Factors Controlling Antibacterial Properties of Bioactive Glasses

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Abstract. Factors controlling the antibacterial ability of three bioactive glasses were studied by comparing the changes in the SBF during immersion of the glasses with their response to four microorganisms. After immersion of 100 mg/ml fine powder (<45μm) of the glasses in the SBF for 1, 2, 4, 8, 16, 27 and 48 hours, the immersion solutions were filtered and the pH in the bulk solution was measured. Ionic concentrations of Na, K, Ca, Mg, P and Si ions in the immersion solutions were determined by ICP-OES. The antibacterial activity of the glasses showed good correlation with the changes of the pH values in the SBF solutions. No correlation was found between the ionic concentration and the antibacterial ability of the three glasses against the four tested microorganisms. The results suggest that, the antimicrobial effect of the glass powder against the microorganisms tested is mostly dependent on the increase of the pH in the solution to values detrimental for the bacteria growth.

Introduction

Infection is the most common reason for implant failure in tissue engineering. Recently, some bioactive glasses have been found to show antibacterial effect when interacting with bacteria. Bioactive glass S53P4 has been reported not to favour adhesion and colonization of microorganism *Klebsiella ozaenae, in-vitro*. This observation was also supported by *in-vivo* findings showing no implant-associated infections or reinfections [1]. In another research the antibacterial activity of particulate 45S5 Bioglass® against supra- and sub-gingival bacteria was investigated [2]. The antibacterial property of the glass was supposed to derive from the changes in the composition of the body fluid due to dissolution of the glass, i.e. both the high pH and the change in the concentration of specific ions. In this work the mechanisms leading to the antimicrobial effect are discussed by comparing the changes in the SBF during immersion of selected bioactive glasses with the previously reported response of the same glasses to four microorganisms [3].

Materials and Methods

Two bioactive glasses known with the codes S53P4 and 13-93 and one novel bioactive glass were studied in this work. The chemical composition of the glasses is given in Table 1.

Table 1. Chemical composition of experimental glasses (wt %).

Glass	Na₂O	K₂O	MgO	CaO	B₂O₃	P₂O₅	SiO₂
S53P4	23	0	0	20	0	4	53
13-93	6	12	5	20	0	4	53
18-04	15	0	4.5	20	2	4	54.5

