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Mineral deposition at dental adhesive resin containing niobium pentoxide

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Abstract

The aim of this study was to asses *in vitro* the potential of methacrylate-based adhesive resins containing niobium pentoxide (Nb_2O_5) for stimulating phosphate deposition. Adhesive resins were obtained by mixing 50 wt% BisGMA, 25 wt% TEGDMA and 25 wt% HEMA, and Nb_2O_5 was added on 2.5 or 5 wt% to the resin. Discs 6.5 mm in diameter and 1.5 mm in height of the resin without Nb_2O_5 and of the resins presenting the oxide were obtained by inserting the resin into a silicon matrix, followed by photo activation. Discs were immersed in simulated body fluid at 36°C for 1, 7 and 28 days, and then their surfaces were examined by Raman spectroscopy. Changes of intensity of the 962 cm^{-1} peak, related to phosphate bond, over the samples' surfaces were used to assess the potential of adhesive resins to stimulate phosphate deposition. Experimental groups containing 2.5 and 5 wt% niobium pentoxide presented a phosphate-rich layer deposition over their surfaces after 7 and 28 days of SBF immersion, and this deposition increased over time. Incorporation of 2.5 or 5 wt% niobium pentoxide provides the potential to promote phosphate deposition on methacrylate-based adhesive resins.

Keywords: Adhesive systems; Bioactive; Calcium phosphates; Niobium pentoxide; Raman

Background

Advances in enamel and dentin bonding enable the predictability of direct restorative treatments [1]. A reliable dentin bond was achieved by the establishment of a hybrid layer, which is defined as a layer composed of collagen fibrillar matrix surrounded by a polymer formed from monomers previously diffused into the collagen matrix [2]. Discrepancies in etching depth and monomer diffusion depth could generate a region of denuded collagen at the bottom of the hybrid layer [2]. Besides, the presence of residual solvent and fluid movement of dentinal tubules into the co-monomer mixtures of adhesives jeopardize the water replacement by resins into the collagen matrix, leading to an incomplete monomer infiltration [3]. With dentin demineralization by acid-etching, matrix metalloproteinases (MMPs) are activated [2,4], which in association with the susceptibility to hydrolytic degradation of polymers [5], primarily on non-

infiltrated collagen regions at the bottom of the hybrid layer [4], could contribute to the degradation of the bonding interface [6].

In order to surpass this problem, one option would be to develop an adhesive system that would induce the release of ions with the goal of filling this exposed collagen fiber region with mineral formation [7-11]. Niobium pentoxide (Nb_2O_5) has shown mineralization induction through mineral deposition in previous studies [12-14], demonstrating promising proprieties. Nb_2O_5 was also evaluated as a filler for adhesive resins and methacrylate-based endodontic cements and enhances the Knoop microhardness and radiopacity of these materials [15,16]. Besides, Nb_2O_5 presents the ability to diffuse into the hybrid layer when inserted into an adhesive resin applied following the etching and primer application in a 3-step etch-and-rinse adhesive system [16]. Therefore, the purpose of this study was to asses *in vitro* the potential of stimulating phosphate deposition of methacrylate-based adhesive resins containing niobium pentoxide.

Methods

Formulation of experimental adhesives

Adhesive resins were obtained by mixing 50 wt% BisGMA, 25 wt% TEGDMA and 25 wt% HEMA (provided by Esstech Inc, Essington, PA, USA). Camphoroquinone (Sigma Aldrich, USA) and ethyl 4-dimethylaminobenzoate (Sigma Aldrich, USA) were added at 1 mol% to the mixture to achieve a photo-activated blend. Niobium pentoxide (Nb_2O_5, Companhia Brasileira de Metalurgia e Mineração, Araxá, MG, Brazil) was added at 2.5 and 5 wt%, in relation to the mass of the experimental adhesives, and one adhesive without Nb_2O_5 was used as a control. Nb_2O_5 was previously silanized using γ-methacryloxypropyltrimethoxysilane (γ-MPTS, Aldrich Chemical Co., Milwaukee, WI, USA) [17]. Reagents were hand mixed and sonicated for 180 s.

Phosphate deposition assay by Raman spectroscopy

Adhesive resin was inserted into a cylindrical silicon matrix of 6.5 mm diameter and 1.5 mm height, covered by a polyester film and photo activated (Radii Cal1200 mW/cm^2, SDI LTD., Bayswater, VIC, Australia) for 20 s on each side. Three discs were obtained for each experimental adhesive. Discs were immersed in simulated body fluid (SBF) prepared according to Kokubo and Takadama [18] for 1, 7 or 28 days at 36°C. The SBF prepared had the following ion concentration in mol/m^3: Na^+ (213.0), K^+ (7.5), Mg^{2+} (2.3), Ca^{2+} (3.8), Cl^- (221.7), HCO^{3-} (6.3), HPO_4^{3-} (1.5) and SO_4^{2-} (0.8), and the pH was adjusted to 7.40. After the storage period, samples were washed with 10 ml of distilled water and dried on desiccators at 36°C.

