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Cytotoxic effects of bioceramic materials on stem cells from human exfoliated deciduous teeth (SHED)

Efeitos citotóxicos de materiais biocerâmicos em células-tronco de dentes decíduos esfoliados humanos (SHED)

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ABSTRACT

Objective: This study aimed to evaluate stem cell from human deciduous teeth (SHED) viability after exposure to different bioceramic materials. **Material and Methods:** Discs were constructed to obtain the material extracts according to the following groups: G1 - Bio-C Repair, G2 - MTA Repair HP, G3 - TheraCal LC, and G4 – Biodentine. Positive and negative control group were respectively maintained with αMEM + 10% FBS and αMEM + 1% FBS. SHED obtained through primary culture were in contact with material extracts for 24, 48, and 72h. MTT assay evaluated cell viability. Groups were plated in triplicate and the cell viability assay were repeated three times. Data were analyzed by two-way ANOVA followed by Tukey test ($p<0.05$). **Results:** The treatment and period comparisons showed statistically significant differences ($p<0.000$). G2 (MTA Repair HP) had greater cell viability values than the other experimental groups and negative control. MTA Repair HP and the control groups exhibited a similar behavior with cell viability values decreasing from 24h to 48h and increasing from 48h to 72h. Bio-C Repair, Biodentine, and Theracal LC did not show statistically significant differences among periods. **Conclusions:** SHED increased viability values after contact with MTA Repair HP in comparison with other bioceramic materials.

KEYWORDS

Cell viability; Cytotoxicity; Materials testing; SHED; Stem cells.

RESUMO

Objetivo: O objetivo desse estudo foi avaliar a viabilidade de células-tronco de dentes decíduos humanos (SHED) após o contato com diferentes materiais biocerâmicos. **Material e Métodos:** Foram confeccionados discos para obtenção dos extratos dos materiais de acordo com os seguintes grupos: G1 - Bio-C Repair, G2 - MTA Repair HP, G3 - TheraCal LC e G4 - Biodentine. Grupo de controle positivo e negativo foram mantidos respectivamente com αMEM + 10% FBS e αMEM + 1% FBS. SHED obtidas por cultura primária entraram em contato com os extratos de materiais por 24, 48 e 72h. O ensaio MTT avaliou a viabilidade celular. Os grupos foram semeados em triplicata e o ensaio de viabilidade celular foi repetido três vezes. Os dados foram analisados por ANOVA a dois critérios seguido pelo teste de Tukey ($p<0,05$). **Resultados:** As comparações de tratamentos e períodos mostraram diferenças estatisticamente significativas ($p<0,000$). O G2 (MTA Repair HP) apresentou maiores valores de viabilidade celular que os demais grupos experimentais e controle negativo. O MTA Repair HP e os grupos controle exibiram um comportamento semelhante com os valores de viabilidade celular diminuindo de 24h

para 48h e aumentando de 48h para 72h. Bio-C Repair, Biodentine e Theracal LC não apresentaram diferenças estatisticamente significativas entre os períodos. **Conclusões:** SHED aumentou os valores de viabilidade após o contato com o MTA Repair HP em comparação com outros materiais biocerâmicos.

PALAVRAS-CHAVE

Viabilidade celular; Citotoxicidade; Teste materiais; SHED; Células-tronco.

INTRODUCTION

Stem cell are undifferentiated cells with high proliferation and self-renewal potential capable of differentiating in many cell types [1]. The possibility of isolating mesenchymal stem cells from dental pulp enables the application of bioengineering for pulp regeneration, allowing promising treatment options [2,3]. Human deciduous teeth are source of stem cells that may be isolated and cultured in vitro. Stem cells from human deciduous teeth (SHED) are a high proliferative cell population with varied differentiation capacity [4]. Pulp therapy repair mechanism occurs through migration, proliferation, and differentiation of stem cells from dental pulp into odontoblasts that account for the synthesis and secretion of tertiary dentin [2,5].

Pulp vital treatment requires biomaterials to protect the exposed vital pulp [6]. For this purpose, the ideal material should be biocompatible; bactericidal; capable of promoting pulp healing; and should not interfere in the normal exfoliation of deciduous teeth [7,8]. To date, different materials for pulp therapy of deciduous teeth are available, including calcium silicate bioceramic cements. These cements are biocompatible and bioinductive, there is, when in contact with the injured pulp, they have the appropriate bioactivity to induce the repair and formation of mineralized tissue [9].

