the present study, no difference was found for Filtek Z350 XT (nanofilled resin) between the groups with finishing and polishing or without (only polyester strip). This may be explained by the size of the inorganic particles present in the composite resin. The larger the size of the particles lost during abrasion, the greater the increase in roughness[18]. Thus, Filtek Z350 XT, a nanofilled resin with particles from 4 to 20 nm, was developed to combine high polishing and gloss retention[19]. When composites with nanoclusters undergo an abrasive process, individual nanometric primary particles may be lost[18]. Hence, the surfaces resulting from wear have smaller defects and better gloss retention when compared to microhybrid compounds that lose larger secondary particles, leading to greater defects on the surface and increased roughness. This difference explains the performance of the Filtek Z350 XT (nanofilled) resin, which exhibits less change in surface roughness after finishing and polishing.

However, after immersing Filtek Z350 XT specimens in water or coffee, the specimens polished with

aluminum oxide discs had greater surface roughness than and those subjected to the sequential use of rubber tips plus felt with diamond paste. This could be explained by the degradation or dissolution of the polymerized organic matrix when exposed to water[19]. The water absorption of the polymeric compound is highly dependent on the chemical structure of the resin monomers[20]. The phenomenon of sorption in composites is a diffusion-controlled process that results in chemical degradation, caused by the release of residual monomer and detachment between the matrix and the filler[21]. A study on the solubility and discoloration of monomers in composites reported that, in terms of absorption, solubility and color change, the monomers were ranked as BisEMA <UDMA<BisGMA[22]. Furthermore, studies indicate that TEGDMA is the monomer that causes the most substantial colour alteration[23]. Specifically, Filtek Z350 XT (nanofilled), whose roughness increased after immersion in water and coffee, contains all four types of monomers in its composition. This may have caused the release of residual monomers and subsequent detachment of fillers, leading to an increase in surface roughness. Despite the lower average pH of coffee (5.39) compared to water (6.0), no significant difference in surface roughness values was observed between specimens according to whether they were immersed in water or coffee. Coffee was selected as the coloring solution for this study due to its rich content of chromogenic substances such as tannin and chlorogenic acid, compared to other beverages[34]. However, the presence of chlorogenic acid in it was not deemed relevant for inducing greater hydrolytic degradation in specimens immersed in coffee.

Although the results of the present research demonstrated that the effect of the polishing system on surface roughness was material-dependent, the literature reports that the lowest Ra values on the surface of composite resins are generally provided by aluminum oxide discs[12]. According to a systematic review, aluminum oxide was one of the most important components for achieving a smooth surface because it is harder than the filler particles in composite resins[19]. Otherwise, the polishing agent would only remove the soft composite resin matrix, leaving protruding filler particles on the surface[24].

In the current study, both polishing systems increased the surface roughness of the specimens to values greater than 0.2 μm, which would lead to the accumulation of biofilm on the restoration and could result in gingival inflammation, surface stains and secondary cavities[25]. Biofilm retention depends on several factors, not just surface roughness, and the surface of polished restorations must have a maximum roughness of 0.50 μm in order not to be perceptible to the patient[26]. Filtek Z350 XT (nanofilled) and Empress Direct (nanohybrid) resins achieved Ra values lower than this threshold, while Estelite Omega (suprananofilled) obtained comparable values when polished both with discs and with rubber tips. The finishing and polishing systems contributed noticeable smoothness.

Considering color stability, the null hypothesis – that the finishing and polishing systems would not significantly impact the color stability of composite resins with high aesthetic performance when subjected to chemical challenge - was rejected. Overall, the L parameter decreased after immersion in water or coffee, as expected for Estelite Omega resin (suprananofilled) immersed in water. The absorption of water or pigment leads to degradation of the organic matrix, as described above, and consequently a decrease in brightness. Increased water absorption results in low color stability due to the increase in the free volume of the polymer formed and greater space for water molecules to diffuse into the polymer network, thus contributing first to its degradation and then to discoloration[20]. Furthermore, color and light reflection are negatively affected by a rough surface. The lower the optical reflection, the rougher is the surface[27]. Thus, the lightness of the control group of Estelite Omega (suprananofilled), which did not undergo any type of finishing or polishing and, therefore, maintained the smoothness of the polyester strip, was not affected by immersion in water.

Considering the finishing and polishing systems, the use of multi-step aluminum oxide discs increased the L values for the Empress Direct (nanohybrid) and Estelite Omega (suprananofilled) resins. Some authors have reported that when surface roughness increases, the degree of random light reflection will increase, resulting in a decrease in brightness[27-29]. For the Filtek Z350 XT (nanofilled) resin immersed in both water and coffee, the highest ΔEab and ΔE00 were observed in the rubber-polished specimens. In Empress Direct (nanohybrid) resins immersed in water, the highest values of ΔEab and ΔE00 were in specimens polished with aluminum oxide discs.

However, in specimens immersed in coffee, there was no difference between polishing with rubber or aluminum discs. Given that the composition of the resin influences its optical properties, materials containing different types of monomers may present differences in color and translucency[30]. Polishing and finishing systems influence color, but this influence is material-dependent. The performance of the two tested finishing and polishing systems differed according to the resin used, making it impossible to conclude that one system, whether disc or rubber, outperforms the other. A single polishing system does not uniformly achieve the same surface quality for all composite resins. This variability is attributable not only to the quality of the polishes, but also to the interaction between the polisher and the composite resin[11].

Furthermore, there are additional considerations regarding the composition of the Empress (nanohybrid) and Estelite (suprananofilled) resins that may elucidate the results obtained in this study. One notable aspect is related to the quality of inorganic fillers. Modified strontium glass, present in Empress Direct, is known to match the index of refraction of the UDMA resin matrix, offering improved aesthetics. In contrast, composites with barium glass, commonly found in some materials, have a higher index of refraction than UDMA resin, resulting in reduced translucency and inferior aesthetics[31]. Additionally, composites with pure silica or quartz fillers tend to be more inert in water, while those containing radiopaque glasses may undergo greater dissolution in water and saline solutions[32]. Empress Direct (nanohybrid) resin, tested in this study, contains barium glass, which may explain its less stable performance in terms of color evaluation compared to Estelite Omega (suprananofilled), which contains silica and zirconia. Another crucial factor is the organic matrix. Estelite (suprananofilled) resin contains the TEGDMA monomer as a diluent. The presence of TEGDMA can enhance surface hardness, elastic modulus, and the degree of polymerization compared to BisEMA, which is present in Empress Direct (nanohybrid) resin. These factors collectively contribute to Estelite's superior resistance against coloring agents[33].

There are two main thresholds for evaluating color differences: the perceptibility threshold and the acceptability threshold[14]. These thresholds differ significantly, with reference values for the perceptibility threshold of ΔEab set at 1.2, and the acceptability threshold at 2.7[14]. For ΔE00, the perceptibility threshold is 0.8, and the acceptability threshold is 1.814. In this study, the Estelite Omega composite resin (suprananofilled), after being polished and immersed in water for all groups, maintained acceptability ΔEab values below 2.7 but presented perceptibility values above 1.2. In other words, although the changes were noticeable to the human eye, they were considered acceptable. The other resins in the study exhibited perceptibility and acceptability values above the reference thresholds.

Other authors have reported that among the composites finished with different systems and immersed in various coloring solutions (coffee, red wine, glue), the surfaces that showed the greatest color change were observed in the groups treated with the polyester strip[28]. They suggest that the surface layer, where the organic matrix is predominant, should be removed to have less influence on color stability. Similarly, in the current study, the unpolished control groups finished with the polyester strip achieved the highest values of ΔEab and ΔE00 after immersion in coffee, indicating greater color change.

CONCLUSION

It can be concluded that the finishing and polishing systems had an impact on the surface roughness and color stability of all composite resins with high aesthetic performance, and their effectiveness differed according to the type of composite resin. Estelite Omega (suprananofilled) demonstrated the highest color stability after immersion in either water or coffee, while Filtek Z350 XT (nanofilled) resin surface roughness increased after immersion in water or coffee.

DECLARATION OF CONFLICTING INTERESTS

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

FUNDING

None.

