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Bioactivity of zinc-doped dental adhesives

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ABSTRACT

Design of restorative materials should be focused on promoting not only adhesion but also dentine self-repair processes.

Objective: To ascertain if ZnO and $ZnCl_2$-doped resins are materials able to induce calcium (Ca) and phosphate (P) deposition.

Methods: 48 resin disks were prepared with the following materials: (1) single bond -3M/ESPE-, (2) single bond + ZnO particles 20 wt% and (3) single bond + $ZnCl_2$ 2 wt%. Specimens were polymerised and polished. Bioactivity was tested through a simulated body fluid solution (SBFS) immersion test. At time 24 h, 7 d and 21 d surfaces were analyzed by stereomicroscope, high resolution scanning electron microscope (HRSEM), energy-dispersive analysis (EDX), confocal laser Raman, and X-ray diffraction (XRD) for morphological and chemical composition.

Results: Under the stereomicroscope, crystal formations were encountered in both zinc-doped resin adhesives after 7 d of immersion. It was, detected by EDX, that the ZnO-doped resin produced Zn, Ca and P deposition (globular formations were observed by HRSEM) after 7 d. Zn and P crystals were detected by HRSEM and EDX in the experimental $ZnCl_2$-doped resin after 7 d and 21 d. Hopeite formation was identified by Raman on both Zn-doped resins. Single bond did not produce mineral or crystal precipitation.

Conclusions: ZnO-doped resin induced Ca and P deposition after SBFS immersion. On $ZnCl_2$-doped resin hopeite formation was detected, if this hopeite may be further converted into apatite, after SBFS immersion, remains to be ascertained.

Clinical significance: Bonding with ZnO-doped resin may facilitate incorporation of Ca and P at the interfacial bonding zone.

1. Introduction

Dentine, even in the absence of cells, is able to actively participate in tissue reparative and regenerative processes. It contains matrix-bonded bioactive molecules, enzymes and growth factors in order to complete reparative processes.[1] Design of restorative materials should be focused on promoting this repair process through a defined and targeted interaction with the host tissue to release these bound bioactive molecules.

During the caries process, the mineral part of dentine is dissolved by bacterial acids, exposing the organic matrix to breakdown by bacterially derived enzymes, and by host-derived enzymes such as the matrix metalloproteinases (MMPs) present within the dentin.[2] Reincorporation of mineral into the demineralised dentine matrix is important since the mineral precipitated may work as a constant site for further nucleation, and the remineralised subsurface of the tissue may be more resistant to subsequent acid attack.[3] This remineralization process that physiologically occurs onto

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demineralised dental surfaces, where the mineral is reabsorbed and damaged crystals are rebuilt[4] should be stimulated by new reparative materials.

Zinc may influence signalling pathways and promote a metabolic effect in hard tissue mineralization[5] and remineralization processes.[6] Zinc has also been shown to inhibit dentine demineralization[7] and somehow facilitates dentine remineralization.[6] These effects make zinc attractive for use as therapeutic agent in the fields of hard and soft tissue engineering. Zinc is widely used in dentistry and it has been experimentally included into a resin adhesive, reducing metalloproteinases-mediated collagen degradation and inducing dentine remineralization at the bonded interface.[8,9]

Bioactivity is defined as a property that elicits a specific biological response at the interface of the material, which results in the formation of a bond between tissue and material. It has been shown that the *in vivo* bioactivity is precisely reproduced by apatite-formining ability in simulated body fluid solution (SBFS). Therefore, evaluating the formation at the surface of a calcium phosphate-rich layer is a good screening test for the *in vivo* bioactivity of materials, resulting in reduction of the need for animal sacrifices and savings in experimental time.[10]

The aim of this study is to ascertain if two novel zinc-doped resins are able to induce Ca and P deposition after SBFS immersion. The null hypothesis tested was that tested resins are not able to promote Ca and P deposits on the surface after SBFS immersion, therefore are not bioactive materials.