The introduction of bioceramic materials represented a pivotal advancement in the evolution of regenerative endodontic therapy. Currently, the available literature evaluate the differences between recently introduced silicate-based materials such as Biodentine, MTA or even more traditional materials such as calcium hydroxide. However, few studies have evaluated newer materials such as Bio-C Repair and Theracal LC. Additionally, there is more extensive literature available on stem cells originating from the dental pulp of permanent teeth, revealing a knowledge gap regarding pulp from deciduous teeth. The introduction of new bioceramic materials combined with additives requires up-to-date research in the area. The evaluation of the bioactivity of these materials concerning dental pulp stem cells relies on in vitro tests or animal studies. These methods, while imperfect, are crucial for mimicking relevant clinical scenarios to a certain extent [10].

In this context, previous studies evaluate the cytotoxicity of vital pulp treatment materials on stem cells from the pulp of permanent teeth, but few used SHED [2,4,11]. Thus, this study aimed to evaluate stem cell from human deciduous teeth (SHED) viability after exposure to four different bioceramic materials: Bio-C Repair, MTA Repair HP, Biodentine, and Theracal. The null hypothesis is that the materials would show similar biocompatibility.

MATERIALS AND METHODS

Study design

This was a two-factor study: treatment (6 levels: Bio-C Repair, MTA Repair, Theracal LC, Biodentine, negative control, and positive control) and periods (3 levels: 24, 48, and 72 horas).

Ethical issues

This study was submitted and approved by the Institutional Review Board (protocol CAAE: 29177820.9.0000.5417). All participants and their legal guardians read and signed a free and clarified consent form to donate the exfoliated deciduous teeth.

Cell culture and isolation

The cells were obtained from primary cell culture and characterized following a previous study [12]. SHED were plated on 25-cm^2 culture

flask (Corning, Union City, CA) containing Alpha-MEM supplemented with 20% FBS, incubated at 37°C and 5% CO_2. The cells were cultivated at 80% flask confluence (passage O). The cells were washed with phosphate buffered saline solution (PBS) (Gibco Invitrogen) and detached with 0.25% trypsin-EDTA (Gibco Invitrogen) for 5 min at 37°C. Culture medium was added to inactivate trypsin activity. Finally, the cells were centrifugated at 1200 rpm, for 5 min and plated on de 75-cm^2 flasks at density of 1×10^4 cm^2, for cell expansion. SHEDs at passages 4^{th} and 8^{th} were used in the experiments.

Pulp capping materials

Four bioactive materials were analyzed: Bio-C Repair (Angelus, Londrina, PR, Brazil), MTA Repair HP (Angelus, Londrina, PR, Brazil), Theracal LC (Bisco Inc., Schaumburg, IL, USA), and Biodentine (Septodont, Saint-Maur-des-Fosses, France) (Table I).

Sample preparation

The cements extracts were prepared following previous studies and ISO standard 10993 [11,13-16]. The materials were prepared according to the manufacturer's instructions. MTA Repair pack content (0.17g) was mixed with two drops of the liquid for 40 seconds to obtain a homogenous cement. Five drops of liquid were added to Biodentine capsule and agitated at 4000 rpm for 30 seconds. Bio-C Repair and TheraCal LC are ready for use. All samples were prepared in aseptic conditions, with the aid of sterile rubber molds (5 mm diameter, 3 mm height) and incubated at 37° C for 6 h. Elapsed that time, the samples were removed from the molds and sterilized by ultraviolet light for 1h inside biosafety cabinets. Each sample was immersed into 1 mL of αMEM (Invitrogen, Carlsbad, California) + 10% FSB (Thermo Fisher Scientific, USA) + 1% antibiotics and antifungals (Anti-Anti - Gibco, Grand Island, NY, USA) and incubated at 5% CO_2 for 3 days. Elapsed that period, the material discs were discarded and the supernatants were collected and filtered with 0.22-mm sterile filter (Sigma-Aldrich, St Louis, MO). The collected supernatants were referred as extracts (1:1).

Cell viability assay

Cell viability was analyzed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). SHED at density of 1 x 10^4 were seeded in 96-well plates (Corning #3595) with 1 mL of culture medium and incubated for 24h, at 37ºC and 5% CO_2 for cell adhesion. Elapsed that period, the culture medium was changed by the materials extracts. SHED was exposed to material extracts for 24, 48, and 72h. Positive and negative control cells were maintained in αMEM + 10% FSB and 1% SFB, respectively. Elapsed the study times, the culture medium was removed, and the cells were washed with PBS 1x. Next, 110ul of MTT (0.5mg/mL) was added to each well. The plates were covered with aluminum foil and incubated at 37º C, 5% CO_2, for 4 h. After that, MTT solution was discarded and 200 ul of Dimethyl sulfoxide (DMSO, Fisher Scientific, Hampton, VA, EUA) were added per well. After 30 minutes, absorbance was measured by spectrophotometer (Synergy Mx; BioTek Instruments, USA), at 570 nm wavelength. Groups were plated in triplicate and the cell viability assay were analyzed in three independent experiments. All statistical analyses were obtained

