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EFFECT OF DIFFERENT TREATMENTS ON DENTIN HYPERSENSITIVITY: EVALUATION OF CYTOTOXICITY AND RANDOMIZED CLINICAL STUDY

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Tese apresentada à Faculdade de Odontologia de Araçatuba da Universidade Estadual Paulista (Unesp),, como parte integrante dos requisitos para obtenção do título de Doutora, pelo Programa de Pós-Graduação em Odontologia, área de Concentração em Dentística.

Orientadora: Profa. Dra. Ticiane Cestari Fagundes

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Resumo Geral

Ramos FSS. Efeito de diferentes tratamentos na hipersensibilidade dentinária: avaliação da citotoxidade e estudo clínico randomizado [tese]. Araçatuba: Universidade Estadual Paulista (Unesp), Faculdade de Odontologia; 2023.

RESUMO GERAL

Objetivos: O objetivo deste estudo foi investigar os efeitos de diferentes categorias de tratamentos para hipersensibilidade dentinária em relação ao desgaste e citotoxicidade usando os seguintes protocolos: verniz placebo (PLA), verniz fluoretado (FLU), verniz de fluoreto de sódio (NaF) + Trimetafosfato de sódio (TMP), verniz adesivo universal (SBU), verniz S-PRG (SPRG), biosilicato (BIOS) e solução de amelotina (AMTN). Adicionalmente, FLU, SBU, SPRG e BIOS tiveram sua longevidade clínica avaliada. *Metodologia:* Setenta blocos de dentina radicular bovina foram seccionados. Metade da superfície de cada espécime não foi tratada (controle) e a outra metade foi imersa em EDTA e tratada com os materiais dessensibilizantes. Após a aplicação, os corpos de prova foram submetidos a um desafio erosivo-abrasivo e o desgaste foi analisado por perfilômetro óptico. Diluições seriadas de extratos obtidos a partir do meio de cultura contendo discos impregnados com esses dessensibilizantes foram aplicadas em culturas de fibroblastos e células odontoblásticas. A citotoxicidade e a produção de proteína total (PT) por ensaios colorimétricos foram determinadas após 24h. Em relação ao ensaio clínico, 192 exposições radiculares não cavitadas foram analisadas usando escala visual analógica (VAS) e visual computadorizada (CoVAS), antes dos tratamentos (*baseline*) e após 7, 15, 30 dias, 6 e 12 meses. Os dados foram analisados usando Kruskal-Wallis, Dunn's para desgaste, One-way ANOVA, Tukey e modelo de regressão linear com efeitos fixos e pós-teste usando contrastes ortogonais ($p \leq 0.05$). *Resultados:* O SBU foi o único material que não apresentou desgaste. O menor desgaste foi observado para AMTN, sendo estatisticamente semelhante ao TMP. A viabilidade celular foi significativamente reduzida para PLA, FLU, TMP e SBU em a fibroblastos e TMP e SBU em células semelhantes a odontoblastos considerando o extrato não diluído. PT mostraram níveis variados de concentrações de proteína sem diferença entre os grupos para fibroblastos. No estudo clínico, todos os dessensibilizantes foram eficazes na redução da HD, em comparação com os dados iniciais para ambas as escalas. Na escala VAS, observou-se redução significativa de HD a partir de 7 dias para BIOS e SBU, e a partir de 15 dias para FLU e SPRG. Para a escala CoVAS, todos os dessensibilizantes foram capazes de reduzir significativamente a HD após 7 dias, exceto o SPRG, que apresentou essa redução após 15 dias. Nenhum dessensibilizante retornou ao nível de sensibilidade inicial após 12 meses para ambas as escalas. Não foram encontradas diferenças

estatísticas entre os dessensibilizantes para todos os momentos de avaliação, para ambas as escalas. *Conclusão:* O adesivo universal protegeu a superfície dentinária após o desafio erosivo-abrasivo. Extratos não diluídos de adesivos universais e vernizes fluoretados apresentam citotoxicidade, principalmente para fibroblastos. Todos os tratamentos em consultório foram eficazes na redução da HD ao longo de 12 meses, com níveis de dor semelhantes.

Palavras-chave: Abrasão Dentária. Biologia Celular. Dessensibilizantes Dentinários. Ensaio Clínico. Ensaios Clínicos Randomizados. Erosão Dentária. Sensibilidade da Dentina.

General Abstract

Ramos FSS. Effect of different treatments on dentin hypersensitivity: evaluation of cytotoxicity and randomized clinical study [tese]. Araçatuba: Universidade Estadual Paulista (Unesp), Faculdade de Odontologia; 2023.

GENERAL ABSTRACT

Objectives: The aim of this study was to investigate the effects of different categories of treatments for dentin hypersensitivity in relation to wear and cytotoxicity using the following protocols: placebo varnish (PLA), fluoride varnish (FLU), sodium fluoride varnish (NaF) + sodium trimetaphosphate (TMP), universal adhesive (SBU), S-PRG varnish (SPRG), biosilicate (BIOS) and amelotin solution (AMTN). Additionally, FLU, SBU, SPRG and BIOS had their clinical longevity evaluated. *Methods:* Seventy bovine root dentin blocks were sectioned. Half of the surface of each specimen was untreated (control) and the other half was immersed in EDTA and treated with the desensitizing materials. After application, the specimens underwent an erosive-abrasive challenge and the wear was analyzed by optical profilometer. Serial dilutions of extracts obtained from the culture medium containing discs impregnated with those desensitizers were applied on fibroblasts and odontoblasts-like cells cultures. Cytotoxicity and production of total protein (TP) by colorimetric assays were determined after 24h. Regarding clinical trial, 192 non-cavitated root exposures were analyzed using visual analogue (VAS) and computerized visual scales (CoVAS), before treatments (baseline) and after 7, 15, 30 days, 6 and 12 months. Data were analyzed using Kruskal-Wallis, Dunn's for wear, One-way ANOVA, Tukey, and linear regression model with fixed effects and post-test using orthogonal contrasts for clinical ($p \leq 0.05$). *Results:* SBU was the only material that did not show wear. The lowest wear was observed for AMTN, being statistically similar to TMP. Cell viability was significantly reduced for PLA, FLU, TMP and SBU in fibroblasts-like cell and TMP and SBU in odontoblast-like cell considering undiluted extract. PT showed varied levels of protein concentrations with no difference between groups at 24h for fibroblasts-cell. For clinical study, all desensitizers were effective in reducing DH, compared with baseline data for both scales. On the VAS scale, a significant reduction in DH was observed after 7 days for BIOS and SBU, and after 15 days for FLU and SPRG. For the CoVAS scale, all desensitizers were able to significantly reduce DH after 7 days, except for SPRG, which showed this reduction after 15 days. No desensitizer returned to initial sensitivity level after 12-months for both scales. No statistical differences were found among desensitizers for all times of evaluation, for both scales. *Conclusion:* Universal adhesive protected the wear of DH after challenge. Undiluted extracts of universal adhesives and fluoride varnishes show cytotoxicity,

mainly for fibroblasts. All in-office treatments were effective for reducing DH over 12 months, with similarity levels of pain.

Keywords: Cell Biology. Clinical Trial. Desensitizing Agents. Dental Abrasion. Dental Erosion. Dentin Sensitivity. Randomized Clinical Trial.

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LISTA DE ABREVIATURAS, SÍMBOLOS E SIGLAS

%	percentage
µM	Micromole
Al_3^+	Aluminum
AMTN	Amelotin
ANOVA	Analysis of variance
BIOS	Biosilicate
BO_3^{3-}	Borate
C	control
$Ca(NO_3)_2.4H_20$	calcium nitrate tetrahydrate
$CaCl_2$	Calcium chloride
CaF_2	Calcium fluoride
cm	Centimeters
CO_2	Carbon dioxide
CONSORT	Consolidated Standards of Reporting Trials
CoVAS	Computerized visual scale
DH	Dentin hipersensitivity
DMEM	Dulbecco´s Modified Eagle´s Medium
DMFT	Missing and filled permanent teeth
EDTA	ethylenediaminetetraacetic acid
ETW	Erosive tooth wear
F	Fluor
F^-	Fluoride
FLU	Fluoride varnish
GBI	Gum bleeding index
h	Hour
H2O	Water
HCl	Hydrogen chloride

HTC	hypersensitive (EDTA immersion), treated (with desensitizing agents), challenged (erosive-abrasive cycles)
KCl	Potassium chloride
KH_2PO_4	Potassium dihydrogen phosphate
M	Mol
MDP	Methacryloyloxydecyl dihydrogen phosphate
MDPC-23	Odontoblast-like cell
min	Minutes
min	Minutes
ml	Milliliters
mm	Millimeter
mmol/L	millimol per liter
mW/cm^2	milliwatts per square centimeter
Na^+	Sodium
NaCl	Sodium chloride
NaF	Sodium fluoride
$NaH_2PO_4.2H_2O$	monobasic sodium phosphate
NCLC	Non-carious cervical lesions
NIH/3T3	ATCC CRL-1658 = Fibroblast-like cell
nm	Nanometer
pH	hydrogen potential
PLA	Placebo varnish
REBEC	Brazilian Registry of Clinical Trials
s	Seconds
SBU	Universal adhesive
SiO_3^{2-}	Silicate
SPRG	S-PRG varnish
Sr_2+	Strontium
TEGDMA	Triethylene glycol dimethacrylate
TMP	Sodium trimetaphosphate
TP	Total protein
UI/mL	International unit per milliliter
VAS	Visual analogue scale
VPI	Visible plaque index

w/w	weight/weight
X	Times
μg/mL	Micrograms per milliliter
μL	Microliters
μm	Micrometers

SUMÁRIO

General Introduction

1 GENERAL INTRODUCTION

Dentin hypersensitivity (DH) is defined as short-term pain caused by thermal, tactile, chemical or osmotic stimuli (QUE *et al*., 2013; FAVARO ZEOLA; SOARES; CUNHA-CRUZ, 2019; FELIX; OUANOUNOU, 2019). Among the mechanisms that explain DH, the hydrodynamic theory assumes that external stimuli move the fluid inside the dentinal tubules, causing contraction and distension of odontoblastic processes, stimulating the nerve fibers of the dentin-pulp interface (CHUNG; JUNG; OH, 2013). This condition is closely related to root exposure, with or without non-carious cervical lesions (NCLC) and the erosion is probably the major predictor of dentin hypersensitivity (ALCANTARA *et al*., 2018).

The proposed treatments include surgical procedure for root coverage, adhesive restorations in cases of cavitation or desensitizing treatments (PEUMANS; POLITANO; VAN MEERBEEK, 2020). Desensitizing products act by obliterating dentinal tubules or reducing nervous excitability; however, DH reduction may have short-term effectiveness (OZEN *et al*., 2009; YILMAZ; KURTULMUS-YILMAZ; CENGIZ, 2011). Currently, three product categories have been tested in for the treatment of DH: fluoride varnishes, products with photocuring agents, and experimental solutions of bioactive products.

The high fluoride concentration in the varnishes makes them one of the materials of choice for DH; however, due to limitations regarding the duration of its effects, studies have indicated that the addition of calcium salts and/or organic or inorganic phosphates can optimize the action on the dental tissue (DANELON *et al.*, 2020; FAVRETTO *et al*., 2018). Among them, sodium trimetaphosphate (TMP) has demonstrated remineralizing action on the dental tissue when associated with fluorides through adsorption to the dentin surface, promoting obliteration of the dentinal tubules, protection of the collagen matrix and deposition of calcium phosphate-apatite (DANELON *et al.*, 2020; FAVRETTO *et al*., 2018).

Currently, particles in powder form have been developed for bioactive purposes. Among them, biosilicates have been introduced with the objective of promoting the remineralization of hard tissues by the precipitation of calcium phosphate (PINTADO-PALOMINO; TIRAPELLI, 2015; RENNO *et al*., 2013; TIRAPELLI *et al.*, 2011). Such properties give biosilicate a potential obliterating effect on dentinal tubules, in addition to remineralization and preventing demineralization in dentin. (PINTADO-PALOMINO; TIRAPELLI, 2015; TIRAPELLI *et al.*, 2010, 2011). Scientific advances have also led to the discovery of an enamel matrix protein secreted by ameloblasts, knowledge as amelotin (AMTN). This protein influences the

biomineralization of dental structure, promoting the precipitation of calcium phosphate, formation of hydroxyapatite and collagen matrix (ABBARIN *et al*., 2015; IKEDA *et al*., 2018); however, this action and results in the dentin was weakly investigated.

Considering the photocuring products, universal adhesive systems have been used as an option for promoting a desensitizing effect by sealing the dentinal tubules and forming a hybrid layer (ASKARI; YAZDANI, 2019; RAVISHANKAR *et al.*, 2018), presenting an 80% reduction in sensitivity after three months of application (ASKARI; YAZDANI, 2019).

