

## Reactions and Surface Transformations of a Bone-Bioactive Material in a Simulated Microgravity Environment

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03-11-11

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A comprehensive program to investigate the expeditious *in vitro* formation of three-dimensional bone-like tissue is currently underway at the University of Pennsylvania. The study reported here forms a part of that program. Three-dimensional bone-like tissue structures may be grown under the simulated microgravity conditions of NASA designed Rotating Wall Bioreactor Vessels (RWV's). Such tissue growth will have wide clinical applications. In addition, an understanding of the fundamental changes that occur to bone cells under simulated microgravity would yield important information that will help in preventing or minimizing astronaut bone loss, a major health issue with travel or stay in space over long periods of time.<sup>[1]</sup> The growth of three-dimensional bone-like tissue structures in RWV's is facilitated by the use of microcarriers which provide structural support. If the microcarrier material additionally promotes bone cell growth, then it is particularly advantageous to employ such microcarriers.

We have found that reactive, bone-bioactive glass (BBG) is an attractive candidate for use as microcarrier material.<sup>[2]</sup> Specifically, it has been found that BBG containing Ca- and P- oxides upregulates osteoprogenitor cells to osteoblasts.<sup>[2]</sup> This effect on cells is preceded by BBG reactions in solution which result in the formation of a Ca-P surface layer. This surface further transforms to a bone-like mineral (i.e., carbonated crystalline hydroxyapatite (c-HA)). At normal gravity, time-dependent, immersion-induced BBG reactions and transformations are greatly affected both by variations in the composition of the milieu in which the glass is immersed and on the immersion conditions.<sup>[3]</sup> However, the nature of BBG reactions and phase transformations under the simulated microgravity conditions of RWV's are unknown, and must be understood in order to successfully use BBG as microcarrier material in RWV's. In this paper, we report some of our recent findings in this regard using experimental and numerical methods.

BBG composition 45S5, the most reactive among known bone-bioactive glasses,<sup>[4]</sup> was chosen for the study. BBG 45S5 behavior in physiological solutions was tested in simulated microgravity and compared with that at normal gravity. On the basis of our numerical study,<sup>[5]</sup> we have chosen the BBG granule size to be in the range 40-70  $\mu\text{m}$ , and a RWV rotational speed of 10 rpm. Our numerical study has shown that these parameters enable the microcarrier to remain suspended in the medium without experiencing collisions with the wall of the vessel. Immersion-induced changes in the solution composition and the material surface were analyzed after immersion.

### **Materials and Methods**

Immersion experiments were conducted in tris(hydroxymethyl)aminomethane buffered solution (pH 7.4 at 37 °C) both in the simulated microgravity environment of a High Aspect Ratio Vessel

(HARV-50 ml, Synthecon, Houston, Texas) and in normal gravity. Melt-derived bone-bioactive glass (BBG) 45S5 (W, %: 45.3 % SiO<sub>2</sub>, 23.9 % CaO, 24.8 % Na<sub>2</sub>O and 6.2 % P<sub>2</sub>O<sub>5</sub>) granules (MO-SCI Co., Rolla MO) were immersed at 1 mg/ml weight-to-solution volume (W/V) ratio for 1, 3, 6, 10 and 24 hours.

Prior to studying the immersion-induced reactions of BBG under the simulated microgravity conditions, we conducted an experiment with gold coated BBG particles in order to observe the trajectories of the particles in the HARV at 10 rpm and the 1 mg/ml W/V ratio. The particles were sputter coated with a thin (about 200 Å) layer of gold to make them visible in solution. This experiment with gold-coated particles confirmed that most of the particles, in an inertial frame, circled around the center of the rotating chamber during immersion up to 24 hours. Particles employed for the actual immersion study were without gold coating.

After immersion, the solutions were analyzed for changes in the Si, Ca and P-concentrations using atomic absorption spectroscopy (5100, Perkin-Elmer, Norwalk CT) and colorimetry (Ultraspec Plus Spectrophotometer, Pharmacia LKB, Piscataway NJ). The BBG surface was analyzed prior to and after immersion using Fourier transform infrared spectroscopy (FTIR, 5DXC, Nicolet, Madison WI).