Table I - Main components of the study materials

Material	Manufacturer	Composition
Bio-C Repair	Angelus, Londrina, PR, Brazil	Calcium silicates, calcium aluminate, calcium oxide, zirconium oxide, iron oxide, silicon dioxide, and dispersing agent.
MTA Repair HP	Angelus, Londrina, PR, Brazil	Powder: Tricalcium silicate; Dicalcium silicate; Tricalcium aluminate; Calcium Oxide; Calcium Tungstate. Liquid: Water and Plasticizer.
Theracal LC	BiscoInc, Schaumburg, IL, USA	Tricalcium silicate particles; Urethane-Dimethacrylate (UDMA); Bisphenol A-glycidyl Methacrylate (Bis-GMA) and Triethylene Glycol Dimethacrylate (TEDGMA); Hydroxyethyl Methacrylate (HEMA) and Polyethylene Glycol Dimethacrylate (PEDGMA).
Biodentine	Septodont, Saint- Maur-des-Fosses, France	Powder: Tricalcium Silicate; Zirconium Oxide; Calcium Oxide; Calcium Carbonate; Yellow Pigment; Red Pigment; Brown Iron Oxide. Liquid: Calcium Chloride Dihydrate; polycarboxylate. Purified Water.

with Statistica 10.0 software for Windows. Data were analyzed by two-way ANOVA followed by Tukey test ($p = 0.05$).

RESULTS

Cell viability assay showed statistically significant differences between materials and times ($p<0.000$). At 24h, MTA Repair HP showed statistically significant higher cell viability values than all the other materials and negative control (C-), followed by Biodentine, Bio-C Repair, and Theracal LC (Table II). Positive control (C+) had the greatest statistically significant viability means than that of the other groups ($p<0,05$), except for MTA Repair HP. Negative control group (C-) exhibited statistically significant differences with all studied groups (Table II)

At 48h, SHED viability values significantly decreased with differences between MTA Repair HP and the other materials and negative control group (Table II). At 72h, the cell viability increased, as follows: MTA Repair HP>Biodentine>Bio-C Repair>Theracal LC (Table II). We observed that SHEDs in contact with MTA Repair HP, negative and positive controls exhibited similar patterns of statistically significant viability decreasing from 24h to 48h, followed by a statistically significant increasing at 72h (24h>48h; 72h>48h; 24h=72h). SHED in contact with Bio-C Repair, Theracal LC, and Biodentine did not show statistically significant differences between periods (Table II).

DISCUSSION

Biocompatibility is an important property to be considered when selecting a material for pulp therapy due to the direct contact with vital tissues [11,17]. As science advances, current bioinductive materials show promising outcomes in pulp therapy for deciduous and permanent teeth. Previous studies have demonstrated the effects of Bio-C Repair, MTA Repair HP, Theracal LC, and Biodentine on human pulp tissue alone or together with other materials with variable success rates [9,15,17,18]. However, the literature lacks studies on the direct contact of these materials with SHEDs.

Cell culture techniques are an excellent choice for analyzing the biocompatibility of different materials. Many quantitative and qualitative in vitro methods evaluate the cytotoxicity of biomaterials and the potential side effects on cellular mechanisms [11]. In vitro tests offer a comprehensive analysis of the biological properties of the materials cultured with cells aiming at predicting the clinical behavior [19]. Minimum Essential Medium (MEM) is a widely recognized medium utilized for culturing mammalian cells. MEMα, as per the manufacturer, does not contain any proteins, lipids, or growth factors. Therefore, MEMα requires supplementation, usually with 10% Fetal Bovine Serum (FBS). MEMα uses a sodium bicarbonate buffer system (2.2 g/L), requiring a 5–10% CO_2 environment to maintain physiological pH (Thermo Fisher Scientific, USA – Safety Data Sheet). Selecting the appropriate culture medium and determining the appropriate percentage of Fetal Bovine Serum (FBS) are crucial steps in establishing an optimal environment that does not interfere with the differentiation of stem cells [20]. According to the manfacturer and previous studies, FBS 10% concentration can maintain the growth of the cells with no interference on the proliferation, which could be a bias in the study [12,21]. A reduced percentage of FBS can create a distinct environment for the cells, potentially mimicking suboptimal conditions for cell growth and proliferation, thereby serving as a negative control [12,21].

