Calcium Phosphates Formation On Bioactive Glasses In HEPES Solutions

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Introduction *In vitro* calcium phosphates formation on bioactive ceramics is often studied in a simulated body fluid (SBF) [1]. SBF is buffered at body pH using 50mM Tris and 45mM HCl. Previous studies have shown that the *in vitro* apatite formation ability of A/W glass ceramics in SBF can be correlated to their *in vivo* bone bonding ability [1]. Because SBF has higher chlorine and lower bicarbonate concentrations than those in human blood plasma, recipes for revised SBF (r-SBF) were proposed recently [2]. HEPES/NaOH was the buffering system in the new recipes [2]. However, independent effects of HEPES solutions on the soaking behavior of bioactive ceramics/glasses (BGs) have not been studied.

In this study, a series of comparison experiments on different soaking periods in three HEPES solutions were conducted. The structural and compositional variations of BG particles soaked in HEPES solutions with different buffering capacities were investigated.

Materials and Methods BG particles with a composition of 4.6MgO, 44.7CaO, 34.0SiO_2, 16.2P_2O_5 and 0.5CaF_2 (wt%) were purchased from Specialty Glass, Inc. The particles were separated using sieves to achieve a particle size between 600 and 1000 microns. The BET surface area of the BG particles is 0.0368 m^2/g (as measured by a Gas Sorptometer, Micromeritics Inc.). N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic or 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, $C_8H_{18}N_2O_4S$), hemisodium HEPES (0.5Na-HEPES, $C_8H_{18}N_2O_4SNa_{0.5}$) and sodium-HEPES (Na-HEPES, $C_8H_{18}N_2O_4SNa$) powders were purchased from Sigma Inc.

HEPES, 0.5Na-HEPES and Na-HEPES solutions (all 0.1 M) were prepared by dissolving the corresponding powders in deionized water. A/W BG particles were immersed at a soaking ratio (i.e., amount of BG particles/volume of soaking solution) of 0.01g/mL in polypropylene bottles maintained at 37˚C. X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy (Diffusive Reflectance mode, DRIFTS), scanning electron microscopy (SEM), and transmission electron microscopy (TEM) were used to characterize specimens after soaking for different time periods (0, 1 d, 3 d, 7 d and 14 d). The concentrations of Si, P, and Ca in the solutions both before and after soaking were analyzed using an inductively coupled plasma (ICP) atomic emission spectroscopy.

Results and Discussion After soaking in Na-HEPES for 14 d, the pH of the solution was relatively stable (before soaking: 10.34 and after 14d: 10.42). SEM showed that cracks appeared on A/W BG particles. However, both XRD and FTIR results did not show the formation of new materials on the surface of the BG particles.

After soaking in 0.5Na-HEPES, pH of the solution changed from 7.45 (before soaking) to 7.62 (14 d). SEM showed that new materials with needle-like structures started to develop on the BG surface after 3 d. These new materials were revealed to be apatitic materials with XRD. The electron diffraction (ED) pattern of the 14 d sample indicated that the new materials have a similar structure to whitlokite (β-tricalcium phosphate).

The pH of the HEPES solution changed from 4.45 (before soaking) to 6.29 (after 14 d). SEM showed that new materials with needle-like structures started to develop on the BG surface after 1 d. These new materials grew into plate-like materials after 14 d (see Figure 1). The ED pattern of the 14 d sample indicated that the new materials have similar structure to octa-calcium phosphate (OCP).

Results of this study are related to reactions at glass-solution interface (e.g., glass dissolution, supersaturation and ion re-precipitation) at different pH ranges (acidic, neutral and basic).

Summary HEPES solutions interacted with BG. Crystalline calcium phosphates developed on BG surface in acidic and neutral HEPES solutions. OCP developed on the BG particles after soaking for two weeks in the acidic HEPES solution.

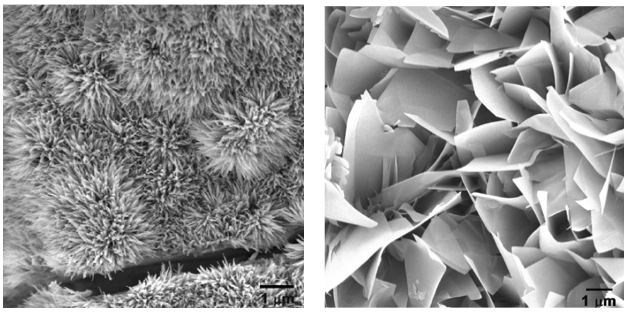

Figure 1. SEM images of the new materials developed on BG after soaking in HEPES for 7 (left) and 14 d (right) (scale bar: 1 µm).

References

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