Nelly Xiomara Alvarado Lemus

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RESUMO

RESUMO

Esse estudo objetivou comparar o manchamento dentário de incisivos bovinos simulando fraturas complicadas com exposição pulpar em dentes imaturos, tratados por pulpotomia total e parcial usando Biodentine (BD) e Bio-C repair (BCR) e avaliou a citotoxicidade dos materiais às células pulpares (hDPCs). Setenta e dois incisivos bovinos extraídos foram preparados para simular fraturas coronárias em um dente imaturo. Na simulação do tecido pulpar, as raízes foram preenchidas com uma mistura de ágar e sangue (grupo controle), sobre as quais foram colocados os materiais BD ou BCR com aproximadamente 2 mm de espessura. As análises de avaliação de cor das amostras foram realizadas antes e imediatamente após a pulpotomia e repetidas aos 30, 60 e 90 dias. As hDPCs foram colocadas em contato com diferentes diluições de meios de cultura previamente expostos a tais materiais e testadas quanto à viabilidade celular usando o ensaio MTT. Posteriormente, as hDPCs foram estimuladas com lipopolissacarídeos (LPS) e expostas aos extratos, para medição quantitativa de espécies reativas de oxigênio (EROS) e óxido nítrico (ON). Os dados foram avaliados por ANOVA seguido do teste de Tukey e Dunnet ($\alpha = 0,05$). Quanto à alteração de cor, houve diferenças significantes para “material de pulpotomia” ($P<0,001$) e “tempo de avaliação” ($P<0,001$) e para a interação “material*tempo de avaliação” ($P=0,004$). Na análise de viabilidade celular, BD e BCR foram semelhantes entre si em todas as diluições avaliadas e semelhantes ao controle ($P=0,3660$). BCR apresentou menor produção de EROS e ON em relação ao grupo LPS (C+) ($P<0,05$) e semelhante ao DMEM (C-) ($P>0,05$). BD apresentou valores de EROS e ON semelhantes aos dois grupos controle ($P>0,05$). Tanto BCR quanto BD quando utilizados em pulpotomia total ou parcial podem alterar significativamente a cor dos dentes. Embora ambos materiais sejam biocompatíveis, BCR apresentou uma redução na produção de EROS e ON em hDPCs estimuladas com LPS.

Palavras-chave: silicato de cálcio, viabilidade celular, pulpotomia total, óxido nítrico, pulpotomia parcial, espécies reativas de oxigênio.

ABSTRACT

ABSTRACT

This study aimed to compare tooth discoloaration in immature teeth mimicking total and partial pulpotomy using Biodentine (BD) and Bio-C repair (BCR) and evaluated the cytotoxicity of the materials to pulp cells (hDPCs). Seventy-two bovine mandibular incisors were prepared to simulate complicated coronary fractures in an immature tooth. In the pulp tissue simulation, the roots were filled with a mixture of agar and blood (control group), and BD and BCR were placed on it with approximately 2 mm of thickness. The color evaluation analyzes of the samples were performed before and immediately after the pulpotomy and repeated at 30, 60 and 90 days. The hDPCs were contacted with different dilutions of culture media previously exposed to such materials and tested for cell viability using the MTT assay. Subsequently, hDPCs were stimulated through contact with lipopolysaccharides (LPS) and exposed to extracts, for quantitative measurement of reactive oxygen species (ROS) and nitric oxide (NO). Data were evaluated by ANOVA followed by the Tukey and Dunnet test ($\alpha = 0.05$).For tooth descoloration, there were significant differences for “pulpotomy material” ($P<0.001$) and “evaluation time” ($P<0.001$) and for the interaction “material*evaluation time” ($P=0.004$). In the cell viability analysis, BD and BCR were similar to each other in all evaluated dilutions and similar to the control ($P=0.3660$). BCR showed lower production of ROS and ON in relation to the LPS group (C+) ($P<0.05$) and similar to DMEM (C-) ($P>0.05$). BD showed ROS and NO values similar to the two control groups ($P>0.05$). Both BCR and BD when used in total or partial pulpotomy can significantly change the color of teeth to a perceptible degree. Although both materials are biocompatible, BCR decreased the production of ROS and NO in hDPCs stimulated with LPS.

Keywords: calcium silicate, cell viability, full pulpotomy, nitric oxide, partial pulpotomy, reactive oxygen species.

INTRODUÇÃO E REFERENCIAL TEÓRICO

1. INTRODUÇÃO E REFERENCIAL TEÓRICO

Fraturas coronárias complicadas envolvendo esmalte e dentina com exposição pulpar devem ser preferencialmente tratadas com terapiaspulpares conservadoras (TPC) , mesmo em adultos e particularmente em crianças com dentes permanentes imaturos (Bourguignon *et al.,* 2020; Abuelniel *et al.,* 2020; Oliveira *et al.,*2021). As TCPs incluem capeamento pulpar e pulpotomia realizados em diferentes níveis de acordo com os sinais clínicos da contaminação pulpar observados após a exposição. A pulpotomia total (PT) envolve a remoção da porção coronal da polpa até o nível dos orifícios do canal (Taha *et al.,* 2018), mantendo o tecido remanescente. A pulpotomia parcial (PP) também denominada pulpotomia de Cvek, consiste na amputação de 2 a 3 mm de tecido pulpar coronal inflamado, mantendo o tecido pulpar remanescente na porção coronal e radicular (Fong & Davis 2002, Ojeda-Gutierrez *et al.,* 2013, Bimstein & Rotstein 2016, Yang *et al.,* 2020).

A decisão de realizar pulpotomia total ou parcial é empírica, uma vez depende da capacidade de controlar o sangramento após a amputação pulpar, tem sido consideradofator decisivo na determinação do grau de inflamação e potencial de cicatrização do tecido remanescente (Stanley 1989, Matsuo *et al.,* 1996, Fong & Davis 2002). O material de proteção pulpar escolhido é um fator determinante para o bom prognóstico do caso, uma vez que o controle da inflamação do tecido pulpar pode ser afetado pela composição de cada material (Kim *et al.,*2018; Giraud *et al.,*2018; Giraud *et al.,* 2019), e que preservar a

vitalidade pulpar é crucial para o desenvolvimento radicular completo (Abuelniel *et al.,* 2020; Bakhtiar *et al.,* 2017; Oliveira *et al.,* 2021) e. Além disso, como a inflamação pulpar causa um aumento do estresse oxidativo intracelular, levando a eventos destrutivos (Sanz *et al.,* 2021, Zorov *et al.,* 2014;Wu *et al.,* 2018), a escolha de materiais capazes de modular a liberação de mediadores inflamatórios pelas células pulpares se torna interessante.

O medicamento utilizado na pulpotomia idealmente deve ser bactericida, biocompatível e promover a cicatrização da polpa radicular (Vargas KG *et al.,* 2016; Elbahary S *et al.,* 2020). Por muitos anos, o $Ca(OH)_2$ foi considerado o material de escolhaem TCPs. Contudo, como esse material possui algumas desvantagens como a baixa capacidade de selamento, baixa adesão e alta solubilidade (Rao Q *et al.,* 2020), outros materiais foram ganhando espaço dentro deste cenário, entre os quais podemos destacar oscimentos bioceramicos.

O primeiro cimento reparador biocerâmico a ser utilizado na odontologia, foi o agregado trióxido mineral (MTA), um biocerâmico à base de silicato de cálcio que ganhou amplo uso por apresentar excelentes propriedades de vedação e biocompatibilidade e possuir diversas aplicações clínicas (Parirokh *et al.,* 2010; Hannah Beatty *et al.,* 2015). Contudo, algumas desvantagens como preço, características de manuseio e potencial de descoloração (Parirokh *et al.,* 2010; Hannah Beatty *et al.,* 2015) incentivaram o lançamento de novos produtos a fim de suprir tais características.

Dentre eles, pode-se citar o Biodentine (BD; Septodont, Saint Maur-des-Fosses, França), um produto que foi introduzido no mercado como um cimento reparador com capacidade desubstituir a dentina (Kunert & Lukomska-Szymanska, 2020), o qual apresenta propriedades clínicas e biológicas comparáveis aos do MTA (Abuelniel *et al.,* 2020; Bakhtiar *et al.,* 2017). Entre algumas das vantagens do BD estão o tempo de presa mais rápido, facilidade de manuseio e menor descoloração coronal (Kaur *et al.,* 2017). Atualmente, outros materiais à base de silicatos com a característica de serem prontos para uso foram desenvolvidos. Entre eles, o Bio-C Repair (BCR; Angelus), o qual é um material com baixa citotoxicidade (Ghilotti *et al.,* 2020; Guerreiro *et al.,* 2021; López-García *et al.,* 2019; Oliveira *et al.,* 2019; Oliveira *et al.,* 2020; Villa *et al.,* 2020), boa biocompatibilidade (Benetti *et al.,* 2019) e capacidade de biomineralização (Benetti *et al.,* 2019; López-García *et al.,* 2019).

Embora diversos estudos tenham avaliado BD e BCR em pulpotomias (Santos *et al.,* 2021) até o momento, nenhum estudo comparou o potencial de descoloração de BD e BCR em função do nível de pulpotomia, parcial ou total. Outro fator importante em relação aos novos materiais de silicato de cálcio utilizados na pulpotomia é determinar o potencial de inibir a produção de estresse oxidativo pelas células pulpares frente a estímulos como o lipopolissacarídeo (LPS).

PROPOSIÇÃO

2- PROPOSIÇÃO: Este trabalho objetivou comparar *in vitro* a descoloração dentária promovida pela pulpotomia parcial e total realizada com BD e BCR, e avaliar a produção de espécies reativas de oxigênio (EROS) e óxido nítrico (ON) induzida por ambos materiais em células da polpa dentária humana (hDPCs).

CAPITULO 1

3. CAPITULO 1

Artigo a ser submetido para INTERNATIONAL ENDODONTIC JOURNAL

Comparison of two bioceramic materials on staining after pulpotomy, viability and oxidative stress of pulp cells: an *in vitro* study

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Keywords: calcium silicate, cell viability, full pulpotomy, nitric oxide, partial pulpotomy, reactive oxygen species.

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Author Contribution

Gisele R. Silva and Camilla Christian G. Moura involved in conceptualization, data curation and writing. Nelly X. Alvarado, Gabriela L. Souza, Thamara Eduarda A. Magalhães and Gabrielle A.N. Freitas involved in investigation, methodology and roles/writing – original draft. Ana Paula T. Hidalgo involved in cells isolation training and (ROS) measurement. Camilla Christian G. Moura supervised the study. All authors contributed to discussions and manuscript review.

Abstract

Aim: This study compared the discoloration on fractured incisors treated by total and partial pulpotomy using Biodentine (BD) or Bio-C repair (BCR) and evaluated the cytotoxicity of the materials to pulp cells (hDPCs).

Methodology: Seventy-two bovine incisors were prepared to simulate fractures with pulp exposure in immature teeth. The roots were filled with agar and blood (control) and BD or BCR were inserted to simulate total and partial pulpotomies. Color assessment was performed before insertion, immediately after, and at 30, 60, and 90 days. hDPCs were stimulated with lipopolysaccharides (LPS) and exposed to material extracts (1:1) in two ways: 1- exposure to extracts and LPS for 24 hrs and 2- exposure to LPS for 24 h + exposure to extracts for 24 hrs. Data were evaluated by ANOVA followed by Tukey and Dunnet's test ($\alpha = 0.05$).

Results: Regarding the color change, there were no statistically significant differences between the two techniques evaluated ($p=0.126$), and after

90 days, the two materials caused an increase in tooth discoloration ($p<0.05$). In scenery 1, BD and BCR released ROS and NO similar to each other ($p>0.05$), with BCR showing lower values than the LPS group ($p<0.05$). In situation 2, ROS release was similar between BD, BCR and LPS ($p>0.05$), which presented higher values when compared to C- ($p<0.05$). The materials caused a reduction in NO release compared to the C+ group ($p<0.05$).

Conclusion: All materials cause tooth discoloration after 90 days, especially BD. The tested materials reduced the release of NO by hDPCs stimulated with LPS.