Recently, a product was launched on the dental market that combines all the mentioned compositions, being composed of a fluoride varnish photocured with pre-reacted surface glass particles. This bioactive technology allows the multifunctional glass particles to be trapped in the polyacid matrix, releasing fluoride, strontium, borate, aluminum, silicate and sodium ions. Thus, there is the neutralization of acids from food and remineralization of tissues, associating with obliteration by means of photoactivated monomers (RAVISHANKAR *et al*., 2018).

Therefore, it is opportune to develop a study that evaluate in vitro and in vivo effect of different treatments on DH.

Capítulo 1

2 CAPÍTULO 1 - Analysis of dentin wear and biological properties promoted by innovative and experimental in-office desensitizing materials*

Fernanda de Souza e Silva Ramos[a], Laryssa de Castro Oliveira[b], Larissa Albertinazzi[c], Sávio José Cardoso Bezerra[d], Vanessa Rodrigues dos Santos[e], Tais Scaramucci[f], Cristiane Duque[g], Bernhard Ganss[h], Marina Trevelin Souza[i], Juliano Pelim Pessan[j], Ticiane Cestari Fagundes[k]

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2.1 Abstract

Objective: This study aimed to evaluate dentin wear and biological performance of desensitizing materials.

Design: Seventy bovine root dentin blocks were sectioned. Half of the surface of each specimen was untreated (control) and the other half was immersed in EDTA and treated with the following desensitizing materials: placebo varnish (PLA), fluoride varnish (FLU), sodium fluoride (NaF) varnish + sodium trimetaphosphate (TMP), universal adhesive (SBU), S-PRG varnish (SPRG), biosilicate (BIOS), and amelotin solution (AMTN). After application, the specimens were submitted to an erosive-abrasive challenge and the wear analyzed by optical profilometer. Serial dilutions of extracts obtained from the culture medium containing discs impregnated with those desensitizers were applied on fibroblasts and odontoblasts-like cells cultures. Cytotoxicity and production of total protein (TP) by colorimetric assays were determined after 24h. Data were statistically analyzed using Kruskal-Wallis, Dunn's, One-way ANOVA, and Tukey ($p \leq 0.05$).

Results: SBU was the only material that not presented wear. The lowest wear was observed for AMTN and TMP. Cell viability was significantly reduced for PLA, FLU, TMP and SBU in fibroblasts cell and TMP and SBU in odontoblast-like cell, considering undiluted extract. TP showed varied levels of protein concentrations with no difference between groups for fibroblasts.

Conclusions: Universal adhesive system protected hypersensitive dentin wear after challenge. Extracts of adhesive and fluoride varnishes presented cytotoxic mainly on fibroblasts.

Key-words: Cell Biology; Dental Abrasion; Dental Erosion; Desensitizing Agents; Dentin Sensitivity.

2.2 Introduction

Erosive tooth wear (ETW) is a dental clinical condition with global prevalence estimated between 20% to 45% in permanent teeth (Schlueter & Luka, 2018). ETW is a gradual loss of dental hard tissues with multifactorial etiology involving chemical, biological and behavioral factors. The exposure of dentinal tubules by ETW is probably the major predictor of dentin hypersensitivity (DH) that is considered one of the most common complaints from

patients (Alcântara et al., Marto et al., 2019, Zeola et al., 2019). DH is characterized as a short, sharp pain that arises from exposed dentin in the cervical region, caused by abrasion, erosion, and/or abfraction (Moraschini et al., 2018). Teeth with DH should be treated considering the related-risk factors and severity (Magalhães et al., 2009, Liu et al., 2020).

Despite the wide range of commercially available products for the treatment, there is no "gold standard" therapy for DH (Moraschini et al., 2018). The preventive approaches recommended for ETW are based on the prevention of erosive acids attacks to the teeth caused by both extrinsic and intrinsic factors (Magalhães et al., 2009). In addition, it is recommended the protection of the tooth structure with dental materials to create an extra mechanical barrier against the erosive acids (Magalhães et al., 2009). In the presence of DH, strategies of treatment have been developed to modify nociceptive response to promote dentinal tubules obliteration (Marto et al., 2019). In this context, fluoride-based varnishes, photocured agents and experimental materials have been used to decrease the DH, by means of the tubular obliteration (Ravishankar et al., 2018; de Castro Oliveira et al., 2022).

Fluoride varnishes represent the most used treatment for DH due to the formation of calcium fluoride precipitates (de Melo Alencar et al., 2019; Pichaiaukrit et al., 2019). However, a reduction in DH up to the first month of treatment have been reported, which is very reduced after three months of application, because those precipitates are not resistant in the oral environment conditions (Wang et al., 2016). The addition of inorganic phosphate salts has been proposed as a method to increase the resistance of fluoride varnishes overtime (Favretto et al., 2018; Danelon et al., 2020). Previous studies have shown the ability of sodium trimetaphosphate (TMP) in protecting the collagen matrix and promoting the deposit of calcium phosphate-apatite, protecting the dentin against erosion by tubules occlusion (Favretto et al., 2018; Danelon et al., 2020, de Castro Oliveira et al., 2022).

Among the photocured agents, universal adhesive promotes a desensitizing effect by dentin tubules sealing by hybrid layer formation, which is able to neutralize the hydrodynamic mechanism of hypersensitivity (Askari & Yazdani, 2019). Recently, an innovative material combining a light-curing fluoride varnish with multifunctional pre-reacted glass particles, S-PRG, promoting dentin tubules obliteration through bioactive technology (Ravishankar et al., 2018; Yamamoto et al., 2021).

Experimental solutions with bioactive ceramics have also shown precipitation of calcium phosphate and hydroxyapatite formation; promoting the obliteration of the dentinal

tubules, and preveting dentin demineralization (Tirapelli et al., 2011; Reis et al., 2021; de Castro Oliveira et al., 2022). Another innovative material is a protein expressed during the maturation phase of tooth enamel formation (amelotin) which is able to bond and form protein complexes, promoting calcium phosphate precipitation, dose-dependent hydroxyapatite formation and collagen matrix mineralization (Abbarin et al., 2015; Ikeda et al., 2018).

Since desensitizers are applied in erosive highly sensitive dentin with exposed tubules, the evaluation of dentin wear and cytotoxicity becomes a requirement before their indication (Elias et al., 2015; Catunda et al., 2017; Garofalo et al., 2019). However, studies have measured dentin wear treated with fluoride varnishes submitted to erosive-abrasive cycling (Magalhães et al., 2012; Garofalo et al., 2019), there is a lack of studies evaluating the wear, by optical profilometry, of innovative and experimental desensitizers on previously eroded dentin. Few studies have also shown that fluoride varnishes, bioactive ceramics and universal adhesive present low cytotoxicity (Kido et al., 2013; Elias et al., 2015; Catunda et al., 2017). Nevertheless, there is a lack of information about biological properties of recently launched and experimental materials that may be indicated to DH therapies.

In this context, the present study aimed to evaluate the effects of different desensitizing agents on dentin wear protection and biological properties. The null hypothesis tested were: (1) there would be no difference among the materials in the protection of dentin erosive wear after erosive-abrasive challenge; and (2) There would be no difference in the among the material considering their effect on the viability and protein production by fibroblasts and odontoblast-like cells.

2.3 Material and methods

This research was conducted after approval by the Local Ethics Committee on Animal Use (Process #00.418–2020).

Study design

This study tested 7 desensitizers agents, in an erosion-toothbrushing cycling model of 5 days, using bovine dentin specimens (n = 10). The response variable was dentin wear (μm), using an optical profilometer. Biological properties were also analyzed by cytotoxicity and total protein production (n = 6), determined for two different cells lines (fibroblasts and odontoblast-like cells) and colorimetric assays (resazurin and Lowry methods), using different extract dilutions in 24h. Figure 1 shows an illustration of the study design.

Regarding sample size calculation, a pilot study was conducted to determine the number of specimens per group for dentin wear analysis. Sample size calculation was performed using n=3 on SigmaPlot 13 software (Systat Software Inc., London, UK) by ANOVA Sample size test, adopting $\alpha = 0.05$ and a power of 0.80, with an expected difference between means of 6.8. A sample size of 10 specimens per group was found.

Dentin wear analysis

Dentin sample preparation

Seventy freshly extracted bovine incisors were collected and teeth with caries, cracks, or gross irregularities of dentin structure were excluded from the study.

From each tooth, a root dentin block was obtained with dimensions of 4 x 4 x 2 mm (n=70). The dentin blocks were polished with waterproof abrasive papers (#600, #800 e #1200 grit), in a polishing machine (AutoMet 250 PRO, Buehler, IL, USA), under running water. The dimensions were checked with a digital micrometer (Mitutoyo America, Dawn, IL, USA). The specimens were ultrasonically cleaned for 5 min between each abrasive paper (Cristófoli, Campo Mourão, PR, Brazil). In order to standardize the specimens, dentin blocks were analyzed with optical profilometry (Proscan 2100, Scantron Ltd, Venture Way, Taunton, UK) to discard specimens with curvature values higher than 0.3 μm (Garofalo et al., 2019). Then, the polished surfaces were protected using an acid-resistant varnish (Colorama, São Paulo, SP, Brazil), leaving a central test area (1 mm) to be exposed to the treatments and two lateral control areas (1,5 mm) (Garofalo et al., 2019). The smear layer was removed in the central area using 0.5 M ethylenediaminetetraacetic acid (EDTA) solution for 5 min, to simulate the open dentin tubules present in cervical hypersensitive areas (de Castro Oliveira et al., 2022). The following areas were created: one central area - HTC – hypersensitive (EDTA immersion), treated one of the desensitizing agents, challenged (erosive-abrasive cycles); and two lateral areas - C – control (no treatment). Only areas C received the acid-resistant varnish.

Experimental groups and modes of application

The samples of dentin were randomly separated in seven experimental groups (n = 10): placebo varnish (PLA), fluoride varnish (FLU); nanoparticulate sodium trimetaphosphate varnish (TMP); universal adhesive (SBU); surface pre-reacted glass-ionomer filler-containing varnish (SPRG); bioactive ceramic solution (BIOS) and protein from enamel solution (AMTN).

All HTD-area received all desensitizers under clean and dry surface with a disposable applicator (KG Sorensen, Cotia, SP, Brazil). In the samples from the groups PLA, FLU and TMP a thin layer was passively applied for 5 s, remaining stable for 10 min. In SBU samples, a thin layer was actively applied for 20 s and photocured using light intensity of 1200 mW/cm^2 (Radii, SDI, Victoria, Australia) for 10 s, without previous acid etching. One drop of varnish was actively mixed with base and applied for 3 s for SPRG group, forming a thin layer. The same light device was used during 10 s. and the uncured layer was removed from surface with the cotton pellet. In the BIOS group a thin layer of bioactive ceramic solution was applied for 5 s, remaining stable for 10 min. In AMTN samples, 5 μL of solution were applied for 10 s, remaining for 10 min.

A single researcher performed all applications only once and the specimens were immediately stored in artificial saliva (1.649 mmol/L $CaCl_2 \cdot H_2O$, 5.715 mmol/L KH_2PO_4, 8.627 mmol/L KCl, 2.950 mmol/L NaCl, l.92 mmol/L Tris buffer, pH adjusted to 7 with HCl) for 6 h at 37 °C (de Castro Oliveira et al., 2022).

Erosive-abrasive challenge

During the experimental period, specimens were subjected to a 5-day erosive-abrasive challenge. Erosive cycles were performed four times daily, and abrasive challenges were applied after the first and last erosive cycles. The samples were eroded by immersion in 2 ml/block of citric acid (pH=3.2) for 2 min under an orbital shaking table (Tecnal TE – 420, Piracicaba, SP, Brazil) (de Castro Oliveira et al., 2022) with 1 h immersion in artificial saliva (1.649 mmol/L $CaCl_2$ H_2O, 5.715 mmol/L KH_2 PO_4, 8.627 mmol/L KCl, 2.950 mmol/L NaCl g/l.92 mmol/L Tris buffer, pH adjusted to 7 with HCl) between the cycles (de Castro Oliveira et al., 2022).

Abrasive challenge was performed by brushing the specimens for 15 s using an automated machine (MSET, Elquip, São Carlos, SP, Brazil), at 150 strokes/min during 2 min and axial load of 150 g (de Castro Oliveira et al., 2022). For all groups, brushing was performed with a slurry made from Colgate Total 12 (Colgate-Palmolive, São Paulo, SP, Brazil) dentifrice and artificial saliva (1:3 w/w) (de Castro Oliveira et al., 2022). At the end of experiment period, the samples were stored under 100% humidity until analysis.

Final wear analysis

After erosive-abrasive cycles, the acid-resistant varnish was removed, and the dentin wear was determined with optical profilometer programmed to scan a central area of the specimen 2 mm long (x-axis) by 1 mm wide (y-axis), being 1 mm reading from the HTC area and 0.5 mm from the C areas on each side (Figure 1). The equipment was set to go 200 steps of 0.01 mm on the x-axis, and 20 steps of 0.05 mm on the y-axis, using a specific software (Proscan Application software v. 2.0.17, Scantron, Venture Way, Tauton, United Kingdom). The dentin wear was calculated based on subtracting the mean height of the test area (HTC) from the mean height of the two reference areas (C). For this analysis, a 3-point height tool was applied. To avoid collagen shrinkage of dentin, specimens were scanned in a moistened condition. The result was expressed in micrometers (Viana et al., 2022).