Fine glass powder (< 45 μm) was immersed in the SBF with a concentration of 100 mg/ml at 37 °C without stirring for 1, 2, 4, 8, 16, 27 and 48 hours. After each immersion time the powder was filtered with 0.2 μm filters and the concentration of Na, K, Ca, Mg, P and Si ions in the solution was measured with inductively coupled plasma-optical emission spectrometer (ICP-OES). The pH values in the bulk solutions were measured with a normal glass electrode. The *in-situ* pH in the interface of the glass powder and the solution was determined during 48 hours with a microelectrode which was inserted inside the sample of powdered glass. The results of the *in-situ* pH values and cultivating the same glasses with four microorganisms: *S. epidermidis, S. aureus, E. faecalis* and *S. mutans* have been reported previously [3].
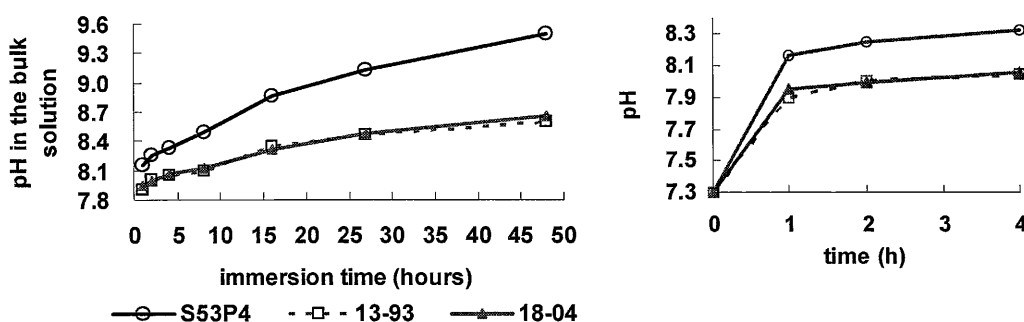
Figure 1. pH in the bulk solutions of the three glasses as a function of immersion time: left: during 48 hours; right: 0-4 hours.
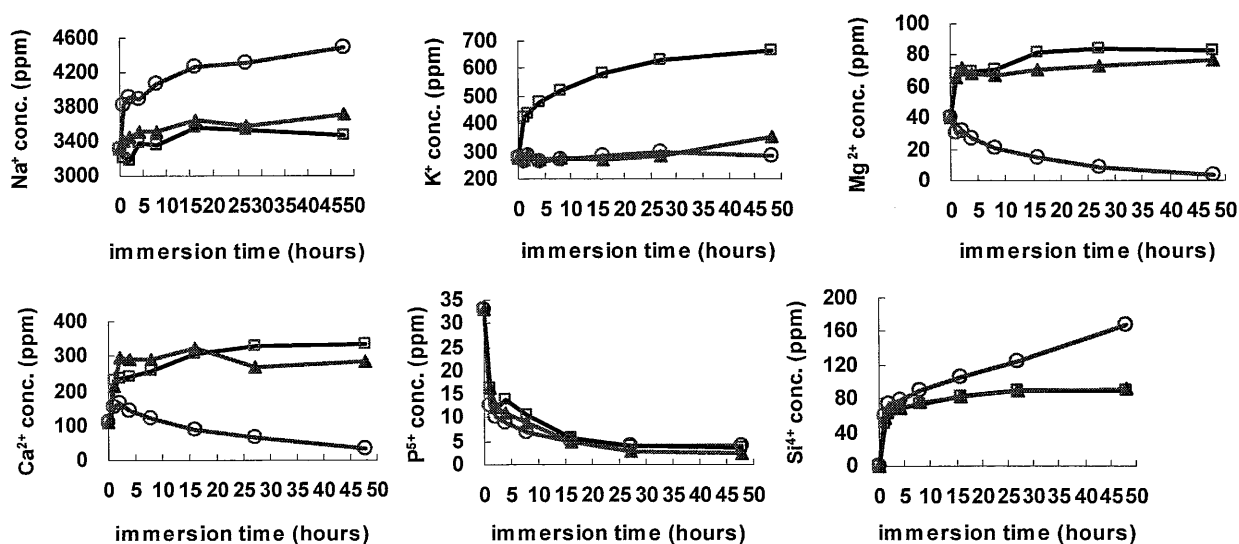
Figure 2. Ionic concentration in the bulk solutions after immersion in the SBF for the three glasses: —⊖— S53P4; —☐— 13-93; —▲— 18-04.

Results

All three glasses showed the same trend of pH change in their bulk immersion solutions, i.e. a rapid increase within the first two hours after which the increase of pH slowed down (Figure 1). As the

observed *in-situ* pH behaviour in the glass-solution interface [3], the pH in the bulk solution also showed much higher value (9.5) for Glass S53P4 than for the other two glasses. Though Glass 13-93 and 18-04 showed clear difference in the interface pH of glass powder and solution, the pH in their bulk solutions gave very similar values.

The ion concentrations in the bulk solutions of the three glasses showed very different values depending on the ion species, immersion time and glass composition (Figure 2). As the in-situ pH-increased, a fast change in the ionic concentration was also found within the first two hours of immersion. The concentration of Na, P and Mg ions in the solutions strongly correlated with the original oxide content in the glass. Though the three glasses contain the same content of CaO, the calcium ion concentration in their immersion solutions showed very different values. In the solutions of all the three glasses, the P-concentration decreased and the Si-concentration increased with immersion time. Detailed information of the ionic changes in the solution is given in Table 2. In the same table is also presented the antibacterial effects of the three glasses against four microorganisms [3]. After cultivation, good growth (+++) of the bacteria was found only for the control condition which was carried out without adding bioactive glass powder.

Table 2. Observations (ICP-OES) of the ionic concentration in the bulk solutions of the powders during immersion in the SBF, and antibacterial activity of the glasses after 1 to 6 days cultivation with four microorganisms: +++ good growth; ++ medium growth; + weak growth; - no growth.