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Introduction

Pulp contamination by saliva and oral bacteria is expected after complicated crown fractures (Abuelniel *et al.*, 2020). Removal of the contaminated parts of the pulp during the first 24-48 hours following the fracture, and prevention of any further contamination by placing a biocompatible agent is critical for the healing (Abuelniel *et al.,* 2020). Calcium–silicate-based cements (CSCs) have been used in order to reduce pulp inflammation, down-regulating pro-inflammatory mediators as cytokines (Giraud *et al.,* 2018; Kim *et al.,* 2018; Weekate *et al.,* 2021), reactive oxygen species (ROS) (Wu *et al.,* 2018) and nitric oxide (NO), which may help to promote pulp healing in vital pulp treatment (VPT) (Weekate *et al.,* 2021).

The primary objective of VPT in immature teeth is to maintain the pulp, promoting continued root development and apical closure (Ghoddusi *et al.,* 2013; Asgary *et al.,* 2015; Abuelniel *et al.,* 2020). Pulpotomy is the gold standard VPT in traumatized anterior immature permanent teeth (Bimstein & Rotstein 2016;

Rao *et al.,* 2020). Pulpotomy includes pulp amputation performed at different levels based on the amount of perceived contamination of the pulp after its exposure (Bimstein & Rotstein 2016). Full pulpotomy (FP) ortotal pulpotomy, involves the removal of the coronal portion of the pulp to the level of canal orifices (Taha *et al.,* 2018), maintaining the remaining tissue. Partial pulpotomy (PP) also named Cvek pulpotomy, consists of the amputation of 2 – 3 mm of damaged, inflamed, coronal pulp tissue, maintaining the remaining pulp tissue on the coronal and radicular portion (Fong & Davis 2002; Ojeda-Gutierrez *et al.,* 2013; Bimstein & Rotstein 2016; Yang *et al.,* 2020). Clinically, the decision to perform partial or total pulpotomy has been based on pulp's appearance and the ability to control bleeding after pulp amputation, which is considered a decisive factor in determining the degree of inflammation and healing potential of the remaining tissue (Stanley1989; Matsuo *et al.,* 1996; Fong & Davis 2002). Additionally, other factors such as the time elapsed between the accident and treatment, size of the exposure and age of patient may contributed to the choice of cervical pulpotomy (Fong & Davis 2002; Bimstein & Rotstein 2016). Although some authors consider that the FP provides a higher chance of removing the infected and irreversibly inflamed tissue compared to PP (Santos *et al.,* 2021), there are clinical studies that support this assertion. A possible benefit of PP technique would be the possibility to preserve the cell-rich coronal pulp tissue and maintain the physiologic deposition on the crown and cervical third of the root, strengthening the tooth (Fong & Davis 2002; Bimstein & Rotstein 2016). However, a concern to be raised is the potential of tooth discoloration, as in this technique the material remains in the crown. New CSCs, as Biodentine-BD (Septodont, Saint-Maur des-Foss es, France) and BiocRepair-BCR (Angelus, Londrina, PR, Brazil) show a lower tendency to promote crown staining than Mineral trioxide aggregate (MTA) (Palma *et al.,* 2020).

Although CSCs materials are correlated with a high rate of clinical success, a current question is related to the effect of different CSCs materials on the control of inflammation on injured pulp tissue, as it may be affected by the composition of each material (Kim *et al.,*2018; Giraud *et al.,*2018; Giraud *et al.,*

2019). Upon bacterial infection, dental pulp cells produce excessive levels of intracellular ROS, which result in oxidative stress (Sanz *et al.,* 2021) and cellular damage (Zorov *et al.,* 2014;Wu *et al.,* 2018), affecting the cells' architecture and integrity (Zhang *et al.,* 2021). NO mediates the process of tissue inflammation, regulating cell differentiation and growth (Korhonen *et al.,* 2005). Since progression of inflammation causes destructive events, such as increased oxidative stress (Bullon *et al.,* 2011) and nitric oxide production, which may act destructively, materials able to modulate the release of inflammatory mediators by pulp cells are desirable.

In this way the aim of the referred study was to compare *in vitro* tooth discoloration promoted to partial and full pulpotomy performed using BD and BCR, and evaluate the ROS and NO production induced by both materials in human dental pulp cells (hDPCs). The null hypothesis was that no significant differences would be found in the cell viability, ROS and NO production, and coronal discoloration associated with the techniques and evaluated materials.

Materials and methods

The manuscript of this laboratory study has been written according to Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) 2021 guidelines (Nagendrababu *et al.* 2021). Figure 1 summarize the key steps in the reporting of the present laboratory study.

Selection and preparation of samples for colour assessment

Considering crown discoloration (ΔE00) as primary outcome, the data from (Oliveira *et al.,*2020) were used to sample calculation which was performed using the statistical software package BioEstat version 5.0.1 (Mamirauá Institute, Tefé, AM, Brazil). A sample size of 72 teeth (n=12 per group) was established to have a 95% chance of detection, significant at the 5% level (2-sided test), with a minimum detectable difference in means of 2.2 with an expected standard deviation of 1.0. Central incisors from zebu cattle, aged 30–36 months were

initially obtained from a local abattoir (Real, Uberlândia, MG, Brazil), cleaned with scaler, inspected, and selected in four stages according to the method previously described by (Rosatto *et al.,* 2020; Oliveira *et al.,* 2021). The selected teeth were stored in distilled water at 4°C until use.

It was performed a simulation of a complicated crown fracture in an immature tooth (Figure 2) (Oliveira *et al.,* 2020; Oliveira *et al.,* 2021). Part of the crown (8 mm above the amelocemental region) and the root (12 mm below the apical region) were removed perpendicular to its long axis, enlarged using #1 -5 peeso reamers followed by a drill PM 82 (KG, Sorensen) to standardize the samples. Next, each root was flushed with 20 mL of 2.5% sodium hypochlorite followed by 20 mL of distilled water, and the apical region was closed with composite resin (3M ESPE Z250, Brazil). Then, the samples were embedded in a polystyrene resin (Cristal, Piracicaba, SP, Brazil) and polyether impression material (Impregum F, 3M-Espe, Seefeld, Germany) (Soares *et al.,* 2005).

To simulate pulp tissue, 8 mL of agar was prepared (Kasvi, São José dos Pinhais, Brazil) using 1600 µL of fresh uncoagulated bovine blood and inserted until the amelocemental region (80 µL per tooth) or up to 3 mm above it (100 µL per tooth), to mimic FP or PP, respectively. The specimens were randomly assigned to two experimental groups (BCR, and BD) and two control group (FP/agar + blood and PP/agar + blood). The materials were placed at approximately 2 mm thickness above the agar + blood. Finally, the crown canals were closed using modified glass ionomer (Riva Light Cure, Australia). The teeth were stored in a humified chamber at 37°C during the experimental periods.

Randomization

The randomization process (blocked random scheme) was carried out using the website www.sealedenvelope.com. The identification of the treatment to be applied on samples followed the sequentially-numbered previously. Randomization and allocation were made by the same researcher, who was not

involved in the implementation and evaluation process. Because they didn't know the coding method, those who assessed color measures and statically evaluated the data were blinded.

Colour assessment

Colour values of the samples were determined using a spectrophotometer (Easyshade Compact Advance 4.0; Vita-Zahnfabrik, Bad Sackingen, BW, Germany). For reproducible and standardized readings, an individual silicone index (Precise SX; Dentsply, Petropolis, RJ, Brazil) with a 6-mm hole was created for each sample to reposition the Easyshade tip at each time-point (Oliveira *et al.,* 2020; Oliveira *et al.,* 2021). Three colour measurements were performed on each tooth, and the average was recorded. Six sessions of colour assessments were conducted at the following intervals: baseline (sound tooth); T0, after cavity preparation; T1, immediately after pulpotomy; T2, 30 days; T3, 60 days and T4, 90 days pulpotomy. The colour readings were quantified in terms of the L*, a* and b* coordinate values, established by the Commission Internationale de l'Eclairage (CIELAB system) for each specimen. The colour difference of the same specimen was calculated using CIEDE2000 colour difference (DE00)1, which was calculated as follows: $\Delta E00 = [(\Delta L/KLSL)2+ (\Delta C/KCSC)2+ (\Delta H/KHSH)2 + RT(\Delta C/KCSC)(\Delta H/KHSH)]1/2$, where ΔL, ΔC and ΔH are the lightness, chroma and hue differences between colour measurements. KL, KC and KH are the parametric factors that influence the viewing conditions and illuminating conditions. RT is the function for the hue and chroma interaction differences in the blue region. SL, SC and SH are the weighting functions for the colour difference adjustment, considering the location variation of the L*, a* and b* coordinates1. The whiteness index (WI) was calculated using the following formula: WI = 0.551*L −2.324*a −1.1*b 2. Moreover, Yellowness Index (YI) per ASTM Method E313 was calculated as follows: YI=(100(CxX - CzZ))/Y. Where X, Y, and Z are the CIE Tristimulus values, and the coefficients used was based on the illuminant/observer D65/2°.

Isolation of hDPCs

The study protocols were approved by the Ethical Committee of the Federal University of Uberlândia (protocol number 09016219.1.0000.5152). hDPCs were obtained from healthy permanent third molars teeth (*n* = 3). Cells were isolated as previously described (Oliveira *et al.,* 2020; Oliveira *et al.,* 2021). Briefly, the fragments of pulp dental tissue were digested using the combination of Collagenase I and Dispase II (Sigma-Aldrich, St. Louis, MO, USA) for 1 h at 37℃, and cultured in Dulbecco's modified Eagle's medium (DMEM) (LGC Biotechnology, Cotia, SP, Brazil) containing 10% foetal bovine serum (FBS; Gibco, Invitrogen, Carlsbad, CA, USA) and 100 µg/mL penicillin/streptomycin (Sigma-Aldrich) at 37℃ in a humified atmosphere of 95% air and 5% CO_2. The culture medium was changed every three days. Cells from passages 4-5 were used in the experiments.

Preparation of BD and BCR extracts

The protocol for preparing the BD and BCR extracts were previously described by (Oliveira *et al.,* 2021). The materials were loaded into 5 mm x 2 mm (Width x Height) sterile cylindrical polyethylene moulds, sterilized using ultraviolet irradiation for 60 min, and immediately after this period, were stored DMEM (LGC Biotechnology) for 24 h at 37℃ with 95% humidity and 5% CO_2 to create the extracts. The supernatant was then collected, filtered with sterile 0.22 µm membrane filter, and diluted with the complete media at a ratio of 1: 16.

Cell cytotoxicity assay

Cytotoxicity was assessed by means of a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. 2×10^4 hDPCs/well were seeded in 96-well plates (Corning, New York, NY, USA) containing 10% DMEM and maintained at 37℃ at 95% humidity and 5% CO_2 for 24 h. After this period, the media was removed and fresh extracts (1:1-1:16) were placed in contact with the hDPCs for 24 h. Cells cultured in 10% DMEM without any extract were used as

the control. MTT reagent (Sigma-Aldrich) was added to the wells at a concentration of 5 mg/mL and incubated for 4 h. Colorimetric quantitative changes were measured using a microplate reader (Biochrom, Cambourne, UK) at a wavelength of both 570 nm. Cell viability was evaluated in proportion to the absorbance and expressed as the percentage of viable cells. Considering that a threshold of 70% cell viability is considered cytotoxic according to ISO 10993-5 guideline, the materials tested were not considered cytotoxic even at the lowest dilutions. Thus, 1:1 dilution was used for the quantitative reactive oxygen species (ROS) measurement and nitric oxide (NO) assay.

hDPCs lipopolysaccharide-induced stress and exposure to extracts

The hDPCs were plated on 96 well plates ($2x10^4$ cell/well) and allowed to adhere overnight. Then, to mimicking the inflammatory pulp condition, hDPCs were exposed to 20 µg/mL of LPS (Ultrapure grade, Escherichia coli O111:B4; Invitro Gen, San Diego, CA, USA) in two different ways: (1) constant exposure of hDPCs to LPS (20 µg/mL) and extracts (1:1) for 24 hours [LPS/Extract (24 hrs)] and, (2) transient exposure of hDPCs to LPS (20 µg/mL) for 24 hours, followed by exposure to extracts (1:1) for 24 hours [LPS (24 hrs) + Extract (24 hrs)]. Positive control groups were formed by cells stimulated by LPS by 24 h [(LPS/Extract (24 hrs)]; and cells cultured in LPS for 24 h followed by 24 h in regular complete medium [(LPS (24 hrs) + Extract (24 hrs)]. Negative control group was mantained in DMEM (not LPS-stimulated).