## **Results and Discussion**

Figures 1 a-c show changes in the P, Ca- and Si-concentrations as a function of immersion time of BBG granules in tris buffered solution both under simulated microgravity (HARV) and unit gravity conditions. The data indicate that the following reactions have occurred in both environments: an initial leaching of all ions, subsequent uptake of P-ions and solution saturation with Si-ions. Although the sequence of these reactions was similar under both conditions, the kinetics were different as indicated by a remarkable increase in the rate and amount of both release and uptake under simulated microgravity conditions. Specifically, whereas the amounts of P- and Ca-ions released during the initial stage (prior to uptake) under unit gravity were 10 ppm (0.32 mM) and 80 ppm (2 mM) respectively, a two-fold increase to 180 ppm Ca (4.5 mM) and 70 % increase to 17 ppm P (0.55 mM) were observed under simulated microgravity. These observations suggest that the enhanced release of Ca- and P-ions in the HARV environment led to a dramatic rise in solution supersaturation with regard to Ca-P phases. The data also showed that the time to detectable P-uptake, indicative of precipitation of Ca-P phases, was reduced from 3 hours under unit gravity to 1 hour under simulated microgravity. Moreover, the amount of P-uptake, i.e. the difference between the highest and the lowest concentrations measured prior to uptake and at 24 hours respectively, was increased from 8.7 ppm at unit gravity to 14.4 ppm at simulated microgravity suggesting a significant increase of the amount of Ca-P precipitates. The HARV environment also affected the pattern of Ca-uptake; it was observed after 1 hour immersion in HARV, but was not detected under unit gravity. Concerning Si-release, the saturation concentration of 65 ppm was reached after 3 hours in the HARV environment in comparison to 6 hours in normal gravity.

Chemical and structural changes of the BBG surface resulting from the immersion-induced reactions are indicated by the FTIR spectra shown in Figure 2. The FTIR spectrum of unreacted (before immersion) particles shows absorption bands characteristic of silica network of unreacted 45S5 glass.<sup>[4]</sup> Bands assigned to P-O and C-O vibrations were present in the spectra after 6 hours of immersion under both unit gravity and simulated microgravity conditions. The appearance of these bands suggests the formation of a carbonated Ca-P phase on the glass surface. Splitting of the lower energy P-O band indicates that the phase was crystalline and as such is carbonated hydroxyapatite (c-HA). The analysis also points out that, although the formation of the c-HA layer on the BBG surface occurred under both conditions, the layer formed under simulated microgravity was significantly thicker than that formed under unit gravity. This was indicated by a greater P-O/Si-O intensity ratio in the lower energy region of the spectrum of BBG after immersion in the HARV environment compared to that in unit gravity.

The combined solution and surface analyses suggest that the following events occurred upon immersion of BBG granules under both testing conditions: dissolution of BBG (indicated by the release of Si- Ca- and P-ions); saturation of solution with Si-ions and supersaturation of originally Ca-P-free solution with these ions followed by precipitation of Ca-P phases (indicated by P- and Ca-uptake); growth of Ca-P precipitates with immersion (suggested by continuous uptake). The analysis also revealed that, although the sequence of the events was similar under both conditions, the kinetics were significantly affected by the simulated microgravity environment, i.e. both dissolution and precipitation reactions were enhanced. In addition, the surface analysis (FTIR) pointed out that a layer (~ 1 µm thick) of bone-like apatite, i.e. carbonated crystalline hydroxyapatite (c-HA) was formed on glass granules after 6 hours of immersion in both conditions; however, this surface layer was significantly thicker on granules which reacted under simulated microgravity in comparison to those in unit gravity. This finding suggests that crystallization of Ca-P precipitates, essential for the bone-bioactive behavior of BBG, was also enhanced by the simulated microgravity conditions.

## Conclusions

The study has demonstrated that the simulated microgravity environment remarkably enhances the kinetics of BBG reactions that are associated with bone-bioactive behavior.