Biological properties

Growth cell conditions

To evaluate the cell response to experimental materials, immortalized cells of the gingival fibroblast cells line (NIH/3T3 – ATCC CRL-1658) and odontoblast-like cells line (mouse dental papila cells - MDPC-23) were used. These cells were cultured (Costar Corp., Cambridge, Massachusetts, USA) in Dulbecco´s Modified Eagle´s Medium (DMEM, SIGMA Chemical Co., St. Louis, Missouri, USA) containing 10% fetal bovine serum (SFB, Cultilab, Campinas, SP, Brazil), 100 UI/mL e 100 μg/mL, penicillin and streptomycin, respectively (GIBCO, Grand Island, Nova York, USA). The cells were incubated at 37°C in a humidified atmosphere of 5% of CO_2 and 95% air (Caiaffa et al., 2019). Culture media were renewed every 2 days until cells reach 80% confluence. Then, cells were detached with using 25% trypsin-EDTA (GIBCO, Grand Island, Nova York, USA) and their concentration was adjusted to $1x10^4$ cells per well using an automatic cell counter (TC20 Automated Cell Counter, Bio-Rad, Santo Amaro, São Paulo, SP, Brazil) to be seed in 96-well plates (Kasvi, São José dos Pinhais, PR, Brazil) (Caiaffa et al., 2019; Marques et al., 2019).

Experimental groups and modes of application

Paper discs with 6 mm diameter were sterilized and impregnated with 5 μL of each material (Table 1) such as described previously (Elias et al., 2015). Subsequently, the discs were inserted in microtubes containing 500 μL of DMEM and kept at 37°C for 24h according to ISO10993-12. After this period, the undiluted extract (100%) of each material as well as dilutions at 1:2 (50%), 1:4(25%) and 1:8 (12.5%) in DMEM prepared to be applied to cell cultures (Lopes Garcia et al., 2021). The culture medium in each well was subsequently

removed and 100ul of each extract was added to the cell cultures and incubated at 37°C for 24h (Pichaiaukrit et al., 2019).

Cytotoxicity tests

For cytotoxicity assessment, NIH/3T3 and MDPC-23 cells were exposed to different extracts (undiluted and diluted from 1:2 to 1:8 in DMEM). After 24h of cell exposure, 125µL of culture medium containing resazurin solution (at 70µM) was added to each well. Viable cells reduced resazurin (blue color) to resorufin (pink color), and the production of resorufin was proportional to the metabolic activity of the viable cells. After 4h, 100 µL of the resazurin-culture medium solution was transferred to a 96-well plate for reading in a spectrophotometer (Spectra Max 190; Molecular Devices, Sunnyvale, California, USA) at 570 and 600 nm. (Müller et al., 2020). According to the other study, the cell viability will be discussed considering the parameters cited as follow: non-cytotoxic (more than 90% cell viability), slightly cytotoxic (60-90 % cell viability), moderately cytotoxic (30-59% cell viability) and severely cytotoxic (less than 30% cell viability) (Catunda et al., 2017).

Total protein production (TP)

To determine the total protein (TP) production, NIH/3T3 and MDPC-23 cells were also seemed in a 96-well plate and exposed to extracts (undiluted and diluted in DMEM) of each desinsitizer agent for 24h, as described previously. After these periods, the culture medium was removed and 150 µL of 0.1% sodium lauryl sulfate in deionized water (Sigma / Aldrich Corp., St. Louis, MO, USA) was added to each well and kept for 40 min at room temperature to produce cell lysis. Then, 100µL of this solution was pipetted into a 96-well plate and 50 µL of Lowry Reagent Solution (Sigma / Aldrich Corp., St. Louis MO, USA) was inserted to each well and incubated for 20 min at room temperature. Afterwards, 25 µL of Folin-Ciocalteu Phenol Reagent Solution (Sigma / Aldrich Corp., St. Louis MO, USA) was added to each well and kept for 30 min. The absorbance values of the wells were determined at a wavelength of 655 nm in a spectrophotometer. TP production was calculated from a standard curve using pre-determined bovine serum albumin (BSA) concentrations (Caiaffa et al., 2019)

Statistical analysis

Normal distribution and homoscedasticity of the data was checked using Shapiro–Wilk and Brown-Forsythe tests, respectively. Data from dentin wear did not present a normal

distribution; thus, comparisons were performed using Kruskal-Wallis and Dunn's tests. The cytotoxicity data were expressed in percentage of cell viability in relation to the control with no treatment (DMEM medium - 100% of cell growth). Data from TP were expressed in U/mg. Cytotoxicity and TP were evaluated by ANOVA One-Way and Tukey tests. The software used for statistical analysis was Jamovi version 2.2.5 (Sydney, Australia), with a significance level of 5%.

2.4 Results

Dentin wear

The results for dentin wear can be observed in Table 2 and representative images in Figure 2. SBU was the only desensitizer that not presented wear, being statistically different the other materials ($p < 0.001$). The lowest wear was obtained for AMTN, being statistically similar to TMP ($p > 0.05$).

Cytotoxicity analysis

Data from cytotoxicity assays are presented in Figures 3 and 4 for NIH/3T3 and MDPC-23 cells, respectively.

Low cell viability was observed for FLU, SBU and TMP ($p \leq 0.05$) with no differences between TMP and PLA ($p > 0.05$), when NIH/3T3 were exposed to material's extracts with no dilution (100%). No differences were found among materials when diluted extracts were evaluated ($p > 0.05$).

SBU and TMP desensitizers also caused higher cytotoxicity for MDCP-23 compared to the other materials, when cells were exposed to undiluted extract (100%) ($p < 0.001$). Considering 50% dilution, BIOS demonstrated significantly less cell viability than SPRG; however, BIOS was statically different from the other materials, except by SBU. There was no difference between materials for the 12.5% dilution ($p > 0.05$).

TP analysis

Figures 5 and 6 show the total protein concentrations determined for NIH/3T3 and MDPC-23 cells, respectively.

Considering NIH/3T3 cells (Figure 5), there were no statistical differences among the materials tested ($p > 0.05$). Considering MDPC-23 cells, undiluted TMP showed higher concentration of TP than other groups except by FLU ($p = 0.006$). FLU, TMP, SBU and SPRG groups at 50% also showed the highest TP concentrations compared to the other groups ($p < 0.001$). At 25% dilution, the highest protein expression was observed in the FLU and TMP groups ($p = 0.005$), being statistically similar to SBU and SPRG ($p > 0.05$). In 12.5% dilution, SBU showed highest TP concentration ($p = 0.004$) with no statically difference compared to TMP and SPRG ($p > 0.05$).

2.5 Discussion

Several materials have been studied in the treatment of ETW, which should be able to reduce DH, as well as protect dentin from erosive/abrasive challenges (Magalhães et al., 2009; Moraschini et al., 2018). Some analysis has been proposed to analyze the in vitro performance of desensitizers (Chinelatti et al., 2017), among them 3D optical measurements have been suggested how a more accurate assessment than other techniques for examining surface specimen alteration (Chinelatti et al., 2017).

As expected, PLA showed the worst results in preventing dentin wear if compared to the other groups. In contrast, the SBU not showed wear of the material itself, rejecting the first null hypothesis. The literature has shown that varnishes containing different sources of fluoride can reduce dentin wear compared to PLA (Magalhães et al., 2012; Danelon et al.,2020; Garofalo et al., 2019; Alencar et al., 2022). The FLU showed no differences compared to TMP, BIOS and SPRG. The FLU varnish may promote the precipitation of a layer similar to calcium fluoride (CaF_2) (de Melo Alencar et al.; 2019, Garofalo et al., 2019). CaF_2 acts as a physical barrier which can prevent acid action on dentin surface (de Melo Alencar et al., 2019). However, the effectiveness of fluoride varnish was compromised after erosive/abrasive challenge, such as demonstrated by the results found in this study and in another previous study (de Melo Alencar et al., 2019). The TMP was used to improve the effectiveness of fluoride varnish because it is a cyclophosphate which produces a negative surface polarity, increasing the deposition of CaF_2 (Favretto et al., 2018; Danelon et al., 2020). The similarity between FLU and TMP can be explained because the effect of fluoride on dentin does not depend only on the deposition of large amounts of fluoride. When the collagen matrix is removed, hydrogen can easily penetrate in the porous dentin, causing severe mineral loss even in the presence of

fluoride. (Schlueter et al., 2016). In contrast, TMP associated with fluoride varnish promoted less dentin wear after erosive/abrasive challenge compared to 5 and 2.5% NaF varnish using contact profilometry (Danelon et al., 2020.

Concerning photoactivated products, SBU presented a superior result compared to SPRG, with formation of a positive curvature, which indicates permanence of material on the dentin surface even after erosive/abrasive challenge. This can be explained because acidic monomers of self-etch adhesives promotes the simultaneous dissolution of smear layer and creates a hybrid layer without exposing the collagen fibrils, reducing the risk of collapse of the collagen network, and sealing the dentin tubules (Askari & Yazdani, 2019; Penha et al., 2020). In another study of our research group, only the SBU under the same challenge, maintaining hydraulic conductance (de Castro Oliveira et al., 2022). In a previous study, SPRG material demonstrated the ability to protect the dentin surface from demineralization after immersion in acid medium (Shiiya et al., 2021); however, when the erosion is associated to abrasion, this material did not resist on the dentin surface such as observed in the present study. According to the manufacturer, this material was developed to be applied on both enamel and dentin, and due to the phosphonic acid monomers, its retention to the tooth surface occurs by a chemical interaction with hydroxyapatite crystals (Mosquim et al., 2022). In this study, dentin specimens were gently dried with an absorbent paper, which better represents represents one of the steps of the procedures used in dental clinical for treatment of DH. SPRG contains TEGDMA with other monomers that increase the product's viscosity (Mosquim et al., 2022). It is also worth mentioning that TEGDMA is an ester-based hydrophilic monomer, susceptible to hydrolytic degradation (Gonzalez-Bonet et al., 2015). This hydrolysis results in disruption of the inter-molecular bonds, plasticizing the polymer chain over time which could lead to leaching of monomers within the dentinal tubules (Gonzalez-Bonet et al., 2015). In another study, show high permeability, since the dentin surface became rapidly wet when specimens were kept in a machine that simulated the dental pulp pressure (Mosquim et al., 2022). SPRG was not efficient in protect dentin surface after erosive/abrasive challenge probably due the detached polymeric layer on the surface that still allowed water flow underneath.

In relation to experimental materials, BIOS showed a higher dentin wear than AMTN. AMTN acts by biomineralization and dentogingival attachment, promoting calcium phosphate precipitation, formation of dose-dependent hydroxyapatite and collagen matrix (Abbarin et al., 2015; Ikeda et al., 2018) which appeared able to protect the surface from wear. Regarding the BIOS, this material was capable of controlling the progression of erosion lesion when submitted

to erosive challenge (Chinelatti et al., 2017). Comparing BIOS and AMTN in other study, no differences were found between them when mean length of occluded dentinal tubules were analyzed (de Castro Oliveira et al., 2022). However, when dentin permeability and scanning electron microscopy analysis were performed, AMTN showed better results than BIOS (de Castro Oliveira et al., 2022).

It is important to highlight that desensitizers agents should also present biocompatibility with adjacent tissues in order to be safe and effective (Elias et al., 2015; Catunda et al., 2017). The evaluation of the cytotoxicity of these materials, improves the understanding of their mechanism of action, considering that two of these materials are experimental. In this context, cells used in this study are related to the cervical area where these materials are applied, specifically gingival fibroblasts (due to the presence of gingival tissue in these regions) and odontoblast-like cells (due to the presence of odontoblastic extensions inside the dentinal tubules) (López-García et al., 2021). Additionally, the present study used dilution of extracts to simulate the interposition of the dentinal barrier, since it is known that the desensitizers did not reach cells in original concentration (Elias et al., 2015; López-García et al., 2021).

Nowadays, there is a degree of opposition to fluoridation due to the risk of possible toxicity (Aoun et al., 2018). PLA, FLU and TMP showed a severely cytotoxicity in undiluted extract, corroborating with previous studies that demonstrate that indiluted extract of fluoride varnishes are toxic to fibroblast cells (Lopez-Garcia et al., 2021; Larpbunphol et al., 2022). A recent study demonstrated that TMP can exert a physicochemical effect in inducing the formation of hydroxyapatite crystals, however, it interferes negatively in the gene expression of odontoblastic cells, which may justify the reduction in their metabolism (Carvalho et al., 2020). A previous study suggests that other components present in fluoride varnishes may also influence their biological responses, which would justify the results of PLA (López-García et al., 2021). However, these materials are considered safe, because they were non-cytotoxic in both cells from the 50% dilution (Lopez-Garcia et al., 2021; Larpbunphol et al., 2022).