Quantitative reactive oxygen species (ROS) measurement

hDPCs were seeded in 96-well plates ($2x\ 10^4$ cells/well) in DMEM supplemented with 10% SFB, and exposed to experimental treatments, as previously described. At the end of the time point, the supernatant was collected to further NO quantification. Then, hDPCs exposed to experimental treatments

were washed with phosphate-buffered saline (PBS) and incubated with PBS containing 10 µM of 2',7'-dichlorodihydrofluorescein diacetate (DCFH2-DA, Sigma–Aldrich, St. Louis, MO) for 45 min at 37°C to allow the probe to enter cells. After that, cells were washed three times with PBS to remove excess probe, and DCFH2-DA fluorescence intensity was detected using Glomax (Promega, Wisconsin, USA), with excitation at 475 nm and emission at 500-550 nm. The mean values obtained for the positive control group were considered 100% ROS production.

Nitric oxide (NO) production

NO production was quantified by Griess method using a microplate reader (Biochrom, Cambourne, UK). 100-µL aliquots of the supernatant of each sample were placed with equal quantities of Griess reagent composed of 1 g of sulfanilamide (Merck KGaA, Darmstadt, Germany), 0.1 g of N (1-naphthyl) ethylenediamine dichloride (Merck KGaA), 2.5 mL of orthophosphoric acid (Mallinckrodt Chemical, St. Louis, MO, USA), and 100 mL of deionized water. After a 10-min incubation at room temperature protected from light, the absorbance was determined in a microplate reader equipped with a 540 nm filter (Biochrom). The absorbance of this reaction at 540 nm is linearly proportional to the nitrite concentration in the samples. The mean values obtained for the positive control group were considered 100% NO production (Bonvicini *et al.,* 2021a; Bonvicini *et al.,* 2021b.)

Statistical analysis

One-way ANOVA was used to compare whiteness index at baseline (sound tooth) to standardizing the colour samples among groups. Repeated measures ANOVA and Tukey's tests were used to compare the colour parameters (ΔE00, WI and YI), where 'time assessment' was used as a repetition factor. Cell viability, ROS measurements and NO production data were analyzed

for normality using Kolmogorov-Smirnov's test. Two-way ANOVA and Tukey's tests were used to compare data of cell viability intragroup amongst dilutions and amongst the materials at each of the dilutions tested. Dunnet's test was used to comparisons with the control group. The multiple T-test was used to determine if differences observed among the effects of materials in the release of ROS and NO were statistically significant. Statistical analysis was performed using Jamovi 2.0 statistical software package (dev.jamovi.org) and GraphPad Prism v.6 (GraphPad Software, La Jolla, CA, USA). The significance level was set at $\alpha = 0.05$ for all data analyses.

Results

Colour assessment

One-way ANOVA showed similar whiteness index on samples among groups (P = 0.414) before experiment. The table 1 presents the results of the overall color changes (ΔE00) according to pulpotomy and assessment time. RM ANOVA showed statistically significance to “pulpotomy material” (P<0.001) and “assessment time” (P< 0.001) and for the interaction “material*assessment time” (P=0.004). However, there was not a statistically significance for “pulpotomy technique” (P=0.126) or interactions between “assessment time and pulpotomy technique”, “pulpotomy technique*material” (P=0.832) and “assessment time*pulpotomy technique*material” (P=0.258).

Immediately after the procedures and after the periods of 30 and 60 days, regardless of the technique used for pulpotomy, there was a greater change in colour with the use of BCR compared to the control group. For these periods, BD showed similar discoloration to the control group and BCR. However, after 90 days, it was observed that both materials caused an increase in tooth discoloration compared to the control group, with BD showing greater discoloration than BCR (Table 1).

The behavior of L*, a*, and b* parameters according to materials used to pulpotomy and assessment times are shown in Figure 2. Some decreasing on lightness occurs after all pulpotomy techniques. BCR seems to have more linear behavior in this parameter. In general, the materials resulted in redness and yellowness increasing.

The WI values are presented on figure 3. RM ANOVA showed statistically significance to “assessment time” (P<0.001) and for the interaction “assessment time*material” (P< 0.001), “assessment time*pulpotomy technique (P<0.001). No statistically significance difference was observed to “pulpotomy technique” (P=0.281), “material” (P=0.584) and for interaction “assessment time*pulpotomy technique*material” (P=0.553).

There was a reduction in the whiteness index of the teeth immediately after total and partial pulpotomy, regardless of the material used. However, in total pulpotomy, there was an increase in WI after 30 and 60 days of pulpotomy, reaching baseline values. In turn, at 90 days, the WI significantly reduces. For partial pulpotomy, the WI remains lower than the initial at 30 and 60 days, reaching the lowest values at 90 days (Figure 3). The use of agar + blood (control group) was able to decrease WI and also the immediate application of the pulpotomy materials seems to be able to decrease significantly WI in experimental groups. However, at 90 days WI remains stable in control group remains stable after 90 days.

The figure 4 shows YI values. RM ANOVA showed statistically significance to “assessment time” (P<0.001) and for the interaction “assessment time*material” (P< 0.001), “assessment time*pulpotomy technique (P=0.002). No statistically significance difference was observed to “pulpotomy technique” (P=0.591), “material” (P=0.733) and for interaction “assessment time*pulpotomy technique*material” (P=0.722).

Immediately after total pulpotomy, both materials increased YI values, which were similar to the results of 90 days. In partial pulpotomy, YI increased immediately after material insertion, remaining constant until 60 days later. At 90

days, there was a new increase in YI, which was statistically significant compared to the other periods evaluated. Considering the time*material factors, YI of the control group remained constant over time. For the BCR, YI increases significantly only after 90 days and for the BD the YI increased immediately after the insertion of the material, reaching the highest values after 90 days (Figure 4).

Cell cytotoxicity assay

Figure 5 presents the cell viability evaluated by MTT formazan assay after contact between hDPCs and extracts diluted to 1:1, 1:2, 1:4, 1:8 and 1:16. BD and BCR were similar to each other in all dilutions evaluated (P=0.3660). There were no statistically significant differences between the tested dilutions (P>0.05) and between the dilutions and the control group (P>0.05) for both BD and BCR. In addition, it could be observed by the cell survival mean values, that all materials maintained cell viability levels above 80% for all dilutions.

Quantitative reactive oxygen species (ROS) measurement

Figure 6 presents the amounts of ROS produced by hDPCs after contact between the cells and extracts diluited to 1:1. In the group in which cells were constantly exposed to LPS [LPS/Extract (24 hrs)], BD and BCR had similar ROS release to each other (P=0.66). BCR showed lower ROS production compared to the LPS group (positive control) (P=0.02) and similar to the DMEM (negative control) (P=0.90). BD showed ROS values similar to both control groups (P>0.05) (Figure 6a). In cells in which LPS was removed followed by contact with extracts of materials [LPS (24 hrs) + Extract (24 hrs)], the BD, BCR and LPS (positive control) groups showed a similar percentage of ROS release to each other (P>0.05), and higher compared to the DMEM group (negative control) (P<0.05) (Figure 6b).

Nitric oxide (NO) production

Figure 7 presents the percentage of NO produced by hDPCs after contact between the cells and extracts diluted to 1:1. Similar to the results observed in the measurement of ROS, the evaluation of NO production demonstrated that in the cells constantly exposed to LPS [LPS/Extract (24 hrs)], BD and BCR had similar NO production to each other (P=0.85). BCR showed lower NO values compared to the LPS (positive control) (P=0.01) and similar to the DMEM (negative control) (P=0.19). BD showed NO production values similar to the control groups (P>0.05) (Figure 7a). On the other hand, in cells in which LPS was removed [LPS (24 hrs) + Extract (24 hrs)], the subsequent contact with the materials caused a reduction in NO production compared to the LPS group (positive control) (P<0.05). BD, BCR and DMEM showed a similar percentage of NO production to each other (P>0.05) (Figure 7b).

Discussion

On the current study the null hypothesis was rejected, since there were no differences among the pulpotomy procedures for CSCs in the assays carried out. The experimental model presented here was designed to simulate the clinical situation of full pulpotomy and partial pulpotomy, by applying the materials on a mixture of agar containing blood, which has a gelatinous consistency similar to that of pulp tissue (Oliveira *et al.,* 2020; Oliveira *et al.,* 2021).

In the present study, the color stability of BD (ΔE00) over time compared to BCR in FP differ from previous data (Oliveira *et al.,* 2021). The colour changes of BD could be related to the amount of fluid/blood uptake during setting reaction and the total setting time of the material. Since the BD has a shorter setting time around 12 minutes, a strong hydration reaction occurs during initial setting and hence a higher amount of fluid is uptaked, which could explain why the YI increases immediately after BD insertion (Chen *et al.,* 2020). Furthermore, it has been shown that voids and pores may entrap blood components and cause discoloration of the material (Lenherr *et al.,* 2012). BCR is a premixed material and therefore may present better homogeneity compared to BD, which needs to be mixed in a specific device before use (Slaboseviciute

et al., 2021). Although, to the authors' knowledge, there are no studies comparing the porosity of the two materials, analyzes by scanning electron microscopy demonstrate that BD exhibits an irregular surface organization when compared to BCR (Ghilotti *et al.,* 2020).

Previous studies evaluating BD in pulpotomies in association with blood, corroborate the results found, demonstrating the ability to change color promoted by the material in total pulpotomy (Yoldas *et. al.,* 2016; Oliveira *et al.,* 2020) and partial pulpotomy (Oliveira *et al.,* 2021). However, few studies have evaluated the discoloration potential promoted by BCR. It was previously demonstrated that the presence of BCR does not result in a greater color change immediately after the insertion of material and after 90 days (Oliveira *et al.,* 2021), differing from the results presented here. This divergence of results could be associated with the type of agar used and the amount of blood diluted in the medium. In the present study, Mueller Hinton agar was applied to the methodology. As this agar has a soft yellow colour, twice the blood volume recommended in the study by (Oliveira *et al.,* 2021) was used to achieve a color similar to that of the dental pulp. Furthermore, it is important to note that BCR contain iron and aluminum in its composition (Benetti *et al.,* 2019; Ghilotti *et al.,* 2020; López-García *et al.,* 2019). Oxidation of the iron content remaining in the set material is considered a possible mechanism for tooth discolouration (Felman & Parashos, 2013; Shokouhinejad *et al.,* 2016).

Considering that in PP, only part of the coronal pulp is removed, it was expected that crown staining caused by biomaterials could become more noticeable compared with FP. However, there was no statistical difference between materials and techniques. Beside of that, it is important to note that the control group, in the technique of FP presented the difference of the WI (1.8) smaller than the whiteness acceptability threshold (2.60 WI units) (Pérez *et al.,* 2016). Therefore, it is possible to suggest that the presence of a greater amount of coronary pulp, mimicked by blood agar mixture in PP technique are related to tooth staining. The mechanism of discoloration of blood is attributed to penetration of erythrocytes into dentine, their hemolysis leading to accumulation

of hematin molecule in the dentinal tubules (Felman & Parashos 2013; Lenherr *et al.,* 2012; Yoldas *et al.,* 2016).

In the current study, the potential of these materials to modulate oxidative stress in hDPCs, under inflammatory-induced conditions were investigated. hDPCs have been identified as the major cell type in dental pulp tissue (Song *et al.,* 2017) playing a key role in regulating host defense and dental pulp regeneration (Eramo *et al.,* 2018). In order to simulate the clinical situation of VPT, extracts from the materials in different dilutions (1:1–1:16) were evaluated, as the concentration decreases with the elimination of leachable components by the extracellular fluid (Mestieri *et al.,* 2015). The results with BCR and BD are consistent with those from other studies (Benetti *et al.,* 2019; Ghilotti *et al.,* 2020; Oliveira *et al.,* 2021; Sanz *et al.,* 2021; Oliveira *et al.,* 2021; Ghilotti *et al.,* 2020), demonstrating excellent cytocompatibility, higher than 80% in all periods and dilutions and similarity to DMEM group.

The analysis of cell viability at 24 h using MTT is a classical assay that is widely used to evaluate the cytotoxic effects of new materials (Ghilotti *et al.,* 2020; Oliveira *et al.,* 2020; Oliveira *et al.,* 2021; Weekate *et al.,* 2021; Mestieri *et al.,* 2015), is most sensitive compared to other *in vitro* cytotoxicity methods and is considered gold standard for in vitro cytotoxicity test (Pintor *et al.,* 2020).