REFERENCES

1. Ferracane JL. Composite resin--state of the art. Dent Mater. 2011;27(1):29-38. https://doi.org/10.1016/j.dental.2010.10.020
2. Kılıç V, Gök A. Effect of different polishing systems on the surface roughness of various bulk-fill and nano-filled resin-based composites: An atomic force microscopy and scanning electron microscopy study. Microsc Res Tech. 2021;84(9):2058-2067. https://doi.org/10.1002/jemt.23761
3. Jung M, Eichelberger K, Klimek J. Surface geometry of four nanofiller and one hybrid composite after One-step and multiple-step Polishing. Oper Dent. 2007;32(4):347-55. https://doi.org/10.2341/06-101
4. Guo X, Yu Y, Gao S, Zhang Z, Zhao H. Biodegradation of Dental Resin-Based Composite-A Potential Factor Affecting the Bonding Effect: A Narrative Review. Biomedicines. 2022;10(9):2313. https://doi.org/10.3390/biomedicines10092313
5. Yanikian C, Yanikian F, Sundfeld D, Lins R, Martins L. Direct Composite resin Veneers in Nonvital Teeth: A Still Viable Alternative to Mask Dark Substrates. Oper Dent. 2019;44(4):E159-E166. https://doi.org/10.2341/18-220-T
6. Demarco FF, Collares K, Coelho-de-Souza FH, Correa MB, Cenci MS, Moraes RR, Opdam NJM. Anterior composite restorations: A systematic review on long-term survival and reasons for failure. Dent Mater. 2015;31(10):1214-24. https://doi.org/10.1016/j.dental.2015.07.005
7. Egilmez F, Ergun G, Cekic-Nagas I, Vallittu PK, Lassila LVJ. Short- and long-term effects of additional post curing and polishing systems on the color change of dental nano-composites. Dent Mater J. 2013;32(1):107-14. https://doi.org/10.4012/dmj.2012-251
8. Bansal K, Gupta S, Nikhil V, Jaiswal S, Jain A, Aggarwal N. Effect of Different Finishing and polishing Systems on the Surface Roughness of Composite resin and Enamel: An In vitro Profilometric and Scanning Electron Microscopy Study. Int J App Basic Med Res Int J Appl Basic Med Res. 2019;9(3):154-158.7. https://doi.org/ 10.4103/ijabmr.IJABMR_11_19
9. Attar N. The effect of finishing and polishing procedures on the surface roughness of composite resin materials. J Contemp Dent Pract. 2007;8(1):27-35
10. Senawongse P, Pongprueksa P. Surface Roughness of Nanofill and Nanohybrid Composite resins after Polishing and Brushing J Esthet Restor Dent 2007;19:26573. https://doi.org/10.1111/j.1708-8240.2007.00116.x
11. St-Pierre L, Martel C, Crepeau H, Vargas M. Influence of Polishing Systems on Surface Roughness of Composite Resins: Polishability of Composite Resins Operative Dentistry, 2019;44(3):122-132. https://doi.org/10.2341/17-140-L
12. Aytac F, Karaarslan EŞ, Agaccioglu M, tastan E, Buldur M, Kuyucy E. Effects of novel finishing and polishing systems on surface roughness and morphology of nanocomposites. J Esthet Restor Dent. 2016;28:247-261. https://doi.org/10.1111/jerd.12215
13. Nascimento HOD, Vieira Junior WF, Basting RT, Turssi CP, Amaral FLB, França FMG. Effect of different polishing systems and staining solution on surface roughness and color stability of bulk fill composite resins. Am J Dent. 2022;35(1):37-42.
14. Paravina RD, Ghinea R, Herrera LJ, Bona AD, Igiel C, Linninger M, et al. Color difference thresholds in dentistry. J Esthet Restor Dent. 2015;Suppl 1:S1-9. https://doi.org/10.1111/jerd.12149
15. Daud A, Adams AJ, Shawkat A, Gray G, Wilson NHF, Lynch CD, et al. Effects of toothbrushing on surface characteristics of microhybrid and nanofilled composite resins following different finishing and polishing procedures. J Dent. 2020;99:103376. https://doi.org/10.1016/j.jdent.2020.103376
16. Başeren M. Surface roughness of nanofill and nanohybrid composite resinand ormocer-based tooth-colored restorative materials after several finishing and polishing procedures. J Biomater Appl. 2004;19(2):121-34. https://doi.org/10.1177/0885328204044011
17. Kumari RV, Nagaraj H, Siddaraju K, Poluri RK. Evaluation of the Effect of Surface Polishing, Oral Beverages and Food Colorants on Color Stability and Surface Roughness of Nanocomposite Resins. J Int Oral Health. 2015;7(7):63-70.
18. Mitra SB, Wu D, Holmes BN. An application of nanotechnology in advanced dental materials. J Am Dent Assoc. 2003;134(10):1382-90. https://doi.org/ 10.14219/jada.archive.2003.0054
19. Giannini M, Di Francescantonio M, Pacheco RR, Boaro LCC, Braga RR. Characterization of water sorption, solubility, and roughness of silorane- and methacrylate-based composite resins. Oper Dent. 2014;39(3):264-72. https://doi.org/10.2341/12-526-L
20. Ferracane JL. Hygroscopic and hydrolytic effects in dental polymer networks. Dent Mater. 2006;22(3):211-22. https://doi.org/10.1016/j.dental.2005.05.005
21. da Silva EM, Gonçalves L, Guimarães JG, Poskus LT, Fellows CE. The diffusion kinetics of a nanofilled and a midfield composite resin immersed in distilled water, artificial saliva, and lactic acid. Clin Oral Investig. 2011;15(3):393-401. https://doi.org/10.1007/s00784-010-0392-z
22. Fonseca ASQS, Moreira ADL, de Albuquerque PAC, Menezes LR, Pfeifer CS, Scheneider LFJ. Effect of monomer type on the CC degree of conversion, water sorption and solubility, and color stability of model dental composites. Dent Mater. 2017;33:394-401. https://doi.org/10.1016/j.dental.2017.01.010
23. Güler AU, Güler E, Yücel AÇ, Ertan E. Effects of polishing procedures on color stability of composite resins. J Appl Oral Sci. 2009;17:108-112. https://doi.org/10.1590/s1678-77572009000200007
24. Avsar A, Yuzbasioglu E, Sarac D. The Effect of Finishing and polishing Techniques on the Surface Roughness and the Color of Nanocomposite resin Restorative Materials. Adv Clin Exp Med. 2015;24(5):881-890. https://doi.org/10.17219/acem/23971
25. Bollen CM, Lambrechts P, Quirynen M. Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: a review of the literature. Dent Mater. 1997;13(4):258-69. https://doi.org/10.1016/s0109-5641(97)80038-3
26. Jones CS, Billington RW, Pearson GJ. The in vivo perception of roughness of restorations. Br Dent J. 2004;196(1):42-45; discussion 31. https://doi.org/10.1038/sj.bdj.4810881
27. Yerliyurt K, Sarıkaya I. Color stability of hybrid ceramics

exposed to beverages in different combinations. BMC Oral Health. 2022;22(1):180. https://doi.org/10.1186/s12903-022-02206-1

28. Aydın N, Topçu FT, Karaoğlanoğlu S, Oktay EA, Erdemir U. Effect of finishing and polishing systems on the surface roughness and color change of composite resins. J Clin Exp Dent. 2021;13(5):e446-e454. https://doi.org/10.4317/jced.58011
29. Watanabe T, Miyazaki M, Takamizawa T, Kurokawa H, Rikuta A, Ando S. Influence of polishing duration on surface roughness of composite resins. J Oral Sci 2005;47(1):21–25. https://doi.org/10.2334/josnusd.47.21
30. Lehtinen J, Laurila T, Lassila LV, Vallittu PK, Raty K, Hernberg R. Optical characterization of bisphenol-A-glycidyldimethacrylate-triethyleneglycoldimethacrylate (BisGMA/TEGDMA) monomers and copolymer. Dent Mater. 2008;24(10):1324-1328. https://doi.org/10.1016/j.dental.2008.02.012
31. Mikhail SS, Schricker SR, Azer SS, Brantley WA, Johnston WM. Optical characteristics of contemporary dental composite resin materials. J Dent. 2013;41(9):771-778. https://doi.org/10.1016/j.jdent.2013.07.001
32. Söderholm KJ. Filler leachability during water storage of six composite materials. Scand J Dent Res. 1990;98(1):82-88. https://doi.org/10.1111/j.1600-0722.1990.tb00944.x
33. Ardu S, Duc O, Di Bella E, Krejci I, Daher R. Color stability of different composite resins after polishing. Odontology. 2018;106(3):328-333. https://doi.org/10.1007/s10266-018-0350-9
34. Alaqeel S. Effect of Grit-blasting on the Color Stability of Zirconia Ceramics Following Exposure to Beverages. Cureus. 2020;12(3):e7170. https://doi.org/10.7759/cureus.7170

https://doi.org/10.54589/aol.37/2/172

Primary tooth wear in children from different social environments

Silvina G Cortese, Lucia Babino, Ana M Biondi

Universidad de Buenos Aires, Facultad de Odontología, Cátedra Odontología Integral Niños, Argentina

ABSTRACT

Bilingual schools have more hours and high levels of academic demands. ***Aims:*** *To compare the degree of dental wear and frequency of severe wear facets between children from public rural schools (RG) and children from private bilingual schools in Buenos Aires City (PG). To compare the presence of facets to parents' reports on bruxism and their opinion on the importance to health of bruxism and snoring.* ***Materials and Method:*** *The sample (n=90) consisted of 5- and 10-year-old children. Their parents/guardians were asked to complete a structured questionnaire on bruxism and snoring. Children's degrees of dental wear on primary incisors, canines and molars were identified and recorded. The data were analyzed statistically.* ***Results:*** *The relative risk of wear between PG and RG was 1.82. Bruxism and snoring were reported by 22.9% of the parents/guardians of 5-year-olds and 8.8% of the parents/guardians of 10-year-olds. In 10-year-olds, significant differences were found between RG and PG for canine wear degree 3 (p=0.01).* ***Conclusions:*** *Children from highly demanding schools presented more dental wear. Higher frequency of severe dental wear was observed in primary canines and molars late in the tooth replacement period regardless of whether sleep bruxism was reported. Parents/guardians from different social conditions considered that bruxism and snoring are important to health to similar degrees.*

Keywords: *bruxism - children - dental wear*

Desgaste de piezas dentarias primarias en niños de diferente ámbito social

RESUMEN

Las escuelas bilingües tienen mayor carga horaria y altos niveles de exigencia académica. ***Objetivos:*** *Comparar en niños preescolares y escolares de escuela pública rural (GR) y de colegios privados bilingües de la Ciudad Autónoma de Buenos Aires (GP) el grado de desgaste dentario y la frecuencia de facetas de desgaste severo. Comparar la presencia de facetas con el reporte de los padres sobre el bruxismo y su opinión sobre la importancia de bruxar y roncar.* ***Materiales y Método:*** *Muestra (n=90) conformada con niños de 5 y 10 años, cuyos responsables completaron un cuestionario estructurado. Fueron registrados y analizados estadísticamente los grados de desgaste dentario en incisivos, caninos y molares primarios.* ***Resultados:*** *El riesgo relativo de desgaste entre GP y GR fue 1,82. El 22,9% de los responsables de los niños de 5 años y el 8,8% de los de 10 años reportaron que bruxan y roncan. En relación a la muestra de 10 años, se hallaron diferencias significativas para caninos desgaste grado 3 entre GR y GP (p=0.01).* ***Conclusiones:*** *Los niños de escuelas con alta exigencia presentaron más desgaste. Se observó mayor frecuencia de desgaste dentario severo en caninos y molares primarios al final del recambio dentario independiente al reporte de bruxismo nocturno. Los cuidadores de diferente condición social revelaron valoración semejante sobre la importancia en la salud del bruxismo y el ronquido.*

Palabras clave: *bruxismo - niños - desgaste dental*

To cite:

Cortese SG, Babino L, Biondi AM. Primary tooth wear in children from different social environments. Acta Odontol Latinoam. 2024 Aug 30;37(2):172-179. https://doi.org/10.54589/aol.37/2/172

Corresponding Author:
Silvina G Cortese
silvina.cortese@odontologia.uba.ar

Received: April 2024.
Accepted: June 2024.

INTRODUCTION

At different times over the years, and according to different specialties, bruxism has been considered a habit, a parafunction, and a parasomnia (according to sleep medicine). In 2013, an international group of experts published a consensus with the aim of proposing a definition and diagnostic classification system for bruxism which could be adopted by researchers and clinical professionals. They defined bruxism as a repetitive jaw-muscle activity characterized by clenching or grinding teeth and/or thrusting the mandible. They distinguished two different circadian manifestations: sleep and awake bruxism. With regard to diagnostic criteria, they established the terms "possible" bruxism when it is self-reported, "probable" when complemented by clinical findings, and "definitive" when confirmed by studies such as polysomnography and/or electromyography[1]. However, polysomnography and electromyography are expensive and invasive, and Berrozpe et al. consider them excessive for diagnosing parasomnias, which can usually be detected clinically[2]. In the same document, Lobbezoo et al. distinguish primary or idiopathic bruxism as being that which is not associated to medical comorbidities, and secondary bruxism when it is related to psychosocial or medical conditions such as breathing-related sleep disorders, neurological problems, psychiatric conditions and drug or medication use[1].