Considering photocured agents, moderately cytotoxicity and severely cytotoxicity in the SBU group in both cells were found in undiluted extracts, corroborating with the literature (Elias et al., 2015; Lee et al., 2016; Caldas et al., 2022). Cells cultivated with self-etching adhesives tend to show an increase in apoptotic activity, which can be explained by the high acidification of the medium due to the presence of monomers methacryloyloxydecyl dihydrogen phosphate (MDP) (Lee et al., 2016; Caldas et al., 2022). A previous study demonstrated low values of cell viability for SBU, with means around of 2%, corroborating

with this study which has an average of 1.4% (Caldas et al., 2022). A significant reduction in cell metabolism after 24 h of contact with the extract obtained from impregnated filter paper discs also was found in a study that evaluated the cytotoxicity of experimental adhesive with different degrees of hydrophilicity in odontoblast cell culture (Bianchi et al., 2013). The use of universal adhesives had not been recommended for deep dentin due to their high toxic potential to pulp cells (Leite et al., 2018). In relation to SPRG, it is difficult to compare the results of present study with the literature because only the S-PRG filler elute have been studied in relation to the cytotoxicity (Ishigure et al., 2021; Kashiwagi et al., 2021). These studies suggested that this material could be applied in dental practice because the safety of SPRG eluate was identified for fibroblast and odontoblast-like cells (Ishigure et al., 2021; Kashiwagi et al., 2021).

AMTN and BIOS groups showed a non and slightly cytotoxic in both analyzed cells. Probably, the AMTN results occurred due to the composition of this material is based on the protein expressed in the maturation of enamel (Abbarin et al., 2015). Regarding the BIOS, previous study also demonstrated that this material has no cytotoxic effects (Kido et al., 2013). These materials are experimental solutions, without the components present in the other analyzed materials, which can produce an apoptotic cellular response, as in the PLA group (Lopez-Garcia et al., 2021; Larpbunphol et al., 2022, Elias et al., 2015; Lee et al., 2016; Caldas et al., 2022).

TP analysis aims to investigate the functionality of the materials tested (Caiaffa et al., 2019; Reis et al., 2021). The release of TP in the cell culture supernatant can provide information on cell physiology and also on its productivity, being a biocompatibility marker complementing the results that are obtained by cytotoxicity analysis (Reichelt et al., 2016). In this study, the secretion of proteins by NIH/3T3 cell was homogenous among the groups. However, protein assay showed varied levels of protein concentrations at some points for MDPC-23 cell. TMP showed a significant higher protein expression for undiluted extract, which suggests higher metabolic activity (Reis et al., 2021).

In the current study, some aspects might be considered as limiting factors, such as the clinical buffering capacity and presence of proteins of dentin and saliva; then, the enzymatic and microbiological effects could not be expected (Garofalo et al., 2019). Additionally, the extracts used in this study were applied directly on the cells, without the dentin protection. Furthermore, care must be taken when extrapolating our results to the clinical setting, where several local factors may influence the results. Thus, a long-term clinical trial is necessary to

define DH reduction and dentin protection of these materials. Besides, different bio-active polymers have been launched in the market, as alternative to fluoride mediated desensitization, being possible the at home (patient-applied) therapy (PradeepKumar at al., 2019).

2.6 Conclusions

Considering the limitations of this study, universal adhesive system protected the wear of hypersensitive dentin after erosive-abrasive challenge. However, this adhesive system and fluoride varnishes were cytotoxic in the undiluted extract, mainly for fibroblast cell.

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2.7 References

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Tables

Table 1 Characteristics and mode of application of in-office desensitizing materials used in this study.

Materials	Main ingredients	Manufacturer	Batch #	Application
Placebo varnish (PLA)	Artificial resin, solvent, essence, saccharine, and deionized water.	SS White Dental Products	-	A thin layer was passively applied for 5 s under the clean and dry surface with a disposable applicator, remaining stable for 10 min.
Fluoride varnish (Duraphat – FLU)	5% NaF (22.600 ppm), colophony; solvent, shellac; mastic; saccharine and others.	Colgate-Palmolive Company	022001	
TMPnano varnish (TMP)	NaF 5%+5% TMPnano (22.7 nm); artificial resin, solvent, essence, saccharine, and deionized water.	SS White Dental Products and Sigma-Aldrich	-	
Universal Single Bond (SBU)	BISGMA; HEMA; UDMA; DPIHFP, 10-MDP; solvent; water; silane; and others.	3M ESPE	1833100782	A thin layer was actively applied for 20 s on the clean and dry surface with a disposable applicator and light-cured for 10 s. No previous acid etching was performed.
Barrier Coat (S-PRG filler varnish – SPRG)	S-PRG filler (3.0 μm): TEGDMA; Bis-MPEPP; fluorine boron aluminosilicate; MAA; phosphonic acid; and others.	Shofu INC.	121901	A thin layer of one drop active mixed with base in the base container was applied for 3 s on the clean and dry surface with specific applicator and light-cured for 10 s. The uncured layer was removed from surface with a water-moistened cotton pellet.
Biosilicate solution (BIOS)	The solution was composed of Biosilicate powder (P_2; O_5-Na_2; O-CaO-SiO_2 1-10 μm) and distilled water (1:10 ratio) and for simulation of the professional-use products, the particles were mixed immediately before application	Laboratory of Vitreous Materials at the Federal University of São Carlos	-	A thin layer was applied for 5 s on the clean and dry surface with a disposable applicator, remaining stable for 10 min.
Amelotin solution (AMTN)	Protein derived from dental enamel. The solution was prepared with 100 μL of pure water added to 500 μg of AMTN powder. The result was 200 μL of solution (5 μg/μL concentration).	Institute of Biomedical Engineering, University of Toronto	-	5 μL of solution were applied for 10 s on the clean and dry surface with a disposable applicator, remaining stable until no visible liquid left.

Abbreviations: TMPnano (nanoparticulate sodium trimetaphosphate) TEGDMA (triethylene glycol dimethacrylate); BISGMA (diglycidildimethacrylate A); HEMA (Hydroxyethylmethacrylate); UDMA (1,3 glycol dimethacrylate) DPIHFP (Diphenyliodonium hexafluorophosphate); 10-MDP (10-decanediol phosphate methacrylate); Bis-MPEPP (bisphenol A polyethoxy methacrylate); MAA (methacrylic acid)

Table 2 Data referring to optical profilometry (μm; Median 25%/75%) of the different *in-office* desensitizers after the erosive-abrasive challenge.

Materials	**Curvature**	
PLA	-9.42 (-10.25/-8.9)	D
FLU	-4.66 (-6.41/-4.44)	C
TMP	-3.19 (-3.82/-2.75)	BC
SBU	25.89 (22.46/51.96)	A
SPRG	-4.05 (-4.28/-2.94)	C
BIOS	-4.14 (-4.74/-3.02)	C
AMTN	-0.75 (-2.15/-0.72)	B

Different letters indicate a statistically significant difference ($p \leq 0.05$).

Figures

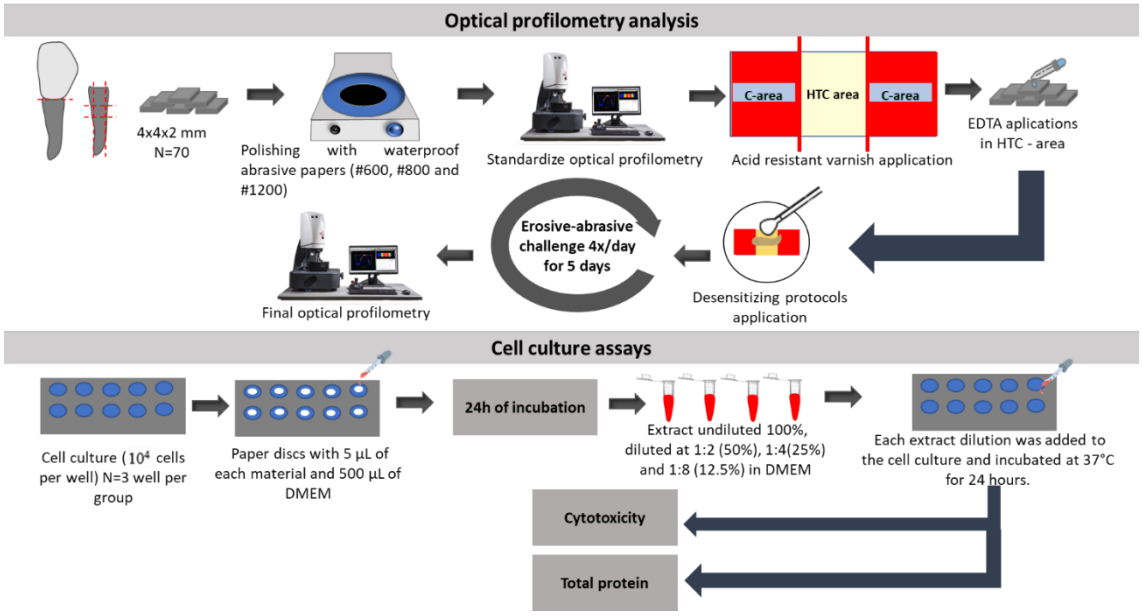

Figure 1 Study design illustration. Dentin wear surface were assessed using an optical profilometer. Control (C), HTC – hypersensitive (EDTA immersion), treated (with desensitizing agents), challenged (erosive-abrasive cycles).

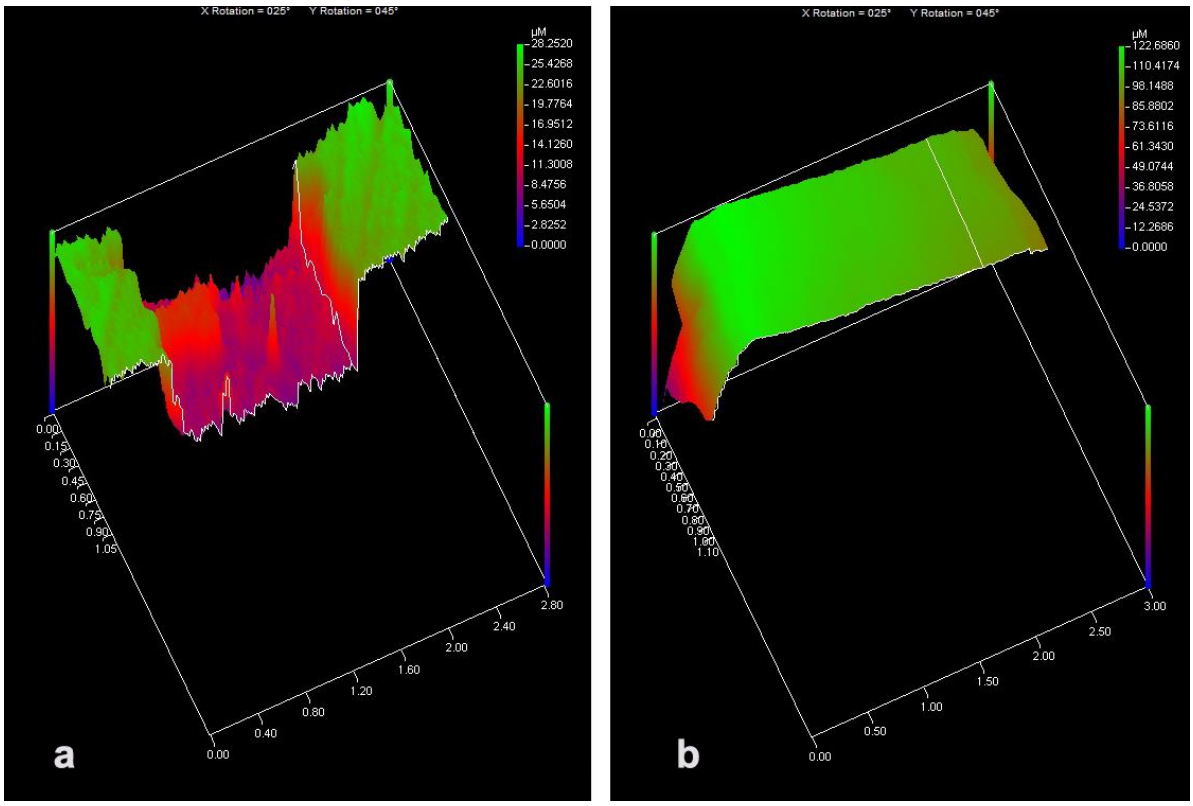

Figure 2 Representative image of the 3-D plot of surface loss (a) and surface preservation (b) after erosive-abrasive challenge.