In this study, *Escherichia coli* LPS was selected to simulate the inflammatory environment often found in teeth submitted to pulpotomy since the main cause of pulp injury is bacterial infection. LPS is one of the most common virulence factors of gram-negative bacteria and has been used to treat hDPCs to establish experimental models examining pulp inflammation (Oliveira *et al.,* 2020; Wang *et al.,* 2021; Weekate *et al.,* 2021). Full pulpotomy involves the removal of the entire coronal portion of the vital pulp to the level of canal orifices while maintaining the health of the remaining radicular portion. This procedure provides a higher chance of removing the infected and irreversibly inflamed tissue compared to partial pulpotomy (Taha *et al.,* 2018). In this sense two models of LPS-stimulation were used. In the model in which exposure to the extracts was performed after stimulation with LPS, the intention was to represent the total

pulpotomy, through the complete removal of the inflammatory stimulus to later perform the exposure of the cells to the material. In the model in which the exposure to extracts was performed simultaneously with stimulation with LPS, the objective was to simulate a situation similar to what happens in partial pulpotomies, in which there may be residues of infected/inflamed tissue concomitant with the presence of the material.

The results demonstrated, as in previous studies (Souza *et al.,* 2020; Bonvicini *et al.,* 2021a; Bonvicini *et al.,* 2021b; Weekate *et al.,* 2021), that LPS induces an increase in ROS and NO production. The present finds indicated that the presence of BCR simultaneously with LPS prompts the cells to be resistant to LPS-induced inflammatory and toxic stress. On the other hand, when cells are first stimulated with LPS for 24 hours and then exposed to the materials, ROS levels remain similar to those of the LPS group (positive control). These results disagree with previous studies, which demonstrated adequate levels of ROS in cells treated with BD (Sanz *et al.,* 2021; Weekate *et al.,* 2021). However, the results cannot be directly compared since in the present study extracts from fresh material were evaluated, and the aforementioned studies evaluated extracts from after the complete setting of the material. Regarding the NO levels, the contact of LPS-stimulated hDPCs with the material extracts reduced NO release to basal levels. A previous study evaluating the effect of BD on hDPCs demonstrated that this material does not evoke severe inflammation and cellular or tissue destruction, keeping nitric oxide production at low levels (Chang *et al.,* 2014). Our results suggest the anti-inflammatory effect of the tested materials, since NO is considered an indirect marker of inflammation, and BD and BCR reduced NO production by LPS-stimulated hDPCs to values similar to those found in unstimulated cells. Therefore, the adequate levels of NO exhibited by BD and BCR-treated groups further support their use in VPT procedures in which the regenerative potential of hDPCs with an appropriate stress response is exploited.

Conclusion

BCR and BD used in total or partial pulpotomy can significantly change the color of the teeth, reducing the whiteness index and increasing the yellowing index, with the greatest changes in BD group. Both total pulpotomy and partial pulpotomy promote significant changes in tooth color, with no differences between the two techniques evaluated. The similarity between the results of viability in BD, which has been considered the new gold standard in pulpotomies, and BCR, makes this material suitable for pulpotomies, with the advantage of ready-to-use formulations. Additionally, BCR makes pulp cells resistant to LPS-induced inflammation and decreases NO production at baseline levels.

REFERENCES

Abuelniel GM, Duggal MS, Kabel N (2020) A comparison of MTA and Biodentine as medicaments for pulpotomy in traumatized anterior immature permanent teeth: A randomized clinical trial. *Dental Traumatology* **36**, 400-410. https://doi.org/10.1111/edt.12553

Asgary S, Eghbal MJ, Fazlyab M, Baghban AA, Ghoddusi J (2015) Fiveyear results of vital pulp therapy in permanent molars with irreversible pulpitis: a non-inferiority multicenter randomized clinical trial. *Clinical Oral Investigation* **19**, 335–41. https://doi.org/10.1007/s00784-014-1244-z

Benetti F, Queiroz ÍOA, Cosme-Silva L, Conti LC, Oliveira SHP, Cintra LTA (2019) Cytotoxicity, Biocompatibility and Biomineralization of a New Ready-for-Use Bioceramic Repair Material. *Brazilian Dental Journal*. **30**, 325-332. https://doi.org/10.1590/0103-6440201902457

Bimstein E, Rotstein I (2016) Cvek pulpotomy - revisited. *Dental Traumatology.* **32**, 438-442. https://doi.org/10.1111/edt.12297

Bonvicini JFS, de Souza GL, Basso FG, de Souza Costa CA, Moura CCG, Silva MJB, et al. (2021) Specific parameters of infrared LED irradiation promote the inhibition of oxidative stress in dental pulp cells. *Archives of Oral Biol.* **131**:105273. https://doi.org/10.1016/j.archoralbio.2021.105273

Bonvicini JFS, Basso FG, de Souza Costa CA, Soares CJ, Turrioni AP (2022) Photobiomodulation effect of red LED (630 nm) on the free radical levels produced by pulp cells under stress conditions. *Lasers in Medical Science.* **37**, 607-617. https://doi.org/10.1007/s10103-021-03309-x

Bullon P, Cordero MD, Quiles JL, Morillo JM, del Carmen Ramirez-Tortosa M, Battino M. (2011) Mitochondrial dysfunction promoted by Porphyromonas gingivalis lipopolysaccharide as a possible link between cardiovascular disease and periodontitis. *Free Radical Biology and Medicine.* **50**, 1336-43.

https://doi.org/10.1016/j.freeradbiomed.2011.02.018

Chang, Seok-Woo; Lee, So-Youn; Ann, Hyo-Jung; Kum, Kee-Yeon; Kim, Eun-Cheol (2014). Effects of Calcium Silicate Endodontic Cements on Biocompatibility and Mineralization-inducing Potentials in Human Dental Pulp Cells. *Journal of Endodontics,* **40**, 1194–1200.

https://doi.org/10.1016/j.joen.2014.01.001

Chen SJ, Karabucak B, Steffen JJ, Yu YH, Kohli MR (2020) Spectrophotometric Analysis of Coronal Tooth Discoloration Induced by Tricalcium Silicate Cements in the Presence of Blood. *Journal of Endodontics.* **46**, 1913-1919.

https://doi.org/10.1016/j.joen.2020.09.009

de Souza GL, Moura CCG, Silva ACA, Marinho JZ, Silva TR, Dantas NO, et al. (2020) Effects of zinc oxide and calcium-doped zinc oxide nanocrystals on cytotoxicity and reactive oxygen species production in different cell culture models. *Restorative Dentistry and Endodontics.* **45**, e54.

https://doi.org/10.5395/rde.2020.45.e54

Felman D, Parashos P.(2013) Coronal tooth discoloration and white mineral trioxide aggregate. *Journal of Endodontics.* **39**, 484-7.
https://doi.org/10.1016/j.joen.2012.11.053

Fong CD, Davis MJ. (2002) Partial pulpotomy for immature permanent teeth, its present and future. *Clinical Oral Investigation.* **24**, 29-32.
https://doi.org/10.1016/j.joen.2012.11.053

F. Song, H. Sun, Y. Wang, H. Yang, L. Huang, D. Fu, et al. (2017) Pannexin3 inhibits TNF-α-induced inflammatory response by suppressing NF-κB signalling pathway in human dental pulp cells, *Journal of cellular and Molecular Medicine* **21**, 444–455.

https://doi.org/10.1111/jcmm.12988

Ghilotti J, Sanz JL, López-García S, Guerrero-Gironés J, Pecci-Lloret MP, Lozano A, et al. (2020) Comparative Surface Morphology, Chemical Composition, and Cytocompatibility of Bio-C Repair, Biodentine, and ProRoot MTA on hDPCs. *Materials (Basel).***13**, 2189.
https://doi.org/10.3390/ma13092189

Ghoddusi J, Forghani M, Parisay I. (2013) New approaches in vital pulp therapy in permanent teeth. *Iranian Endodontic Journal*. **9**, 15–22.

Giraud T, Jeanneau C, Rombouts C, Bakhtiar H, Laurent P, About I. (2019) Pulp capping materials modulate the balance between inflammation and regeneration. *Dental Materials* **35**, 24-35.
https://doi.org/10.1016/j.dental.2018.09.008

Giraud T, Jeanneau C, Bergmann M, Laurent P, About I (2018) Tricalcium silicate capping materials modulate pulp healing and inflammatory activity in vitro. *Journal of Endodontics* **44**, 1686–91
https://doi.org/10.1016/j.joen.2018.06.009

Kim DH, Jang JH, Lee BN et al. (2018) Anti-inflammatory and mineralization effects of ProRoot MTA and endocem MTA in studies of human and rat dental pulps in vitro and in vivo. *Journal of Endodontics* **44**, 1534–41.
https://doi.org/10.1016/j.joen.2018.07.012

Korhonen R, Lahti A, Kankaanranta H, Moilanen E (2005) Nitric oxide production and signaling in inflammation. *Current Drug Targets - Inflammation & Allergy* **4**, 471-479.

https://doi.org/10.2174/1568010054526359

Lenherr P, Allgayer N, Weiger R., Filippi A, Attin T, Krastl G (2012) Tooth discoloration induced by endodontic materials: A laboratory study. *International Endodontic Journal* **45**, 942–949.

https://doi.org/10.1111/j.1365-2591.2012.02053.x

López-García S, Lozano A, García-Bernal D, Forner L, Llena C, Guerrero-Gironés J, et al. (2019) Biological Effects of New Hydraulic Materials on Human Periodontal Ligament Stem Cells. *Journal of Clinical Medicine* **8**, 1216.

https://doi.org/10.3390/jcm8081216

Matsuo T, Nakanishi T, Shimizu H, Ebisu S (1996) A clinical study of direct pulp capping applied to carious-exposed pulps. *Journal of Endodontics* **22**, 551-6.

https://doi.org/10.1016/S0099-2399(96)80017-3

M. C. Wang;H. F. Tu;K. W. Chang;S. C. Lin;L. Y. Yeh;P. S. Hung; (2021). The molecular functions of Biodentine and mineral trioxide aggregate in lipopolysaccharide-induced inflamed dental pulp cells . *International Endodontic Journal* **54**, 1317-1327.

https://doi.org/10.1111/iej.13513

Mestieri, L.B, Gomes-cornélio A.L, Rodrigues E.M., Salles L.P, Bosso-martelo R., Guerreiro-tanomaru, J.M. et al. (2015) Biocompatibility and bioactivity of calcium silicate-based endodontic sealers in human dental pulp cells. *Journal Applied of Oral Science* **23**, 467–471.

https://doi.org/10.1590/1678-775720150170

Nagendrababu V, Murray PE, Ordinola-Zapata R, OA Peters, IN Rôças, JF Siqueira Jr, et al. (2021) PRILE 2021 guidelines for reporting laboratory studies in Endodontics: a consensus-based development. *International Endodontic Journal* **54**, 1482-1490.

https://doi.org/10.1111/iej.13542

Ojeda-Gutierrez F, Martinez-Marquez B, Arteaga-Larios S, Ruiz-Rodriguez MS, Pozos-Guillen A (2013) Management and followup of complicated crown fractures in young patients treated with partial pulpotomy. *Case Reports in Dentistry* **13**, 597563.

