Abstract

Bioactive glass surface functionalization to develop enamel remineralization materials

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Introduction

White spot lesions, the most frequent iatrogenic effect of fixed appliance orthodontics, result from enamel demineralization due to an imbalance in the natural dynamic demineralization–remineralization process. New methods to prevent and reverse this sequela of orthodontic treatment are based on bioactive glasses (BGs) and peptides. BGs, increase mineral saturation. Peptides are consider as biomimetic materials characterized to simulate the natural formation of hydroxyapatite prisms.

BGs are versatile materials capable of true enamel remineralization. BG surface functionalization enhances bioactivity and biological interactions. The functionalization of the outer layer of BG opens up the possibility of developing a new material type with unique properties and the ability to respond to external stimuli. However, to date, surface functionalization of BGs has focused on the immobilization of molecules such as peptides and proteins, as well as controlled drug delivery.

Biomimetic materials, such as peptides, continue to attract attention due to their size and easy synthesis. Peptide functionality, according to the therapeutic need, can be customized in terms of the chain length, amino acid type involved, and associated sequence. For example, some peptides cannot fold. Biomimetic materials, such as foldamers, can mimic the structural behavior of peptides and conform to various three-dimensional secondary structures, thus providing a larger molecular space, enhanced functionality, and increased resistance to enzymatic activity.

Despite the wide variety of BG formations and peptides used for enamel remineralization, the mechanical characteristics of enamel have not been fully clarified. Thus, practical materials that allow for control over enamel remineralization are desirable.

Photosensitive materials can be used to address this functionality issue as a molecular ‘machine’, similar to the approach used in medical fields for drug delivery (which can be combined with therapies capable of repairing and producing hydroxyapatite at the nanometer scale). In dentistry, photosensitive materials are needed to prevent enamel demineralization and enhance the regeneration necessary to protect dental health.

The aim of the present research was to design a new material capable of controlling the Ca^{2+} release and promoting enamel remineralization based on the surface functionalization of BGs. The research was divided into two major experiments, as described below.

Experiment 1:

Surface modification of BG and loading of foldamers with enamel remineralization potential.

Experiment 2:

Synthesis of a photosensitive hybrid material based on bioactive glass nanoparticles (BGNPs) for the Ca^{2+} release control and enamel remineralization.

Materials and methods.

A. Experiment 1:

i. Design and synthesis of foldamers

Dynamic foldamer design was realized using Spartan 18 software (Wavefunction Inc., Irvine, CA, USA), based on hybrid peptides with nitrogenous pyridine at positions 2 and 3 of the aromatic ring. The foldamer configuration was developed with the intention of imparting the ability to chelate Ca^{2+} ions. All foldamers were synthesized using traditional methods in solution, and were characterized by proton (1H) and carbon 13 (13C) nuclear magnetic resonance (NMR) and Fourier-transform infrared spectroscopy (FTIR), coupled with attenuated total reflection (ATR) measurements.

ii. Synthesis and characterization of mesoporous BG nanoparticles

The quinary composition of BGNPs was synthesized using the sol-gel method described by Leite (2015) Tetraethyl orthosilicate (TEOS, 99.90% pure, Sigma-Aldrich) was dissolved in 60 mL of EtOH and mixed with a solution of Calcium nitrate tetra hydrate (99%, Sigma-Aldrich), Sodium nitrate (99%, Nakalai tesque), and Strontium nitrate (99%, Sigma-Aldrich), the pH of the mixture was adjusted to 1.5 adding citric acid anhydrous (98%, Wako) and left stirring until mixture became transparent (around 20 min). The mixture was added drop-by-drop to a 1500 mL aqueous solution containing ammonium dihydrogen phosphate (99%, Nacalai Tesque). The pH was adjusted to 11.5 with ammonium solution (25%. Nacalai Tesque) and left stirring for 48 h, followed by a 24 h resting period. The precipitates were washed with deionized water until no smell of ammonia was present (around four times). A 200mL, 2% wv^{-1} aquos solution of Plutonic 123 (Sigma-Aldrich) was added to the precipitated and left stirring for 30 min, followed by freeze drying for 72 h. The gel power was calcinated at 700ºC for 5 h to remove the surfactant.