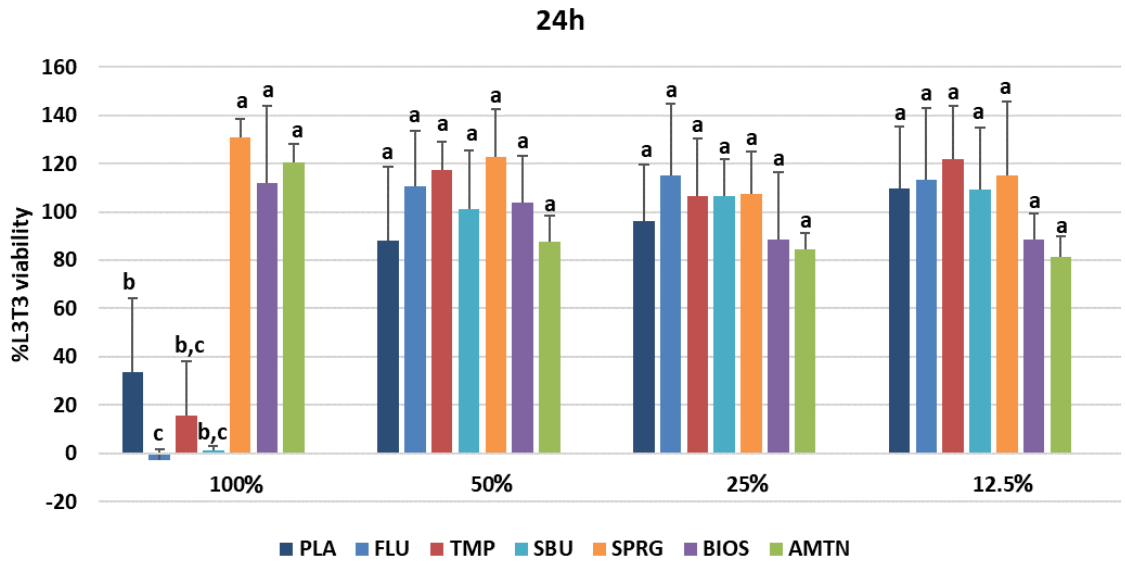

Figure 3 Percentage of NIH/3T3 cell viability (mean+standard deviation) after treatments with different materials. Different uppercase letters show statistical difference among the groups, considering each extract dilution separately (100% - undiluted extract, 50% diluted, 25% diluted, 12.5% diluted in DMEM)

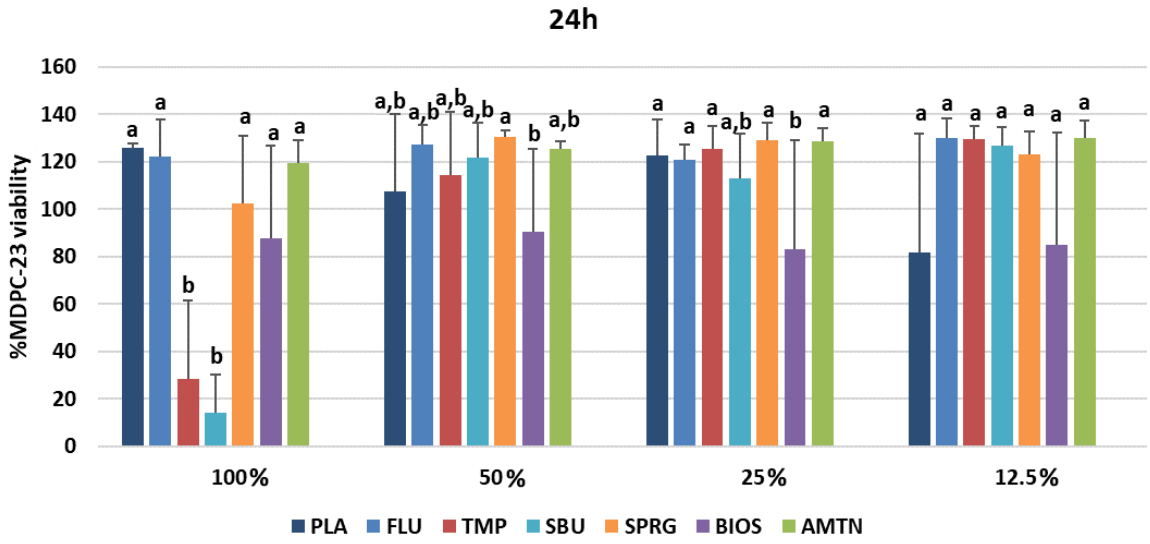

Figure 4 Percentage of MDPC-23 cell viability (mean+standard deviation) after treatments with different materials. Different uppercase letters show statistical difference among the groups, considering each extract dilution separately (100% - undiluted extract, 50% diluted, 25% diluted, 12.5% diluted in DMEM)

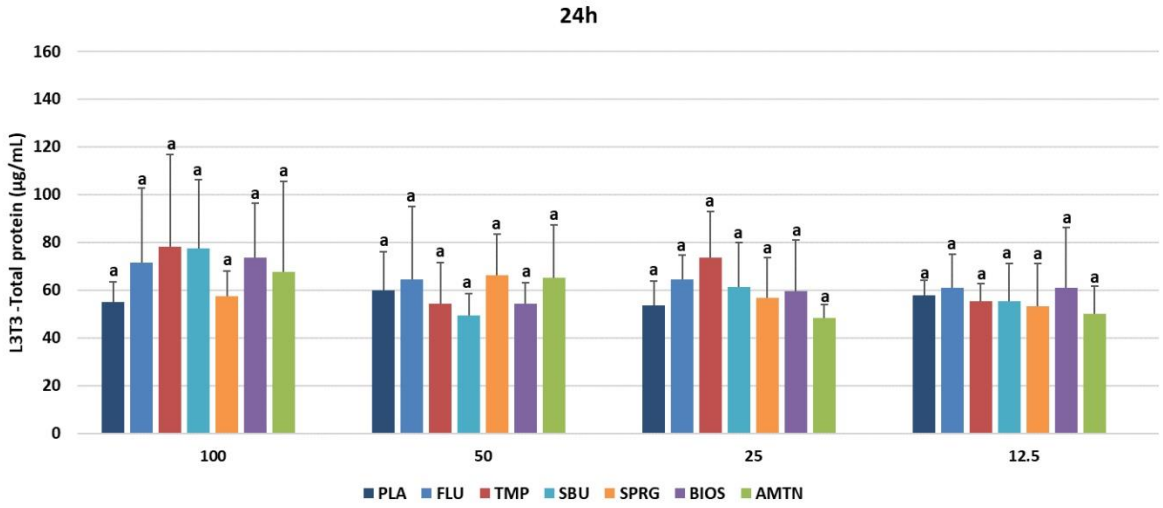

Figure 5 Total protein concentrations (ug/mL) obtained by NIH/3T3 after treatments with different materials. Different uppercase letters show statistical difference among the groups, considering each extract dilution separately (100% - undiluted extract, 50% diluted, 25% diluted, 12.5% diluted in DMEM). Values are expressed in means/standard deviations.

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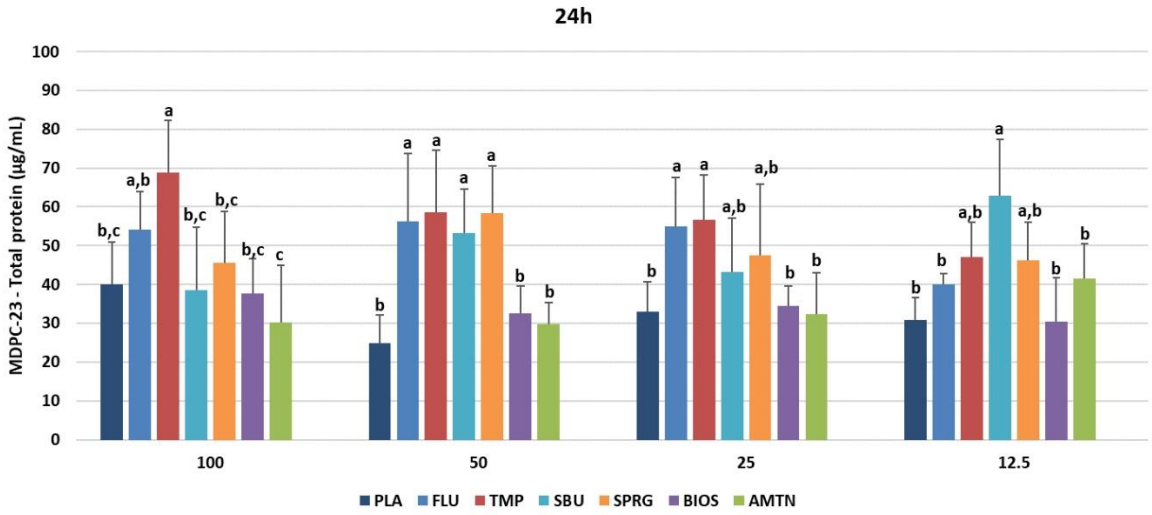

Figure 6 Total protein concentrations (ug/mL) obtained by MDPC-23 after treatments with different materials. Different uppercase letters show statistical difference among the groups, considering each extract dilution separately (100% - undiluted extract, 50% diluted, 25% diluted, 12.5% diluted in DMEM). Values are expressed in means/standard deviations.

Capítulo 2

3 CAPÍTULO 2 - Longevity of different in-office treatments for dentin hypersensitivity: a 12-month randomized and parallel clinical trial†

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3.1 Abstract

Objectives: This longitudinal, randomized, and parallel clinical trial aimed to analyze the longevity of different in-office treatments for dentin hypersensitivity (DH) after 12 months. *Materials and Methods:* One-hundred ninety-two teeth with DH on root exposures were treated using different desensitizers: fluoride varnish (Duraphat – FLU); bioactive ceramic solution (Biosilicate - BIOS); universal self-etching adhesive (Single Bond Universal – SBU); bioactive photoactivated varnish (PRG filler - SPRG). DH was analyzed using visual analogue scale (VAS) and computerized visual scale (CoVAS), before treatments (baseline) and after 7, 15, 30 days, 6 and 12 months. Data were submitted to linear regression model with fixed effects and post-test using orthogonal contrasts ($p \leq 0.05$). *Results:* VAS scale showed a significant reduction of DH was observed from 7 days for BIOS and SBU, from 15 days for FLU and SPRG. For CoVAS analysis all desensitizers were able to significantly reduce DH after 7 days, except for SPRG, which showed this reduction after 15 days of the initial application. No desensitizer returned to initial sensitivity level after 12 months, for both scales. No statistical differences were found among desensitizers for all times of evaluation, for both analyses. Representative curves of 12 months appeared difference from curves of baseline, showing less steep curves in the last follow-up.

Conclusions: All in-office treatments were effective in reducing DH after 12 months, with similarity levels of pain.

Clinical relevance: In-office treatments were effective in control DH after 12-month follow-up.

Keywords: Clinical Trial; Dentin Desensitizing Agents; Dentin Sensitivity; Randomized Clinical Trial

3.2 Introduction

Dentin hypersensitivity (DH) is defined as a sharp short pain, arising from exposed dentin, caused by thermal, tactile, chemical or osmotic stimuli [1-3]. DH has a prevalence between 11% to 33% in adults [2]. At the same time, DH is one of the dental pathologies most associated with pain and with less successful treatment over time [4]. According to the hydrodynamic theory, DH results from the movement of the fluid within the dentinal tubules [1,4]. DH treatment of non-cavitated root exposure can be perform using desensitizing products, laser irradiation, restorative treatments, and periodontal surgeries, which aim to control the

hydrodynamic mechanisms of pain [5]. Desensitizers can act modifying nervous by preventing or reducing neuronal transmission response and occluding the dentinal tubules [3,4]. Furthermore, neural agents must be associated to dentin obliteration products in order to promote longevity of reduced DH [5].

Regarding obliterating materials, the literature presents three types: fluoride varnishes, experimental solutions of bioactive agents and photocuring products, as well as the combination of these types [5]. Fluoride varnishes characterize most of the protocols used, since they have the ability to reduce the movement of fluids in the dentinal tubules through the formation of calcium-fluoride precipitates on dentin surface [6-8]. Experimental solutions of bioactive agents include materials with crystalline bioactive ceramics, biosilicates, with the aim of promoting the remineralization of hard tissues by precipitating calcium phosphate and forming hydroxyapatite [9,10]. The formed apatite layer lead to physically occluding the dentin tubules, resulting in remission or pain reduction [4]. In relation to photocuring products, universal adhesives offer a treatment option by promoting the sealing of the dentinal tubules and the formation of a hybrid layer, neutralizing the hydrodynamic mechanism and promoting an immediate reduction of DH [11,12]. Combining all the aforementioned compositions, a light-activated varnish with surface pre-reacted glass particles was developed [13]. This bioactive technology has multifunctional glass particles trapped in the polyacid matrix, which release fluoride (F^-), strontium (Sr_2^+), borate (BO_3^{3-}), aluminum (Al_3^+), silicate (SiO_3^{2-}), and sodium (Na^+) [14]. This material acts by neutralizing the acids derived from food and liquids, promoting also the obliteration of dentinal tubules by means of photocuring monomers [14].