2. Materials and methods

48 resin disks (6 mm diameter and 2 mm thickness) were prepared with the following materials: (1) single bond -SB- (3M ESPE, St. Paul, MN, USA), (2) SB with ZnO microparticles addition (Panreac Química S.A., Barcelona, Spain) 20 wt% –SBZnO– and (3) SB doped with $ZnCl_2$ (Sigma–Aldrich, St. Louis, MO) 2 wt% –SB$ZnCl_2$–. To get complete dissolution of $ZnCl_2$ and dispersion of ZnO microparticles, adhesive mixtures were vigorously shaked during 1 min in a tube agitator (Vortex Wizard, Ref. 51075, Velp Scientifica, Milan, Italy). The complete process was performed in darkness. Resins were placed in teflon ring splits, and evaporation was performed, air-drying for 60 s at a distance of 10 cm to remove solvent. Specimens were then polymerised by means of a halogen unit (Bluephase, Ivoclar Vivadent AG, Schaan, Liechtenstein) from different sides until the entire area was exposed (light energy density: 750 mW/cm^2). After 24 h, the surfaces were polished through SiC abrasive papers from 800 up to 4000 grits (Struers LaboPol-4; Struers GmbH, Hannover, Germany). The specimens were treated in an ultrasonic bath containing deionised water for 5 min at each polishing step.

Disks were immersed in simulated body fluid solution (SBFS: NaCl 8.035 g, $NaHCO_3$ 0.355 g, KCl 0.225 g, $K_2HPO_4{\cdot}3H_2O$ 0.231 g, $MgCl_2{\cdot}6H_2O$ 0.311 g, 1.0 M – HCl 39 ml, $CaCl_2$ 0.292 g, Na_2SO_4 0.072 g, Tris 6.118 g, 1.0 M – HCl 0–5 ml) (pH 7.45), for 24 h, 7 d and 21 d. The complete procedure was performed as reported in Kokubo and Takadama 11. After each storage period, surfaces were analyzed by stereomicroscope, scanning electron microscope including elemental analysis, Raman, and X-ray diffraction (XRD) analyses.

Stereomicroscope imaging: Images of resin surfaces at time 0 and after 24 h, 7 d and 21 d of SBFS immersion were acquired in a stereomicroscope Olympus SZ-60 (Olympus, Japan) at 60× and 120× magnifications.

High resolution scanning electron microscopy analysis (HRSEM) and energy dispersive (SEM/EDX) analyses: Specimens were desiccated and then sputter-coated with carbon by means of a sputter-coating Nanotech Polaron-SEMPREP2 (Polaron Equipment Ltd., Watford, UK) and observed with a high resolution scanning electron microscope (HRSEM Gemini, Carl Zeiss, Oberkochen, Germany) at an accelerating voltage between 10 kV and 20 kV. Energy-dispersive analysis was performed in selected points using an X-ray detector system (EDX Inca 300, Oxford Instruments, Oxford, UK) attached to the HRSEM.

Raman and X-ray diffraction (XRD) analyses: Surfaces were analyzed with a computer-controlled confocal laser Raman apparatus equipped with a Zeiss optical microscope with a 100× objective and CCD detector attached to a modular research spectrograph (JASCO/NRS-5100, JASCO Europe S.R.L., Milan, Italy). A near-infrared diode laser spot size of $<1\ \mu m$ operating at 785 nm was used to induce the Raman scattering effect. The spectral coverage of this model ranged from 200 cm^{-1} to 1800 cm^{-1}. The calibration of the wavelength and intensity was performed according to manufacturer's specification using a silicon standard and the calibration system integrated with the software (JASCO Spectra Analysis).

The crystal phases on the specimens were analyzed using X-ray diffraction analysis under a diffractometer Bruker D8 Advance (XRD Bruker Corporation, Wien, Austria) conditions were CuKα radiation in θ–θ scan, in a range 2 theta from 0° to 60° (WL = 1.54060).

3. Results

Stereomicroscope analysis: Representative images taken with the stereomicroscope are shown in Fig. 1. SB disks did not present crystal formations (Fig. 1a). On SBZnO resin, a concentric needle shape crystal formations were sporadically evidenced at the surface, after 7 d of SBFS immersion (Fig. 1b). After 7 d, SB$ZnCl_2$ surfaces were completely covered by needle-like crystal formations (Fig. 1c).

High resolution scanning electron microscopy analysis (HRSEM) and energy dispersive (SEM/EDX) analysis: HRSEM images and representative spectra acquired by EDX analyses at the tested resin surfaces are presented in Fig. 2.

Mineral deposition or crystal formation was not observed in SB specimens at any time point of the study. EDX spectrum (Fig. 2a, Ep1) evidenced the presence of silica (Fig. 2a). Crystal formations were detected on the surface of SBZnO specimens after 7 d of water immersion. Isolated bundles of prismatic crystals were encountered at the resin surface (Fig. 2b and c), and after EDX analysis zinc and phosphate were identified as main crystal components (Fig. 2d, Ep2). At higher magnification, the resin surface was covered by bundle-shaped particles (Fig. 2d); these particles were identified by EDX analysis as calcium, zinc, silica and phosphate (Fig. 2d, Ep3). After 21 d of