Serum Proteins Limit the Kinetics of Surface Reactions in Novel Porous Bioactive Nanocomposites

Gautam Gupta, Ahmed El-Ghannam
Center for Biomedical Engineering, University of Kentucky, Lexington, KY, USA. 40506

Introduction: The presence of protein in physiological solutions affects the dissolution kinetics of biomaterials. However, data in the literature shows contradiction regarding the role of protein in the dissolution-precipitation reactions occurring at the interface between the tissue fluids and bioactive ceramics. Recently, a novel porous, resorbable, bioactive and mechanically compatible silica-calcium phosphate nanocomposite (SCPC) has been proposed as a tissue engineering scaffold for bone regeneration in orthopedic and maxillofacial surgeries. In this study, we examined the effect of serum protein concentration on the dissolution kinetics and surface transformations of SCPC.

Methods: Three SCPC compositions with Si/CaP ratios 1:10 (SCPC10), 30:70 (SCPC30) and 50:50 (SCPC50) were prepared as previously reported [1]. To synthesize porous templates, SCPC particles (45-150µm) were mixed with 40 % polyethylene glycol (PEG) of particle size range (90-425µm) and compressed uniaxially at 150 MPa into discs (12 mm dia. x 1.5 mm). The samples were then subjected to a two-step thermal treatment at 350°C/24h to remove the porogen (PEG), and then at 800°C/1h for sintering. The discs were incubated in tissue culture media containing different concentrations of serum proteins (P-0: 0%, P-10: 10% and P-30: 30 %). for 0.5, 1, 3, 5, 7, 9, 14, 21 and 28 days at 37°C. The medium was completely refreshed after every time point. The ionic concentration (Ca, P, Si and Na) of the solutions was analyzed by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). All concentrations were normalized to the unit surface area of the SCPC samples. Changes in the surface chemistry of SCPC post-immersion were analyzed by Fourier transformed infrared spectroscopy (FTIR). For all experiments, five replicates of each sample were used and data was analyzed using ANOVA with unequal variance; statistical significance was at $p < 0.05$.

Results: The Ca^{+2} concentrations of P-0 and P-10 incubated with SCPC samples decreased significantly at all incubation times. On the other hand, when SCPC samples were incubated in P-30, there was a net increase in Ca^{+2} concentrations of the solutions (Fig.1). Si-rich SCPC50 incubated in P-10 exhibited significantly greater ($p<0.005$) Ca-uptake compared to SCPC10 or SCPC30. SCPC50 incubated in P-0 exhibited a Ca-uptake of 90 ppm up to 3d, which was significantly higher than that by SCPC30 (76 ppm) or SCPC10 (72 ppm). However, after 3d, the Ca-uptake by the three SCPC samples in P-0 was not significantly different. In conjunction with Ca-uptake, the P-uptake by SCPC samples decreased markedly as the protein concentration of the solutions was increased. Moreover, SCPC10 or SCPC30 exhibited significantly higher P-uptake compared to SCPC50 in all solutions. All SCPC samples showed a sustained Si release over the 28d period, with Si-rich SCPC50 and SCPC30 exhibiting significantly higher Si release compared to SCPC10. Between 4-28 d, the Si release for SCPC30 incubated in P-0 was significantly higher than that incubated in P-10 or P-30. However, for SCPC10 and SCPC50, the Si release did not change significantly as the protein concentration of TCM was varied. FTIR analysis revealed that all SCPC samples exhibited formation of a hydroxyapatite surface layer, as indicated by the twin orthophosphate (570 and 600 cm^{-1}) and carbonate (870 cm^{-1}) peaks (Fig. 2). However, as the concentration of protein in the solution increased, the intensity of these signals decreased.

Discussion: Ca-uptake by the ceramic was reduced significantly as the concentration of serum proteins increased. This can be attributed to the protein molecules chelating the Ca^{+2} in the solution. As a result, the precipitation of the biological apatite layer on the ceramic surface is inhibited. It is also possible that the adsorbed protein layer would inhibit the interaction between the ceramic and the physiologic solution. The Si-rich SCPC50 and SCPC30 exhibited significantly higher Si release compared to SCPC10, which correlated well to the higher surface area of SCPC50 (0.84 m^2/g) and SCPC30 (0.56 m^2/g) as compared to SCPC10 (0.12 m^2/g).

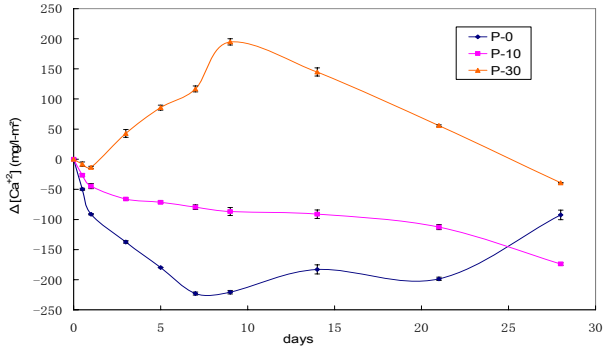

Fig.1. Change in the Ca^{+2} conc. (normalized to unit surface area) of TCM after incubation with SCPC30.

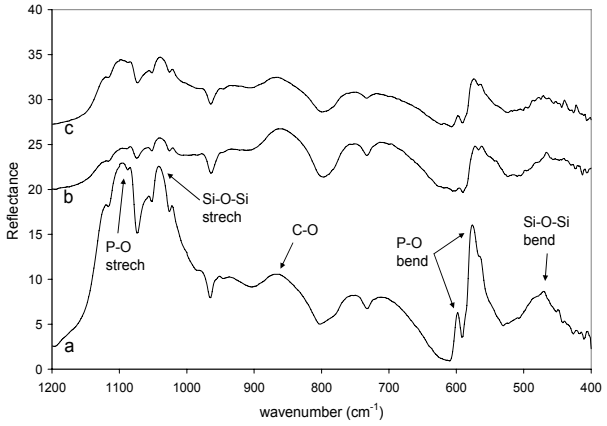

Fig.2. FTIR spectra of SCPC50 incubated in (a) P-0; (b) P-10 and (c) P-30 for 12 h.

References: 1. El-Ghannam A; JMBR 2004; 69(3):490-501.

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