Chemical changes on the sample surface were analyzed by Raman spectroscopy (Senterra, Bruker Optics, Ettlingen, Germany). An area of 200x200μm was irradiated three times for 5 s by a 785-nm laser of 50 mW on 100 equidistant points. Spectra were obtained at 1800 to 440 cm^{-1} Raman band. Using spectroscopy software (Opus 7.5, Bruker Optics, Ettlingen, Alemanha), the integral of the 962 cm^{-1} peak absorbance (symmetric stretching υ_1 mode of phosphate) [19] and of the 1610 cm^{-1} peak (vibration mode of carbon-to-carbon stretching of the aromatic ring of BisGMA) [20], as indicated in Figure 1, was calculated. Increases in the ratio between the absorbance of the 962 and 1610 cm^{-1} peaks ($A^{-1}_{962cm}/A^{-1}_{1610cm}$) indicate the deposition of calcium phosphates on the samples' surfaces.

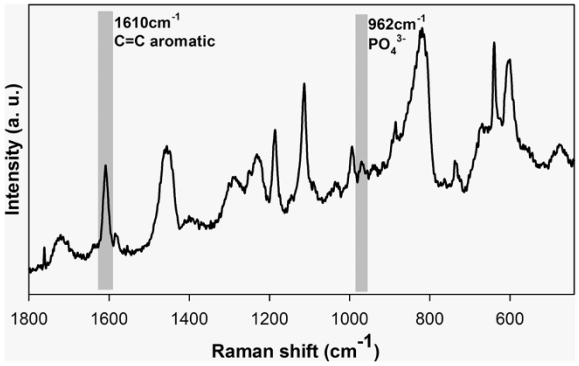

Figure 1 Raman spectrum of adhesive resin containing 5% of Nb_2O_5 after 28 days immersed in SBF. Absorbance bands with 1610 and 962 cm^{-1}peaks, related to aromatic carbon-to-carbon double bonds and phosphate groups, respectively, were highlighted.

Results and discussion

Bioactive materials should interact with tissues, evoking positive responses to the host body [21], as this stimulates hard tissue formation. Figure 2 shows phosphate content changes over the sample surface after distinct periods immersed in SBF, assessed by variation in the absorbance of the phosphate Raman peak. Phosphate content increases over time for all groups, being more pronounced in the groups containing 2.5 and 5% Nb_2O_5 than in the control group. Adhesive resins with niobium pentoxide incorporation exhibited phosphate deposition after 7 days of immersion. The presence of niobium pentoxide stimulates the deposition of calcium phosphate from the fluids [12] and allows the material to be bioactive.

Higher content of phosphates ($A^{-1}_{962cm}/A^{-1}_{1610cm}$) measured at 100 points on each sample after 28 days of SBF immersion reached 0.27 for the control resin, 0.42 for resin with 2.5% Nb_2O_5 and 0.40 for resin with 5% Nb_2O_5. Phosphate deposition on the control resin could be attributed to immersion of a solid sample that could be partially solubilized, evoking pH changes, altering local supersaturation of SBF and causing spontaneous precipitation of calcium and phosphate ions [22]. Despite this drawback of SBF testing, the adhesive resins containing Nb_2O_5 exhibited a more pronounced deposition of phosphates than the control resin. The use of SBF testing to predict bioactivity of biomaterials has been severely questioned [22,23]. It is stated that use of SBF testing leads to false-positive results and cannot reliably mimic physiological conditions [22,23]. A review discussing the *in vitro* and *in vivo* bioactivity of calcium silicate cements showed that a material that produced an apatite layer over its surface after interacting with the ions derived from SBF did not exhibit a bone bond when inserted in living tissues [24]. Three different mechanisms of interaction with tissues could contribute to the bioactivity of materials: chemical bonds to host tissues, influence on cellular pathways and stimulus to cell differentiation by topographical features of biomaterial. From these mechanisms, the SBF test simulates only the chemical bond to tissues which could be predicted by their apatite-forming ability on samples surface [25]. Nevertheless, the protocol for *in vitro* testing of implants using SBF was described

