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Original article

Mineral formation on dentin induced by nano-bioactive glass

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ABSTRACT

The object of this study was to evaluate the effect of bioactive glass (BG) size on mineral formation on dentin surfaces. Totally demineralized dentin discs were treated using BG suspensions with different particle sizes: *i.e.*, microscale bioactive glass (*m*-BG), submicroscale bioactive glass (*sm*-BG) and nanoscale bioactive glass (*n*-BG). Field-emission scanning electron microscopy and 3D profile measurement laser microscopy were used to observe the surface morphology and roughness. It was found that all BG particles could promoted mineral formation on dentin surfaces, while plug-like depositions were observed on the dentin discs treated by *n*-BG and they were more acid-resistant. The present results may imply that *n*-BG has potential clinical application for dentin hypersensitivity treatment.

1. Introduction

Dental hypersensitivity (DH) occurs when dentin is exposed to various types of stimuli such as thermal, evaporative and tactile. It manifests as a short sharp pain without other forms of dental defect or pathology [1]. Occlusal wear, toothbrushing, dietary erosion, and gingival recession may all lead to this problem [2]. At present, two main methods are used in the treatment of DH: tubule occlusion [3] and nerve activity blockage [4]. The reduction of dentin permeability through tubule sealing is expected to be an effective way of DH treatment [5].

Bioactive glass (BG) is calcium phosphosilicate cement that has been proven to relief DH by yielding hydroxyapatite deposition on the dentin surface [6]. Early forms of BG were synthesized with a traditional melting method and their diameters were relatively large [7]. As regards DH treatment, Mitchell et al. stated that application of BG with an average diameter of 1–20 μm to a demineralized dentin surface could reduce the fluid conductance through the dentin tubules [8]. Furthermore, Vollenweider et al. compared nanoscale BG (n-BG) synthesized using flame spray with traditional 45S5 BG. Their research demonstrated that the n-BG released Ca^{2+} and Si^{2+} more quickly than its counterpart, which was beneficial for dentin mineralization [9]. However, the flame-spray-synthesized BG had a broad size distribution [9] and different chemical composition compared to the 45S5 [9,10], and thus the effect of chemical composition on mineral formation cannot be excluded. Recently, the sol–gel method has been widely applied in BG synthesis [11,12]. Compared with the melting method, the sol–gel method requires relatively lower temperatures of approximately 600–700 °C, or even lower when calcium methylethoxide is used as the calcium precursor [13]. Further, the sol–gel BG may have uniform composition, mesoporous surface, and higher specific surface area. In addition, the form, size, and dissolution rate of sol–gel BG may be easily controlled [14].

In recent years, Chen et al. have fabricated new *n*-BG [15] and submicroscale BG (*sm*-BG) [16] using sol–gel technique. In this study, the application of these newly developed BG particles in the mineral formation on dentin surface were explored and particular attention was paid to the effect of BG particle size.

2. Experiment

2.1. Materials

The composition of all BG particles used in this study were same: 58% SiO_2, 33% CaO, and 9% P_2O_5 (w/w) [15,17,18]. The following particle sizes were employed: microscale BG (*m*-BG) synthesized by acid-catalyzed sol–gel method: 2–20 μm (Fig. 1a) [17,19]; mono-dispersed sm-BG synthesized by alkali-catalyzed sol–gel method: approximately 500 nm (Fig. 1b) [18,20] and n-BG

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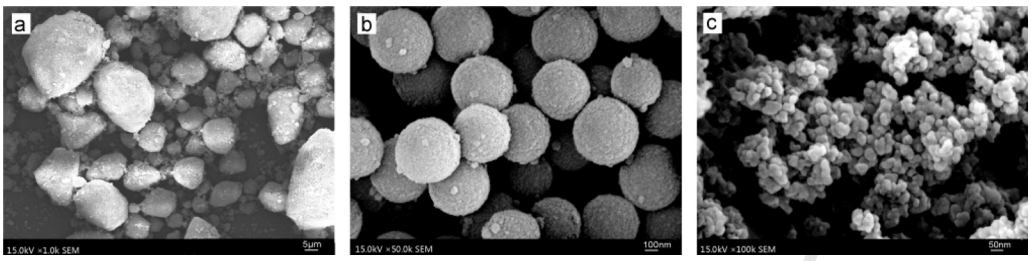

Fig. 1. Surface morphology of BG. Surface morphology of (a) *m*-BG, (b) *sm*-BG and (c) *n*-BG by FE-SEM analysis.

synthesized by acid-alkali-catalyzed sol–gel method: 20–30 nm (Fig. 1c) [15,17]. Both the sm-BG and *n*-BG particles were regularly spherical, whereas the m-BG particles were irregularly agglomerate. Our early studies [17,18] have reported the surface areas, total pore volumes and average mesopore sizes evaluated *via* N_2 adsorption experiments, the data was shown in Table 1. All the BG particles used in this study were prepared by the National Engineering Research Center for Human Tissue Restoration and Reconstruction, South China University of Technology.