A meta-analysis that evaluated randomized clinical trials with various DH treatments concluded that in-office treatments are effective for immediate DH reduction [1]. However, the literatures is scarce of high-quality randomized controlled trials on the long-term use of these agents [1,15].

The aim of this randomized and parallel clinical trial was to evaluate the longevity of different in-office treatments for DH. The primary outcome was the longevity of desensitizers. Secondary outcomes measured the influence of patient's characteristics and habits on DH, as well as the difference among desensitizers. The tested hypotheses were that 1) there are no influence of characteristics from patients and teeth, harmful habits, and oral treatments/instructions on level of DH pain; 2) there are no significant difference among times of evaluation for each tested desensitizing protocol; 3) there are no significant differences among desensitizing protocols, regardless of the time of analysis.

3.3 Material and methods

Experimental design and ethical aspects

This research project was approved by the local ethics committee in human research (N° 30122220.1.0000.5420) and registered in clinical trial database (REBEC: U1111-1251-1091). The study was designed as recommended by CONSORT, the flowchart showing the distribution of patients is presented in Figure 1. The research was planned as an interventional, randomized, prospective, single-center, single-blind (subjects), and parallel clinical trial. The study was conduct from July 2021 to March 2023.

Sample size, subjects' selection and groups

The calculation of the sample size was performed based on a previous study [16], using the software Sigma Plot 12.0 (Systat Software Inc., London, UK), with an expected difference between means of 0.210. of Visual Analogue Scale (VAS). The parameters used were: significance level (α) = 0.05; test power (1-β) = 0.80; dropout (β)=0.2, resulting in a final sample number of 48 teeth per group. Patients were selected at the undergraduate clinic of the local Faculty of Dentistry.

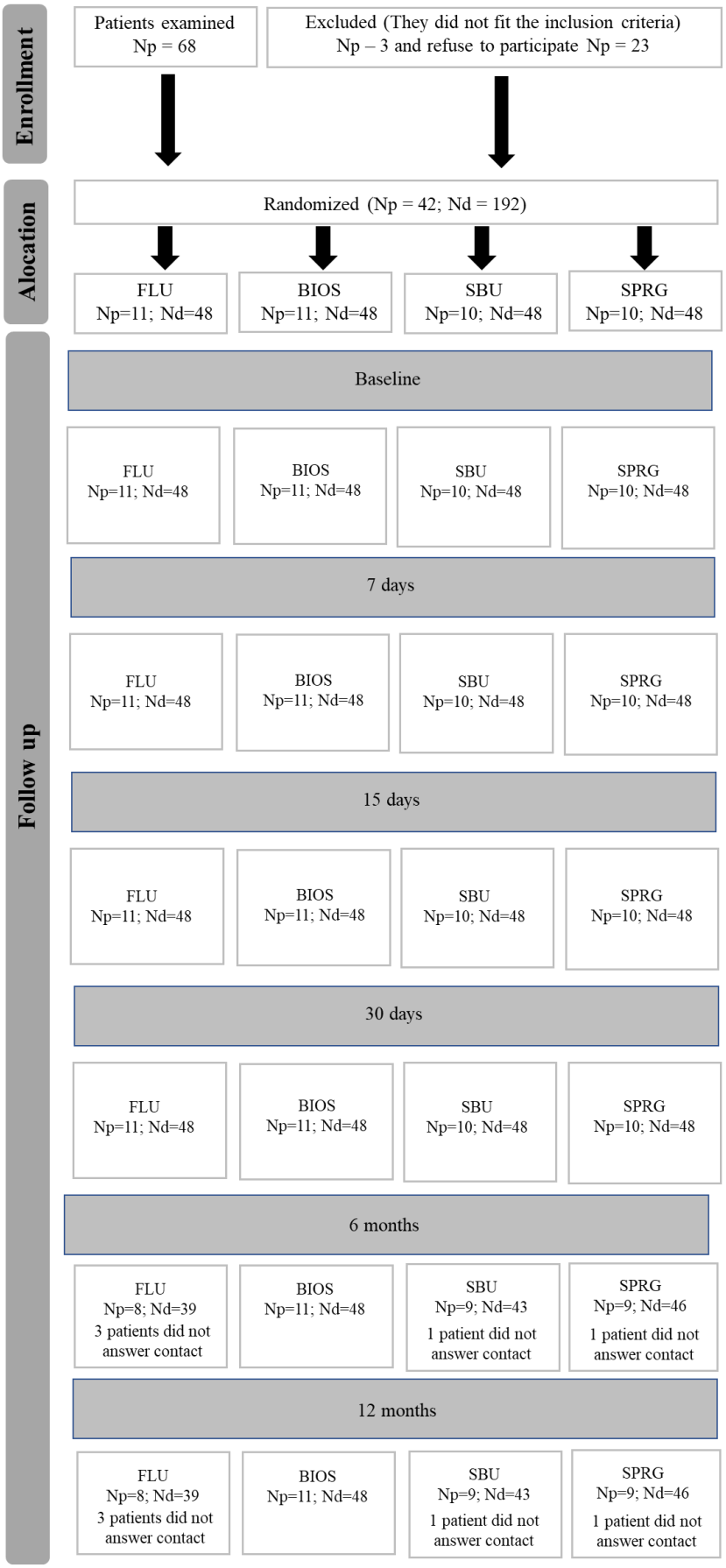

Fig. 1 Consort flowchart. Np: patient number, Nd: number of teeth

Relative cottom roll isolation was performed with cotton rolls. The stimuli were performed using means of an air jet, with the triple syringe positioned 1 centimeter from the cervical region, with a duration of 5 s. Immediately, the patient indicates the sensitivity level by VAS, consisting of a 10 centimeters horizontal line. The pain was identified by the patient from 1 to 10 points, where 0 means “no pain”, 1-3 “light pain”, 4-6 “moderate pain” and 7-10 “severe pain”. The patient made a vertical line crossing the horizontal line of the scale to identify the number of intensities of the DH [17,18]. All teeth with DH less than 3 on the VAS scale were excluded from the study.

Sixty-eight patients were examined and 42 were selected. One hundred ninety-two teeth with DH in non-cavitated root exposures, were enrolled in this study according to inclusion and exclusion criteria (Table 1) [17,19]. The patients were randomly allocated to the four groups according to the in-office treatments: fluoride varnish (FLU), Biosilicate solution (BIOS), universal adhesive (SBU), and varnish with S-PRG particles (SPRG) (Table 2) [20].

Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion Criteria
- Age between 20 and 60, regardless of gender - Good health, no history of allergies to dental products - Presence of at least 1 non-cavitated root exposure or with the beginning of cavitation (up to 1mm deep, without the need for restoration), which present sensitivity of at least grade 3 (VAS) to the air jet at a distance of 10mm - Absence of active periodontal disease	- Pregnant women, nursing mothers or smokers - Presence of active carious lesions - Use of desensitizing agents in the last 6 months - Active and untreated periodontal disease -Use of orthodontic appliance or removable partial denture with clamp on the tooth to be evaluated - Parafunctional habits and occlusal trauma - Use of analgesic/anti-inflammatory

Clinical exams, randomization, and baseline evaluations

Informed consent was obtained from all selected subjects. The conditions of the patient's oral environment were analyzed through the index of decayed, missing and filled permanent teeth (DMFT), visible plaque index (VPI) and gum bleeding index (GBI). Each participant answered a questionnaire for identifying characteristics and harmful habits, including data on lifestyle, dietary and oral health behavior, that can be associated with DH [21].

Then, measurements of the exposure deep and height were performed with the aid of a millimeter probe. The deep of the root exposure could not exceed 1 millimeter. The height of the root exposure was measured considering the distance between the most apical end of the

cement-enamel junction and the highest point of the free marginal gingival, no limit was established [22]. The height of root exposure and the sensitivity score by the VAS scale were stratified variables in the randomization process. The tooth was considered the sample unit. Eligible teeth were recorded in an Excel spreadsheet in order to have a homogeneous distribution of these two factors. Teeth were ordered by the VAS score and height to be divided into four conglomerates: (a) lower sensitivity scores (3-7 on the VAS scale) and smaller exposure height (up to 3 millimeters), (b) lower sensitivity scores (3-7 on the VAS scale) and larger exposure height (greater than 3 millimeters), (c) higher sensitivity scores (8-10 on the VAS scale) and smaller exposures height (up to 3 millimeters), and (d) higher sensitivity scores (8-10 on the VAS scale) and larger exposures height (greater than 3 millimeters). This stratified method of randomization was based on another parallel clinical study [23].

Computerized Visual Scale (CoVAS) scale was then used by applying a constant stimulus, by means of an air jet, at a distance of 10 millimeters, on the buccal surface of the dental elements for 30 seconds. During this period, the patient recorded the intensity of discomfort on a scale from 0 to 100 using a manually controlled potentiometer [24]. The responses obtained were also synchronized, to compare the dental sensitivity by the different treatments in volunteers during the seconds [24]. This time of analyze was consider as baseline.

Interventions

After the previous evaluations, prophylaxis was performed and a buccal retractor was positioned in order to separate the lips and cheek. The tooth that received the desensitizer was isolated with cotton rolls and dried with a jet of air, with the humidity being controlled with a sucker. All protocol steps of each in-office desensitizer treatment were described in Table 2. The FLU desensitizer was applied in once-weekly session, during three weeks. The BIOS solution was mixed using 0.15 mg of powder in 1.35 mL of distilled water and applied such as the previous desensitizer [10]. Light-curing products (SBU and SPRG) were applied and light-cured (LED Radii-cal, SDI Brazil Industry and Commerce LTDA, SP, Brazil) only in the first section. After interventions, cotton rolls and buccal retractor, were removed.

Outcomes

The intensity of DH was evaluated before treatments (baseline) and after 7, 15, 30 days, 6 and 12 months. VAS and Computerized Visual Scale (CoVAS) were used. DH analysis was measured before the reapplication for FLU and BIOS groups, in each session.

Table 2 Characteristics and mode of application of in-office desensitizing materials used in this study

Material	Manufacturer	Batch #	Main ingredients	Application
Duraphat (FLU)	Colgate-Palmolive Company	022001	5% NaF (22.600 ppm), colophony; solvent, shellac; mastic; saccharine and others.	A thin layer was passively applied under the clean and dry surface with a disposable applicator, remains for 5 min, a second layer is applied, remaining for another 5 min.
Biosilicate(BIOS)	Laboratory of Vitreous Materials at the Federal University of São Carlos	-	The solution was composed of Biosilicate powder (P_2; O_5-Na_2; O-CaO-SiO_2 1-10 μm) and distilled water (1:10 ratio) and for simulation of the professional-use products, the particles were mixed immediately before application	A thin layer was applied for 5 s on the clean and dry surface with a disposable applicator, remaining stable for 10 min.
Single BondUniversal (SBU)	3M ESPE	1833100782	BISGMA; HEMA; UDMA; DPIHFP, 10-MDP; solvent; water; silane; and others.	A two thin layer was actively applied for 20 s on the clean and dry surface with a disposable applicator and light-cured for 10 s. No previous acid etching was performed.
Barrier Coat (S-PRG filler varnish – SPRG)	Shofu INC.	121901	S-PRG filler (3.0 μm): TEGDMA; Bis-MPEPP; fluorine boron aluminosilicate; MAA; phosphonic acid; and others.	A two thin layer of one drop active mixed with base in the base container was applied for 3 s on the clean and dry surface with specific applicator and light-cured for 10 s. The uncured layer was removed from surface with a water-moistened cotton pellet.

Abbreviations: TMPnano (nanoparticulate sodium trimetaphosphate) TEGDMA (triethylene glycol dimethacrylate); BISGMA (diglycidildimethacrylate A); HEMA (Hydroxyethylmethacrylate); UDMA (1,3 glycol dimethacrylate) DPIHFP (Diphenyliodonium hexafluorophosphate); 10-MDP (10-decanediol phosphate methacrylate); Bis-MPEPP (bisphenol A polyethoxy methacrylate); MAA (methacrylic acid)

* According to the manufacturer § de Castro Oliveira et al. [20]

Statistical analyzes

VAS and CoVAS data were evaluated with the assumptions of normality and homogeneity with the SAS 9.4 software (SAS Institute Inc. NC, USA). Data from CoVAS were transformed adding a constant (0.5) to avoid the number zero. The application of the Neperian logarithm was also used in order to normalize the residuals. Data were submitted to the linear regression model with mixed effects (random and fixed effects). The post-test using orthogonal contrasts was used. Significance level of 5% was adopted. The study was carried out per protocol and in parallel. Representative curves of CoVAS analysis were presented.