ATR-FTIR and X-ray diffraction (XRD; Rint-2500; Rigaku, Tokyo, Japan) measurements over the 2θ range of 10–70° were used to characterize the nanoparticles. Field emission-scanning electron microscopy (FE-SEM) and transmission electron microscopy (TEM) were used to determine the nanoparticle morphology; images were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

iii. Surface functionalization and foldamer immobilization

Surface functionalization was performed with 3-amimopropyl triethoxysilane (ATPS). The BGNPs were cleaned under sonication for 5 min in acetone, and then dried at 100ºC in vacuum for 1 h. The BGNPs were added to the ATPS mixture and a 95%:5% EtOH:H_2O solution. ATPS was preactivated by stirring the solution for 1 h. Stirring was then performed for 1 additional hour. The BGNPs were then washed with EtOH and dried at 100ºC under vacuum for 1 h.

Immobilization of the foldamer was realized by immersing bioactive glass functionalized nanoparticles (BGNPsF) in a citrate phosphate buffer solution (pH: 5) with a foldamer concentration of 2 mg/mL, and stirring the resulting solution for 1 h. BGNPsF characterization was carried out using SEM, energy dispersive X-ray spectroscopy (EDS), XRD, and X-ray photoelectron spectroscopy (XPS).

iv. Changes in Ca^{2+} concentration, pH, and the bioactivity of BGNPs with an immobilized foldamer

The foldamer BGNP B2 was immersed in artificial saliva. The Ca^{2+} ion concentration and pH were evaluated over time with a sensor. The samples were centrifuged, and the precipitates were collected, washed, and dried prior to characterization by XRD, SEM, and FTIR.

v. Enamel remineralization test

An enamel block (dimensions: 2 mm × 2 mm) was obtained from first premolars extracted for orthodontic purposes, imbibed in epoxy resin and demineralized by acid etching in orthophosphoric acid for 60 s. The specimens were rinsed and cleaned by sonication for 5 min in distilled water. The specimens were then treated in a foldamer solution (2 mg/mL) posteriorly and submerged in artificial saliva for 14 days. The mechanical properties of the specimens were assessed using a nanoindenter.

B. Experiment 2:

i. Design and synthesis of the crown ether-type molecule

Two ether ligands (L1 and L2) were conformed by an azobenzene chromophore bound to a chain of carbon groups with alkaloid groups. All ether ligands were synthesized in solution using the liquid method. 1H and 13C NMR, ATR-FTIR, and ultraviolet-visible (UV/Vis) spectroscopy were used for characterization. The isomerization of the heterogeneous material was evaluated by NMR after exposure to UV light (370 nm) and light from a blue light-emitting diode (LED; wavelength: 400–500 nm).

ii. Ether ligand immobilization on bioactive glass surface and isomerization of EL1 and EL2 immobilized on BGNPs

Immobilization was performed in toluene under heating. The nanoparticles were dried and characterized by SEM, EDS, XPS, and UV/Vis spectroscopy. The new materials were names as EL1 and EL2.

Isomerized EL1 and EL2 (2 mg/mL) were diluted in acetonitrile and irradiated with light to obtain the *Trans* and *Cis* conformations, as indicated in the UV/Vis spectra.

iii. Changes in Ca^{2+} concentration, pH, and the bioactivity behavior of EL1 and EL2

EL1 and EL2 were immersed in artificial saliva. Changes in the Ca^{2+} concentration and pH were evaluated over time using a sensor. All samples were centrifuged, and the precipitates were collected, washed, and dried for characterization by XRD, SEM, EDS, FTIR, and XPS.

iv. Switch on /off

EL1 and EL2 were immersed in artificial saliva. The solution was constantly irradiated with UV (370 nm) at different times (15 min and 30 min) to subsequently be change to LED (400-500nm). Changes in the Ca^{2+} concentration and pH were evaluated over time using a sensor. All samples were centrifuged, and the precipitates were collected, washed, and dried for characterization by XRD, SEM, EDS, FTIR, and XPS. Data analysis

v. Enamel remineralization

An enamel block (dimensions: 2 mm × 2 mm) was obtained from first premolars extracted for orthodontic purposes, imbibed in epoxy resin and demineralized by acid etching in orthophosphoric acid for 15 s. The specimens were rinsed and cleaned by sonication for 5 min in distilled water. The specimens were submerged in artificial saliva containing the different materials (BG, EL1 and EL2) for 7 days. The mechanical properties of the specimens were assessed using a nanoindenter.

vi. Statistical analysis

The obtained Ca^{2+} concentrations and enamel mechanical properties (nano-hardness and elastic modulus) were analyzed by SPSS software (ver. 26.0; IBM Corp., Armonk, NY, USA) to identify significant differences in Ca^{2+} concentration. The mechanical properties of the enamel were assessed by one-way analysis of variance (ANOVA).