2.2. Dentin disc preparation

In total, 18 intact human third molars were collected with ethical approval and informed donor consent, and were stored in distilled water containing 0.9% NaCl at 4 °C. 1-mm-thick dentin discs were created using a hard histotome (SP1600, Leica, Germany) to discard the enamel and pulp and then the dentin surfaces were then wet ground using SiC paper (600 grit) so as to increase their smoothness. The dentin discs were subsequently wiped to remove the surrounding enamel and divided into four parts using diamond burrs. The dentin discs were immersed in 17% ethylenediaminetetraacetic acid (EDTA) (pH 7.4) for 1 wk, so that completely demineralized discs were obtained. The BG particles were mixed with deionized water to form BG suspensions with a liquid/powder ratio of 0.1 mL/0.05 g.

The dentin discs were randomly divided into four groups labeled m-BG, sm-BG, n-BG, and CTR (control). Each group contained 18 discs. The m-BG, sm-BG, and n-BG discs were separately embrocated with the m-BG, sm-BG, and n-BG suspensions, respectively, using a brush for 20 s. Next, they were washed with deionized water for 20 s and then placed into artificial saliva (AS). The CTR discs were placed into the AS directly. The AS was composition of 1.5 mmol/L of $CaCl_2$, 50 mmol/L of KCl, 0.9 mmol/L of KH_2PO_4, and 20 mmol/L Tris, with a pH of 7.4 [21].

2.3. Evaluation of sealing ability

Field-emission scanning electron microscopy (FE-SEM; S4800, JEOL, Tokyo, Japan) was used to observe the surface morphology of the dentin discs. After one week, six discs were collected randomly from each group and rinsed with deionized water for 20 s. The discs were then observed using FE-SEM for the cross sectional and longitudinal sectional views. Prior to the microscopic observation, the discs were coated with gold (5 min, 50 mTorr). The cross sectional surface elemental compositions of the 1-wk discs were evaluated using an energy-dispersive X-ray spectroscope (EDS) attached to the FE-SEM. FE-FEM and Image-J software (Version 1.48, National Institutes of Health, USA) were used together to assess the sealing ratios of the dentin tubules. The Image-J software was used to observe six 2000× magnified FE-SEM images of the dentin disc surfaces from each group. Hence, the overall open dentin tubule area (*S*), the average area of a single dentin tubule (*s*), and the number of dentin tubules (*N*) were measured in each case. The sealing ratios were calculated as

$$\text{Sealing ratio} = \left(\frac{1-S}{Ns}\right)\times 100\%.$$

After two weeks, the remaining twelve discs were collected from each group and rinsed with deionized water for 20 s. Six discs were observed the morphological changes on the disc surfaces using FE-SEM.

Table 1
The surface areas, total pore volumes and average pore diameters of BG.

Sample	Surface area ($m^2 g^{-1}$)	Total pore volume ($cm^3 g^{-1}$)	Average pore diameter (nm)
m-BG	28.020 ± 0.1	0.113 ± 0.01	15.5042 ± 0.02
sm-BG	38.087 ± 0.1	0.382 ± 0.01	4.015 ± 0.03
n-BG	63.545 ± 0.2	0.230 ± 0.01	15.0104 ± 0.03

2.4. Study of acid resistance

The other six discs immersing in BG suspensions for two weeks were used to evaluate the roughness. A 3D profile measurement laser microscope (LM, VK-X200, Keyence, Japan) was used to measure the initial roughness (*Ra*) at two locations on each disk. The discs were then submerged in cola (pH 2.45) for 2 min and again rinsed with deionized water for 20 s. The roughness was measured for a second time (*Ra′*). After drying, the FE-SEM was used to observe the morphological changes on the disc surfaces.

2.5. Statistical analysis

The sealing ratio and *Ra* data were reported as mean ± standard deviation (SD) values. Comparisons of the dentin tubule sealing ratios of the 1-wk groups were conducted with *t*-tests. The dentin surface *Ra* and *Ra′* values of the four groups before and after the cola immersion, and the corresponding roughness variance values, were also compared using *t*-tests. Differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Dentin-tubule sealing

The FE-SEM analysis showed that, following immersion in 17% EDTA for one week, the dentin tubules of the initial demineralized discs were completely open (Fig. 2a and b). Moreover, the EDS spectra indicated that the surface elements present on the demineralized dentin discs were carbon, oxygen and gold (Fig. 2c). Therefore, calcium and phosphorus were absent; this demonstrated that the discs were completely demineralized.

As regards the 1-wk specimens treated with the three different BG suspensions, all the disc surfaces exhibited material and

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