Oliveira LV, de Souza GL, da Silva GR, Magalhães TEA, Freitas GAN, Turrioni AP, et al. (2021) Biological parameters, discolouration and radiopacity of calcium silicate-based materials in a simulated model of partial pulpotomy. *International Endodontic Journal* **54**, 2133-2144.
https://doi.org/10.1155/2013/597563

Oliveira LV, da Silva GR, Souza GL, Magalhães TEA, Barbosa GLR, Turrioni AP, et al. (2020) A laboratory evaluation of cell viability, radiopacity and tooth discoloration induced by regenerative endodontic materials. *International Endodontic Journal* **53**, 1140-1152.
https://doi.org/10.1111/iej.13616

Palma, P. J., Marques, J. A., Santos, J., Falacho, R. I., Sequeira, D., Diogo, P, et al. (2020). Tooth discoloration after regenerative endodontic procedures with calcium silicate-based cements—An ex vivo study. *Applied Sciences* **10** , 5793.
https://doi.org/10.3390/app10175793

Pérez MDM, Ghinea R, Rivas MJ, Yebra A, Ionescu AM, Paravina RD, et al. (2016) Development of a customized whiteness index for dentistry based on CIELAB color space. *Dental Materials* **32**, 461- 7.

https://doi.org/10.1016/j.dental.2015.12.008

Pintor, A.V.B, Queiroz, L.D, Barcelos, R., Primo, L.S.G, Maia, L.C. & Alves, G.G. (2020) MTT versus other cell viability assays to evaluate the biocompatibility of root canal filling materials: a systematic review. *International Endodontic Journal* **53**, 1348–1373.
https://doi.org/10.1111/iej.13353

Rao Q, Kuang J, Mao C, Dai J, Hu L, Lei Z ,et al. (2020) Comparison of iRoot BP Plus and Calcium Hydroxide as Pulpotomy Materials in Permanent Incisors with Complicated Crown Fractures: A Retrospective Study. *Journal of Endodontics* **46**, 352-357
https://doi.org/10.1016/j.joen.2019.12.010

Rosatto CMP, Oliveira LV, Ferraz DC, Soares PBF, Soares CJ, Moura CCG. (2020) Apical Displacement and Residual Root Canal Filling with Single-Cone After Post Space Preparation: A Micro-CT Analysis. *Brazilian Dental Journal.* **31**, 25-31.
https://doi.org/10.1590/0103-6440202002597

Santos JM, Pereira JF, Marques A, Sequeira DB, Friedman S (2021) Vital Pulp Therapy in Permanent Mature Posterior Teeth with Symptomatic Irreversible Pulpitis: A Systematic Review of Treatment Outcomes. *Medicina (Kaunas)* **57**, 573.
https://doi.org/10.3390/medicina57060573

Sanz JL, Soler-Doria A, López-García S, García-Bernal D, Rodríguez-Lozano, FJ, Lozano A, et al. (2021) 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. *Journal of Endodontics* **47**, 1896-1906
https://doi.org/10.1016/j.joen.2021.08.001

Eramo S, Natali A, Pinna R, Milia E (2018) Dental pulp regeneration via cell homing. *International Endodontic Journal* **51**, 405-419.
https://doi.org/10.1111/iej.12868

Shokouhinejad N, Nekoofar MH, Pirmoazen S, Shamshiri AR, Dummer PM (2016) Evaluation and Comparison of Occurrence of Tooth Discoloration after the Application of Various Calcium Silicate-based Cements: An Ex Vivo Study. *Journal of Endodontics* **42**, 140-4.
https://doi.org/10.1016/j.joen.2015.08.034

Slaboseviciute M, Vasiliauskaite N, Drukteinis S, Martens L, Rajasekharan S (2021) Discoloration Potential of Biodentine: A Systematic Review. *Materials (Basel)* **14**, 6861.
https://doi.org/10.3390/ma14226861

Soares CJ, Pizi EC, Fonseca RB, Martins LR (2005) Influence of root embedment material and periodontal ligament simulation on fracture resistance tests. *Brazilian Oral Research* **19**, 11-6.
https://doi.org/10.1590/S1806-83242005000100003

Stanley HR (1989) Pulp capping: conserving the dental pulp--can it be done? Is it worth it? *Oral Surgery, Oral Medicine, Oral Pathology* **68**, 628-39.
https://doi.org/10.1016/0030-4220(89)90252-1

Taha NA, Abdelkhader SZ (2018) Outcome of full pulpotomy using Biodentine in adult patients with symptoms indicative of irreversible pulpitis. *International Endodontic Journal* **51,** 819-828.
https://doi.org/10.1111/iej.12903

Weekate K, Chuenjitkuntaworn B, Chuveera P, Vaseenon S, Chompu-Inwai P, Ittichaicharoen J, et al. (2021) Alterations of mitochondrial dynamics, inflammation and mineralization potential of lipopolysaccharide-induced human dental pulp cells after exposure to N-acetyl cysteine, Biodentine or ProRoot MTA. *International Endodontic Journal* **54**, 951-965.
https://doi.org/10.1111/iej.13484

Wu B, Li J, Ni H, Zhuang X, Qi Z, Chen Q, et al. (2018) TLR4 Activation Promotes the Progression of Experimental Autoimmune Myocarditis to Dilated Cardiomyopathy by Inducing Mitochondrial Dynamic Imbalance. *Oxidative Medicine and Cellular Longevity* **26**, 3181278.
https://doi.org/10.1155/2018/3181278

Yang Y, Xia B, Xu Z, Dou G, Lei Y, Yong W (2020) The effect of partial pulpotomy with iRoot BP Plus in traumatized immature permanent teeth: A randomized prospective controlled trial. *Dental Traumatology* **36**, 518-525.
https://doi.org/10.1111/edt.12563

Yoldaş SE, Bani M, Atabek D, Bodur H (2016) Comparison of the Potential Discoloration Effect of Bioaggregate, Biodentine, and White Mineral

Trioxide Aggregate on Bovine Teeth: In Vitro Research *Journal of Endodontics* **42**,1815-1818.
https://doi.org/10.1016/j.joen.2016.08.020

Zhang J, Lan T, Han X, Xu Y, Liao L, Xie L, et al. (2021) Improvement of ECM-based bioroot regeneration via N-acetylcysteine-induced antioxidative effects. *Stem Cell Research & Therapy* **12**, 202
https://doi.org/10.1186/s13287-021-02237-5

Zorov DB, Juhaszova M, Sollott SJ (2014) Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiological Reviews* **94**, 909-50.
https://doi.org/10.1152/physrev.00026.2013

TABLES

Table 1- Mean and standard deviation of discolouration (ΔE_{00}) after pulpotomy techniques using different materials and assessment times.

Pulpotomy technique	Material	Assessment time			
		T1*	30 days	60 days	90 days
Total	Control	1.05 ± 0.48 ^{Bc}	2.11 ± 1.20 ^{Bc}	1.60 ± 0.77 ^{Bc}	1.67 ± 0.80 ^{Bc}
	BCR	2.55 ± 1.84 ^{Ab}	3.25 ± 2.22 ^{Ab}	3.68 ± 2.11 ^{Ab}	3.70 ± 2.68 ^{Ab}
	BD	2.23 ± 0.90 ^{ABb}	2.10 ± 0.98 ^{ABb}	2.43 ± 0.90 ^{ABb}	3.77 ± 1.35 ^{Aa}
Parcial	Control	1.74 ± 1.23 ^{Bc}	1.91 ± 1.04 ^{Bc}	2.12 ± 0.79 ^{Bc}	2.28 ± 0.99 ^{Bc}
	BCR	3.01 ± 1.78 ^{Ab}	3.50 ± 1.57 ^{Ab}	3.64 ± 1.53 ^{Ab}	4.00 ± 1.72 ^{Ab}
	BD	2.24 ± 0.85 ^{ABb}	2.99 ± 1.34 ^{ABb}	3.53 ± 1.65 ^{ABb}	4.41 ± 1.81 ^{Aa}

*Different capital letters in columns indicate significant differences between groups in the same assessment time, and different lowercase letters in rows indicate significant intragroup differences between the periods analysed (Repeated measures ANOVA and Tukey's test – p< .05). Pulpotomy technique comparison (P=0.126).*T1, immediately after pulpotomy.*

Figures

Figure 1: PRILE 2021 Flowchart

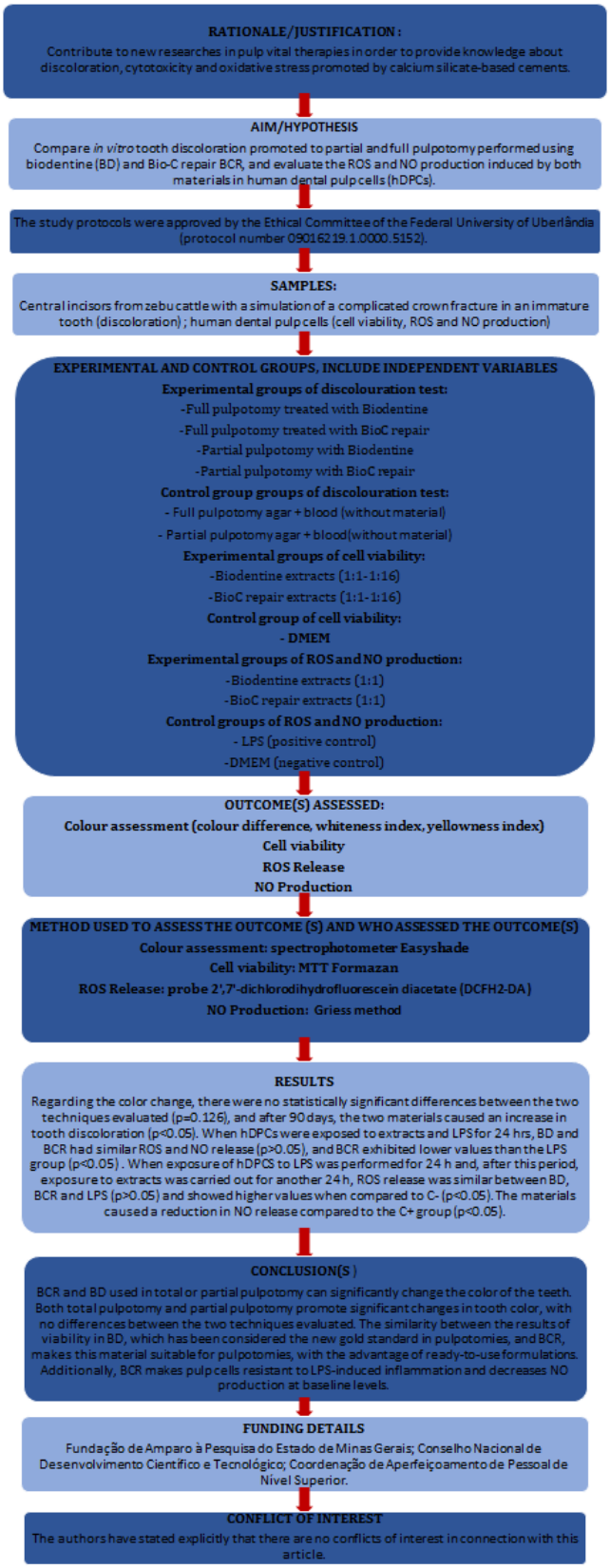


Figure 2: Graphs show the trends in the *L, *a, *b parameters of groups over time. B, baseline (sound tooth); T0, after cavity preparation; T1, immediately after application of the root-end filling material; T2, 30 days and T3, 60 days, T4, 90 days after pulpotomy.

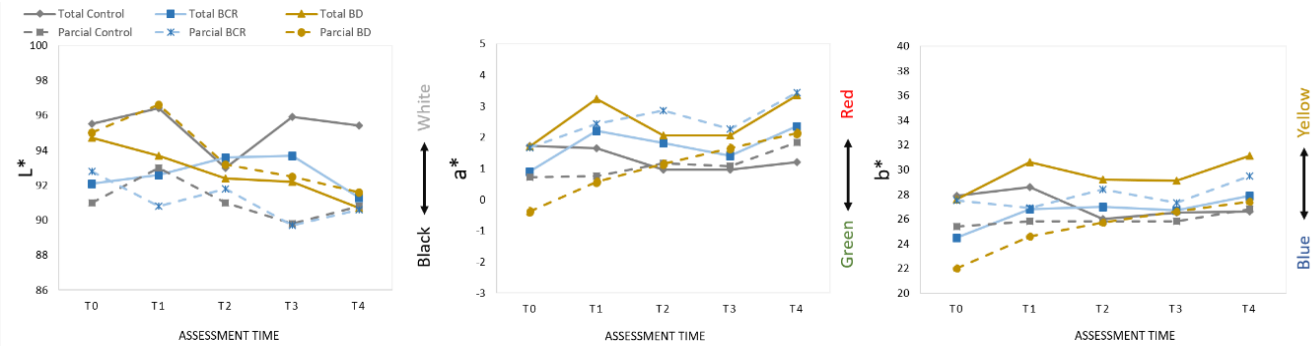


Figure 3: Whiteness index (WI) on different assessment time after pulpotomy techniques using different materials. Capital letter to compare assessment time*pulpotomy technique and lowercase to compare assessment time*material. There was no difference to pulpotomy technique" (P=0.281).