3.4 Results

The descriptive results about number of patients per group, demographic characteristics, root exposure measurements, number and type of tooth per group, pattern of disocclusion, and presence of antagonist teeth are presented in Table 3. Answers about harmful habits and oral treatments/instructions are presented in Table 4. No significant difference was found when distribution of descriptive data among groups was analyzed ($p > 0.05$). Some descriptive data influenced the DH: depth of root exposure, type of tooth and presence of antagonist teeth ($p < 0.05$). The smaller the depth of the root exposures, the lower the dentin sensitivity, since 60.6% and 70.8% of the exposures with a depth of up to 0.4 mm presented sensitivity up to 6 on the VAS scale and 50 on the CoVAS scale, respectively. Regarding the type of tooth, 44.4% and 32.8% of posterior teeth presented sensitivity over to 6 on the VAS scale and 50 on the CoVAS scale, respectively. In contrast, 42.9% and 28.6% of anterior teeth presented sensitivity over to 6 on the VAS scale and 50 on the CoVAS scale, respectively. The presence of antagonist tooth promoted more DH because 72% and 71% without antagonist showed VAS up to 6 and CoVAS up to 50; in contrast to 55% and 66% with antagonist. The patient's harmful habits, oral treatments and instructions did not significantly influence the initial DH ($p > 0.05$). However, 21% had reflux problems, 90.4% had the habit of consuming acidic substances, 28.5% declared having dental clenching and bruxism and 90.4% had already performed periodontal treatment.

Table 3 Descriptive results from characteristics of patients and teeth, described as mean (±standard deviation) or number (percentage %) per group

Groups	**FLU**	**BIOS**	**SBU**	**SPRG**	P value (group)	Confidence level 95%		P value (DH)
						Lower	Upper	
Number of patients	11	11	10	10	0.26	-	-	-
Demographic characteristics								
Age	41.5±7.2	39.9±11.7	41.5±11.1	43.9±8.4	0.80	-0.03	0.08	0.32
Male	1(9.1%)	6(54.5%)	3(30%)	4(40%)	0.14	-0.80	1.25	0.66
Female	10(90.9%)	5(45.4%)	7(70%)	6(60%)				
Oral conditions								
DMFT	12.2±5.7	13.3±4.8	10±7.6	11.9±6.9	0.70	-0.05	0.11	0.41
VPI	0±0	0±0	0±0	0±0	-	-	-	-
GBI	6.1±3.8	5.2±2.7	6.1±4.2	5.5±1.8	0.16	-0.03	0.27	0.12
Root exposure measurements								
Height	1.6±0.7	1.7±0.9	1.6±0.9	1.6±0.9	0.36	-0.10	0.31	0.32
Depth	0.3±0.3	0.3±0.2	0.4±0.3	0.3±0.2	0.46	0.46	2.04	<0.01
Type of tooth								
Canine	4 (8.3%)	4(8.3%)	1(2.1%)	1 (2.1%)	0.57	-0.71	0.95	<0.05
Central Incisor	2 (4.2%)	1(2.1%)	1(2.1%)	0 (0%)				
Lateral incisor	2 (4.2%)	1(2.1%)	0 (0%)	1 (2.1%)				
Molar	10 (20.8%)	15 (31.2%)	12 (25%)	12 (25%)				
Premolar	30 (62.5%)	27(56.3%)	34 (70.8%)	34 (70.8%)				
Pattern of disocclusion								
Canine guidance	35(72.9%)	42(87.5%)	42(87.5%)	28(58.4%)	0.10	-0.57	1.63	0.34
Group function	13(27.1%)	6(12.5%)	6(12.5%)	20(41.6%)				
Antagonist teeth								
No	3(6.2%)	2(4.2%)	2(4.2%)	0	0.99	0.56	2.46	<0.01
Yes	46(93.8%)	46(95.8%)	46(95.8%)	48(100%)				

Table 4 Descriptive results of harmful habits and oral treatments/instructions, described as number (percentage %) per group

Groups		FLU	BIOS	SBU	SPRG	P value (group)	Confidence level 95%		P value (DH)
							Lower	Upper	
Harmful habits									
Do you have gastric problem with reflux?	No	8(72.7%)	7(63.6%)	9(90%)	9(90%)	0.38	-0.96	1.42	0.71
	Yes	3(27.3%)	4(36.3%)	1(10%)	1(10%)				
How often during the week do you have gastric problems with reflux?	No	1(9.1%)	1(9.1%)	2(20%)	0(0%)	0.09	-	-	-
	Yes, once a week	1(9.1%)	0(0%)	0(0%)	2(20%)				
	Yes, 2 × a week	1(9.1%)	0(0%)	2(20%)	2(20%)				
	Yes, 3 × a week	2(18.2%)	3(27.3%)	1(10%)	1(10%)				
	Yes, every day	6(54.5%)	7(63.6%)	5(50%)	5(50%)				
Do you consume acidic foods or drinks?	No	1(9.1%)	1(9.1%)	2(20%)	0(0%)	0.58	-1.16	2.24	0.53
	Yes	10(90.9%)	10(90.9%)	8(80%)	10(100%)				
How often do you consume acidic foods or drinks during the week?	No	1(9.1%)	1(9.1%)	2(20%)	0(0%)	0.75	-	-	-
	Yes, once a week	1(9.1%)	0(0%)	0(0%)	2(20%)				
	Yes, 2 × a week	1(9.1%)	0(0%)	2(20%)	2(20%)				
	Yes, 3 × a week	2(18.2%)	3(27.3%)	1(10%)	1(10%)				
	Yes, every day	6(54.5%)	7(63.6%)	5(50%)	5(50%)				
Do you have dental clenching?	No	9 (81.8%)	4 (36.4%)	6 (60%)	5 (50%)	0.18	-1.72	0.18	0.11
	Yes	2 (18.2%)	7 (63.6%)	4 (40%)	5 (50%)				
Do you have bruxism?	No	8 (72.7%)	7 (63.6%)	8 (80%)	7 (70%)	0.96	-1.43	0.43	0.32
	Yes	3 (27.3%)	4 (36.4%)	2 (20%)	3 (30%)				
Do you usually put objects in your mouth?	No	10 (90.9%)	10 (90.9%)	9 (90%)	9 (90%)	0.99	-1.36	-1.36	-1.36
	Yes	1 (9.1%)	1 (9.1%)	1 (10%)	1 (10%)				
Oral treatments and instructions									
Have you ever had periodontal treatment?	No	2 (18.2%)	0 (0%)	0 (0%)	2 (20%)	0.28	-2.13	1.13	0.55
	Yes	9 (81.8%)	11 (100%)	10 (100%)	8 (80%)				
Have you ever received oral hygiene guidance?	No	1 (9.1%)	0 (0%)	1 (10%)	0 (0%)	0.86	-2.08	2.42	0.88
	Yes	10 (90.9%)	11 (100%)	9 (90%)	10 (100%)				

Table 5 and 6 presented the results for the VAS and CoVAS analysis. The observation of pain values according to scales during the follow-up periods was interpreted as the longevity of treatment success. The pain values observed at 12 months did not differ significantly from that observed at 30 days for FLU and BIOS ($p > 0,05$); in contrast, these similarities were observed at 15 days for SPRG and 7 days for SBU ($p > 0,05$). Regarding CoVAS analysis, the DH values at 12 months did not differ significantly from that observed at 30 days for FLU,

BIOS and SBU ($p > 0,05$); however, SPRG demonstrated a gradual reduction in DH over time, with the lowest value at 12 months ($p < 0,05$). The desensitizers did not differ statistically for both analyses ($p > 0,05$). Figure 2 shows that the desensitizers treatments produced curves with peaks that indicate the moment of the highest intensity of sensitivity that the patient experienced. In this case, representative curves of 12 months appeared difference from curves of baseline, showing less steep curves in the last follow-up, that also demonstrate that the patients took longer to reach the peak.

Table 5 Mean (± standard of deviation) presented by different desensitizing materials according to the VAS scale

Material / Time	FLU	BIOS	SBU	SPRG
Baseline	6.0±2.0 ^{d}	5.5±2.6 ^{c}	6.3±2.3 ^{b}	5.5±2.3 ^{c}
7 days	5.7±1.7 ^{cd}	4.0±2.9 ^{b}	4.5±2.9 ^{a}	4.9±2.5 ^{bc}
15 days	4.9±2.1 ^{bc}	4.2±3.0 ^{b}	4.1±2.7 ^{a}	4.2±2.9 ^{ab}
30 days	4.4±2.5 ^{ab}	3.4±3.3 ^{ab}	3.7±2.9 ^{a}	3.9±2.5 ^{ab}
6 months	3.6±2.5 ^{a}	2.9±2.6 ^{a}	4.6±3.7 ^{a}	3.8±2.6 ^{a}
12 months	4.0±2.6 ^{a}	3.2±3.0 ^{a}	4.1±3.7 ^{a}	3.3±2.6 ^{a}

Lowercase letters represent differences between analysis times. There was no significant difference between the analyzed desensitizers ($p > 0.05$).

Table 6 Mean (± standard of deviation) presented by different desensitizing materials according to the CoVAS scale

Material / Time	FLU	BIOS	SBU	SPRG
Baseline	45.9±33.9 ^{c}	44.1±40.9 ^{c}	42.0±37.3 ^{c}	38.6±36.7 ^{d}
7 days	34.4±34.5 ^{b}	38.19±41.5 ^{ab}	34.0±38.7 ^{b}	36.7±38.6 ^{cd}
15 days	35.5±33.9 ^{bc}	41.25±42.9 ^{bc}	33.0±40.5 ^{b}	35.7±39.0 ^{cd}
30 days	33.1±35.2 ^{ab}	38.0±43.5 ^{a}	27.7±38.3 ^{ab}	34.5±41.6 ^{bc}
6 months	24.5±33.4 ^{a}	38.5±44.9 ^{a}	26.19±37.3 ^{a}	34.2±40.9 ^{b}
12 months	28.5±32.0 ^{ab}	38.4±43.7 ^{a}	32.23±41 ^{a}	22.6±36.5 ^{a}

Lowercase letters represent differences between analysis times. There was no significant difference between the analyzed desensitizers ($p > 0.05$).

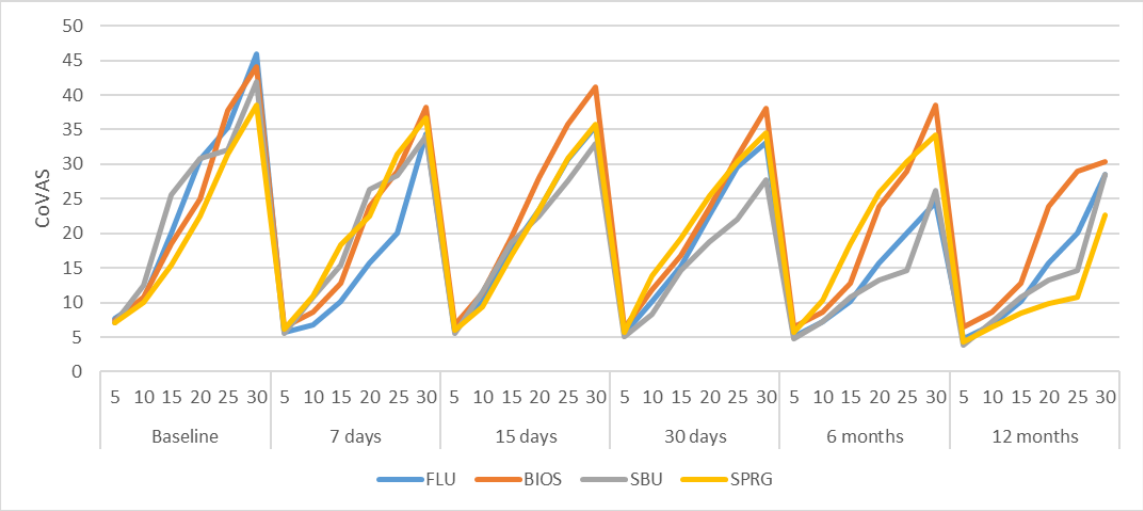

Fig. 2 Curves with peaks showing the moment of the highest intensity of sensitivity by group each second (s) for CoVAS analysis and time of evaluation

3.5 Discussion

The impact of DH on patients' daily routine has led researchers to search for an effective and long-lasting treatment [1,2]. Clinical studies are better to evaluate the longevity of treatments for DH [10]. Among the different categories of studies, the parallel has the advantage of a treatment not influence in other one, especially when bioactive properties are investigated [13,25]. Randomization was done by conglomerates so that the individual characteristics and/or teeth did not influence in the results, permitting a homogeneous distribution per group [23]. Before evaluating the effectiveness of desensitizing treatments, it is also important to highlight that DH is a subjective condition that is difficult to quantify [1,26]. VAS and CoVAS analysis were chosen because of their easy application and good patient tolerance [18,24]. The VAS scale was also selected due to its wide use in the literature [1,10-13,15,17-19,24,26-28]; however, the CoVAS scale was added because it is computerized, allowing patient's pain threshold [18,24]. The evaporative method was used due to its more precise than the tactile method for assessing DH, in addition to promoting the stimulation of a larger area of dentin and being more physiologically controllable [1,26].