Table II - Cell viability intragroup and intergroup comparisons

Periods	Materials					
	Bio-C Repair (mean ± SD)	MTA Repair HP (mean ± SD)	Theracal LC (mean ± SD)	Biodentine (mean ± SD)	Negative control (mean ± SD)	Positive control (mean ± SD)
24 h	0.074 ± 0.004^{a}	0.366 ± 0.029^{e}	0.078 ± 0.004 ^{a}	0.088 ± 0.004 ^{a}	0.287 ± 0.016 ^{d}	0.378 ± 0.041 ^{e}
48 h	0.098 ± 0.006^{a}	0.242 ± 0.007^{c}	0.091 ± 0.001 ^{a}	0.110 ± 0.007 ^{a}	0.197 ± 0.011 ^{b}	0.264 ± 0.019 ^{cd}
72 h	0.102 ± 0.011^{a}	0.351 ± 0.008^{e}	0.095 ± 0.004 ^{a}	0.108 ± 0.004 ^{a}	0.244 ± 0.012 ^{cd}	0.355 ± 0.037 ^{e}

Different superscript letters in the same column and row indicate statistically significant differences in intragroup and intergroup comparisons. (two-way ANOVA followed by Tukey test; $p<0.05$). Standard Deviation.

New biomaterial formulations have been constantly launched into the market for clinical use. Currently, silicate and calcium phosphate-based materials have been used due to their capability of stimulating tissue repair through the deposition of mineralized tissue. Thus, they have been studied regarding their cytotoxicity and bioactivity on cell cultures [15,22].

Ghilotti et al. [17] tested the cytotoxicity of Biodentine, Bio-C Repair, and ProRoot MTA on human dental pulp cells from permanent teeth and reported through the production of formazan that the materials were not cytotoxic to hDPCs. Unexpectedly, undiluted Biodentine exhibited significantly higher levels of relative formazan formation than the control group at all tested time points. Bio-C Repair showed formazan production similar to that of untreated cells. The more diluted ProRoot MTA extracts showed higher formazan formation than control group only at 72 hours. The studies of Youssef et al. [23] showed that the cell viability of DPSCs was measured using MTT assay, exhibiting variable cytotoxicity against DPSCs compared to the control; MTA was more cytocompatible than Biodentine, which showed significant cytotoxicity against DPSCs compared to the control, corroborating the results of the present study [24,25]. To achieve tooth regeneration, a comprehensive understanding of tooth stem cells is essential for better application of tissue engineering. A systematic review carried out by Sanz et al. [10], evaluated the bioactivity of bioceramic materials in relation to dental pulp stem cells (DPSCs), a total of 37 articles were included in the review. A systematic review carried out by Sanz et al. [10], evaluated the bioactivity of bioceramic materials in relation to dental stem cells (DSC), a total of 37 articles were included in the review. The authors concluded that the differences between DSC justifies the need for individual assessment of the biological response of dental biomaterials to different DSC variants. The study points out that SHED generally exhibited adequate levels of cell viability, proliferation, migration and an increase in the formation of mineralized nodules after incubation with various calcium silicate-based compositions, acting as supporting evidence for their use in endodontic procedures [10,17,23].

In this present study, the tested materials showed statistically significant different cell viability results. MTA Repair HP was biocompatible while TheraCal LC significantly decreased cell viability values. Therefore, the null hypothesis of this in vitro study was rejected. Although using different methodologies, other studies showed comparable results [17,22,26].

The biocompatibility of MTA HP Repair has been shown by previous studies, suggesting its beneficial clinical use [16,27,28]. MTA HP Repair main components (tricalcium silicate, calcium oxide, tricalcium oxide, silicate, and aluminate) are similar to dentinal tissue components and account for its low cytotoxicity [29]. In this study, SHED in contact with MTA HP Repair behaved better at 24 and 72h, showing the highest cell viability values. Previous studies demonstrate that MTA cements are biocompatible with dental pulp stem cells. Tomás-Catalá et al. [30] studied the effects of MTA HP Repair on dental pulp stem cells, through MTT assay, and found high cell viability rates, corroborating our results.

