Matrix hydrophobicity affects templated mineralization of hydrogel scaffold materials

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Statement of Purpose: We incorporated acryloyl modified amino acids with varying numbers of methylene groups into hydrogels to produce pendent side chains of varying length with identical terminal carboxyl groups, thereby allowing for studying the effect of changes in surface hydrophobicity (as determined by pendant side chain length) on *in vitro* mineralization of anionic hydrogels, independent of surface functionality. We observed an increase in mineralization with an increase in number of methylene groups in the pendent side chains of up to 5 groups; a further increase in side chain length showed a decrease in mineralization, confirmed by calcium content. We also confirmed the ability of the mineralized hydrogel surfaces to support adhesion and proliferation of human mesenchymal stem cells (hMSCs).

Methods: N-acryloyl 2-glycine (A2AGA), N-acryloyl 4-aminobutyric acid (A4ABA), N-acryloyl 6-aminocaproic acid (A6ACA) and N-acryloyl 8-aminocaprylic acid (A8ACA) were synthesized as described by Badiger et al[1]. 1M solutions of each of these co-monomers in 1 M NaOH were cross-linked with poly(ethylene glycol) diacrylate (PEGDA-6K, MW: 6000 Da) using ammonium persulfate as initiator and tetramethylene diamine as accelerator. After soaking in DI water for 36 hours followed by drying, these gels were re-swollen in simulated body fluid (SBF), prepared as specified by Oyane et al[2] and immersed in a solution of 40 mM $CaCl_2$ and 24 mM K_2HPO_4 for 30 min. Following the soaking, the gels were replaced in simulated body fluid for four hours. This procedure was repeated twice. Gels were also immersed in simulated body fluid, supplemented with 10% fetal bovine serum (fSBF), with daily solution exchange to ensure a continuous supply of ions. Gels were lyophilized and analyzed with SEM-EDS and calcium assay kit (Pointe Scientific) to study the microscopic morphology, composition and calcium content of the mineralized phase respectively. PEGDA 6K/A6ACA hydrogels mineralized by both of the above methods were also seeded with P5 hMSCs at a density of 5,000 cells/cm^2 and cultured in growth medium to evaluate cellular response.

Results: Gels immersed in 40 mM Ca^{2+}/25 mM PO_4^{3-} showed formation of a white mineral phase upon soaking in the solution. An increase in calcium binding was observed with increase in length of the pendant side chain; maximum binding was observed for A6ACA with a decrease in binding for A8ACA (Figure 1). Upon analysis with SEM-EDS, hydrogels mineralized in this manner showed the presence of spherulites, ranging from approximately 1-10 µm in diameter. The calcium phosphate ratio (Ca/P ratio) for these particles was 1.38, 1.39, 1.61 and 1.58 for A2ACA, A4ABA, A6ACA and A8ACA respectively.

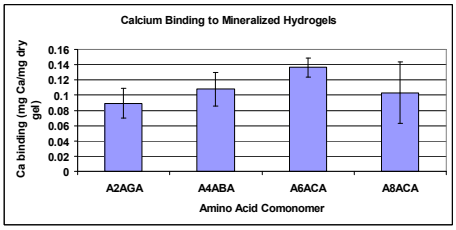

Figure 1: Calcium binding to hydrogels immersed in 40 mM Ca^{2+}/25 mM PO_4^{3-}

Gels immersed in fSBF showed formation of a white opaque mineral layer within 48 hours of immersion. Interestingly, a similar but much more pronounced trend was observed, with PEGDA/A6ACA showing maximum calcium binding again. PEGDA/A8ACA on the other hand, showed the lowest binding out of all the co-monomers tested. (Figure 2). Upon analysis with SEM-EDS, the opaque mineral layer was seen to consist of aggregations of spherulites approximately 0.2-1 µm in diameter. Elemental analysis with SEM-EDS yielded calcium/phosphate ratios of 1.72, 1.66 and 1.58 for A2AGA, A4ABA and A6ACA, respectively. A8ACA did not show detectable calcium-phosphate deposits in SEM-EDS study. Thus, the effect of proteins on extent of mineralization was found to be quite significant.

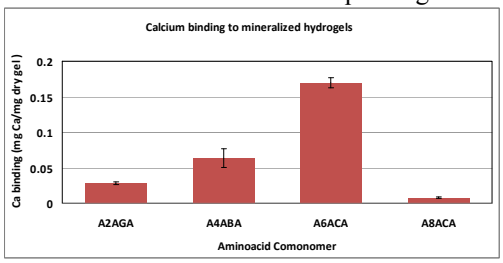

Figure 2: Calcium binding to hydrogels immersed in simulated body fluid with 10% fetal bovine serum

Immunofluorescent actin staining of monolayer-cultured hMSCs on mineralized A6ACA/PEGDA 6K hydrogels at 4 days, 8 days and 14 days showed that the mineralized A6ACA/PEGDA 6K hydrogels supported adhesion and proliferation of human mesenchymal stem cells.

Conclusions: Templated mineral formation on hydrogels with identical surface functionality was found to vary with surface energy. Beyond a side chain length of five alkyl groups, a decrease in binding was seen, possibly due to the inward collapse of the low solubility side chains of A8ACA in aqueous medium (as determined in a previous study). This study thus demonstrates the importance of surface hydrophobicity in addition to surface functionality for the design of composite polymer/mineral materials to be used for bone regeneration and as cell scaffolds.

References: 1. Badiger M. J Chem Phys. 1998;109 (3): 1175-1184
2. Oyane A. J Biomed Mat Res A. 2003; 65A (2):188-195