The multifactorial etiology of bruxism involves factors related to the central nervous system and possible influence of socioenvironmental factors.

Sleep and awake bruxism are currently considered to be different muscular activities. Sleep bruxism may be rhythmic or non-rhythmic. It should be noted that in healthy individuals it should not be considered disorder, although in some situations, bruxism may be a risk behavior, and in other situations a protective factor, mainly against sleep apneas. Individual diagnosis is therefore necessary[3].

Most studies on children report prevalence of sleep bruxism (SB) as 14 to 36.8%. However, and given the difficulties in diagnosing awake bruxism (AB), its prevalence has been estimated only among adults as 5 to 31%[4].

In a previous study on children of mean age 11 years, we found reports of 35.3% SB, 35.3% SB + AB, and 29.4% AB. Subjects with both types of bruxism had high emotional instability[5], which is a personality trait involving anxiety, a high degree of worry, and distorted perception of negative situations, causing changes in neurotransmitters such as dopamine and serotonin, which are the main causes of bruxism[6-7].

Esparza and Rodríguez studied a sample of 6- to 11-year-old children by applying the Reynolds & Kamphaus Multidimensional Behavior Assessment Scale, concluding that academic demand is a factor associated to the presence of states of anxiety. Serra Negra et al. found higher prevalence of sleep bruxism in children from better socioeconomic conditions[7-8].

With regard to wear, our findings in a previous study showed that primary tooth wear should be considered according to age and series. The presence of exposed dentin at early ages could be considered as an indicator of parafunction[9]. In a recent study on 48 children, Martins et al. concluded that those with more severe facets have possible sleep and awake bruxism[10].

The aims of this study were (a) to compare degree and frequency of severe wear facets in preschoolers and schoolchildren from a half-day public rural school to those in children from a full-day private bilingual school who visit private pediatric dentistry practices in Buenos Aires City, and (b) to compare the presence of facets with parent/guardian-reported bruxism and opinion on the importance to health of bruxism and snoring.

MATERIALS AND METHOD

This was a cross-sectional study. It was approved by the FOUBA Ethics Committee (009/2022-CETICA FOUBA).

A structured questionnaire was answered on a voluntary basis by parents/guardians of patients from private bilingual schools seeking care at two practices in Buenos Aires City (PG), and parents/guardians of children enrolled at a public rural school in Buenos Aires Province (RG). The questionnaire consisted of 3 items: reporting on bruxism, reporting on snoring, and providing an opinion on whether bruxism and snoring are important to the child's health. The questionnaire had been used previously Fridman et al. in a study on degree of wear in children´s teeth before and after completing the tooth replacement period[11] (Fig. 1).

The sample consisted of 5-year-olds whose first permanent molars had not yet erupted and 10-year-olds with mixed dentition, whose parents/guardians

Department of Children's Comprehensive Dentistry
5 years (first molars not erupted) and 10 years.

Sex: Age:

Child's family name and given name: .....

Phone number: .....

1) *Does the child make noise with teeth while asleep?*

Never ☐	1 to 3 nights/week ☐	4 to 7 nights/week ☐	Don't know ☐

2) *Does the child snore while asleep?*

Never ☐	1 to 3 nights/week ☐	4 to 7 nights/week ☐	Don't know ☐

3) *Do you think that either of the following are important to the child's health?*

	Yes	No	Don't know
Grinding teeth			
Snoring			

Tooth wear
Specify the highest degree of tooth wear observed in each of the following tooth groups, considering *0= no wear, 1= facet on enamel, 2= facet with exposed dentin, 3= facet in deep dentin.*

	Degree
Incisors	
Canines	
Molars	

Fig. 1: Form used for recording

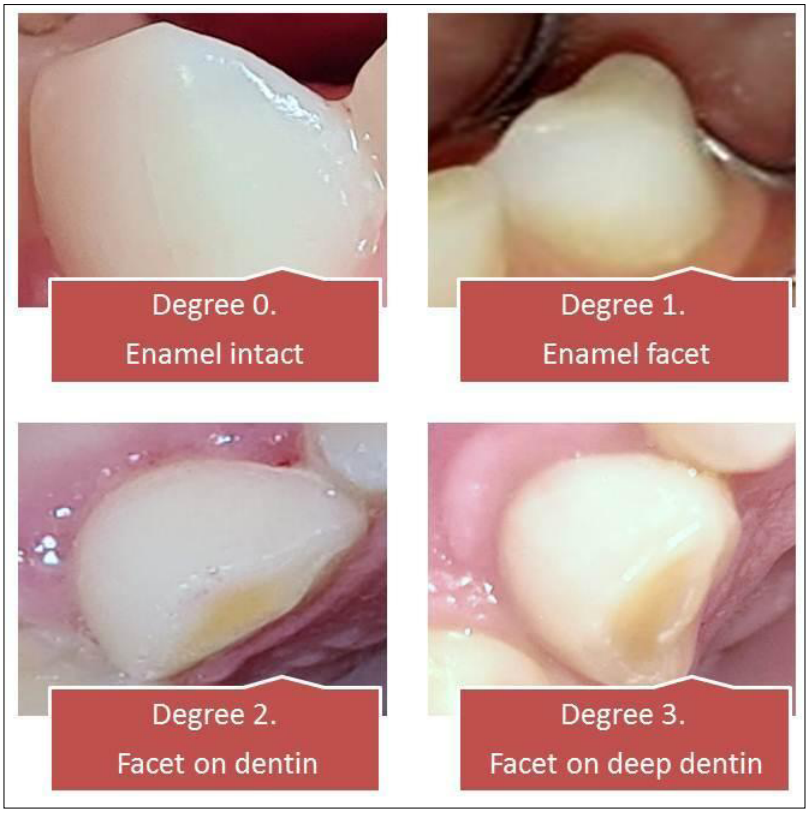

Fig. 2: Degrees of wear according to Smith and Knight's Index

provided consent. Data were collected from May to July 2023.

Three pediatric dentists, with Kappa concordance coefficient 0.92 for determining the Smith and Knight index, recorded degrees of wear for incisors, canines and primary molars in both groups for both 5-year-olds (PG-5 and RG-5) and 10-year-olds (PG-10 and RG-10) (Fig. 2). Any children with multiple caries that interfered with the evaluation of wear in any of the tooth groups, children medicated with neuroleptics, or medically compromised children were excluded.

Data were analyzed using R software (https://www.R-project.org/) and VGAM package.

Relative risk (RR) was used to compare the presence of wear between PG and RG. The degree of wear for each tooth group (incisors, canines and molars) was compared between PG and RG for each age using ordinal logistic regression with proportional odds.

Categorical variables were compared using the chi square test when at least 80% of the cells had an expected value greater than 5 and all of them had an expected value of at least 1.

RESULTS

Samples PG-5 and PG-10 consisted of 26 and 24 children, respectively, while RG-5 and RG-10 comprised 20 children each.

- The RR for presence of wear between PG and RG was 1.82.
  - For incisors, there were significant differences in degree of wear between PG-5 and RG-5 ($p = 0.002$). In PG-5, all teeth had wear degree 1 or 2, while in RG-5, there was predominance of teeth without wear or teeth with worn enamel only (Table 1).

Table 1. Percentages of different degrees of incisor wear in both groups.

			Incisor wear			
Age	Group	N	G0 %	G1 %	G2 %	G3 %
5 years **(p= 0.02)**	PG	26	0	65.4	34.6	0
	RG	20	45	50	0	5

  - For canines, there were significant differences at both ages ($p < 0.001$), with lower degrees of wear in RG (Table 2).

Table 2. Percentages of different degrees of canine wear in both groups at ages 5 and 10 years.

			Canine wear			
Age	Group	N	G0 %	G1 %	G2 %	G3 %
5 years **(p < 0.001)**	PG	26	7.69	34.6	57.7	0
	RG	20	50	50	0	0
10 years **(p < 0.001)**	PG	24	0	8.70	60.9	30.4
	RG	20	50	10	40	0

  - For molars, no significant difference was found in degree of wear between RG-5 and PG-5 ($p = 0.1863$). In both groups, there was predominance of unworn teeth, followed by teeth with wear degree 1. At age 10 years, the differences were significant ($p = 0.004$), with predominance of teeth with wear degree 1 in PG-10, and unworn teeth in RG-10 (Table 3).
- Among parents/guardians, 22.9% reported that 5-year-olds presented bruxism and snoring, 8.8% reported that 10-year-olds did so, and 10.4% said they did not know.
- There was no difference in reported grinding between groups with or without tooth wear in RG at both ages (RG-5 $p = 0.068$, RG-10 $p = 0.582$). It was not possible to analyze this in PG because all the children presented facets. Fig. 3, 4, 5 and 6 show the association between wear recorded by dentists and tooth grinding reported by parents/guardians in the four subgroups.
- There was no difference between PG and RG regarding the importance assigned by parents/guardians to bruxism ($p = 0.58$) or snoring ($p = 0.68$).
- The frequencies of degree 3 facets were: 30.4%

Table 3. Percentages of different degrees of molar wear in both groups at ages 5 and 10 years.

			Molar wear			
Age	Group	N	G0 %	G1 %	G2 %	G3 %
5 years ($p = 0.1863$)	PG	26	50	42.3	7.69	0
	RG	20	70	25	5	0
10 years **(p = 0.004)**	PG	24	9.09	54.5	27.3	9.09
	RG	20	52.9	35.3	11.8	0

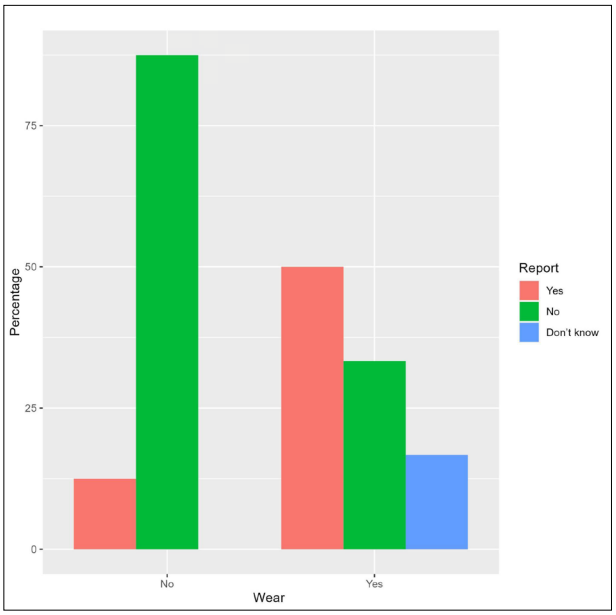

Fig. 3: Comparison of tooth grinding reported by parents/ guardians of rural school 5-year-olds with and without wear