Results and Discussion

A. Experiment 1:

1. Design and synthesis of foldamers

1H and 13C NMR spectra and FTIR measurements confirmed the structure of five foldamers with pyridine in different positions.

2. Synthesis and characterization of BGNPs

The BGNPs were analyzed by XRD, in which an amorphous phase was confirmed. The average particle size was 40 nm. EDS analysis was used to verify the BG composition (SiO_2:CaO:NaO_2:SrO:P_2O_5 = 46.1:19.3:27.0:5:2.6 mol%).

3. Surface functionalization and foldamer immobilization

Nitrogen was detected in EDS and XPS analyses, confirming surface functionalization through the detection of nitrogen binding energy. The morphological characterization by SEM revealed a plate-like structure, indicating foldamer immobilization on the surface. No significant changes were found in the XRD spectra of BGF and BG2 materials.

4. Changes in Ca^{2+} concentration, pH, and the *in-vitro* bioactivity of BG 2

Bioactivity was evaluated after immersion in artificial saliva. XRD results showed a peak at 32º, characteristic of an apatite-like formation. No significant changes in the BG2 or BGNP XRD spectra were found.

5. Enamel remineralization test

Foldamer 2 had the highest nano-hardness and elastic modulus values. A statistically significant difference was found from the control group. Under microscopic observation, the sample treated by foldamer 2 showed a smoother surface.

B. Experiment 2:

1. Design and synthesis of the crown ether-like molecule

The synthesis of the two ligand types was confirmed by the NMR spectra. The interconversion of the ligands was verified by NMR, thus establishing the reversibility of the

compounds.

2. Ether ligand immobilization and isomerization of L1 and L2 immobilized on BGNPs

The immobilization of L1 and L2 was confirmed by XPS, based on the nitrogen binding energy. The nitrogen peak intensity increased with the L concentration of the BGNPs. The new materials were named as EL1 and EL2. The amount of ether ligand immobilized on the surface of bioactive glass was 5mg, 10mg, and 30mg obtaining 6 types of materials (EL1-5, El2-5, EL1-10; EL2-10, EL1-30 and EL2-30).

The interconversion of EL1 and El2 immobilized on the BGNPs was evaluated by UV/Vis spectroscopy, with signals seen at 301 and 324 nm, respectively, similar to those observed for the heterogeneous material. No significant changes were observed in the FTIR or XRD measurements.

3. *In-vitro* bioactivity behavior of the modified nanoparticles

The Ca^{2+} concentration was lower for EL1 and EL2 than the BGNPs. XRD showed peaks related to the apatite layer in all samples evaluated.

4. Switch on/off

The Ca^{2+} concentration decreased when EL1-30 and EL2-30 were irradiated with UV light, and increased when irradiated with blue LED light, confirming isomerization of the material and the capture and release of Ca^{2+}. Thirty-minute activation provided better control over the Ca^{2+} concentration.

5. Enamel remineralization

The BGNPs had higher nano-hardness and elastic modulus values. EL2 showed a statistically significant difference from the results obtained for the etching and control groups.

Conclusions

A. Experiment 1:

The size of peptide has been described as one of the major factors to induce a proper enamel remineralization. In the current research the peptides were combine with foldamers to be able to have a ß-sheet formation but with a short chain of amino acid.

Surface modification and foldamer immobilization did not affect the bioactivity or buffering capacity of the BG.

Foldamer 2 has potential for enamel remineralization.

B. Experiment 2:

Controlling the bioactivity has gain attention in the medical area. The uses of light for this purpose has advantages such as reversibility, and the control of the application spatio-temporal. In this research the photosensitive materials were successfully synthetized and immobilized on the bioactive glass surface.

EL1 and EL2 can control the availability of Ca^{2+}, depending on its conformation. However, EL1 showed better enamel remineralization than EL2.

The results of the present investigation suggest that surface functionalization of BG is promising for the development of BG materials.