On the other hand, Theracal LC was the most cytotoxic cement because it significantly reduced SHED viability values in comparison with negative control, at all studied times, in agreement with the literature [16,31-33]. The literature has indicated that TheraCal LC has shown worst results than MTA and Biodentine, due to low quality of the dentin barrier, great inflammatory effect, less favorable odontoblastic layer formation, and small capacity of calcium release. The non-polymerized resin monomer accounts for such results, leading to inflammation and toxicity to pulp tissue [34-38]. Moreover, heating during photopolymerization can potentially induce unfavorable pulp reactions [39]. The study of Camilleri et al. [40] on calcium hydroxide releasing of pulp capping materials found a relation between calcium hydroxide releasing and pulp tissue regeneration. These authors reported that Theracal LC calcium releasing directly depends on its hydration, so enough calcium hydroxide may be not produced and consequently released. pH is another issue. The material pH is an essential physical property that is related with pulp response [41]. The releasing of hydroxyl ions increases the surrounding environment pH leading to pulp tissue inflammation, but it accounts for the bactericidal effect of the material, which may explain the grater cytotoxicity values [32].

Biodentine showed smaller viability values than MTA Repair HP and positive and negative control groups, which is in agreement with previous studies [9,17,24]. The literature reports

that cell viability may significantly depend on the extracts' concentration, that is, less extraction dilution leads to small cell viability values [15]. Thus, we consider Biodentine concentrations lower than that used in this present study (1:1) would be ideal to increase SHED regenerative potential [25]. Sequeira et al. [42] analyzed apical papilla cell viability after exposure of 100% Biodentine and found results similar to those of this present study, with significantly small viability values at 24h, 48h, and 72h. Possible explanations for this result would be that 1) all newly-prepared calcium silicate cements (resin free) are initially cytotoxic mainly due to their alkalinity [43]; 2) this material may increase stem cell differentiation, which may change outcomes dependent on cell proliferation, but it may show the regenerative potential of the material. Therefore, further studies are necessary to find the bioactive properties influencing on differentiation because they can indirectly impact on proliferation and interfere in cell viability. We emphasize that the material dilution is justified to mimic its contact with the tissue resulting in dilution by the extracellular fluids and progressively decreasing of its concentration.

According to Ghilotti et al. [17], Bio-C Repair biocompatibility is similar to that of Biodentine, which agrees with the results of this present study. However, Bio-C Repair cell viability values were significantly smaller than that of MTA Repair HP. We hypothesize that longer setting time and high solubility favored cytotoxicity because they indicate the greater releasing of toxic components. Remnants of some biomaterials in the extracts may negatively influence cell cultures [44]. Youssef et al. [23] emphasizes that the mechanisms involved in cytotoxicity are not clearly understood, suggesting the initial calcium ion releasing, ionic activity, presence of toxic components, or pH changing may affect the cell behavior. This would explain the different cytotoxic effect of the tested materials.

The proper cement choice should consider not only the biological behavior but also other parameters, such as antimicrobial, physical, and chemical properties. Although these results cannot be directly applied to clinical situations in humans, they are scientifically significant because they represent an appropriate prototype for evaluating various initial features of dental materials. Further research using in vivo animal models is necessary to confirm the results, and enable direct outcome comparisons and clinical application, aiming at the best cement choice.

CONCLUSION

In conclusion, SHED increased viability values after contact with MTA Repair HP in comparison with other bioceramic materials. The results suggested that MTA Repair HP is still considered the gold standard among the materials studied and can be indicated for use in clinical regenerative procedures of the dentin-pulp complex.

Author's Contributions

BLS: Data curation, Methodology, Investigation, Writing – Original Draft Preparation. ABVS: Supervision, Writing – Original Draft Preparation. MTOP: Conceptualization, Writing – Review & Editing. ECPA: Formal Analysis, Writing – Review & Editing. PKJ: Formal Analysis, Validation, Writing – Review & Editing. MB: Methodology, Writing – Original Draft Preparation. NLN: Conceptualization, Methodology, Writing – Review & Editing. MAAMM: Conceptualization, Writing – Review & Editing. TMO: Conceptualization, Fundation Acquisition, Supervision, Project Administration, Writing – Review & Editing.

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of: Research Ethics Committee of the Faculty of Dentistry of Bauru, University of São Paulo. The approval code for this study is: 29177820.9.0000.5417.

REFERENCES

1. Lemischka IR. Stem cell biology: a view toward the future. Ann N Y Acad Sci. 2005;1044(1):132-8. http://doi.org/10.1196/annals.1349.017. PMid:15958706.

2. Araújo LB, Cosme-Silva L, Fernandes AP, Oliveira TM, Cavalcanti BN, Gomes JE Fo, et al. Effects of mineral trioxide aggregate, BiodentineTM and calcium hydroxide on viability, proliferation, migration and differentiation of stem cells from human exfoliated deciduous teeth. J Appl Oral Sci. 2018;26:e20160629. http://doi.org/10.1590/1678-7757-2016-0629.

3. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci USA. 2000;97(25):13625-30. http://doi.org/10.1073/pnas.240309797. PMid:11087820.