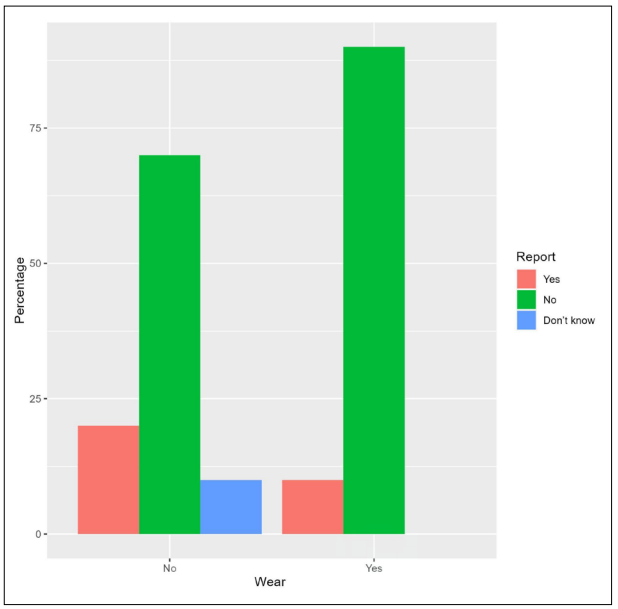

Fig. 5: Comparison of tooth grinding reported by parents/ guardians of rural school 10-year-olds with and without wear

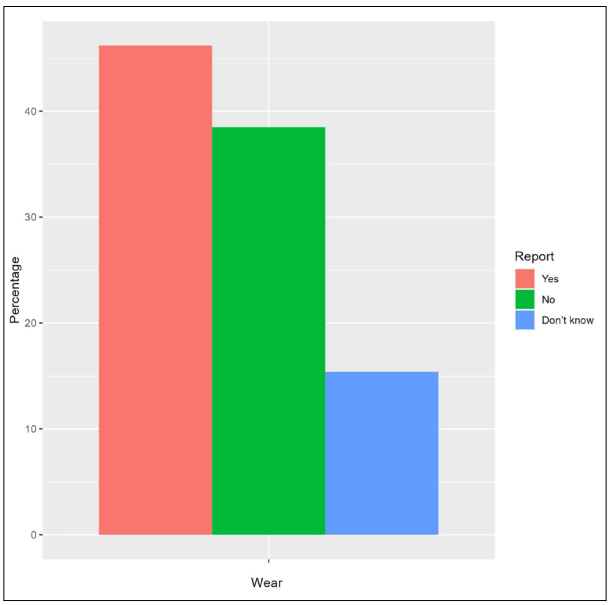

Fig. 4: Tooth grinding reported by parents of bilingual school 5-year-olds with wear. In this group, all the children presented wear.

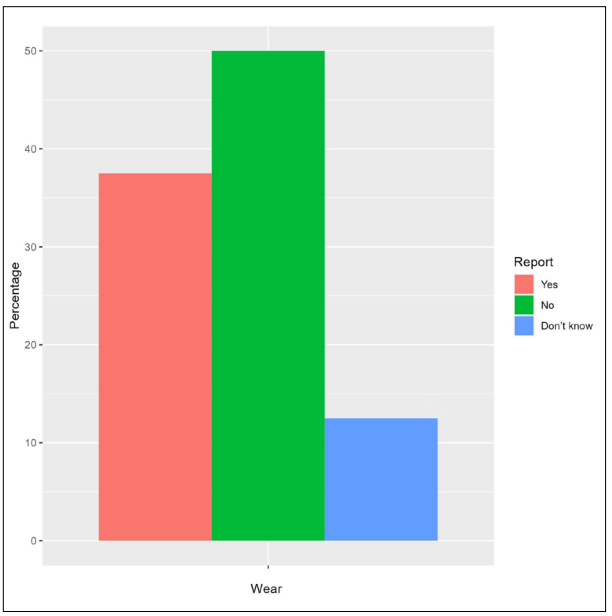

Fig. 6: Tooth grinding reported by parents of bilingual school 10-year-olds with wear. In this group, all the children presented wear.

for canines and 9.1% for molars in PG-10, and 5% for incisors in RG-5. No severe wear was recorded in the other groups. Fisher's test showed significant differences between RG-10 and PG-10 for canines (p=0.01), and non-significant differences between RG-5 and PG-5 for incisors (p=0.43), and between RG-10 and PG-10 for molars (p=0.50).

DISCUSSION

The ages included in the sample were selected because preschoolers (5-year-olds) are frequently reported on, and 10-year-olds have mixed dentition, so it is still possible to evaluate present primary teeth. The decision to compare children according to schooling type arose from the marked socioenvironmental differences observed between sites located less than 100 kilometers away from each other. The rural schoolchildren live in the district Exaltación

de la Cruz, Buenos Aires Province, where over 95% of the population consists of rural workers. In contrast, the children from private bilingual schools have long days at highly academically demanding schools, urban family habits and health insurance.

Bulanda et al. emphasize that socioeconomic and cultural features may be associated with the onset of sleep bruxism, which occurs more frequently in children from families with higher socioeconomic level. This might be related to the greater number of duties and demands these children have compared to children from poor environments. It is consistent with the findings on dental wear in the current study[12].

Different authors consider that SB is related to stress, anxiety, and behavioral and personality disorders, among others, as a result of serotonin and dopamine release which increases brain activity, heart rate and muscle tone, thereby affecting sleep quality. There is also an association between obstructive sleep apnea and bruxism, and it is currently believed that bruxism may be a protective factor that maintains airway patency, which is why snoring has been investigated[12].

Awake bruxism is associated to the inability to express emotions and to states of anxiety[13]. The current study observed significant differences in primary tooth wear between PG and RG. In PG, all children presented at least one facet at age 5 years. Our results found that the major difference was in canines, in agreement with Soares et al., who report canines as being the teeth most highly affected[14].

Adult self-reporting is considered to differ from parental reporting on sleep bruxism, and parents' reports may not always be accurate since they often sleep at a distance from the children and do not hear them. Nevertheless, in the current study, the number of "don't know" answers were low, although reports were not consistent with presence of facets[3].

The use of a questionnaire plus clinical examination is consistent with current recommendations. Research thus has a multifactorial approach, providing greater consistency, thereby identifying useful information for preventing consequences[15].

In the sample used in the current study, children from academically demanding schools had higher degrees of wear and greater frequency of severe wear in primary canines and molars late in the tooth replacement period. These values were independent of reported sleep bruxism. Parents/guardians from different social conditions evaluated the importance of bruxism and snoring to health similarly.

ACKNOWLEDGMENTS

The authors thank Dr. Gladys Banegas for collecting data from the rural school.

REFERENCES

1. Lobbezoo F, Ahlberg J, Glaros AG, Kato T, Koyano K, Lavigne GJ, de Leeuw R, Manfredini D, Svensson P, Winocur E.J Bruxism defined and graded: an international consensus. Oral Rehabil. 2013 Jan;40(1):2-4. https://doi.org/10.1111/joor.12011
2. Berrozpe EC, Folgueira A, Gonzalez Cardozo A, Ponce de León M, Valiensi SM. Polisomnografía nocturna y test múltiple de latencias del sueño. Nociones básicas e indicaciones. Guía práctica. Grupo de sueño – Sociedad Neurológica Argentina. Neurolarg.2023;15(2):108–115 https://doi.org/10.1016/j.neuarg.2012.09.003
3. Restrepo C, Manfredini D, Castrillon E, Svensson P, Santamaria A, Alvarez C, Manrique R, Lobbezoo F. Diagnostic accuracy of the use of parental-reported sleep bruxism in a poly somnographic study in children. Int J Paediatr Dent. 2017 Sep;27(5):318-325 https://doi.org/10.1111/ipd.12262
4. Wetselaar P, Vermaire EJH, Lobbezoo F, Schuller AA. The prevalence of awake bruxism and sleep bruxism in the Dutch adult population. J Oral Rehabil. 2019; 46:617–623. https://doi.org/10.1111/joor.12787
5. Cortese S G, Guitelman I, Biondi, A M. Cortisol salival en niños con y sin bruxismo. Rev Odontopediatr Latinoam. [Internet]. 2021; 9(1). https://doi.org/10.47990/alop.v9i1.163
6. Barbaranelli C, Caprara GV, Rabasca A. Cuestionario "Big Five" de personalidad para niños y adolescentes. Manual. TEA Ediciones, Madrid. 2006
7. Esparza, N, Rodríguez, M C. Factores contextúales del desarrollo infantil y su relación con los estados de ansiedad y depresión. Diversitas: Perspectivas en Psicología, 2009 5(1), 47-64. http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S1794-99982009000100005&lng=en&tlng=es.
8. Serra-Negra JM, Paiva SM, Seabra AP, et al. Prevalence of sleep bruxism in a group of Brazilian schoolchildren. Eur Arch Paediatr Dent. 2010 Aug;11(4):192-5. https://doi.org/10.1007/BF03262743
9. Cortese, SG. Biondi A M. Oliver L M. Desgaste Incisal y Oclusal como Indicador de Patología parafuncional en Dentición Primaria. Bol. Asoc. Argent. Odontol. Niños,

2005 34(4): 10-13
10. Martins IM, Alonso LS, Vale MP, Abreu LG, Serra-Negra JM. Association between the severity of possible sleep bruxism and possible awake bruxism and attrition tooth wear facets in children and adolescents. Cranio. 2022 Jul 25:1-7. https://doi.org/10.1080/08869634.2022.2102708
11. Fridman DE, Biondi AM, Cortese SG. Grado de desgaste dentario en piezas primarias antes y al finalizar el recambio. LV Reunión anual de la Sociedad Argentina de Investigación Odontológica. Buenos Aires, Argentina, 2022. Disponible en: https://saio.org.ar/wp-content/uploads/2022/12/LibroRRAASAIO2022_v3.pdf
12. Bulanda S, Ilczuk-Rypuła, D, Nitecka-Buchta A et al. Sleep Bruxism in Children: Etiology, Diagnosis and Treatment—A Literature Review. Int. J. Environ. Res. Public Health 2021, 18, 9544. https://doi.org/10.3390/ijerph18189544
13. Poojary B, Kanathila H, Pangi, A, Doddamani M. Diagnosis and treatment of bruxism: Concepts from past to present. International Journal of Applied Dental Sciences, 2018 4(1), 290–295. https://www.oraljournal.com/pdf/2018/vol4issue1/PartE/4-1-44-680.pdf
14. Soares JP, Moro J, Massignan C, Cardoso M, Serra-Negra JM, Maia LC, Bolan M. Prevalence of clinical signs and symptoms of the masticatory system and their associations in children with sleep bruxism: A systematic review and meta-analysis. Sleep Med Rev. 2021 Jun;57:101468. https://doi.org/10.1016/j.smrv.2021.101468
15. Lobbezoo F, Ahlberg J, Raphael KG, Wetselaar P, Glaros AG, Kato T, Santiago V, Winocur E, De Laat A, De Leeuw R, Koyano K, Lavigne GJ, Svensson P, Manfredini D. International consensus on the assessment of bruxism: Report of a work in progress. J Oral Rehabil. 2018 Nov;45(11):837-844. https://doi.org/10.1111/joor.12663

https://doi.org/10.54589/aol.37/2/179

Microtomographic and histological evaluation of two bioceramics as pulp capping agents *in vivo*.

