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In situ pH within particle beds of bioactive glasses

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Abstract

The in vitro behavior of three bioactive glasses with seven particle size distributions was studied by measuring the in situ pH inside the particle beds for 48 h in simulated body fluid (SBF). After immersion, the surface of the particles was characterized with a field emission scanning electron microscope equipped with an energy-dispersive X-ray analyzer. In addition, the results were compared with the reactions of the same glasses formed as plates. A similar trend in pH as a function of immersion time was observed for all systems. However, the pH inside the particle beds was markedly higher than that in the bulk SBF of the plates. The pH decreased as power functions with increasing particle size, i.e. with decreasing surface area. The in vitro reactivity expressed as layer formation strongly depended on the particle size and glass composition. The average thickness of the total reaction layer decreased with the increase in sample surface area. Well-developed silica and calcium phosphate layers typically observed on glass plates could be detected only on some particles freely exposed to the solution. No distinct reaction layers were observed on the finest particles, possibly because the layers spread out on the large surface area. Differences in the properties of the bulk SBF and the solution inside the particle bed were negligible for particles larger than 800 μm. The results enhance our understanding of the in vitro reactions of bioactive glasses in various product forms and sizes.

Keywords: Bioactive glass; Particle size; In vitro test; In situ pH; Reaction layers

1. Introduction

Bioactive glasses, developed by Hench represent a group of glasses that can bond to bone by formation of an apatite layer. The formation of the bone-like layer occurs not only in the body, but also in vitro. On immersion of a bioactive glass in an aqueous solution, three general processes occur: leaching, dissolution and precipitation [1]. Fast leaching results in an increase in the pH of the surrounding solution. The pH change has been shown to vary depending on, for example, the glass composition, surface area to volume ratio and sample dosage and agitation rate of the system [2–7]. By studying the pH changes in the solution, a fast and simple determination of the in vitro behavior of the glass can be obtained. After partial leaching and dissolution, silica- and calcium phosphate-rich layers start to form on the glass surfaces. The ability of glass to form a calcium phosphate layer in vitro is often taken as a measure of its bioactivity in vivo. Banchet et al. [8,9] verified the in vitro formation of silica-rich and calcium phosphate layers on particles of powdered bioactive glasses (<40 μm) by elemental analysis at the submicrometer scale by scanning transmission spectroscopy associated with energy-dispersive X-ray spectroscopy. Karlsson et al. [10] reported that the solubility of hydroxyapatite depended on temperature, the concentrations of calcium and phosphate and pH. According to them, the silica gel was not stable when the pH increased to above 9.5. However, the stability of the calcium phosphate precipitation increased with pH [10]. According to Lu and Leng, the nucleation rate of calcium phosphate precipitation in simulated body fluid (SBF) was significantly affected by the pH value, resulting in a different crystallized structure of the precipitate [11]. A high pH environment was favorable for hydroxyapatite nucleation,

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and the hydroxyapatite nucleation rate approached that of octacalcium phosphate at pH 10 [11].

Many in vitro studies of bioactive glasses are carried out with monolithic samples, i.e. disks and plates [4,12–14]. However, various clinical applications also call for other shapes and sizes of bioactive glass products. Glass particulates greater than 100 μm in diameter, which can be used to fill in bone defects, have been investigated by Greenspan et al. and Peltola et al. [16,17]. Bioactive glasses with a porous structure have been studied by Ylänen et al. [18]. In addition, studies on bioactive glass powders with an average particle size significantly smaller than 100 μm have been carried out [19,20]. Fine powders of bioactive glasses have been used as a coating material on high-strength metal implants [21,22]. Fine powders of bioactive glasses in dental paste material have been found to show antibacterial properties for the prevention of infections [23,24]. The antibacterial properties of the powdered bioactive glasses on a wide selection of bacterial species were reported in our previous work [25,26].

The present work discusses the in vitro behavior of various particle size fractions of three bioactive glasses. The goal is to establish the influence of particle size on the in vitro properties of glasses in SBF. The results are relevant in understanding the influence of particle size on in vitro and in vivo reactions of bioactive glasses.

