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Remineralization of human dentin using ultrafine bioactive glass particles

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Abstract

Bioactive glass nanoparticles synthesized by flame spray synthesis were tested for their remineralization capabilities in vitro. After artificial demineralization with EDTA, human dentin was treated with 20–50 nm size bioactive glass nanoparticles or a micrometer-sized, commercial reference material (PerioGlas) for up to 30 days. The degree of remineralization was measured using quantitative gravimetric methods (thermogravimetry, elemental analysis) and element-sensitive scanning electron microscopy imaging to detect new mineral precipitated on or within the demineralized tooth matrix. After treatment with bioactive glass nanoparticles for 10 or 30 days a pronounced increase in mineral content of the dentin samples suggested a rapid remineralization. The mechanical properties of the remineralized dentin samples were well below the stability of natural dentin. It is suggested that this lack of mechanical reconstitution may be attributed to an imperfect arrangement of the newly deposited mineral within the demineralized tooth matrix. Nevertheless, the substantially higher remineralization rate induced by nanometer-sized vs. micrometric bioactive glass particles corroborated the importance of particle size in clinical bioglass applications.

Keywords: Bioactive glass; Nanoparticles; Human dentin; Remineralization

1. Introduction

Dental plaque, i.e. the biofilm adhering to teeth, contains numerous bacteria, which produce organic acids that can dissolve the dental hard tissues enamel and dentin (demineralization). The loss of mineral from these tissues is counterbalanced by the deposition of minerals from saliva or oral fluid (remineralization). The relative magnitude of these two actions determines whether destruction (caries) or regeneration (remineralization) occurs [1].

Dentin accounts for the greatest part of the dental hard substance. Unlike enamel, dentin is a tissue with an organic matrix of collagen and other proteins, in which crystalline apatite mineral is embedded. A well-defined system of tubules stretches from the pulp space towards the dentin–enamel and dentin–cementum junctions. Dentinal tubules contain projections of odontoblast cells that line the inside of the pulpo-dentinal junction. Odontoblasts orchestrate mineralization processes in dentin, not only during dentinogenesis, but also after teeth have been formed. Mineralizing processes are conveyed via a liquid similar to extracellular fluid [2].

Bioactive glasses are known for in vivo responses including osteoconductivity and bonding to bone via release of ions and formation of an apatite layer in contrast to inactive glasses such as soda-lime and borosilicate glass [3,4]. Bioactive glasses are therefore used for bone reconstitution and tissue engineering [5,6] but are also interesting candidates for mineralization in dentistry [7]. In earlier in vitro

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studies, bioactive glasses have been reported to induce mineralization of dentin disc surfaces [7–9]. These results suggest that bioactive glass could be instrumental in the remineralization of human dentin and that it has potential as a filler component in mineralizing restorative materials [10]. Unfortunately, relatively long reaction times have limited or inhibited the application of bioactive glass as a remineralization agent in dental practice. The remineralization process may be described as a slow dissolution/precipitation process of mineral constituents into the dentin matrix. The present study therefore investigated the use of 20–50 nm bioactive glass [11] for dentin remineralization, and compared this new form of bioactive glass to a micron-sized reference material. It was hypothesized that due to its high specific surface area the novel nanoparticulate bioactive glass could facilitate the dissolution of ions from the glass and thereby accelerate the remineralization of dentin.

