Biomineralization of A Peptide Hydrogel Scaffold Functionalized with Hydroxyapatite-Binding Peptide

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Introduction: A major ongoing challenge in the field of reconstructive and regenerative medicine is successful repair or replacement of hard tissue, which has been lost due to disease, congenital defects or trauma. To overcome the inadequacies of current tissue repair and reconstruction strategies, tissue engineering approaches have been developed with focus on utilizing synthetic tissue scaffolds to replace or regenerate the lost tissue. Most of the currently investigated scaffolds serve only as a physical support for the cells and lack the cell signaling or mineral modulating properties of the native extracellular matrix (ECM) in the hard tissues. Therefore, scaffolds with the ability to influence and guide the structural properties of the newly formed inorganic mineral in a similar fashion with the native ECM remains as a major challenge. We have developed an *in situ* forming scaffold sequence with a high, inherent propensity for calcium phosphate mineralization by conjugating a self assembling peptide hydrogel [1] and a combinatorially selected hydroxyapatite (HA) binding peptide (HABP) that regulates the calcium phosphate mineralization. [2] With the developed scaffold, we hope to exploit and join the the bio-friendly properties of the peptide hydrogel and the molecular control of the HABP on the mineralization

Methods: The peptides were synthesized via automated solid phase peptide synthesis and purified by reverse phase HPLC up to a purity of >99%. The gelation properties of the hybrid and the control peptides were investigated via circular dichroism (CD) temperature scans at physiological conditions. To assess the mineralization capabilities of the hybrid hydrogel, an alkaline phosphatase (AP) based solution mineralization model was used where the AP is entrapped in the hydrogel and the diffusion of Ca and β-glycerophosphate ions are allowed into the gel. The AP then releases the PO_4^{-3}groups which react with the Ca ions to form HA. The mineralization properties of the hybrid and the control gels were further investigated by scanning electron microscopy (SEM) and X-ray diffraction spectroscopy (XRD).

Results: CD analyses demonstrated that the peptide hydrogels containing the HABPs retain the self assembly capability. Although a structural interference by the HABP was observed, the gelation was achieved under the same conditions with both the hybrid and the control peptides under the same conditions. (Figure 1) SEM analysis on the mineralized gels showed that the mineral deposited on MAX8 formed a poorly crystallized layer on the gels (Figure 2a-b) while the mineral deposited on MAX8-HABP1 contained elongated, plate-like crystals. (Figure 2c-d) Results obtained from the XRD analysis were consistent with the SEM observations. The major peaks observed with the mineral deposited on MAX8-HABP1 were at 2θ = 31.8° (d = 2.81Å) and 32.1° (d = 2.78Å) corresponding to (211) and (300) planes, respectively. (Figure 2e) The mineral deposited on MAX8 yielded only a weak and broad peak at 2θ = 31.8° (d = 2.81Å) indicating a small amount of poorly crystalline HA. (Figure 2e)

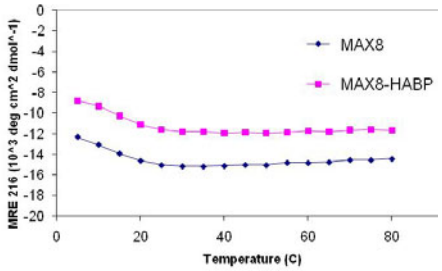

Figure1: Gel transition temperatures of the native hydrogel (MAX8) and HABP containing hydrogel (MAX8-HABP) obtained by CD temperature scans.

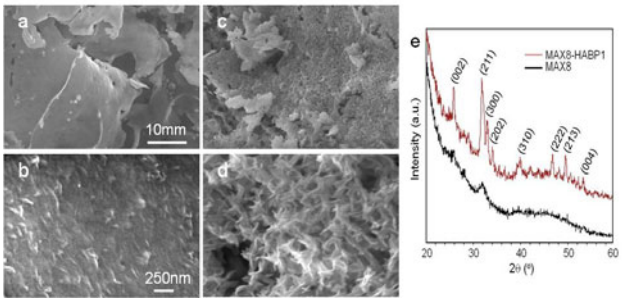

Figure3: SEM micrographs of the mineral deposited on MAX8 (a-b) and MAX8-HABP1 (c-d) at different magnifications and the XRD spectrum of the minerals deposited on MAX8 and MAX8-HABP1 (e)

Conclusions: We have designed and synthesized a bi-functional fusion peptide (MAX8-HABP1) with the ability to form a mechanically rigid hydrogel structure and regulate the apatite mineral morphology deposited on. The three major advantages of the designed scaffold are the ability to form *in situ*, the simple mechanism of the gelation requiring no complex or biologically hostile chemistry and the inherent propensity to regulate the products of the biomineralization. The *in vitro* studies presented here indicate that combinatorially selected inorganic binding peptides such as HABP may find substantial use in tissue engineering applications for successful restoration and regeneration of the hard tissues.

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References

1. Veerman, C., et al., Gelation kinetics of beta-hairpin peptide hydrogel networks. Macromolecules, 2006. **39**(19): p. 6608-6614.

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