Ions	S53P4	13-93	18-04
Na^+	Highest. Very fast increase during the first hour, then slow increase.	Lowest. Slight decrease within the 2 first hours, then increase to the initial value.	Higher value than for 13-93. Slow increase with a somewhat higher increase rate during the four first hours.
K^+	Almost constant.	Sharp increase during the first hour, then slow increase to a two-fold value.	Almost constant.
Mg^{2+}	Slow decrease to near zero.	Very sharp increase in one hour to 1.5-fold of the initial concentration, then slow increase to 2-fold at 15 hours.	Very sharp increase in the first hour to a value 1.5-fold of the initial conc., then almost constant.
Ca^{2+}	Increase during the first two hours, then slow decrease.	Fast increase in two hours to 2-fold of the initial conc., then slight increase.	Fast increase in the first two hours to 3-fold of the initial conc., then decrease to 2-fold at 16 hours.
P^{5+}	Similar trend and values for all glasses: very sharp decrease within two hours to about 1/3 ($\sim$ 10 ppm) of the initial concentration, and then slow decrease to less than 5 ppm.		
Si^{4+}	Fast increase in two hours from 0 to 80 ppm, then increase to 160 ppm.	Similar trend and values: fast increase in two hours from 0 to 80 ppm, then keep constant.	

Time	1d	2d	3d	4d	5d	6d	1d	2d	3d	4d	5d	6d	1d	2d	3d	4d	5d	6d
S. epidermidis	+	-	-	-	-	-	++	++	++	+	+	+	+	+	+	+	+	-
E. faecalis	-	-	-	-	-	-	++	++	++	+	+	+	++	++	++	++	++	+
S. aureus	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	+	-	-
S. mutans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Discussion

For the glass powders tested the fastest dissolution rates were observed during the two first hours of immersion. The first two hours were critical for the fast dissolution and reaction of the glasses powder in-vitro.

Glass S53P4 showed higher dissolution than the other two glasses. Glass 13-93 and 18-04 gave very similar pH values in their bulk solutions, but the pH in the glass-solution interface of Glass 18-04 was much higher than that of Glass 13-93. This could be due to the difference in the ions (species and concentrations) releasing from the glasses in the interface of the two glass systems. In the beginning stage, more alkali ions were released in the glass-solution interface of Glass 18-04 than Glass 13-93, thus leading to a higher pH in the interface of the former glass.

The ion concentrations suggest that the pH was controlled by the dissolved amount of alkaline and alkaline earth ions in the solutions. The concentrations of Na, P and Mg ions in the solutions strongly correlated with the original oxide content in the glasses. The observed concentration decrease of P and Ca ions indicates their precipitation, and thus to formation of a CaP-layer on the glass powder surface. The large increase in the Si^{4+}-concentration and the decrease in the Ca^{2+}-concentration in the solution of Glass S53P4 indicate that the glass powder was rapidly dissolved in the SBF. However, no correlation was found between the ionic concentration and the antibacterial ability of the glass powders tested. This could be due to either that the concentration of the ions did not reach a detrimental value needed to prevent the growth of the tested microorganisms, or that the influence of the ions was concealed by the strong pH-increase.

The antibacterial activity of the glasses showed a good correlation with the change of the pH values in the SBF solutions, i.e. Glass S53P4 with the highest pH gave the strongest antimicrobial effect. The other two glasses with lower but very close pH values showed weaker and similar antibacterial effect. Besides the pH of the solution, the ionic concentration, especially the high concentration of Ca ion, has been supposed to be essential for the antimicrobial effect of the glass. However, glass S53P4 with the highest ability to prevent growth of microorganisms showed the lowest Ca^{2+}-concentration in the SBF.

Conclusion

The antimicrobial effect of the experimental glasses was found to depend primarily on the increase of the pH in the solution to values detrimental for the bacteria growth. Whether the concentration of some specific ions in the solution is favourable for the growth of some microorganism in the powdered system cannot be deduced from the results obtained in this study.

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