Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting information for

Mineral binding peptides with enhanced binding stability in serum

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Experimental

Peptide synthesis: Fluorescently labeled peptides were manually prepared by standard solid phase peptide synthesis on Fmoc-Rink Amide MBHA resin. Fmoc group of N-terminus of peptide was removed using piperidine (20% in DMF), and Fmoc-protected amino acid (3 equiv) was then coupled after activated with 1-hydroxybenzotriazole (3 equiv) in presence of diisopropylcarbodiimide (3 equiv). The completion of coupling reaction was confirmed by Kaiser test. For fluorescence labeling, 5(6)-carboxyfluorescein (FAM) or 5(6)-carboxytetramethylrhodamine (TAMRA) was conjugated to the N-terminus of peptide chain. After reaction with amino acids and fluorophore, resultant resin was treated with a mixture of trifluoroacetic acid, triisopropylsilane and water (95:2.5:2.5) at room temperature for 4 hours for deprotection of side chain of amino acids and cleavage of peptide samples from resin. The peptide was then extracted, precipitated in cold diethyl ether, centrifuged and dried in air. Final product was purified by a dialysis method (MWCO 1000), lyophilized, and stored at -20^{o}C.

Peptide binding to calcium phosphate biomaterials: Binding of fluorescently labeled peptides was examined on hydroxyapatite (HAP) and β-tricalcium phosphate (β-TCP). HAP or β-TCP particles (~1 mg) were incubated in peptide solution in PBS at 37^{o}C under constant agitation. The incubation was continued for 4 hours to ensure the maximum binding at equilibrium based on our previous results (Lee JS *et al.*, *Angew. Chemie Int. Ed.*, 2009, **121**, 6384; Lee JS *et al.*, *Adv. Mater.*, 2010, **22**, 5494). After binding, supernatants were collected by centrifuging particle

suspension and their peptide concentration was determined by measuring fluorescence intensity (Ex/Em of 494nm/519nm and 546nm/579nm for FAM and TAMRA, respectively) and comparing to those of standard peptide solutions of known concentrations. The amount of bound peptides was calculated from the concentration change of peptide solutions upon incubation and normalized to the mass of particles.

Binding stability of peptides on calcium phosphate biomaterials: The stability of peptides bound to calcium phosphate biomaterials, HAP and β-TCP, was tested in presence of fetal bovine serum (FBS; Gibco). HAP slabs and β-TCP particles were incubated in solutions of fluorescently labeled peptides at 37°C for 4 hours. After washing with deionized water, peptide-bound slabs were incubated in 1 mL of PBS solution with different fetal bovine serum compositions (0, 25 and 50 vol/vol %). At each time point, slabs were copiously rinsed with PBS and fluorescently imaged using Nikon Eclipse Ti inverted microscope. After imaging, the slabs were continuously incubated in a designated solution. To quantify the fluorescence intensity, the average intensity of all pixels in the images was obtained using ImageJ. The average pixel intensity was considered to be proportional to the amount of peptide present.