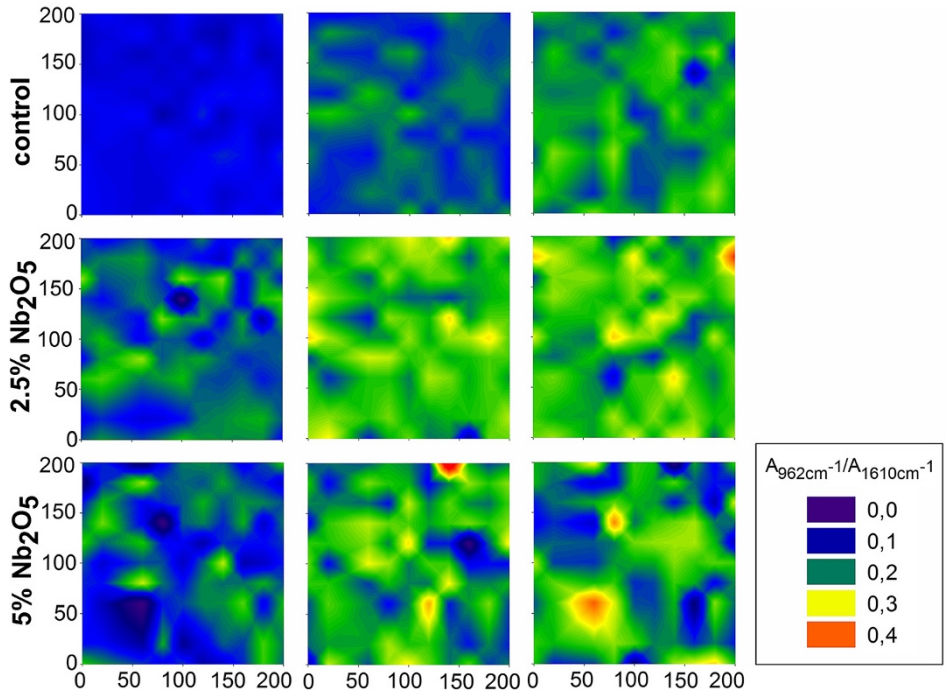

Figure 2 Raman map of phosphate deposition. The intensity is given by the ratio between the 962 and 1610 cm^{-1}peaks ($A^{-1}_{962cm}/A^{-1}_{1610cm}$).

by ISO 23317 [26]. Recently, Zadpoor [25] systematically reviewed the literature, looking for studies that evaluate the bioactivity of two or more biomaterials by SBF immersion and animal models. Of 33 papers included in the analysis, 25 of the results showed that *in vitro* testing matched the *in vivo* results. In three the bioactivity was confirmed by *in vitro* and *in vivo* tests; however, the materials' bioactivity was not ranked in the same way. In the other five papers, the biomaterials showed no apatite layer in an SBF test but bonded to animals' bone tissue. In this review, no false-positive results were noticed. Thus, the lowcost, rapid speed and ease make the SBF test the method of choice for an initial screening of newly developed materials.

Retention of direct restorative materials currently used is achieved by micromechanical interlocking and/or chemical adhesion [2,27]. Nowadays, the adhesive procedures have a reliable clinical effectiveness [1]. However, some issues remain challenging in achieving a durable bond to tooth structure. During restorative procedures using a separate etching step, a discrepancy between the demineralization depth and monomer penetration could occur [2]. This fact makes the non-monomer-infiltrated collagen at the bottom of the hybrid layer more prone to degradation by proteolytic enzymes, as dentinal matrix metalloproteinases and, consequently, the resin-dentin bonding interface could be compromised [2]. The incorporation of bioactive materials has been proposed, attempting to backfill the denuded collagen to prevent hybrid layer deterioration [8-11]. Calcium silicates and calcium orthophosphates have already been experimentally tested on adhesives aiming at this therapeutic/remineralization effect [28,29]. These compounds are highly soluble, and their action is due to an initial solubilization followed by a deposition of minerals [28]. This process does not occur for niobium pentoxide; it is expected that an apatite-like phase directly deposits over its surface

without an initial dissolution of the mineral due to a Nb-OH bond formed on the oxide surface that induces apatite nucleation from SBF ions [14,30]. Besides, this does not require the dissolution of filler from the adhesive matrix niobium pentoxide, which enhances the Knoop microhardness and radiopacity of adhesive resins [16]. Thus, we assume that the developed adhesive resins were capable of promoting calcium-phosphate deposition on their surfaces and could contribute to the maintenance of hybrid layer integrity.

Conclusion

Adhesive resins containing 2.5 and 5% niobium pentoxide stimulated the deposition of a phosphate layer over their surfaces.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
FMC participated in the study design development, manuscript construction and manuscript final review; FFP participated in the study design development, data interpretation and manuscript construction; GCSF participated in the study design development and manuscript construction; SMS, LCBA and ERS participated in laboratory tests; VCBL participated in study design development and manuscript final review; SMWS participated in study design development and manuscript final review. All authors read and approved the final manuscript.

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