4. Athanasiadou E, Paschalidou M, Theocharidou A, Kontoudakis N, Arapostathis K, Bakopoulou A. Biological interactions of a calcium silicate based cement (BiodentineTM) with Stem Cells from Human Exfoliated Deciduous teeth. Dent Mater. 2018;34(12):1797-813. http://doi.org/10.1016/j.dental.2018.09.014. PMid:30316525.

5. Ahmed GM, Abouauf EA, Abubakr N, Dörfer CE, El-Sayed KF. Tissue engineering approaches for enamel, dentin, and pulp regeneration: an update. Stem Cells Int. 2020;2020:1-15. http://doi.org/10.1155/2020/5734539.

6. Prati C, Gandolfi MG. Calcium silicate bioactive cements: biological perspectives and clinical applications. Dent Mater. 2015;31(4):351-70. http://doi.org/10.1016/j.dental.2015.01.004. PMid:25662204.

7. Cuadros-Fernández C, Lorente Rodríguez AI, Sáez-Martínez S, García-Binimelis J, About I, Mercadé M. Short-term treatment outcome of pulpotomies in primary molars using mineral trioxide aggregate and Biodentine: a randomized clinical trial. Clin Oral Investig. 2016;20(7):1639-45. http://doi.org/10.1007/s00784-015-1656-4. PMid:26578117.

8. Bossù M, Iaculli F, Di Giorgio G, Salucci A, Polimeni A, Di Carlo S. Different pulp dressing materials for the pulpotomy of primary teeth: a systematic review of the literature. J Clin Med. 2020;9(3):838. http://doi.org/10.3390/jcm9030838. PMid:32204501.

9. Dahake PT, Panpaliya NP, Kale YJ, Dadpe MV, Kendre SB, Bogar C. Response of stem cells from human exfoliated deciduous teeth (SHED) to three bioinductive materials - an in vitro experimental study. Saudi Dent J. 2020;32(1):43-51. http://doi.org/10.1016/j.sdentj.2019.05.005. PMid:31920278.

10. Sanz JL, Rodríguez-Lozano FJ, Llena C, Sauro S, Forner L. Bioactivity of bioceramic materials used in the dentin-pulp complex therapy: a systematic review. Materials (Basel). 2019;12(7):1015. http://doi.org/10.3390/ma12071015. PMid:30934746.

11. Collado-González M, García-Bernal D, Oñate-Sánchez RE, Ortolani-Seltenerich PS, Álvarez-Muro T, Lozano A, et al. Cytotoxicity and bioactivity of various pulpotomy materials on stem cells from human exfoliated primary teeth. Int Endod J. 2017;50(Suppl 2):e19-30. http://doi.org/10.1111/iej.12751. PMid:28169432.

12. Oliveira Prado Bergamo MT, Vitor LLR, Lopes NM, Lourenço Neto N, Dionísio TJ, Oliveira RC, et al. Angiogenic protein synthesis after photobiomodulation therapy on SHED: a preliminary study. Lasers Med Sci. 2020;35(9):1909-18. http://doi.org/10.1007/s10103-020-02975-7. PMid:32056077.

13. International Standards Organization. ISO 10993-5 Avaliação Biológica de Dispositivos Médicos – Parte 5: Testes para citotoxicidade in vitro. Genebra: ISO; 2005.

14. Cintra LTA, Benetti F, Queiroz ÍOA, Lopes JMA, Oliveira SHP, Araújo GS, et al. Cytotoxicity, biocompatibility, and biomineralization of the new high-plasticity MTA material. J Endod. 2017;43(5):774-8. http://doi.org/10.1016/j.joen.2016.12.018.

15. Benetti F, Queiroz ÍOA, Cosme-Silva L, Conti LC, Oliveira SHP, Cintra LTA. Cytotoxicity, biocompatibility and biomineralization of a new ready-for-use bioceramic repair material. Braz Dent J. 2019;30(4):325-32. http://doi.org/10.1590/0103-6440201902457. PMid:31340221.

16. Rodríguez-Lozano FJ, López-García S, García-Bernal D, Sanz JL, Lozano A, Pecci-Lloret MP, et al. Cytocompatibility and bioactive properties of the new dual-curing resin-modified calcium silicate-based material for vital pulp therapy. Clin Oral Investig. 2021;25(8):5009-24. http://doi.org/10.1007/s00784-021-03811-0. PMid:33638052.