2. Materials and methods

Two well-known bioactive glasses, 45S5 and S53P4, both used in clinical applications, and one experimental bioactive glass, 23-04, were used in this study (Table 1). Glass 23-04 has been discussed in our previous reports [14,15]. The glasses were made from mixtures of analytical grade Na_2CO_3, K_2CO_3, MgO, $CaCO_3$, H_3BO_3, $CaHPO_4 \cdot 2H_2O$ and commercial grade Belgian quartz sand. Batches were melted in a Pt crucible at 1360 °C for 3 h. After casting and annealing, the glasses were crushed and remelted in order to improve homogeneity. Glass plates with dimensions of 2.00 × 1.50 × 0.15 cm were cut from the blocks by a low speed diamond saw. The glass blocks were crushed and sieved to give the following size fractions: <45, 45–90, 90–250, 250–315, 315–500, 500–800 and 800–1000 μm. SBF was prepared according to the protocol of Kokubo et al. [27]. The pH of the solution was adjusted to around 7.3 at 37 °C.

A Teflon container with a cylindrical bottom cavity was used as the container for the in situ pH measurements (Fig. 1). A microelectrode (Orion 9863BN) was inserted inside the cavity. The total tip length of the electrode was 137 mm and the needle length was 40 mm. The diameters of the upper part and the needle tip were 7.6 and 1.7 mm, respectively. About 1.5 g of glass particles was put into the cavity so that the detecting area of the electrode was totally covered. Then 15 ml SBF was carefully poured into the container to give an approximate sample concentration of 100 mg ml^{-1}. The pH in the powder bed was measured in situ at 37 °C for 48 h. The pH in the SBF after removing the particles was also measured at 48 h.

Table 1
Codes and chemical composition (wt.%) of the experimental glasses

Glass	Na_2O	K_2O	MgO	CaO	B_2O_3	P_2O_5	SiO_2
45S5	24.5	0	0	24.5	0	6	45
S53P4	23	0	0	20	0	4	53
23-04	5	11.25	4.5	20	2	1	56.25

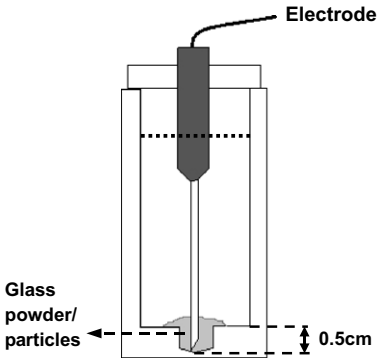

Fig. 1. Experimental scheme showing the in situ measurement for glass particles.

The plate samples were placed in a polystyrene container. About 17 ml of SBF was added to the container to give a ratio of surface area of particles to volume of SBF (SA/V) of 0.4 cm^{-1}, corresponding to an average concentration of 100 mg ml^{-1}. The plates were immersed for 4, 8, 24 and 72 h at 37 °C. The pH in the bulk solutions was measured at 37 °C with a normal glass electrode after removing the glass plates.

After immersion the samples were washed with ultrapure water and ethanol. The concentration of phosphorus ions in SBF after removing the particles was measured with an inductively coupled plasma-optical emission spectrometer (ICP-OES; Optima 5300 DV, Perkin Elmer) for glasses S53P4 and 23-04 at 2, 4, 27 and 48 h for the finest fraction. Particle samples at 48 h and plate samples at 72 h were analyzed by a field emission scanning electron microscope (LEO 1530) equipped with an energy-dispersive X-ray analyzer (EDXA; ThermoNoram). After embedding in resin, the samples were cut from the middle and the cross-sectional surfaces of the samples were observed.

3. Results

The pH of the SBF inside the particle beds and in the bulk solutions after immersing the plates are shown in Fig. 2. The maximum standard deviation of the pH measured was 0.1 pH unit. For both particles and plates, the pH values were highest for glass 45S5 and lowest for 23-04. The pH of the immersion solutions of the plates increased with time, but to a lower degree than for the particle systems. The pH inside the particle beds increased with

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