Predisposing factors for the development of DH are the loss of enamel, as well as the presence of dentin exposure, which result from bruxism, acidic diet habits, gastric reflux, gingival recession, gingivitis, periodontitis (and its treatment), and brushing teeth too hard [29,30]. These factors did not directly influence the intensity of DH in this study; however, they may possible have contributed to the development of DH [5,29,30]. Considering the initial characteristics of patients, the first null hypothesis was rejected because the depth of root

exposure, the type of tooth, and presence of antagonist tooth influenced the initial intensity of DH. In the present study, smaller DH was found in minimal root wear (0 – 0.4 mm) [31]. In contrast, West et al. [21] considered that newly exposed dentin lesions, exhibiting minimal tooth wear at the cemento-enamel junction, can cause sharp sensitivity in young adult individuals. Regarding the group of teeth, there was a high prevalence of premolars in the present study (65%), corroborating with the literature, which states that these teeth are more susceptible to the development of NCCL due to greater occlusive forces, anatomical morphology and easy access to brushing [5,32,33]. Analyzing the results with percentages, posterior teeth presented superior sensitivity than anterior teeth, due to the factors mentioned above. Teeth with the presence of antagonist also were the majority (96.6%), presenting higher DH than teeth without antagonist. The literature has also been demonstrated that the development of DH is associated with the presence of antagonist [5,29,30].

Regarding the longevity, the second null hypothesis was also rejected, because all in-office treatments for DH showed a significance reduction of pain if compared to baseline measurements. The FLU desensitizer showed a significant reduction in sensitivity after 15 days, which continued to decrease up to 30 days, maintaining the values after 12 months in both analyzes performed. Clinical studies have shown that this material is capable of reducing DH in the first weeks of follow-up, by the precipitation of calcium fluoride into the dentinal tubules [26,27]. In an in vitro study that evaluated the obliteration of dentinal tubules, this material remained inside the tubules after simulating erosive/abrasive conditions equivalent to a period of 12 months, presenting better results than the placebo [20]. In clinical trials of 6-month follow-up, FLU was also effective in reducing sensitivity [19,28].

The BIOS experimental desensitizer showed promising results, with significantly lower sensitivity values at 6-12 months when compared to baseline, 7 and 15 days in VAS and CoVAS analysis. Results of in vitro studies also suggest that BIOS is able to promote tubular obliteration through hydroxycarbonateapatite deposition, reducing dentin permeability, even after erosive/abrasive challenges [9,20]. A clinical study that evaluated the efficacy and durability of BIOS concluded that this material is able to reduce DH in a short period of time, maintaining this effect at the 6-month follow-up [10].

When photoactivated materials were evaluated, reduction of DH was found for SBU after 7 days and SPRG after 15 days, for VAS and CoVAS analysis. This rapid reduction in DH presented by the SBU corroborates with other clinical trial which demonstrated that this material is capable of reducing severe sensitivity after the first day of application, maintaining

low values for up to 90 days [12]. This fact can be explained because the SBU acts forming a hybrid layer on the dentin tissue, sealing the dentin tubules and promoting a rapid decrease in DH [12,20]. In the present study, patients were able to maintain significantly lower sensitivity values even after 6 and 12 months of follow-up, however there is a lack of clinical studies in the literature that evaluated long-lasting with adhesive system to treat DH. SBU also resist to erosive/abrasive challenge, demonstrated by dentin permeability analysis and confocal microscopy images [20]. The 10-MDP monomer (10-methacryloyloxydecyl dihydrogen phosphate) can be responsible for this longevity because it is capable to bind to the hydroxyapatite, forming calcium salts, improving the mechanical and structural stability of collagen, leading to stability of the dentin matrix after the infiltration of resinous monomers and consequently reducing the hydraulic conductance [34]. Regarding SPRG, a significant reduction in DH occurred after 15 days, such as Ravishankar et al. [13] found significantly reducing DH in a period of 7 and 30 days. However, this cited clinical trial [13] evaluated only 20 patients with 60 teeth, until 30 days. In vitro studies suggested that this material is able to reduce the permeability of dentin under erosive conditions by mineral deposits on dentin [14,20]. These characteristics is due to the multiple ions present in the composition of the bioactive molecule, being associated with high fluorine release, forming fluorapatite, fluoridated apatite, and/or strontium apatite incorporated into the calcium site in hydroxyapatite. [25,35]. These components may promote a buffer capacity, which contributes to inhibition of the dentin demineralization [25,35]. These factors may have contributed to the longevity of this treatment, which showed significantly lower DH values at 6 and 12 months.

The longevity of desensitizing treatments was demonstrated by the representative curves of time spent to reach the moment of the highest intensity of sensitivity (Figure 2). The curves after 12 months differed from of baseline because they were less steep, confirming that all desensitizers were effective in reduce the DH after 12 months.

When the desensitizers were compared, no difference was observed between them, not rejecting the third null hypothesis. It should be highlighted that the similar results among the in-office treatments evaluated might be also partially attributed to the positive changes in patient behavior during the study. These effects are likely to happened for all treatments and would interfere in their comparisons. Some studies also showed similar results for SBU, BIOS, and SPRG desensitizers when compared to the others categories in clinical trials that evaluated 1, 3, and 6 months, respectively [10,12,13]. In contrast, other studies found statistically significant differences when the materials used in the present study are compared to other

desensitizers [11,15,26-28,36]. SBU demonstrated superior results than other desensitizers in a follow-up of 30 days [36]; however, showed inferior results when compared to other neural action agent [11]. FLU has shown lower results than other desensitizing components in clinical studies of up to 6 months [15,26-28].

A limitation of the present study was the questionnaire should have been applied on the beginning of the study, during patient selection. The second evaluation could have detected the changes of patient's behavior. Furthermore, this positive change in patient behavior can influence the long-lasting results obtained in this study [26], being a suggestion for future clinical trials.

3.6 Conclusions

Considering the experimental design and the findings, this randomized clinical trial demonstrated that some characteristics related to root exposure and tooth may influence the initial intensity of DH. All in-office treatments evaluated were effective in reducing DH over 12 months, with similarity levels of pain among desensitizers. No difference was found between the different desensitizing protocols evaluated.

Conflict of Interest: The authors declare that they have no conflict of interest.

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ANEXOS

ANEXO A – General Introduction References

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ANEXO B - CONSORT 2010 checklist of information to include when reporting a randomized trial

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	56
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	57
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	58
	2b	Specific objectives or hypotheses	59
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	59
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	60
Participants	4a	Eligibility criteria for participants	61
	4b	Settings and locations where the data were collected	61
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	63
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	62
	6b	Any changes to trial outcomes after the trial commenced, with reasons	-
Sample size	7a	How sample size was determined	59
	7b	When applicable, explanation of any interim analyses and stopping guidelines	-
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	61
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	61

Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	61
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	61
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	59
	11b	If relevant, description of the similarity of interventions	62
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	64
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	61
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	65
	13b	For each group, losses and exclusions after randomisation, together with reasons	60
Recruitment	14a	Dates defining the periods of recruitment and follow-up	59
	14b	Why the trial ended or was stopped	59
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	65
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	65
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	67
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	-
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	66
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	65
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	71
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	68
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	68

Other information			
Registration	23	Registration number and name of trial registry	59
Protocol	24	Where the full trial protocol can be accessed, if available	59
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	71

ANEXO C - Certificado do Comitê de Ética no Uso de Animais (CEUA)

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

CAMPUS ARAÇATUBA
FACULDADE DE ODONTOLOGIA
FACULDADE DE MEDICINA VETERINÁRIA

CEUA - Comissão de Ética no Uso de Animais
CEUA - Ethics Committee on the Use of Animals

CERTIFICADO

Certificamos que o Relatório Final do trabalho intitulado **"Avaliação in vitro dos efeitos de diferentes protocolos para hipersensibilidade dentinária após desafio erosivo-abrasivo"**, Processo FOA nº 2020-0418, sob responsabilidade de Ticiane Cestari Fagundes Tozzi e colaboração de Laryssa de Castro Oliveira foi aprovado pela CEUA em 29 de Julho de 2022.

CERTIFICATE

We certify that the study entitled **"In vitro evaluation of the effects of different protocols for dentin hypersensitivity after erosive-abrasive challenge"**, Process FOA nº 2020-0418, under the supervision of Ticiane Cestari Fagundes Tozzi and collaboration of Laryssa de Castro Oliveira had its the Final Report approved by the CEUA on July 29, 2022.

Prof. Associado João Carlos Callera
Coordenador da CEUA
CEUA Coordinator

CEUA - Comissão de Ética no Uso de Animais
Faculdade de Odontologia de Araçatuba
Faculdade de Medicina Veterinária de Araçatuba
Rua José Bonifácio, 1193 – Vila Mendonça - CEP: 16015-050 – ARAÇATUBA – SP
Fone (18) 3636-3234 Email CEUA: ceua.foa@unesp.br

ANEXO D – Parecer consubstanciado de aprovação do Comitê de Ética em Pesquisa (CEP)

UNESP - FACULDADE DE ODONTOLOGIA-CAMPUS DE ARAÇATUBA/ UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: Efeito de diferentes tratamentos na hipersensibilidade dentinária: avaliação da citotoxidade e estudo clínico randomizado

Pesquisador: FERNANDA DE SOUZA E SILVA RAMOS

Área Temática:

Versão: 3

CAAE: 30122220.1.0000.5420

Instituição Proponente: Faculdade de Odontologia do Campus de Araçatuba - UNESP

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.561.259

Apresentação do Projeto:

O objetivo deste estudo será investigar os efeitos de diferentes categorias de tratamentos para hipersensibilidade dentinária em relação à citotoxidade e por meio de estudo clínico randomizado, utilizando os seguintes protocolos: verniz fluoretado (controle positivo, Duraphat – VF); solução com cerâmica bioativa cristalina (Biosilicato® - SS); sistema adesivo autocondicionante (Single Bond Universal – SB); verniz fotoativado bioativo (PRG - VB). A análise de citotoxicidade será realizada utilizando-se o corante rezazurina. Para o estudo in vivo, cento e noventa e dois dentes (48 por tratamento) com raiz exposta com hipersensibilidade dentinária (sem cavidade) serão tratados por meio de um estudo paralelo randomizado. Cada paciente será tratado com o mesmo protocolo dessensibilizante. O grau de sensibilidade dentinária será analisado por meio da escala visual analógica (VAS) e escala visual computadorizada (CoVAS), antes do tratamento (baseline) e 7, 15, 30 dias, 6 e 12 meses após o tratamento. Os resultados serão submetidos a testes estatísticos específicos. Espera-se que o presente estudo contribua para o conhecimento do mecanismo de ação de novos protocolos dessensibilizantes, bem como para a obtenção de um protocolo clínico inovador e eficaz para a hipersensibilidade dentinária.

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UNESP - FACULDADE DE ODONTOLOGIA-CAMPUS DE ARAÇATUBA/ UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"

Objetivo da Pesquisa:

O primeiro objetivo será avaliar os efeitos da aplicação de diferentes protocolos dessensibilizantes em relação à citotoxicidade, em células tipo fibroblastos.O objetivo do estudo in vivo será avaliar a influência de diferentes protocolos para o tratamento da hipersensibilidade dentinária.

Avaliação dos Riscos e Benefícios:

Riscos:

Riscos mínimos, que são próprios de qualquer tratamento odontológico de rotina

Benefícios:

Os pacientes terão os dentes tratados quanto a sensibilidade. Faremos também o encaminhamento para as outras disciplinas, caso necessite de outro tipo de tratamento odontológico.

Comentários e Considerações sobre a Pesquisa:

Pesquisa apresenta-se apta para a sua realização.

Considerações sobre os Termos de apresentação obrigatória:

todos os termos foram adicionados de acordo com a resolução 466/12 do CNS.

Recomendações:

Não há.

Conclusões ou Pendências e Lista de Inadequações:

Pesquisa apresenta-se apta para a sua realização.

Considerações Finais a critério do CEP:

Salientamos que, de acordo com a Resolução 466 CNS, de 12/12/2012 (título X, seção X.1., art. 3, item b, e, título XI, seção XI.2., item d), há necessidade de apresentação de relatórios semestrais, devendo o primeiro relatório ser enviado até 01/08/2021.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1654085_E2.pdf	05/02/2021 09:10:14		Aceito
Projeto Detalhado	Projetodepesquisa.pdf	04/02/2021	FERNANDA DE	Aceito

Endereço: JOSE BONIFACIO 1193
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Plataforma Brasil

Continuação do Parecer: 4.561.259

/ Brochura Investigador	Projetodepesquisa.pdf	08:58:57	SOUZA E SILVA RAMOS	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	04/02/2021 08:58:26	FERNANDA DE SOUZA E SILVA RAMOS	Aceito
Folha de Rosto	Folhaderosto.pdf	04/02/2021 08:57:44	FERNANDA DE SOUZA E SILVA RAMOS	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

ARACATUBA, 26 de Fevereiro de 2021

Assinado por:
Aldiéris Alves Pesqueira
(Coordenador(a))

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