17. Ghilotti J, Sanz JL, López-García S, Guerrero-Gironés J, Pecci-Lloret MP, Lozano A, et al. Comparative surface morphology, chemical composition, and cytocompatibility of Bio-C Repair, biodentine, and ProRoot MTA on hDPCs. Materials (Basel). 2020;13(9):2189. http://doi.org/10.3390/ma13092189. PMid:32397585.

18. Nowicka A, Lipski M, Parafiniuk M, Sporniak-Tutak K, Lichota D, Kosierkiewicz A, et al. Response of human dental pulp capped with biodentine and mineral trioxide aggregate. J Endod. 2013;39(6):743-7. http://doi.org/10.1016/j.joen.2013.01.005. PMid:23683272.

19. Pedano MS, Li X, Yoshihara K, Landuyt KV, Van Meerbeek B. Cytotoxicity and bioactivity of dental pulp-capping agents towards human tooth-pulp cells: a systematic review of in-vitro studies and meta-analysis of randomized and controlled clinical trials. Materials (Basel). 2020;13(12):2670. http://doi.org/10.3390/ma13122670. PMid:32545425.

20. Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. Stem Cell Res Ther. 2019;10(1):68. http://doi.org/10.1186/s13287-019-1165-5. PMid:30808416.

21. Vitor LLR, Prado MTO, Lourenço N No, Oliveira RC, Sakai VT, Santos CF, et al. Does photobiomodulation change the synthesis and secretion of angiogenic proteins by different pulp cell lineages? J Photochem Photobiol B. 2020;203:111738. http://doi.org/10.1016/j.jphotobiol.2019.111738. PMid:31954290.

22. Sanz JL, Soler-Doria A, López-García S, García-Bernal D, Rodríguez-Lozano FJ, Lozano A, et al. Comparative biological properties and mineralization potential of 3 endodontic materials for vital pulp therapy: theracal PT, theracal LC, and biodentine on human dental pulp stem cells. J Endod. 2021;47(12):1896-906. http://doi.org/10.1016/j.joen.2021.08.001. PMid:34425148.

23. Youssef AR, Emara R, Taher MM, Al-Allaf FA, Almalki M, Almasri MA, et al. Effects of mineral trioxide aggregate, calcium hydroxide, biodentine and Emdogain on osteogenesis, Odontogenesis, angiogenesis and cell viability of dental pulp stem cells. BMC Oral Health. 2019;19(1):133. http://doi.org/10.1186/s12903-019-0827-0. PMid:31266498.

24. Zaen El-Din AM, Hamama HH, Abo El-Elaa MA, Grawish ME, Mahmoud SH, Neelakantan P. The effect of four materials on direct pulp capping: an animal study. Aust Endod J. 2020;46(2):249-56. http://doi.org/10.1111/aej.12400. PMid:32129919.

25. Hasweh N, Awidi A, Rajab L, Hiyasat A, Jafar H, Islam N, et al. Characterization of the biological effect of BiodentineTM on primary dental pulp stem cells. Indian J Dent Res. 2018;29(6):787-93. http://doi.org/10.4103/ijdr.IJDR_28_18. PMid:30589009.

26. Manaspon C, Jongwannasiri C, Chumprasert S, Sa-Ard-Iam N, Mahanonda R, Pavasant P, et al. Human dental pulp stem cell responses to different dental pulp capping materials. BMC Oral Health. 2021;21(1):209. http://doi.org/10.1186/s12903-021-01544-w. PMid:33902558.

27. Cintra LTA, Benetti F, de Azevedo Queiroz ÍO, de Araújo Lopes JM, Penha de Oliveira SH, Sivieri Araújo G, et al. Cytotoxicity,

biocompatibility, and biomineralization of the new high-plasticity MTA Material. J Endod. 2017;43(5):774-8. http://doi.org/10.1016/j.joen.2016.12.018. PMid:28320539.

28. Nam OH, Kim JH, Choi SC, Kim Y. Time-dependent response of human deciduous tooth-derived dental pulp cells treated with TheraCal LC: functional analysis of gene interactions compared to MTA. J Clin Med. 2020;9(2):531. http://doi.org/10.3390/jcm9020531. PMid:32075286.

29. Bin CV, Valera MC, Camargo SEA, Rabelo SB, Silva GO, Balducci I, et al. Cytotoxicity and genotoxicity of root canal sealers based on mineral trioxide aggregate. J Endod. 2012;38(4):495-500. http://doi.org/10.1016/j.joen.2011.11.003. PMid:22414836.

30. Tomás-Catalá CJ, Collado-González M, García-Bernal D, Oñate-Sánchez RE, Forner L, Llena C, et al. Biocompatibility of New Pulp-capping Materials NeoMTA Plus, MTA Repair HP, and Biodentine on Human Dental Pulp Stem Cells. J Endod. 2018;44(1):126-32. http://doi.org/10.1016/j.joen.2017.07.017. PMid:29079052.