Diana Torres-Flamenco[1], Reneé Jiménez-Castellanos[1], Lucía Pérez-Sánchez[2], Monserrat Llaguno-Munive[3], Patricia González-Alva[2], Febe Carolina Vázquez-Vázquez[4], Marine Ortiz-Magdaleno[5], Janeth Serrano-Bello[2]

1. *Departamento de Endodoncia, División de Estudios de Posgrado e Investigación, Facultad de Odontología, Universidad Nacional Autónoma de México.*
2. *Laboratorio de Bioingeniería de Tejidos, División de Estudios de Posgrado e Investigación, Facultad de Odontología, Universidad Nacional Autónoma de México.*
3. *Laboratorio de Física Médica, Subdirección de Investigación Básica, Instituto Nacional de Cancerología.*
4. *Laboratorio de Investigación de Materiales Dentales y Biomateriales, División de Estudios de Posgrado e Investigación, Facultad de Odontología, Universidad Nacional Autónoma de México.*
5. *Facultad de Estomatología, Universidad Autónoma de San Luis Potosí, México.*

ABSTRACT

Maintaining pulp vitality and function is a priority of the medicaments employed in pulp therapy to preserve tooth integrity. ***Aim:*** *This study evaluated inflammatory response and reparative dentin bridge formation after direct pulp capping with two different bioceramics.* ***Materials and Method:*** *This was an in vivo controlled experimental study on 12 male Wistar rats. Pulpotomies were performed and the exposed pulps were capped with Biodentine or Neo MTA. After 15, 45 and 90 days, maxillary segments were obtained and prepared for histologic analysis and Micro-CT. Hounsfield Units (HU) were quantified.* ***Results:*** *Micro-CT analysis showed greater mineralization at 90 days with Neo MTA than with Biodentine. HU did not differ significantly ($p > 0.05$) between molars treated with Biodentine and Neo MTA at 15 and 45 days, but at 90 days, there was statistically significant difference ($p < 0.05$) between them. Reparative dentin was observed near the pulp exposure and canal orifice with both bioceramics. At 45 and 90 days, molars treated with Neo MTA showed mineralized tissue filling the canal orifice. Molars treated with Biodentine showed mineralized tissue and dentin bridge at the site of exposure at 45 days, and total pulp exposure coverage and mineralized dentin matrix at 90 days.* ***Conclusions:*** *Biodentine and Neo MTA induce the formation of reparative dentin bridge after 45 days with inflammatory cell infiltrate.*

Keywords: *endodontic repair materials - bioceramics - cellular inflammatory infiltrate - reparative dentin*

To cite:

Torres-Flamenco D, Jiménez-Castellanos R, Pérez-Sánchez L, Llaguno-Munive M, González-Alva P, Vázquez-Vázquez FC, Ortiz-Magdaleno M, Serrano-Bello J. Microtomographic and histological evaluation of two bioceramics as pulp capping agents *in vivo*. Acta Odontol Latinoam. 2024 Aug 30;37(2):179-187. https://doi.org/10.54589/aol.37/2/179

Corresponding Author:
Janeth Serrano-Bello
janserbello@fo.odonto.unam.mx

Received: April 2024.
Accepted: July 2024.

Evaluación microtomográfica e histológica de dos agentes biocerámicos como recubrimiento pulpar *in vivo*

RESUMEN

Mantener la vitalidad pulpar y su función es una de las prioridades de los medicamentos utilizados en la terapia pulpar con la finalidad de preservar la integridad del diente. ***Objetivo:*** *El objetivo de este estudio fue evaluar la respuesta inflamatoria y la reparación del puente dentinario con dos biocerámicas.* ***Materiales y Métodos****: Se realizó un estudio experimental in vivo en 12 ratas Wistar formando dos grupos de estudio (n = 6), en las que se realizaron pulpotomías. Posterior a 15, 45 y 90 días, se obtuvieron segmentos de los maxilares y se prepararon los especímenes para análisis histológicos y cortes microtomográficos. Las Unidades Hounsfield (UH) se cuantificaron.* ***Resultados:*** *El análisis microtomográfico mostró un incremento en la mineralización después de 90 días con Neo MTA comparado con Biodentine. No existió diferencia significativa ($p > 0.05$) entre las UH posterior a 15 y 45 días, sin embargo, a los 90 días hubo diferencia significativa ($p < 0.05$) entre Biodentine y Neo MTA. A los 45 y 90 días los molares tratados con Neo MTA mostraron la formación de tejido mineralizado en el orificio comunicados. Los molares tratados con Biodentine mostraron la formacion de tejido mineralizado, a los 45 dias se observó un puente de dentina en el sitio expuestos y una cobertura total de la exposición pulpar y una matriz de dentina mineralizada a los 90 días.* ***Conclusiones:*** *Biodentine y Neo MTA inducen la formación del puente dentinario reparador posterior a 45 días con infiltrado de células inflamatorias.*

Palabras clave: *materiales de obturación endodóntica - biocerámicos - infiltrado celular inflamatorio - dentina de reparación*

INTRODUCTION

The purpose of pulp therapy is to repair the pulp tissue to preserve its vitality and function after it has been damaged[1]. The treatment consists of removing the inflamed or damaged pulp tissue and sealing the pulp cavity with biocompatible bioactive materials and dental tissues to promote the formation of reparative dentin or dentinal bridge, thereby preserving pulp viability[2].

Treatments to promote dentin bridge formation can still be challenging. The dentin bridge is considered the histological indicator of a subsequent healing process after exposure of the pulp tissue. It should seal the pulp tissue to protect it and prevent microleakage, thereby preventing recurrent infections[1].

Many different materials with appropriate physicochemical and biological properties are used to repair deciduous and permanent teeth. Materials based on calcium hydroxide (CH), such as mineral trioxide aggregate (MTA Plus (Prevest DentPro) and Biodentine (Septodont Ltd)), are considered regenerative. These cements can increase calcium ion concentration, aiding hard-tissue formation. CH has antimicrobial activity and promotes hard-tissue formation, but is highly soluble, has poor sealing ability and lacks adhesion. MTA has good sealing properties and biocompatibility, but becomes discolored, has long setting times and is expensive[3]. MTA-based cement and Biodentine have demonstrated high clinical success rates in treatments which would formerly have had reserved prognoses, such as root resorption, perforation, and direct pulp capping[4]. MTA Plus (Avalon Biomed Inc.) is similar in composition to the original MTA, but has been replaced by Neo MTA Plus, which contains tantalum oxide instead of bismuth oxide as a radiopaque agent, and an excellent inorganic powder of tricalcium and dicalcium silicate, which, when mixed with the water-based gel, initiates the setting reaction[5].

Biodentine, a calcium silicate-based dentin substitute, has a liquid phase and a powder phase. The powder contains tricalcium silicate, calcium carbonate and zirconium oxide, while the liquid contains water, calcium chloride as a setting accelerator, and a modified polycarboxylate[6].

Advances in restorative materials should promote the development of specific therapies and materials designed to regenerate dental pulp, even in complex clinical situations[7]. There is thus a need to evaluate the therapeutic potential of newly available bioactive materials by verifying and assessing their potential to promote tertiary dentin formation and tissue repair and regeneration. However, there is no consensus regarding how long after the application of Biodentine and Neo MTA to observe dentin bridging and inflammatory response.

This study evaluated the inflammatory response and reparative dentin bridge formation in an animal model 15, 45, and 90 days after direct pulp capping with two different bioceramics.

MATERIALS AND METHOD

The study design was approved by the Internal Committee for the Care and Use of Laboratory Animals of the Faculty of Dentistry of the National Autonomous University of Mexico (027-CIC-2019) and followed the parameters established in the Official Mexican Standard NOM-062-ZOO-1999[8].

Pulp capping procedure

Twelve male Wistar rats (weighing 240-300 g) were used for direct pulp capping experiments. Rats were sedated with an intramuscular injection of Ketamine (ANESKET®) (80 mg/Kg) and Xylazine (PROCIN®) (10 mg/Kg).

After cleaning and disinfecting the right and left maxillary first molars with 0.2% chlorhexidine digluconate solution for one minute, a class-I cavity with pulp exposure was prepared on the occlusal surface of each, using a steel round bur (FG1/4 MDT®) with a low-speed electric micromotor (NSK Surgic AP), under constant irrigation with sterile saline solution. One experienced operator performed all the procedures to establish a stable, standard-sized cavity (approximately 0.4 mm diameter and depth). Drilling time for each sample was about 10 seconds (Fig. 1a-b). Bleeding of the pulp communication was controlled by applying light pressure with sterile cotton pellets for a few seconds.

The exposed pulp was directly capped with Neo MTA (NuSmile, Ltd.) on the right maxillary first molars, and Biodentine (Septodont) on the left maxillary first molars, following the manufacturers' protocols. After direct pulp capping, dental etching was performed with Scotchbond™ Etchant Phosphoric Acid (3M ESPE) for 15 seconds. Single Bond Universal (3M™ ESPE) was applied as directed by

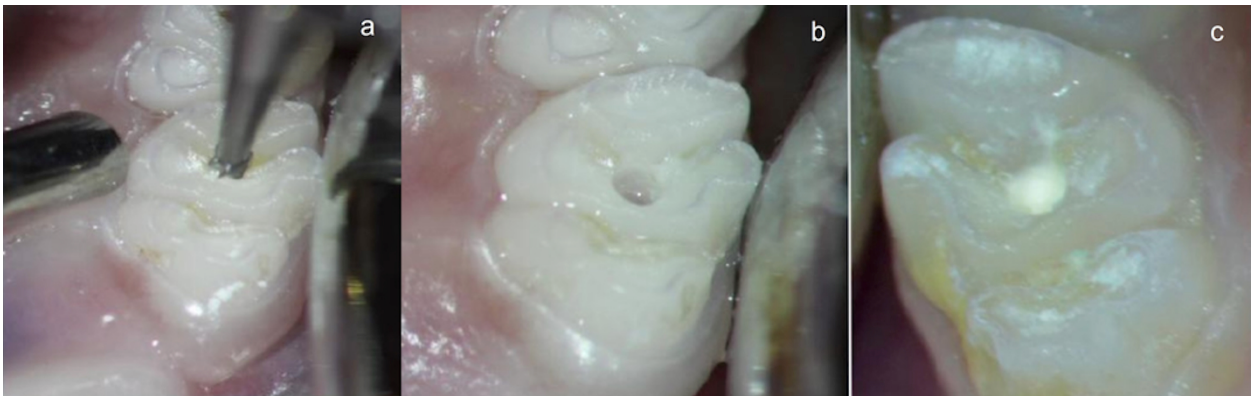

Fig. 1: Vital pulp therapy in a rat molar. ***a)*** *Preparation of the class-I cavity on the occlusal surface,* ***b)*** *Pulp communication,* ***c)*** *Placement of materials as direct pulp capping and resin filling.*

the manufacturer, and the cavity was restored with Filtek Flow Z350 XT (3M ESPE) (Fig. 1c).