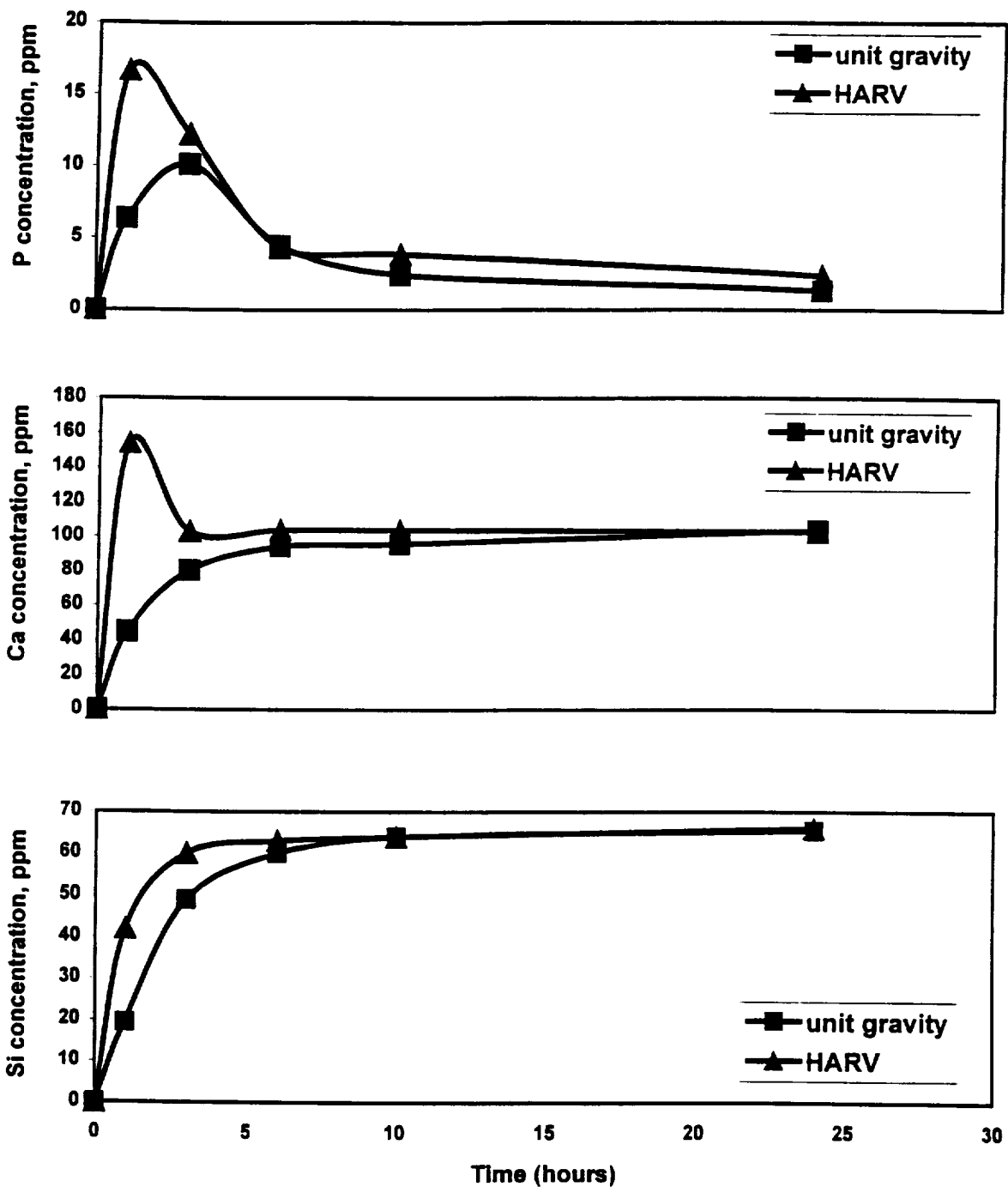
Our ongoing study on the effect of simulated microgravity on the BBG reactions, associated with bone-bioactivity, includes testing BBG in solutions closely mimicking the electrolyte and organic content of plasma, as well as expanding the number of measurements in order to produce statistically significant data in all solutions.

## References

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## Acknowledgment

This work was supported by NASA-NRA grant NAG 8-1483.



Figures 1 a-c. Changes in the concentrations of P-, Ca- and Si-ions as a function of immersion time in tris buffered solution (pH 7.4 at 37°C) under simulated microgravity (HARV) and unit gravity conditions. A remarkable increase in the rate and the amount of initial release of all ions (dissolution) and the following uptake of P- and Ca-ions (precipitation) was observed in the simulated microgravity environment of the HARV.