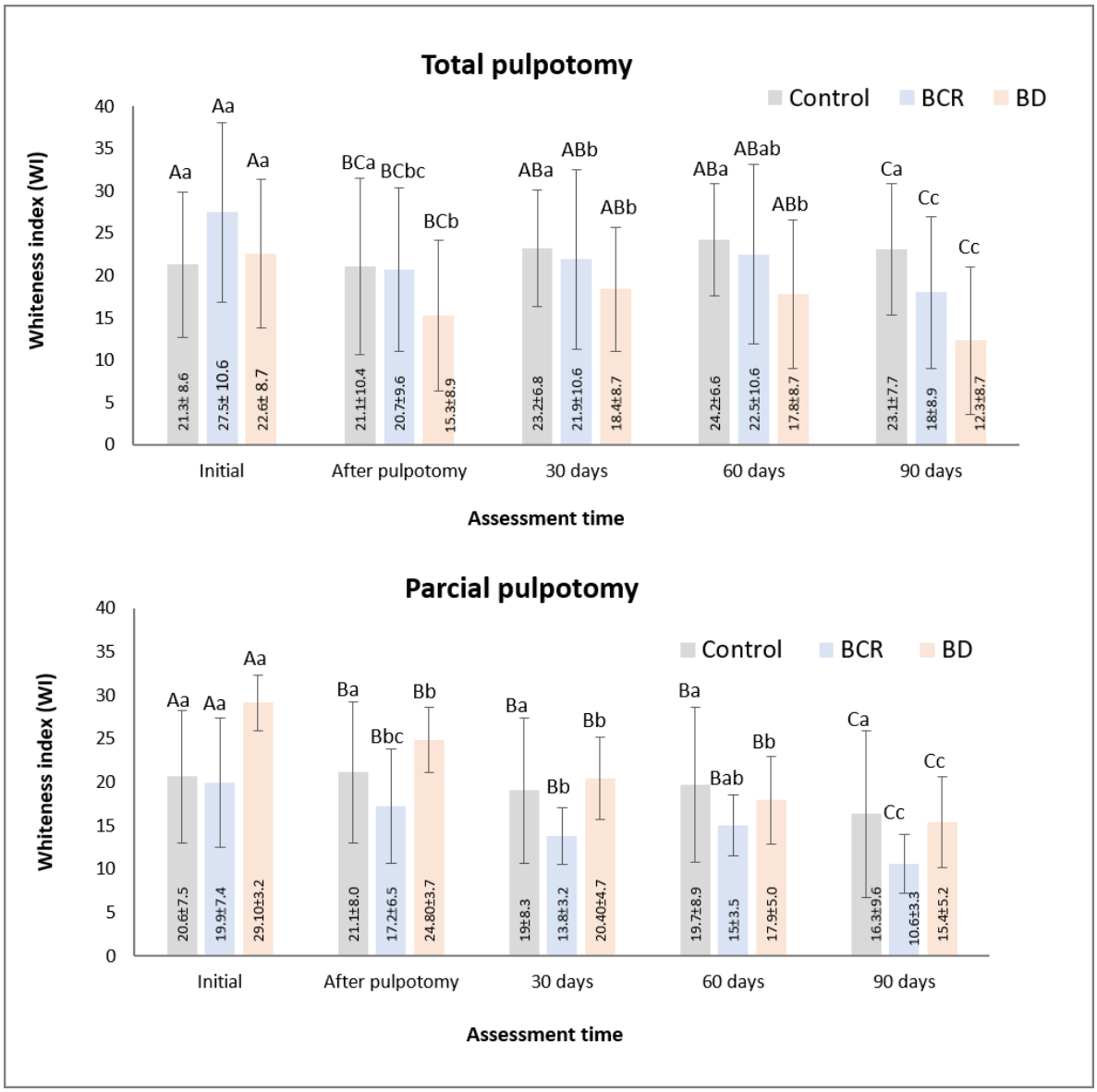


Figure 4: Yellowness index (YI) on different assessment time after pulpotomy techniques using different materials. Capital letter to compare assessment time*pulpotomy technique and lowercase to compare assessment time*material. There was no difference to pulpotomy technique" (P=0.).

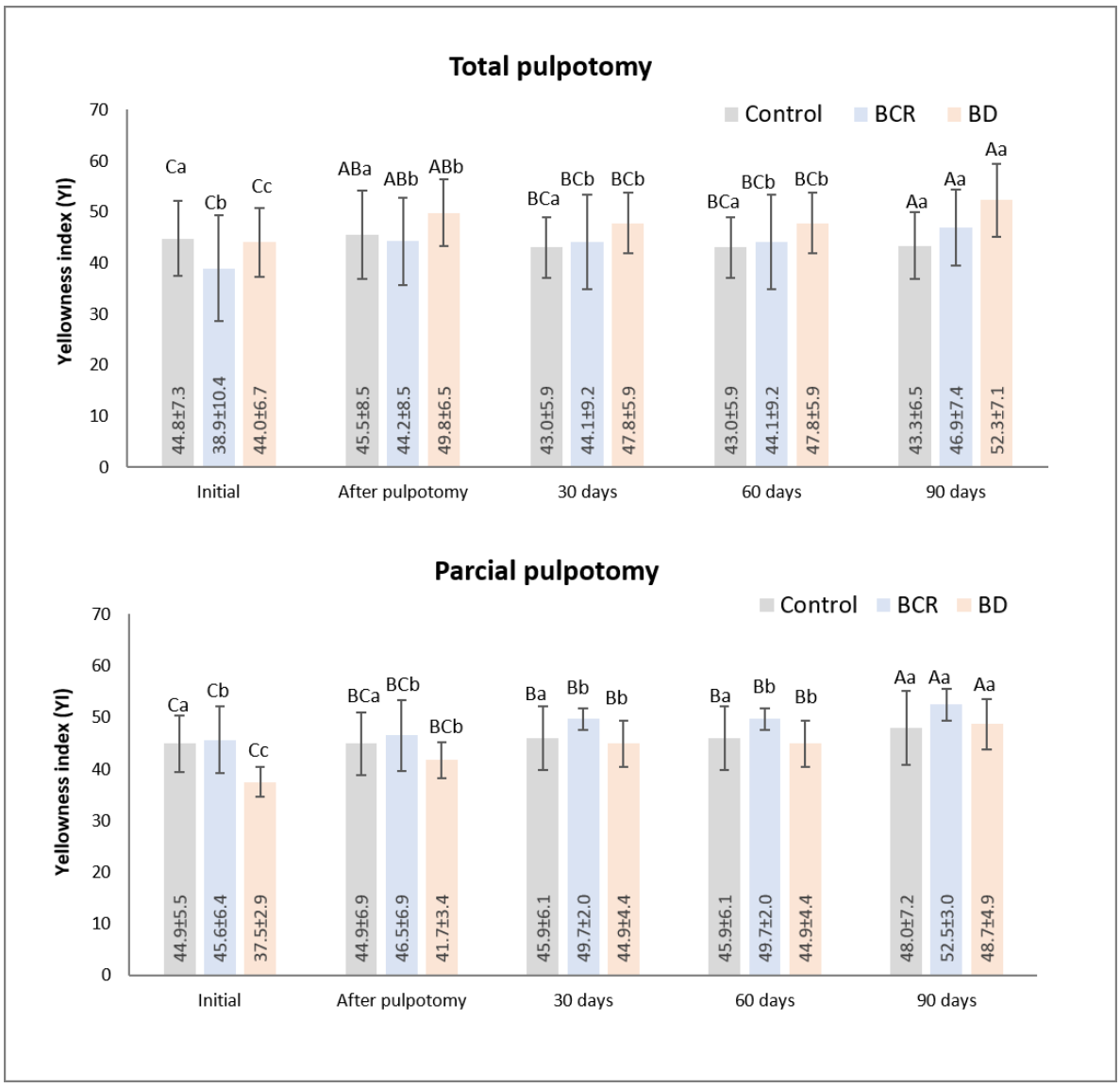


Figure 5: Cell viability evaluated by MTT formazan assay after contact between hDPCs and extracts diluted to 1:1, 1:2, 1:4, 1:8 and 1:16 by 24 h.

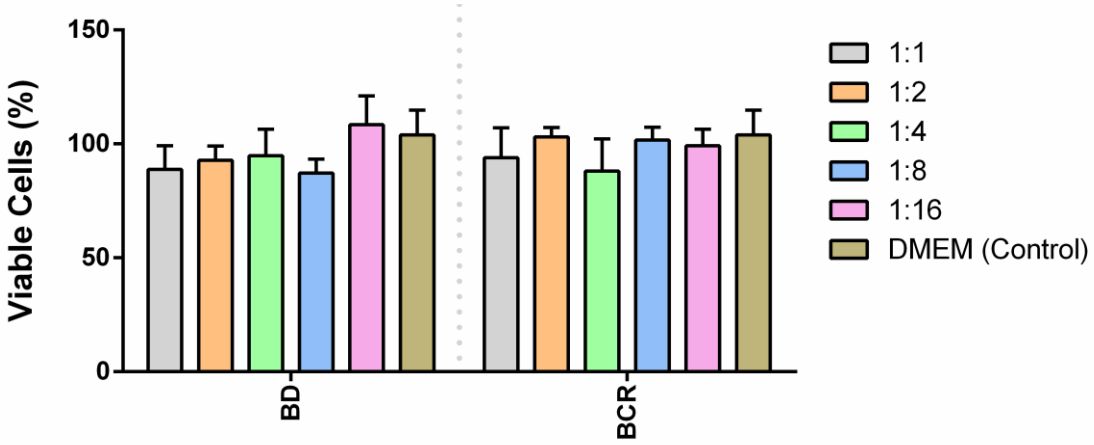


Figure 6: ROS production by hDPCs after contact between the cells and extracts diluited to 1:1. a) cells simultaneously exposed to LPS and extract [LPS/Extract (24 hrs)]; b) cells exposed first to LPS (24 hrs), and after exposed to materials extracts (24 hrs) [LPS (24 hrs) + Extract (24 hrs)]. Different capital letters indicate statistically significant differences between the experimental groups.

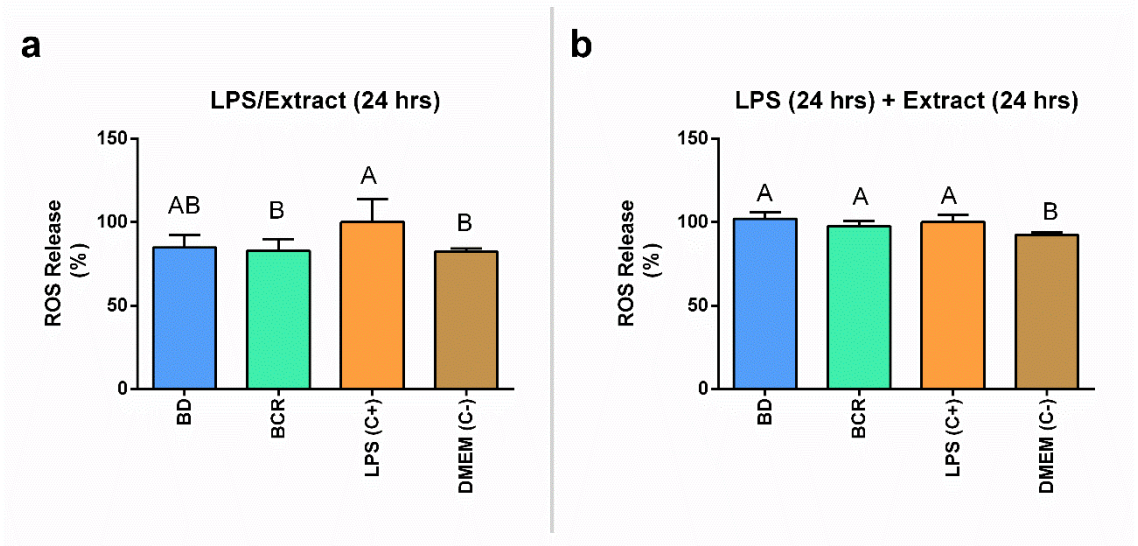
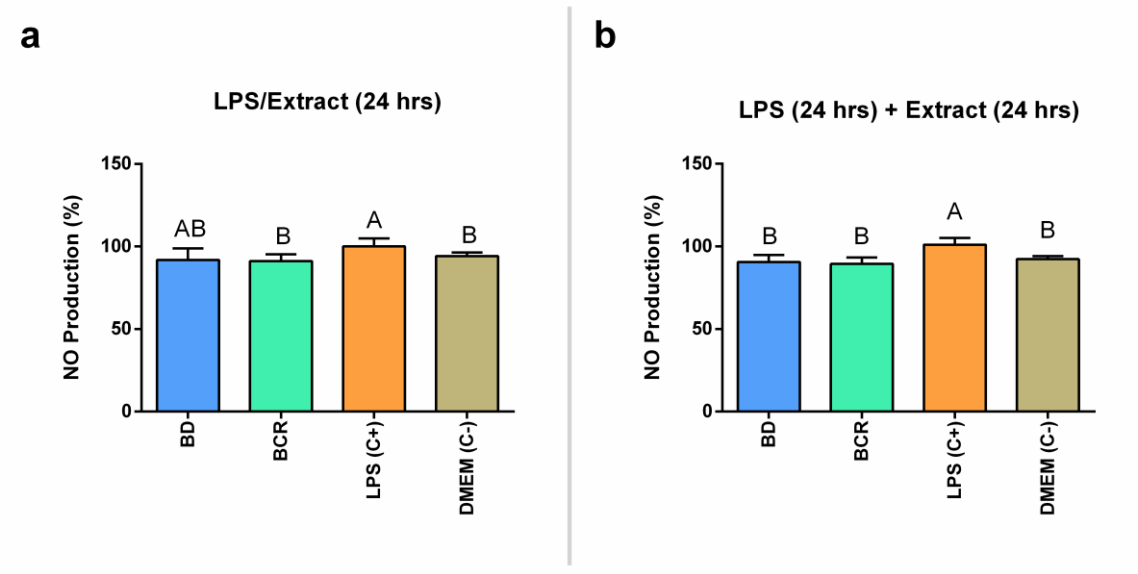


Figure 7: NO produced by hDPCs after contact between the cells and extracts diluted to 1:1. a) cells simultaneously exposed to LPS and extract [LPS/Extract (24 hrs)]; b) cells exposed first to LPS (24 hrs), and after exposed to materials extracts (24 hrs) [LPS (24 hrs) + Extract (24 hrs)]. Different capital letters indicate statistically significant differences between the experimental groups.