After the restorations were completed, all the rats were evaluated clinically and weighed, and it was found that there was an increase in volume, bleeding, fistulization, and dental mobility. The rats were divided into three groups (n = 4) and euthanized at 15, 45 or 90 days after direct pulp capping.

Micro-CT Analyses

Micro-CT images were used to verify the presence of the bioceramic materials in the region of the pulp chamber, and the sealing they provided in the coronal portion of the molars, while the animals were under inhalation anesthesia with Isoflurane at a concentration of 2/100 oxygen. The images were acquired with a micro-CT scanner (Albira ARS II PET/CT). The field of view was the maxillary area, with current 0.4 mA, voltage 45 kV, and 1,000 projections to obtain high-resolution images (CT-High-Resolution). Hounsfield Units (HU) were calculated by tracing a circular ROI of 60 mm^2 on each micro-CT image on the axial plane, using the OsiriX MD DICOM Viewer program in the cavity area per period. Two-way ANOVA with Sidak's multiple comparisons test was performed with a p-value of 0.005. GraphPad Prism 9.4.1 was used.

Histological evaluation

After the established periods, the molars were dissected and fixed in 10% formaldehyde for 24 h at 4 °C. Then, they were demineralized in Evans and Krajian solution for 5 days at 4 °C. Finally, they were dehydrated in a series of alcohol solutions ranging from 50%, 60%, 70%, 80%, 90%, (10 minutes each) to 100% (three 15-minute changes) and embedded in paraffin. Sagittal sections (7-µm thick) were cut and stained with hematoxylin and eosin and evaluated at different magnifications.

RESULTS

Micro-CT analysis of dentin formation

No abnormal radio-dense region indicating the development of a periapical lesion was observed in any of the micro-CT images. The micro-CT images showed similar behavior at 15 and 45 days in the molars treated with Neo MTA (Fig. 2a-b) and Biodentine (Figure 2d-e). At 90 days, molars treated with Neo MTA (Fig. 2c) had greater mineralization than those treated with Biodentine (Fig. 2f).

The HU analysis demonstrated similar behavior at 15 and 45 days in the molars treated with Neo MTA and Biodentine, and a statistically significant difference at 90 days ($p < 0.05$), when HU was higher in molars treated with Neo MTA than in those treated with Biodentine (Fig. 3). In molars treated with Neo MTA, HU increased from an average of 18173.3 ±337.7 on day 15, to 19735.4 ±1588.5 on day 45, and 2209.4 ±2709.6 HU on day 90. In molars treated with Biodentine, HU increased from 19313.9 ±1133.7 on day 15, to 20168.0 ±623.8 on day 45, and decreased to 19450.1 ±2952.1 on day 90 with no statistical difference compared to day 45. Likewise, there were no statistical differences ($p > 0.05$) between molars treated with Neo MTA and those treated with Biodentine at 0,15, 45 and 90 days (Fig. 3).

Morphological findings

At 15 days after pulp capping, hard tissue formation

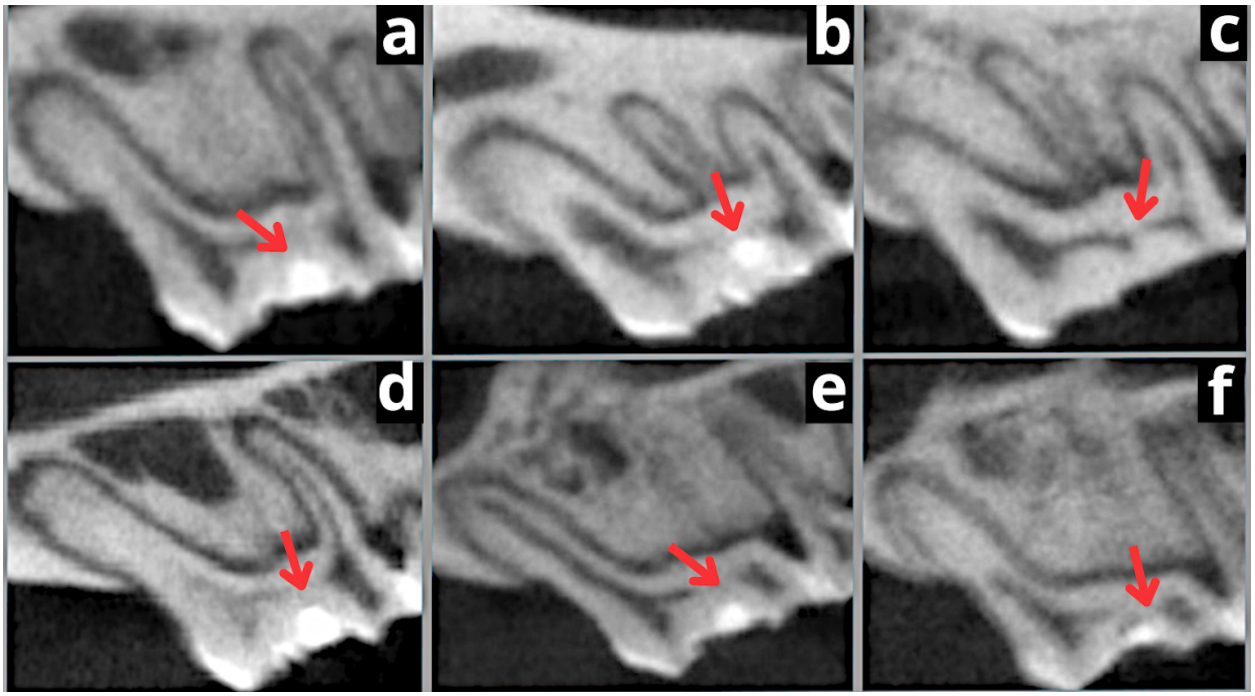

*Fig. 2: Micro-CT images of pulp-capped rat molars with Biodentine after 15 (**a**), 45 (**b**) and 90 (**c**) days. arrows indicate communication and radio-dense areas. images with Neomta after 15 (**d**), 45 (**e**) and 90 (**f**) days. Arrows indicate dental bridge formation.*

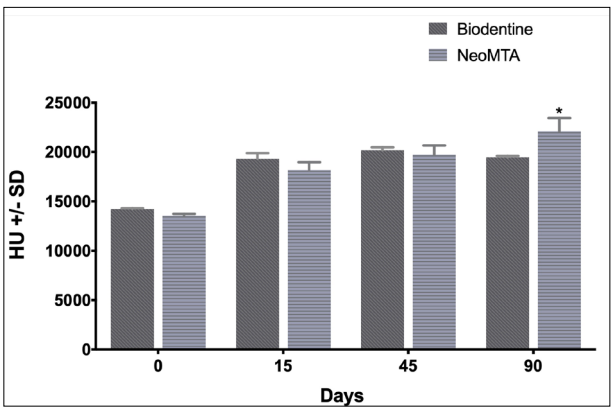

Fig. 3: Average HU values with Biodentine and NeoMTA.

and reparative dentin were observed near the pulp exposure site and adjacent to the existing dentine in molars treated with Biodentine and Neo MTA (Fig. 4a-f and 5a-f, respectively).

At 15 days after treatment, an inflammatory infiltrate with polymorphonuclear cells, and macrophages were observed in both groups. After 45 and 90 days, both groups displayed chronic inflammatory-cell infiltration of the coronal pulp near the border of the newly formed dentin. However, the pulp tissue in other areas, such as the pulp horns, was similar to normal pulp tissue, and there was no sign of pulp necrosis. Molars treated with Biodentine exhibited less inflammation and the best pulp-tissue reaction. Both groups showed incremental lines of dentin throughout the experiment.

At 45 and 90 days, both groups exhibited reparative dentin formation. However, the quality of the reparative dentin was better witeh Biodentine than with Neo MTA.

With Biodentine, the histological results showed that after 15 days, the dentinal bridge of reparative dentin calcified above the pulp tissue. The border between the wound of the pulp chamber and the dentin was covered in incremental lines of reparative dentin (Figure 4a-b), and the pulp tissue under the newly formed tissue below the cavity floor consisted of odontoblast-like cells adjacent to the reparative dentin made of incremental lines of mineralized tissue and possible cell inclusions. The pulp area showed few inflammatory cells (Fig. 4b).

At 45 days after pulp capping, the area of the cavity was visible (Fig. 4c-d), there was an area of irregular mineralized tissue above the pulp wound area, and the pulp area had a rich cell zone of odontoblast-like cells with a degenerative area at the border of the dentin (arrowhead) with congested capillaries, and a mild-to-severe inflammatory reaction above vital pulp tissue. The pulp tissue contained elongated, columnar odontoblast-like cells. Regarding morphology, the cell bodies extending from the