2. Materials and methods

2.1. Bioactive glasses

Flame spray synthesis [12] was applied to synthesize nanometric 45S5-type bioactive glass [11]. Precursors containing the corresponding metal loading of Si, Ca, Na and P were prepared by mixing hexamethyldisiloxane (Lancaster) with 2-ethylhexanoic acid salts of calcium and sodium, and tributyl phosphate [11]. The as-formed nanoparticles of bioactive glass (hereafter referred to as NBG) were then analyzed prior to application. Powder X-ray diffraction (XRD) patterns were recorded on a Stoe STAPI-P2 apparatus (Ge monochromator, Cu Kα1, PSD detector; Stoe Co., Darmstadt, Germany). Transmission electron microscopy (TEM) images were recorded on a CM30 ST (LaB_6 cathode, operated at 300 kV, point resolution ~4 Å; Philips, Eindhoven, the Netherlands). The specific surface area (SSA) was measured on a Tristar 3000 (Micromeritics, Norcross, GA, USA) by nitrogen adsorption at 77 K using the Brunauer–Emmett–Teller (BET) theory. The chemical composition of uniaxially compacted samples was investigated by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) using a 193 nm ArF excimer laser ablation system (Lambda Physik, Göttingen, Germany) coupled to an ICP-MS (DRC II+, Perkin–Elmer, Norwalk, CT, USA) [13]. Conventional 45S5 Bioglass (PerioGlas®, NovaBone, Alachua, FL, USA, hereafter referred to as PG) with particles sizes in the range of 90–710 μm (manufacturer's information) was used as a reference material.

2.2. Dentin bars

Teeth used in this study were provided and prepared by the Department of Preventive Dentistry, Periodontology and Cariology, University of Zürich, Switzerland. Dentin bars were prepared from human third molars following extraction. The treatment plan of any of the involved patients, who had given informed consent that their extracted teeth could be used for study purposes, was not altered by this study, which was conducted in accordance with the ethical guidelines for medical research of Zürich. Immediately after extraction the teeth were stored in a 0.2% thymol solution. The dentin bars were prepared using a saw microtome (SP1600, Leica Microsystems, Glattbrugg, Switzerland) to a profile of 0.8 mm × 1.2 mm; their length varied between 10 and 20 mm. The orientation of the tubuli was perpendicular to the greater bearing surface of the bars and was equal for all samples. The dentin bars were stored in sterile saline solution until further usage. In order to minimize the risk of bacterial contamination the samples were sterilized with ethylene oxide for 5 h before usage.

2.3. Ion release and pH of bioactive glasses

Ion-release profiles of samples of 50 mg bioactive glass in 80 ml water were recorded for 24 h under continuous stirring. The molybdenum blue method was applied to measure aquatic silica concentrations photometrically at 815 nm [14]. The free calcium ion concentrations were measured using an ion-selective electrode (polymer membrane electrode Ca; Metrohm AG, Herisau, Switzerland). Simultaneously, the pH of the dispersions was measured using a pH electrode (SevenEasy, Mettler Toledo, Greifensee, Switzerland).

2.3.1. Demineralization and remineralization

Demineralization of dentin bars was achieved by immersion in sterile 17% EDTA solution (pH 8) [15] for 2 h. Suspensions of nanometric bioactive glass (NBG, 45S4 composition) and PerioGlas® (PG) in ultrapure deionized water (Millipore, Bedford, MA, USA; resistivity >18.2 $M\Omega\,cm^{-1}$) were used as remineralizing agents; 150 mg of NBG or PG were suspended in 0.87 ml water. After demineralization the bars were rinsed with excess amounts of water, and placed in NBG or PG suspensions. Specimens were then stored therein at 37 °C for 1, 10 and 30 days. Control specimens were stored in water. Prior to further experiments, all dentin bars were thoroughly rinsed with water three times. Whenever dentin bars were placed into solution they were positioned so that all surfaces were equally accessible to the surrounding medium.

2.4. Instrumentation and analytical conditions

After treating the dentin with different remineralizing suspensions or water, the bars were dried under vacuum at 110 °C for at least 12 h, and the surface was examined using Raman spectroscopy (EQUINOX 55 spectrometer equipped with a FT-Raman accessory FRA 160/S; Bruker Optics, Faellanden, Switzerland) in backscattering mode as suggested by Tsuda et al. [16]. To examine the mineralization depth profile in the dentin bars, they were dehydrated

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