31. Bakhtiar H, Nekoofar MH, Aminishakib P, Abedi F, Naghi Moosavi F, Esnaashari E, et al. Human pulp responses to partial pulpotomy treatment with TheraCal as compared with biodentine and ProRoot MTA: a clinical trial. J Endod. 2017;43(11):1786-91. http://doi.org/10.1016/j.joen.2017.06.025. PMid:28822566.

32. Arandi NZ, Rabi T. TheraCal LC: from biochemical and bioactive properties to clinical applications. Int J Dent. 2018;2018(1):3484653. http://doi.org/10.1155/2018/3484653.

33. Adıgüzel M, Ahmetoğlu F, Ünverdi Eldeniz A, Tekin MG, Göğebakan B. Comparison of cytotoxic effects of calcium silicate-based materials on human pulp fibroblasts Mehmet. J Dent Res Dent Clin Dent Prospect. 2019;13(4):241-6. http://doi.org/10.15171/joddd.2019.037. PMid:32190206.

34. Manojlovic D, Dramićanin MD, Miletic V, Mitić-Ćulafić D, Jovanović B, Nikolić B. Cytotoxicity and genotoxicity of a low-shrinkage monomer and monoacylphosphine oxide photoinitiator: comparative analyses of individual toxicity and combination effects in mixtures. Dent Mater. 2017;33(4):454-66. http://doi.org/10.1016/j.dental.2017.02.002. PMid:28258769.

35. Hebling J, Lessa FCR, Nogueira I, Carvalho RM, Costa CAS. Cytotoxicity of resin-based light-cured liners. Am J Dent. 2009;22(3):137-42. PMid:19650592.

36. Lee H, Shin Y, Kim SO, Lee HS, Choi HJ, Song JS. Comparative study of pulpal responses to pulpotomy with ProRoot MTA, RetroMTA, and TheraCal in dogs' teeth. J Endod. 2015;41(8):1317-24. http://doi.org/10.1016/j.joen.2015.04.007. PMid:26015158.

37. Poggio C, Beltrami R, Colombo M, Ceci M, Dagna A, Chiesa M. In vitro antibacterial activity of different pulp capping materials. J Clin Exp Dent. 2015;7(5):e584-8. http://doi.org/10.4317/jced.52401. PMid:26644833.

38. Jeanneau C, Laurent P, Rombouts C, Giraud T, About I. Light-cured tricalcium silicate toxicity to the dental pulp. J Endod. 2017;43(12):2074-80. http://doi.org/10.1016/j.joen.2017.07.010. PMid:29032813.

39. Kunert M, Rozpedek-Kaminska W, Galita G, Sauro S, Bourgi R, Hardan L, et al. The cytotoxicity and genotoxicity of bioactive dental materials. Cells. 2022;11(20):3238. http://doi.org/10.3390/cells11203238. PMid:36291107.

40. Camilleri J, Laurent P, About I. Hydration of biodentine, Theracal LC, and a prototype tricalcium silicate-based dentin replacement material after pulp capping in entire tooth cultures. J Endod. 2014;40(11):1846-54. http://doi.org/10.1016/j.joen.2014.06.018. PMid:25154317.

41. Paula A, Laranjo M, Marto CM, Abrantes AM, Casalta-Lopes J, Gonçalves AC, et al. BiodentineTM Boosts, WhiteProRoot®MTA Increases and Life® suppresses odontoblast activity. Materials (Basel). 2019;12(7):1184. http://doi.org/10.3390/ma12071184. PMid:30978943.

42. Sequeira DB, Seabra CM, Palma PJ, Cardoso AL, Peça J, Santos JM. Effects of a new bioceramic material on human apical papilla cells. J Funct Biomater. 2018;9(4):74. http://doi.org/10.3390/jfb9040074. PMid:30558359.

43. Bortoluzzi EA, Niu LN, Palani CD, El-Awady AR, Hammond BD, Pei DD, et al. Cytotoxicity and osteogenic potential of silicate calcium cements as potential protective materials for pulpal revascularization. Dent Mater. 2015;31(12):1510-22. http://doi.org/10.1016/j.dental.2015.09.020. PMid:26494267.

44. Paula AB, Laranjo M, Coelho AS, Abrantes AM, Gonçalves AC, Sarmento-Ribeiro AB, et al. Accessing the cytotoxicity and cell response to biomaterials. J Vis Exp. 2021;2021(173):1. http://doi.org/10.3791/61512. PMid:34309590.

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