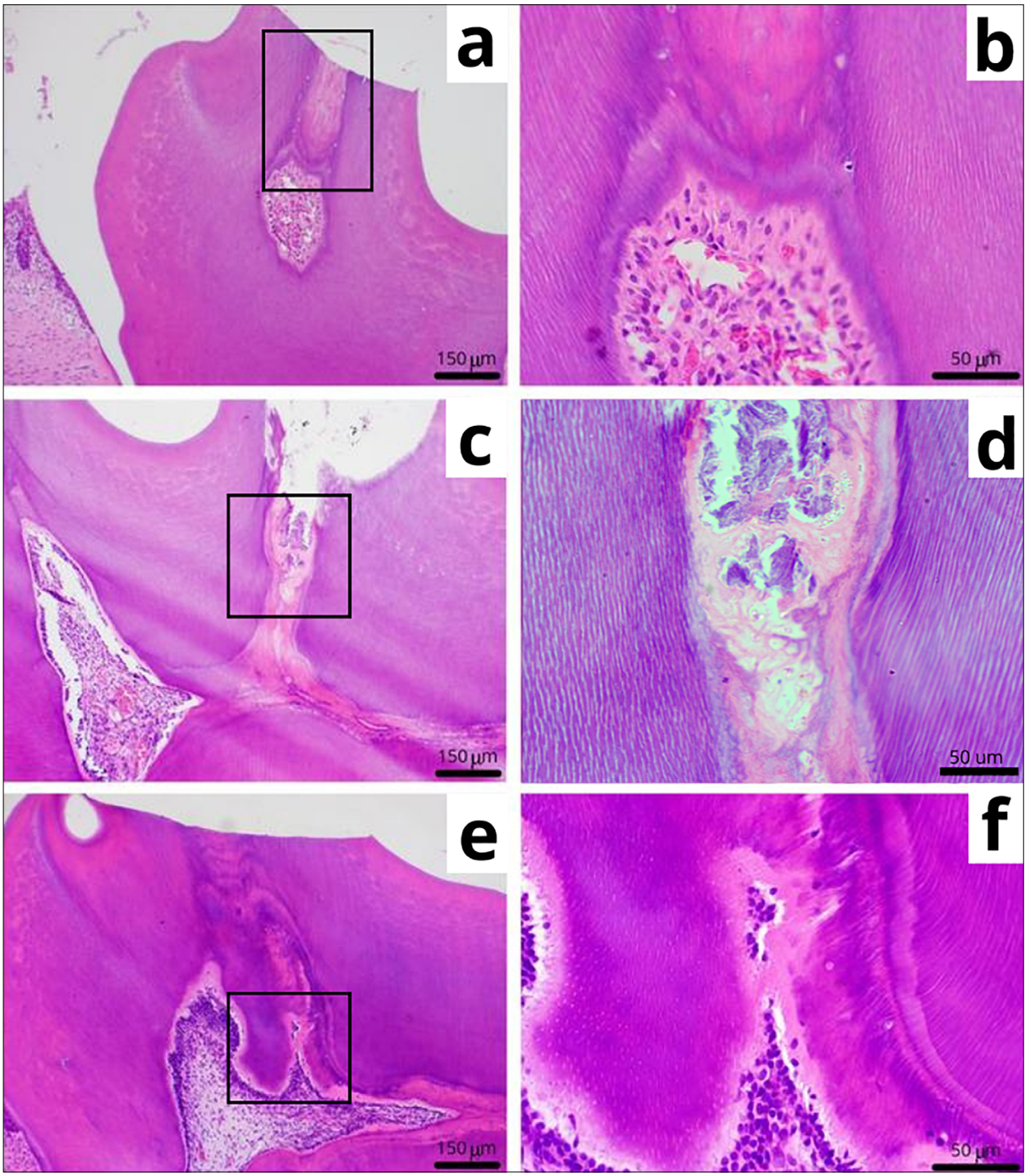

*Fig. 4: Histological features of pulpotomy with Biodentine. The boxes indicate the subsequent magnification (x20 and x40) after 15 days (**a-b**), 45 days (**c-d**) and 90 days (**e-f**). Magnification bar = 150 µm, 50 µm.*

pulp tissue to the dentin-like tissue contained large nuclei, and there were small capillary blood cells, and fibroblasts surrounded by collagen fibers.

At 90 days after direct pulp capping (Fig. 4e-f), there was a mineralized matrix of dentin at the pulp border, with irregular shape and different degrees of mineralization, and with sparse, twisted dentinal tubules in the area of the cavity. The pulp area had decreased in size, and the inflammatory response had finally resolved near the border of the dentin area and the pulp area. A zone comprising dentin, odontoblasts and odontogenic progenitor cells was

observed. The odontogenic-like cell area was a rich cell zone, and the underlying pulp tissue contained progenitor cells, fibroblasts with large oval nuclei, as well as small capillary blood cells, and loosely arranged collagen fibers.

With Neo MTA, at 45 days after direct pulp capping, the histological results showed a bridge of mineralized tissue resembling bone, with different levels of mineralization above the pulp tissue. The pulp chamber had shrunk. At higher magnifications (x20) (Fig. 5c), there were incremental lines of dentin at the pulp-tissue border of dentin resembling bone. Figure 5d (at x40) shows pulp tissue under the newly formed tissue beneath the

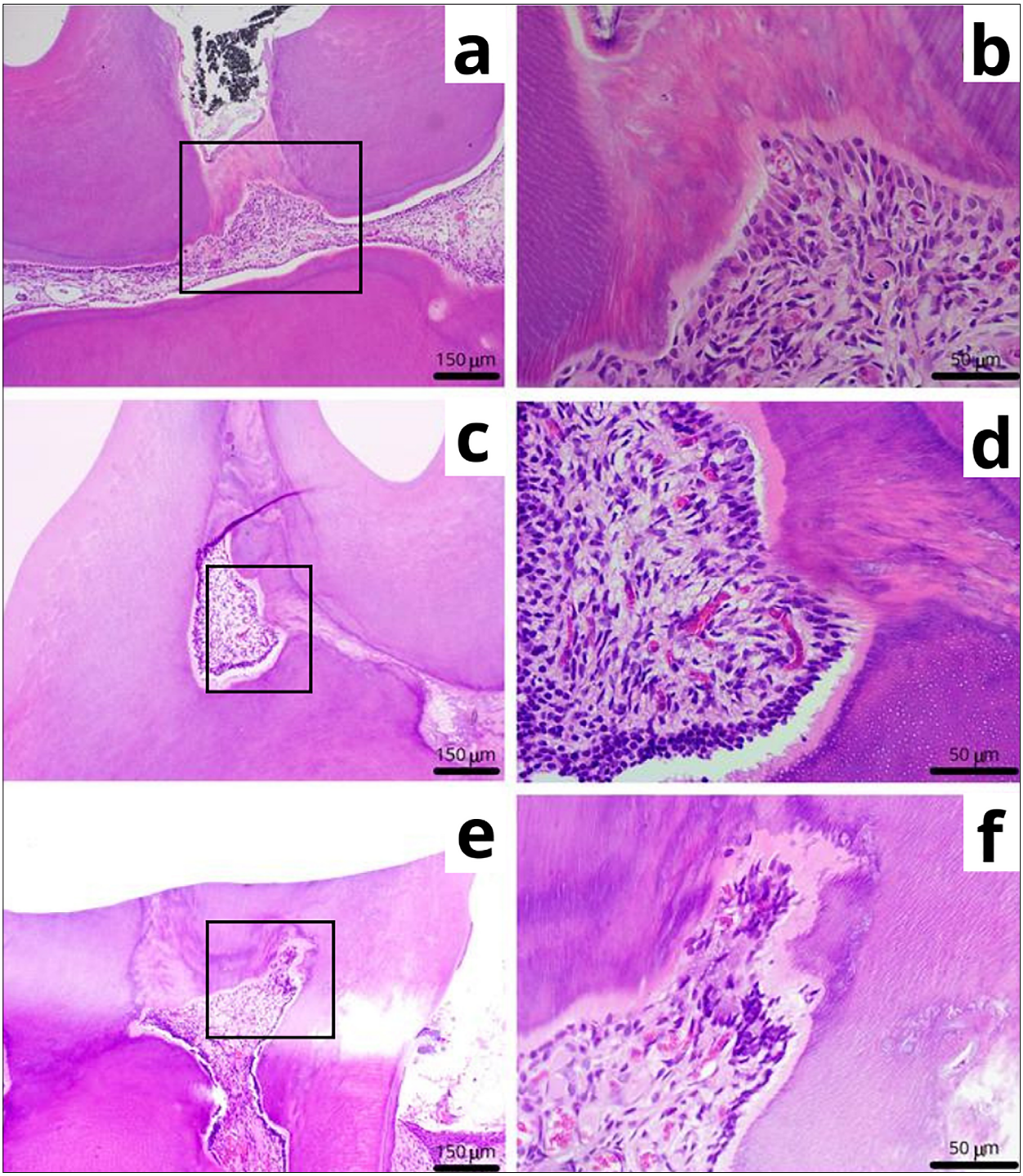

*Fig. 5: Histological features of pulpotomy with NeoMTA. The boxes indicate the subsequent magnification (x20 and x40) after 15 days **(a-b)**, 45 days **(c-d)** and 90 days **(e-f)**. Magnification bar =150 µm, 50 µm.*

cavity floor, consisting of odontoblast-like cells with few inflammatory cells.

At 90 days, reparative dentin was observed in localized areas under the cavity preparation, and the dentin-like tissue appeared irregular with sparse twisted tubules Fig. 5e (x20). Fig. 5G (x20) shows odontoblast-like cells adjacent to reparative dentin with incremental lines. The odontoblast-like cells appear columnar and elongated in morphology. At higher magnification (Fig. 5f x40), an area of incremental lines of dentin with different degrees of mineralization was evident, as well as disorganized pulp tissue with loose connective tissue and small capillary cells with mild congestion in otherwise healthy pulp tissue.

DISCUSSION

Previous studies have examined direct capping materials according to their ability to induce repair by forming mineralized tissue and the dentin bridge, which indicates a pulp healing process[9]. In the present study, the bioceramic materials Biodentine and Neo MTA were utilized as direct capping materials in an in-vivo model. Both materials resulted in the formation of a dentine bridge with an inflammatory cell infiltrate, and absence of bacterial contamination.

The literature has reported that dentin-bridge formation is detected in human teeth at 6 weeks. It involves different mechanisms, including cell response to calcium, differentiation of odontoblasts, odontogenesis, dentinogenesis, and dental mineralization[10]. In animal models, the formation of a dentin bridge has been observed at 120 days after placing Biodentine in furcation perforations in beagle dogs[11]. In the current study on rat teeth, when Neo MTA was used, a dentine bridge was observed at 90 days in Micro-CT images. Other studies have demonstrated that Biodentine effectively induces reparative dentin when it is placed directly on mechanically exposed pulp tissue in rat teeth, exhibiting well-defined characteristics at the damaged site[12]. Kim et al.[13] placed Biodentine in rat teeth for 4 weeks and observed irregular heterogeneous distribution of mineralization nodules within a uniform thickness of a rigid tissue barrier. In contrast to our results, Candeiro et al. found that Biodentine and Neo MTA showed mineralized pulp healing process[9].

In the present study, both Biodentine and Neo MTA provided homogeneous seal (Figure 5a and b). This agrees with Ricucci et al.[14], who demonstrated with histological evidence that when the primary odontoblast dies or is absent due to pulp exposure, it does not regenerate. Therefore, the tissue resulting from reparative dentinogenesis lacks the tubular structure typical of dentin, and fibroblasts from the pulp calcify the new tissue during this repair process. Shayegan et al.[15] examined the pulp response after a pulpotomy in primary pig teeth at 7, 28 and 90 days using Biodentine, white mineral trioxide aggregate (WMTA) or formocresol. They found that Biodentine and WMTA are both suitable biocompatible materials. Nowicka et al.[16] evaluated the volume of reparative dentin bridges formed after direct pulp capping in human third molars and concluded that the complete reparative dentin bridge dependent on the material used demonstrated that Biodentine and MTA resulted in the formation of bridges with a significantly higher average volume compared to dentin bonding systems. Therefore, each material had different degrees of influence on dentin bridge formation, and both Biodentine and MTA generated reparative pulp responses. De Rossi et al.[17] performed pulpotomies on dog teeth, achieving success rates of 96.8% in treatments with Biodentine and 72.2% in those using MTA. Other articles also conclude that there is no significant difference between Biodentine and MTA in terms of clinical success[18,19].