CONCLUSÃO

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BCR e BD utilizados na pulpotomia total ou parcial podem alterar significativamente a cor dos dentes. Tanto a pulpotomia total quanto a pulpotomia parcial promovem mudanças significativas na cor do dente, sem diferenças entre as duas técnicas avaliadas. A semelhança entre os resultados de viabilidade em BD, que tem sido considerado o novo padrão ouro em pulpotomias, e BCR, torna este material adequado para pulpotomias, com a vantagem de formulações prontas para uso.

Além disso, o BCR torna as células pulpares resistentes à inflamação induzida por LPS e diminui a produção de NO nos níveis basais.

REFERÊNCIAS

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Bourguignon C, Cohenca N, Lauridsen E, Flores MT, O'Connell AC, Day PF,et al. International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 1. Fractures and luxations. Dent Traumatol. 2020;36(4):314-330. https://doi.org/10.1111/edt.12578

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. Dental Traumatology, 2020;36:400-410. https://doi.org/10.1111/edt.12553

Oliveira LV, de Souza GL, da Silva GR, Magalhães TEA, Freitas GAN, Turrioni AP, et al. Biological parameters, discolouration and radiopacity of calcium silicate-based materials in a simulated model of partial pulpotomy. International Endodontic Journal, 2021;54:2133-2144. https://doi.org/10.1111/iej.13616

Stanley HR Pulp capping: conserving the dental pulp--can it be done? Is it worth it? Oral Surgery, Oral Medicine, Oral Pathology 1989;68:628-39. https://doi.org/10.1016/0030-4220(89)90252-1

Matsuo T, Nakanishi T, Shimizu H, Ebisu S A clinical study of direct pulp capping applied to carious-exposed pulps. Journal of Endodontics 1996;22:551-6. https://doi.org/10.1016/S0099-2399(96)80017-3

Fong CD, Davis MJ. Partial pulpotomy for immature permanent teeth, its present and future. Clinical Oral Investigation 2002;24:29-32.

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; 43:1786-1791. https://doi.org/10.1016/j.joen.2017.06.025

Kim DH, Jang JH, Lee BN et al. Anti-inflammatory and mineralization effects of ProRoot MTA and endocem MTA in studies of human and rat dental pulps in vitro and in vivo. Journal of Endodontics 2018;44:1534-41. https://doi.org/10.1016/j.joen.2018.07.012

Giraud T, Jeanneau C, Rombouts C, Bakhtiar H, Laurent P, About I. Pulp capping materials modulate the balance between inflammation and regeneration. Dental Materials 2019;35:24-35. https://doi.org/10.1016/j.dental.2018.09.008

Giraud T, Jeanneau C, Bergmann M, Laurent P, About I. Tricalcium silicate capping materials modulate pulp healing and inflammatory activity in vitro. Journal of Endodontics 2018;44:1686-91 https://doi.org/10.1016/j.joen.2018.06.009

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. Journal of Endodontics 2021;47:1896-1906 https://doi.org/10.1016/j.joen.2021.08.001

Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. Physiological Reviews 2014; 94:909-50.
https://doi.org/10.1152/physrev.00026.2013

Wu B, Li J, Ni H, Zhuang X, Qi Z, Chen Q, et al. TLR4 Activation Promotes the Progression of Experimental Autoimmune Myocarditis to Dilated Cardiomyopathy by Inducing Mitochondrial Dynamic Imbalance. Oxidative Medicine and Cellular Longevity 2018; 26:3181-278. https://doi.org/10.1155/2018/3181278

Vargas, K.G., Fuks, A.B., Peretz, B. Pulpotomy Techniques: Cervical (Traditional) and Partial. In: (eds) Pediatric Endodontics 2016. Springer, Cham. https://doi.org/10.1007/978-3-319-27553-6_5

Elbahary S, Bercovich R, Flaisher-Salem N, Azem H. Quantifying Coronal Primary Tooth Discoloration Caused by Different Pulpotomy Materials. J Clin Pediatr Dent. 2020;44:142-147. https://doi.org/10.17796/1053-4625-44.3.2

Rao Q, Kuang J, Mao C, Dai J, Hu L, Lei Z ,et al. Comparison of iRoot BP Plus and Calcium Hydroxide as Pulpotomy Materials in Permanent Incisors with Complicated Crown Fractures: A Retrospective Study. Journal of Endodontics 2020;46:352-357
https://doi.org/10.1016/j.joen.2019.12.010

Taha NA, Abdelkhader SZ Outcome of full pulpotomy using Biodentine in adult patients with symptoms indicative of irreversible pulpitis. International EndodonticJournal2018;51:819-828
https://doi.org/10.1111/iej.12903

Ojeda-Gutierrez F, Martinez-Marquez B, Arteaga-Larios S, Ruiz-Rodriguez MS, Pozos-Guillen A Management and followup of complicated crown fractures in young patients treated with partial pulpotomy. Case Reports in Dentistry 2013; 13:563-597 .
https://doi.org/10.1155/2013/597563

Bimstein E, Rotstein I Cvek pulpotomy - revisited. Dental Traumatology. 2016;32;438-442.
https://doi.org/10.1111/edt.12297

Yang Y, Xia B, Xu Z, Dou G, Lei Y, Yong W The effect of partial pulpotomy with iRoot BP Plus in traumatized immature permanent teeth: A randomized prospective controlled trial. Dental Traumatology 2020;36:518-525. https://doi.org/10.1111/edt.12563

Parirokh M, Torabinejad M. Mineral trioxide aggregate: a comprehensive literature review--Part III: Clinical applications, drawbacks, and mechanism of action. J Endod. 2010;36:400-13. https://doi.org/10.1016/j.joen.2009.09.009

Beatty H, Svec T. Quantifying Coronal Tooth Discoloration Caused by Biodentine and EndoSequence Root Repair Material. J Endod. 2015;41:2036-9.
https://doi.org/10.1016/j.joen.2015.08.032

Kunert M, Lukomska-Szymanska M. Bio-Inductive Materials in Direct and Indirect Pulp Capping-A Review Article. Materials (Basel). 2020;13:1204. https://doi.org/10.3390/ma13051204

Bakhtiar H, Nekoofar MH, Aminishakib P, Abedi F, Naghi Moosavi F, Esnaashari E, Azizi A, Esmailian S, Ellini MR, Mesgarzadeh V, Sezavar M, About I. Human Pulp Responses to Partial Pulpotomy Treatment with TheraCal as Compared with Biodentine and ProRoot MTA: A Clinical Trial. J Endod. 2017;43:1786-1791. https://doi.org/10.1016/j.joen.2017.06.025

Kaur M, Singh H, Dhillon JS, Batra M, Saini M. MTA versus Biodentine: Review of Literature with a Comparative Analysis. J Clin Diagn Res. 2017;8:ZG01-ZG05. doi: 10.7860/JCDR/2017/25840.10374

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;13:2189 https://doi.org/10.3390/ma13092189

Guerreiro JCM, Ochoa-Rodrígez VM, Rodrigues EM, Chavez-Andrade GM, Tanomaru-Filho M, Guerreiro-Tanomaru JM, Faria G. Antibacterial activity, cytocompatibility and effect of Bio-C Temp bioceramic intracanal medicament on osteoblast biology. Int Endod J. 2021;7:1155-1165. https://doi.org/10.1111/iej.13502

López-García S, Lozano A, García-Bernal D, Forner L, Llena C, Guerrero-Gironés J, et al. Biological Effects of New Hydraulic Materials on Human Periodontal Ligament Stem Cells. Journal of Clinical Medicine 2019;8:1216. https://doi.org/10.3390/jcm8081216

Villa N, Santos VVD, Costa UMD, Mendes AT, Duarte PHM, Rosa RAD,et al. A New Calcium Silicate-Based Root Canal Dressing: Physical and Chemical Properties, Cytotoxicity and Dentinal Tubule Penetration. Braz Dent J. 2020: ;6:598-604.
https://doi.org/10.1590/0103-6440202003376

ANEXOS

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    - permission to reproduce material from other sources
    - clinical trial registration
    - Your co-author details, including affiliation and email address. (Why is this important? We need to keep all co-authors informed of the outcome of the peer review process.)
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2.1 Reporting Guidelines

2.1.1 Case reports/case series

Case reports should be written to comply with the Preferred Reporting Items for Case reports in Endodontics (PRICE) 2020 guidelines (Nagendrababu et al. 2020, doi:10.1111/iej.13285).

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A PRICE checklist (for editors/referees) and flowchart (as a Figure to be included in the manuscript for readers) should also be completed and included in the submission

material. The PRICE 2020 checklist and flowchart can be downloaded from: **http://pride-endodonticguidelines.org/price/**.

It is recommended that authors consult the following papers when writing case reports, which explains the rationale for the PRICE 2020 guidelines and their importance:

Nagendrababu V, Chong BS, McCabe P, Shah PK, Priya E, Jayaraman J, Pulikkotil SJ, Setzer FC, Sunde PT, Dummer PMH (2020) PRICE 2020 guidelines for reporting case reports in Endodontics: a consensus-based development. International Endodontic Journal 53, 619-26. (**https://www.ncbi.nlm.nih.gov/pubmed/32090342**)

Nagendrababu V, Chong BS, McCabe P, Shah PK, Priya E, Jayaraman J, Pulikkotil SJ, Dummer PMH (2020) PRICE 2020 guidelines for reporting case reports in Endodontics: Explanation and elaboration. International Endodontic Journal 53, 922-47. (**https://onlinelibrary.wiley.com/doi/abs/10.1111/iej.13300**)

2.2.2. Randomised clinical trials

Randomised clinical trials should be reported to comply with the Preferred Reporting Items for Randomised Trials in Endodontics (PRIRATE) 2020 guidelines (Nagendrababu et al. 2020, doi: 10.1111/iej.13294).

When submitting manuscripts that have been written using the PRIRATE 2020 guidelines, authors should include the following statement in the beginning of "Materials and Methods" section: "This randomised clinical trial has been written according to Preferred Reporting Items for RAndomised Trials in Endodontics (PRIRATE) 2020 guidelines (Nagendrababu et al. 2020, doi: 10.1111/iej.13294).