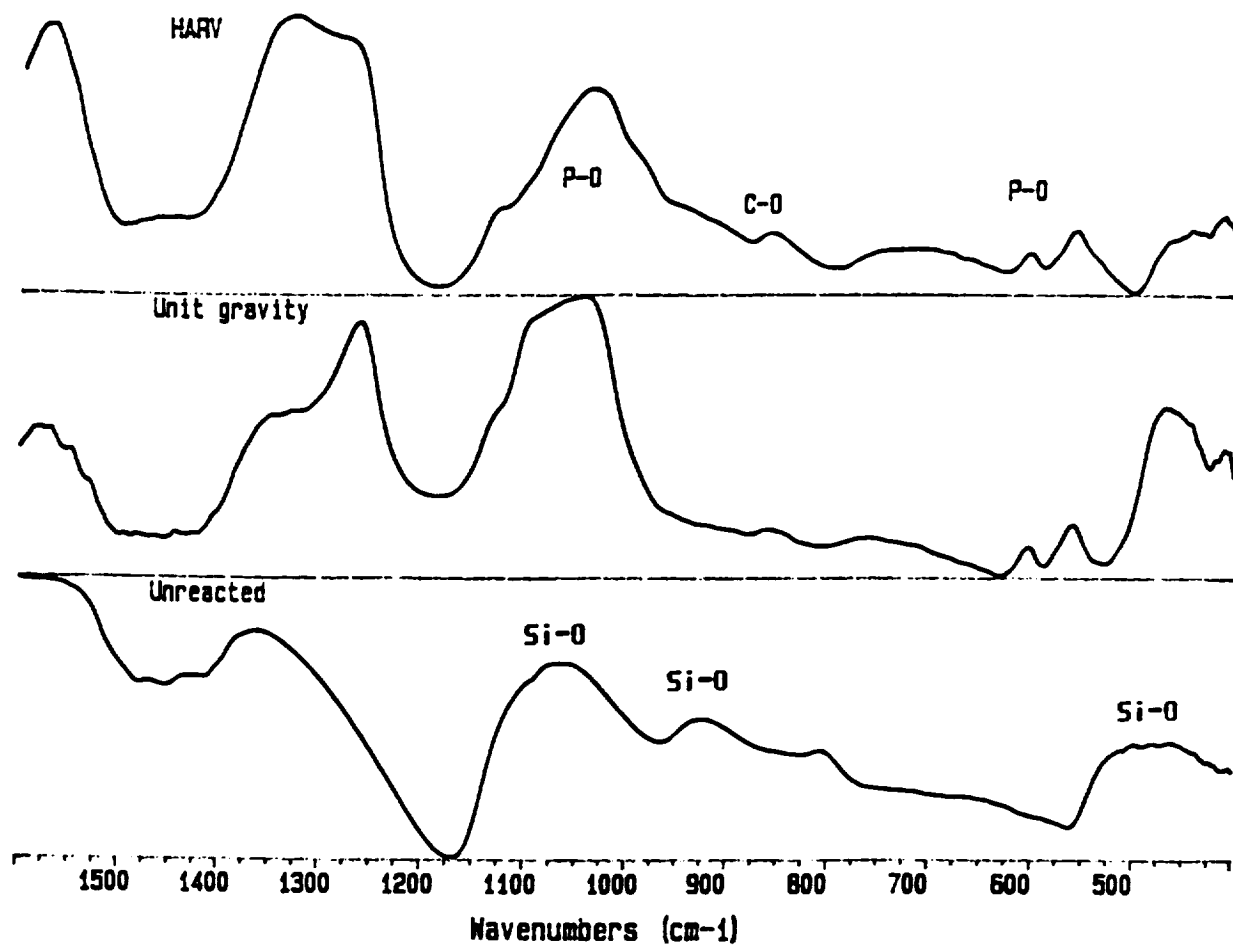


Figure 2. FTIR spectra of BBG granules before immersion (unreacted) and after 6 hours of immersion in tris buffered solution under simulated microgravity (HARV) and unit gravity conditions. The appearance of P-O and C-O bands (which were not present before immersion) on both spectra of reacted granules indicates formation of a layer of crystalline carbonated hydroxyapatite (c-HA), bone-like apatite, on the glass surface. However, the apatite layer formed under simulated microgravity was significantly thicker than that formed under unit gravity (as suggested by a greater ratio of the P-O/Si-O intensity in the lower energy region).