Quiñones et al.[20] compared the biocompatibility of the regeneration of the dentin–pulp complex in a murine model with MTA Angelus, Neo MTA, and TheraCal PT. Histologically, they observed no cellular inflammatory infiltrate, but after 15 days, inflammatory cell infiltrate was slightly higher in teeth treated with Neo MTA than with MTA or TheraCal PT. However, at 30 and 45 days, all three materials had grade 1 of slight inflammatory infiltrate. Tziafa et al.[21] reported in an animal study that the dentin bridge thickness produced with use of Biodentine, at three and eight weeks, was significantly higher than that produced with use of MTA, which is consistent with the findings of the present study.

The differences found between the bioceramic materials could be related to the handling of the materials, which may have affected treatment outcomes. Biodentine presentation is in a capsule, which is eventually mixed with an amalgamator;

whereas the Neo MTA powder and liquid must be mixed by hand, which increases the chance of potential errors in the final preparation of the cement[22]. The setting time of MTA cement is longer, and moreover, the initial setting releases toxic substances into the cells, increasing the toxicity of the cement[23,24].

After setting, Biodentine has denser microstructure and less porosity than MTA cement, enabling more calcium ions to be released, and ultimately, more hard tissue was formed[6]. The results of the present study agree with previous reports that found superior results in teeth treated with Biodentine. Concerning cell biological response, the proposed mechanism of action that regulates the induction of reparative dentin and its quality is currently an active area of research. However, the molecular mechanisms remain poorly understood.

CONCLUSION

Based on the results and methods used in this study, Biodentine and Neo MTA induce the reparative dentin bridge formation with differences in the thickness and morphology of the hard tissue formed, and both produce a cellular inflammatory infiltrate.

ACKNOWLEDGMENTS

The authors thank the Division of Graduate Studies and Research of the UNAM Faculty of Dentistry for the facilities provided for this study.

FUNDING

This study was supported by a grant from the National Autonomous University through UNAM-DGAPA-PAPIIT-IN218223 and IN204124.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

REFERENCES

1. Quiñonez-Ruvalcaba F, Bermúdez-Jiménez C, Aguilera-Galavíz LA, Villanueva-Sánchez FG, García-Cruz S, Gaitán-Fonseca C. Histopathological Biocompatibility Evaluation of TheraCal PT, NeoMTA, and MTA Angelus in a Murine Model. J Funct Biomater 2023;14(4):202. https://doi.org/10.3390/jfb14040202
2. Liu S, Wang S, Dong Y. Evaluation of a bioceramic as a pulp capping agent in vitro and in vivo. J Endod 2015;41(5):652-7. https://doi.org/10.1016/j.joen.2014.12.009
3. Abuelniel GM, Duggal MS, Kabel N. A comparison of MTA and Biodentine as medicaments for pulpotomy in traumatized anterior immature permanent teeth: A randomized clinical trial. Dent Traumatol 2020;36(4):400-410. doi: 10.1111/edt.12553.4 Mohammadi Z, Shalavi S, Soltani MK. Mineral trioxide aggregate (MTA)-like materials: an update review. Compend Contin Educ Dent 2014;35(8):557-61. https://doi.org/10.1111/edt.12553
5. Benetti F, de Azevedo Queiroz ÍO, Oliveira PHC, Conti LC, Azuma MM, Oliveira SHP, Cintra LTA. Cytotoxicity and biocompatibility of a new bioceramic endodontic sealer containing calcium hydroxide. Braz Oral Res 2019;33:042. https://doi.org/10.1590/1807-3107bor-2019.vol33.0042
6. Rajasekharan S, Martens LC, Cauwels RGEC, Anthonappa RP. Biodentine™ material characteristics and clinical applications: a 3 year literature review and update. Eur Arch Paediatr Dent. 2018;19(1):1-22. https://doi.org/10.1007/s40368-018-0328-x
7. Quintana RM, Jardine AP, Grechi TR, Grazziotin-Soares R, Ardenghi DM, Scarparo RK, et al. Bone tissue reaction, setting time, solubility, and pH of root repair materials. Clin Oral Investig 2019;23(3):1359-1366. https://doi.org/10.1007/s00784-018-2564-1
8. Norma Oficial Mexicana. (6 de diciembre de 1999). Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio. [NOM-062- ZOO-1999].
9. Candeiro GT, Correia FC, Duarte MA, Ribeiro-Siqueira DC, Gavini G. Evaluation of radiopacity, pH, release of calcium ions, and flow of a bioceramic root canal sealer. J Endod. 2012;38(6):842-5. https://doi.org/10.1016/j.joen.2012.02.029
10. Rathinam E, Govindarajan S, Rajasekharan S, Declercq H, Elewaut D, De Coster P, et al. The calcium dynamics of human dental pulp stem cells stimulated with tricalcium silicate-based cement determine their differentiation and mineralization outcome. Sci Rep 2021;11(1):645. https://doi.org/10.1038/s41598-020-80096-5
11. Cardoso M, Pires M dos A, Correlo V, Reis R, Paulo M, Viegas C. Biodentine for furcation perforation repair: An animal study with histological, radiographic and micro-computed tomographic assessment. Iran Endod J 2018;13:323-30.
12. Tran XV, Gorin C, Willig C, Baroukh B, Pellat B, Decup F, et al. Effect of a calcium-silicate-based restorative cement on pulp re¬pair. J Dent Res 2012;91(12):1166-71. https://doi.org/10.1177/0022034512460833
13. Kim J, Song YS, Min KS, Kim SH, Koh JT, Lee BN, 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. https://doi.org/10.5395/rde.2016.41.1.29
14. Ricucci D, Loghin S, Lin LM, Spångberg LS, Tay FR. Is hard tissue formation in the dental pulp after the death of the primary odontoblasts a regenerative or a reparative process?

J Dent. 2014 Sep;42(9):1156-70. https://doi.org/10.1016/j.jdent.2014.06.012
15. Shayegan A, Jurysta C, Atash R, Petein M, Abbeele AV. Biodentine used as a pulp-capping agent in primary pig teeth. Pediatr Dent 2012;34(7):202-8.
16. Nowicka A, Wilk G, Lipski M, Kołecki J, Buczkowska-Radlińska J. Tomographic Evaluation of reparative dentin formation after direct pulp capping with Ca(OH)2, MTA, Biodentine, and dentin bonding system in human teeth. J Endod 2015; 41: 1234-40. https://doi.org/10.1016/j.joen.2015.03.017
17. De Rossi A, Silva LA, Gatón-Hernández P, Sousa-Neto MD, Nelson-Filho P, Silva RA, et al. Comparison of pulpar responses to pulpotomy and pulp capping with biodentine and mineral trioxide aggregate in dogs. J Endod 2014;40:1362-9. https://doi.org/10.1016/j.joen.2014.02.006
18. Paula AB, Laranjo M, Marto CM, Paulo S, Abrantes AM, Casalta-Lopes J, et al. Direct pulp capping: What is the most effective therapy? Systematic review and meta-analysis. J Evid-Based Dent Pract 2018;18(4):298-314. https://doi.org/10.1016/j.joen.2014.02.006
19. Matsuura T, KS Kawata-Matsuura V, Yamada S. Long-term clinical and radiographic evaluation of the effectiveness of direct pulp-capping materials. J Oral Sci 2019;61(1):1-12. https://doi.org/10.2334/josnusd.18-0125
20. Quiñonez-Ruvalcaba F, Bermúdez-Jiménez C, Aguilera-Galavíz LA, Villanueva-Sánchez FG, García-Cruz S, Gaitán-Fonseca C. Histopathological biocompatibility evaluation of TheraCal PT, NeoMTA, and MTA Angelus in a murine model. J Funct Biomater 2023;14(4):202. https://doi.org/10.3390/jfb14040202
21. Tziafa C, Koliniotou Koumpia E, Papadimitriou S, Tziafas D. Dentinogenic responses after direct pulp capping of miniature swine teeth with Biodentine. J Endod 2014;40:1967-1971. https://doi.org/10.1016/j.joen.2014.07.021
22. Yaemkleebbua K. Analysis of hard tissue regeneration and Wnt signalling in dental pulp tissues after direct pulp capping with different materials. Int Endod J 2019;52:1605-16. https://doi.org/10.1111/iej.13162
23. Mahmoud SH, El-Negoly SA, El-Din AMZ, El-Zekrid MH, Grawish LM, Grawish HM, et al. Biodentine versus mineral trioxide aggregate as a direct pulp capping material for human mature permanent teeth-A systematic review. J Conserv Dent 2018;21:466-473. https://doi.org/10.4103/JCD.JCD_198_18
24. Ha WN, Nicholson T, Kahler B, Walsh LJ. Mineral trioxide aggregate-A review of properties and testing methodologies. Materials 2017;10:1-18. https://doi.org/10.3390/ma10111261

LVII SAIO 2024 Annual Meeting, City of Buenos Aires - Argentina

We are delighted to announce that the LVII Annual Meeting of the Argentine Society of Dental Research will take place from November 15th to 16th, 2024, in the city of Buenos Aires, Argentina. The event will be hosted by the Faculty of Dentistry at the University of Buenos Aires. Organized by the Organizing Committee of the 2024 Annual Meeting, chaired by Dr. Romina De Lucca, the meeting will include presentations and lectures by prominent experts in contemporary dental research, an awards ceremony, and the presentation of research papers in both oral and poster formats.

LVI Annual Scientific Meeting of the Argentine Society for Dental Research 2023

On November 9th, 10th, and 11th, 2023, the LVI Annual Scientific Meeting of the Argentine Society for Dental Research took place at the Faculty of Health Sciences of the Catholic University of Cordoba and the Faculty of Dentistry of the National University of Cordoba. Dr. Pablo Rodríguez, President of the Society, highlighted during his speech at the opening ceremony the fundamental role of publications and scientific dissemination within the dental community. He also emphasized his interest, along with that of the Board of Directors, in supporting and fostering a new image and wider dissemination of the official publication of SAIO, the journal Acta Odontológica Latinoamericana. Additionally, he underscored the current significance of social media networks and the website in promoting the journal to more researchers, thereby facilitating its expansion and growth.

The Organizing Committee of the Annual Meeting was comprised of the following members: President: Dr. Gabriela Martín; Secretary: Pablo Fontanetti; Assistant Secretary: Valentín Mendoza; Treasurer: Dr. Carlos Rozas; General Abstract Coordinator: Dr. Mariana Rocamundi; Commercial Relations: Dr. Jorgelina Ulloque.