A PRIRATE 2020 checklist (for editors/referees) and flowchart (as a Figure to be included in the manuscript for readers) should also be completed and included in the submission material. The PRIRATE 2020 checklist and flowchart can be downloaded from: **http://pride-endodonticguidelines.org/prirate/.**

It is recommended that authors consult the following papers when writing manuscripts, which explains the rationale for the PRIRATE 2020 guidelines and their importance:

Nagendrababu V, Duncan HF, Bjørndal L, Kvist T, Priya E, Jayaraman J, Pulikkotil SJ, Pigg M, Rechenberg DK, Vaeth M, Dummer PMH (2020) PRIRATE 2020 guidelines for reporting randomised trials in Endodontics: a consensus-based development. International Endodontic Journal 53, 764-73. (**https://onlinelibrary.wiley.com/doi/abs/10.1111/iej.13294**)

Nagendrababu V, Duncan HF, Bjørndal L, Kvist T, Priya E, Jayaraman J, Pulikkotil SJ, Dummer PMH (2020) PRIRATE 2020 guidelines for reporting strials in Endodontics: Explanation and elaboration. International Endodontic Journal 53, 774-03. (**https://onlinelibrary.wiley.com/doi/abs/10.1111/iej.13304**)

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2.2.3. Epidemiological observational trials

Observational studies should be written using the STrengthening the Reporting of OBservational studies in Epidemiology' (STROBE) guidelines. Compliance with this should be detailed in the "Materials and Methods" section. (**www.strobe-statement.org**). A STROBE checklist (for editors/referees) and flowchart (as a Figure to be included in the manuscript for readers) should also be completed and included in the submission material.

It is recommended that authors consult the following papers when writing manuscripts, which explains the rationale for the STROBE guidelines and their importance:

Von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, Strobe Initiative (2014) The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies. International Journal of Surgery 12, 1495-9.

Vandenbroucke JP, von Elm E, Altman DG, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, Poole C, Schlesselman JJ, Egger M, STROBE Initiative. (2014) Strengthening the reporting of observational studies in epidemiology (STROBE): explanation and elaboration. International Journal of Surgery 12, 1500–24

2.2.4. Diagnostic accuracy studies

Diagnostic accuracy studies should be written using the Standards for Reporting of Diagnostic Accuracy Studies (STARD) 2015 guidelines. Compliance with this should be detailed in the "Materials and Methods" section. A STARD checklist (for editors/referees) and flowchart (as a Figure to be included in the manuscript for readers) should also be completed and included in the submission material. The STARD checklist and flowchart can be downloaded from: **https://www.equator-network.org/reporting-guidelines/stard/**

It is recommended that authors consult the following papers when writing manuscripts, which explain the rationale for the STARD guidelines and their importance:

Cohen JF, Korevaar DA, Altman DG, Bruns DE, Gatsonis CA, Hooft L, Irwig L, Levine D, Reitsma JB, de Vet HCW, Bossuyt PMM. (2016) STARD 2015 guidelines for reporting diagnostic accuracy studies: explanation and elaboration. BMJ Open 6, e012799

http://bmjopen.bmj.com/content/6/11/e012799.abstract

2.2.5. Animal studies

Animal studies should be written using the Preferred Reporting Items for Animal Studies in Endodontology (PRIASE) 2021 guidelines (Nagendrababu et al. 2021, doi: 10.1111/iej.13477).

When submitting manuscripts that have been written using the PRIASE 2021 guidelines, authors should include the following statement in the beginning of "Materials and Methods" section: "The manuscript of this animal study has been written according to Preferred Reporting Items for Animal studies in Endodontology (PRIASE) 2021 guidelines (Nagendrababu et al. 2021, doi: 10.1111/iej.13477).

A PRIASE 2021 checklist (for editors/referees) and flowchart (as a Figure to be included in the manuscript for readers) should also be completed and included in the submission material. The PRIASE 2021 checklist and flowchart can be downloaded from: **http://pride-endodonticguidelines.org/priase/**

It is recommended that authors consult the following papers when writing manuscripts, which explain the rationale for the PRIASE 2021 guidelines and their importance:

Nagendrababu V, Kishen A, Murray PE, Nekoofar MH, de Figueiredo JA, Priya E, Jayaraman J, Pulikkotil SJ, Camilleri J, RM S, Dummer PMH (2021) PRIASE 2021 guidelines for reporting animal studies in Endodontology: a consensus-based

development. International Endodontic Journal 54, 848-57. (**https://onlinelibrary.wiley.com/doi/10.1111/iej.13477**)

Nagendrababu V, Kishen A, Murray PE, Nekoofar MH, de Figueiredo JA, Priya E, Jayaraman J, Pulikkotil SJ, Jakovljevic A, Dummer PMH (2021) PRIASE 2021 guidelines for reporting animal studies in Endodontology: Explanation and Elaboration. International Endodontic Journal 54, 858-86. (**https://onlinelibrary.wiley.com/doi/10.1111/iej.13481**)

2.2.6. Laboratory studies

Laboratory studies should be reported using the Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) 2021 guidelines (Nagendrababu et al. 2021, doi: 10.1111/iej.13542).

When submitting manuscripts that have been written using the PRILE 2021 guidelines, authors should include the following statement in the beginning of "Materials and Methods" section: "The manuscript of this laboratory study has been written according to Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) 2021 guidelines (Nagendrababu et al. 2021, doi: 10.1111/iej.13542).

A PRILE checklist (for editors/referees) and flowchart (as a Figure to be included in the manuscript for readers) should also be completed and included in the submission material The PRILE 2021 checklist and flowchart can be downloaded from: **http://pride-endodonticguidelines.org/prile/**

It is recommended that authors consult the following papers when writing manuscripts, which explain the rationale for the PRILE 2021 guidelines and their importance:

Nagendrababu V, Murray PE, Ordinola-Zapata R, OA Peters, IN Rôças, JF Siqueira Jr, E Priya, J Jayaraman, SJ Pulikkotil, J Camilleri, C Boutsioukis, G Rossi-Fedele, PMH Dummer (2021) PRILE 2021 guidelines for reporting laboratory studies in Endodontics: a consensus-based development. International Endodontic Journal May 3. doi: 10.1111/iej.13542. (**https://onlinelibrary.wiley.com/doi/abs/10.1111/iej.13542**)

Nagendrababu V, Murray PE, Ordinola-Zapata R, OA Peters, IN Rôças, JF Siqueira Jr, E Priya, J Jayaraman, SJ Pulikkotil, N Suresh, PMH Dummer (2021) PRILE 2021 guidelines for reporting laboratory studies in Endodontics: Explanation and elaboration. International Endodontic Journal (**https://onlinelibrary.wiley.com/doi/abs/10.1111/iej.13565**)

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The abstract and main body of the systematic review should be reported using the PRISMA for Abstract and PRISMA guidelines respectively (**http://www.prisma-statement.org/**). Authors submitting a systematic review must register the protocol in one of the readily-accessible sources/databases at the time of project inception and not retrospectively (e.g. PROSPERO database, OSF registries). The protocol registration number, name of the database or journal reference should be provided at the submission stage in the "Registration" section in the abstract and 'Methods' section in the main body of the text.

A PRISMA checklist and flow diagram as a Figure (to be included in the manuscript for readers) should also be included in the submission material. Source of funding (grant number, if available) should be added in the 'Acknowledgements' section.

It is recommended that authors consult the following papers, which help in the production of high quality reviews:

Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLOS Medicine 6, e1000097.

Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Kleijnen J, Moher D (2009) The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. Journal of Clinical Epidemiology 62, e1-34.

Nagendrababu V, Duncan HF, Tsesis I, Sathorn C, Pulikkotil SJ, Dharmarajan L, Dummer PMH (2019) PRISMA for abstracts: best practice for reporting abstracts of systematic reviews in Endodontology. International Endodontic Journal 52, 1096-1107. (**https://onlinelibrary.wiley.com/doi/10.1111/iej.13118**)

Nagendrababu V, Dilokthornsakul P, Jinatongthai P, Veettil SK, Pulikkotil SJ, Duncan HF, Dummer PMH (2020) Glossary for systematic reviews and meta-analyses. International Endodontic Journal 53, 232-249.(**https://onlinelibrary.wiley.com/doi/full/10.1111/iej.13217**)

2.2.8 Scoping reviews

Reviews should be reported using the PRISMA guidelines. A checklist for scoping reviews should also be included in the submission material - see: **http://www.prisma-statement.org/Extensions/ScopingReviews.**

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Submission will be assessed according to MIAME and MINSEQE standards. The complete current guidelines are available at

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Appendix

Abbreviations:

The International Endodontic Journal adheres to the conventions outlined in Units, Symbols and Abbreviations: A Guide for Medical and Scientific Editors and Authors. When non-standard terms appearing 3 or more times in the manuscript are to be abbreviated, they should be written out completely in the text when first used with the abbreviation in parenthesis.

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Examples of correct forms of reference

Standard journal article

Jakovljevic, A., Duncan, H.F., Nagendrababu, V., Jacimovic, J., Milasin, J. & Dummer, P.M.H. (2020) Association between cardiovascular diseases and apical periodontitis: an umbrella review*. International Endodontic Journal,* 53, 1374–1386.

Selman, P. (2016) The global decline of intercountry adoption: what lies ahead? Social Policy and Society, 11(3), 381–397.

Corporate author

British Endodontic Society (1983) Guidelines for root canal treatment. *International Endodontic Journal* 16, 192-5.

Department of Health. (2009) Living well with dementia: a national dementia strategy.

Journal supplement

Frumin AM, Nussbaum J, Esposito M (1979) Functional asplenia: demonstration of splenic activity by bone marrow scan (Abstract). Blood 54 (Suppl. 1), 26a.

Holding, M.Y., Saulino, M.F., Overton, E.A., Kornbluth, I.D. & Freedman, M.K. (2008) Interventions in chronic pain management. 1. Update on important definitions in pain management. Archives of Physical Medicine and Rehabilitation, 89 (3, Supplement 1), S38–S40.

Books and other monographs

Personal author(s)

Gutmann J, Harrison JW (1991) Surgical Endodontics, 1st edn Boston, MA, USA: Blackwell Scientific Publications.

Barnes, R. (1995) Successful study for degrees, 2nd edition, London: Routledge.

Chapter in a book

Wesselink P (1990) Conventional root-canal therapy III: root filling. In: Harty FJ, ed. Endodontics in Clinical Practice, 3rd edn; pp. 186-223. London, UK: Butterworth.

Partridge, H. & Hallam, G. (2007) Evidence-based practice and information literacy. In: Lipu, S., Williamson, K. & Lloyd, A. (Eds.) Exploring methods in information literacy research. Wagga Wagga, Australia: Centre for Information Studies, pp. 149–170.

Published proceedings paper

DuPont B (1974) Bone marrow transplantation in severe combined immunodeficiency with an unrelated MLC compatible donor. In: White HJ, Smith R, eds. Proceedings of the Third Annual Meeting of the International Society for Experimental Rematology; pp. 44-46. Houston, TX, USA: International Society for Experimental Hematology.

Wittke, M. (2006) Design, construction, supervision and long-term behaviour of tunnels in swelling rock. In: Van Cotthem, A., Charlier, R., Thimus, J.-F. and Tshibangu, J.-P. (Eds.) Eurock 2006: multiphysics coupling and long term behaviour in rock mechanics: proceedings of the international symposium of the international society for rock mechanics, EUROCK 2006, 9–12 May 2006, Liège, Belgium. London: Taylor & Francis, pp. 211–216.

Agency publication

Ranofsky AL (1978) Surgical Operations in Short-Stay Hospitals: United States-1975. DHEW publication no. (PHS) 78-1785 (Vital and Health Statistics; Series 13; no. 34.) Hyattsville, MD, USA: National Centre for Health Statistics.8.

Wittke, M. (2006) Design, construction, supervision and long-term behaviour of tunnels in swelling rock. In: Van Cotthem, A., Charlier, R., Thimus, J.-F. and Tshibangu, J.-P. (Eds.) Eurock 2006: multiphysics coupling and long term behaviour in rock mechanics: proceedings of the international symposium of the international society for rock mechanics, EUROCK 2006, 9–12 May 2006, Liège, Belgium. London: Taylor & Francis, pp. 211–216.

Dissertation or thesis

Saunders EM (1988) In vitro and in vivo investigations into root-canal obturation using thermally softened gutta-percha techniques (PhD Thesis). Dundee, UK: University of Dundee.

The University Encyclopedia (1985) London: Roydon.

URLs

Full reference details must be given along with the URL, i.e. authorship, year, title of document/report and URL. If this information is not available, the reference should be removed and only the web address cited in the text.

Smith A (1999) Select committee report into social care in the community [WWW document]. URL **http://www.dhss.gov.uk/reports/report015285.html** [accessed on 7 November 2003].

European Space Agency. (2015) Rosetta: rendezvous with a comet. Available at: **http://rosetta.esa.int** [Accessed 15th June 2015].