Surface Modification of Calcium Phosphate Biomaterials and Detection Using Confocal Raman Microscopy

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Statement of Purpose: Biomimetic peptides are becoming increasingly important in the innovation of new biomaterials and biomedical devices. These mimetic devices are able to induce cellular transformations that are similar, if not identical, to the molecules or substrates they are intended to mimic. These induced cellular transformations can lead to directed tissue regrowth, regeneration and repair. A group of small peptides, intended to amplify the cellular adhesion characteristics of Type I collagen, has recently been introduced to the biomaterials community. One particular integrin binding, collagen mimetic peptide, P-15, is currently being utilized commercially in hard tissue regenerative products for orthopedic and dental applications. P-15 has been shown to adsorb onto calcium phosphate (CaP) substrates where it is able to facilitate cellular migration, binding and differentiation, leading to hard tissue regrowth and repair[1]. The nature of the adsorption to CaP is unclear; as is how the adsorbed peptides and their active sites are presented to the anchorage dependent cells they are intended to influence.

Methods: *Peptide Synthesis*. The peptide GTPGPQGIAGQRGVV (P-15) was synthesized as described previously[1]. *Anorganic Bone Mineral (ABM).* Particulate ABM, derived from bovine bone (diameter 250-420 μm), was obtained from Cerapedics (Westminster, CO); purity was verified by x-ray diffraction standard (JCPDS 09-0432). *Preparation of ABM.P-15.* Peptide was adsorbed on ABM by incubating ABM for 24 h in a solution of the peptide in Phosphate-buffered saline (PBS) in a ratio of 1.0 g ABM: 12.0 ml solution containing a concentration of ~125 μg peptide per ml PBS[1]. Incubation was at room temperature on a shaker table to ensure equal coating on all exposed surfaces. After incubation, ABM was washed three times, 8h each, shaking in PBS. *Confocal Raman Microscopy.* Raman scattering experiments were performed using a WiTec Alpha 300 Confocal Raman Microscope equipped with a 30mW Class 3B Laser (λ = 532 nm); 1μm probing spot.

Results: *Raman Results.* ABM spectra matches literature values for HA[2]. P-15 spectrum was compared with previous FTIR results[3] and analyzed using Raman organic wavenumber tables. Characteristic peaks from 1400-3400cm^{-1} were used to detect P-15 coating on the surface of ABM.P-15 (Figure 1). For P-15, the peaks in this region are 1445cm^{-1}, 1660cm^{-1} and 2935cm^{-1} representing CH deformation, Amide I and CH_2 asymmetric stretching, respectively[4]. In Figure 2, P-15 coating peaks are clearly visible above background at approximately 2945cm^{-1} and 1455cm^{-1}.

Conclusions: Literature shows the CaP structure to be that of calcium ions coordinated to oxygen atoms on phosphate groups. Surface calcium ions of ABM may only be coordinated to three oxygen atoms or less, allowing for coordination of P-15. Detection of P-15 on the surface of ABM.P-15 indicates two things: (1) P-15 is present on the surface of the ABM, (2) peak intensity shift (approx. 10 cm^{-1}) indicates P-15 is interacting with the surface. Peak shifts are evident in the CH_2 and Amide I peaks. Uncoordinated Ca and O atoms may be present on the surface of ABM that could interact with H atoms of both of these regions of the peptide backbone.

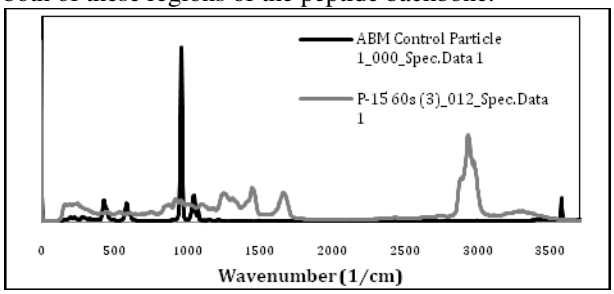

Figure 1. Raman spectra of ABM (black), P-15 (gray).

Specific amino acids in P-15 may also be interacting with the surface such as Glutamine, Arginine and Threonine. All of these interactions would cause P-15 to lie horizontally on the surface of CaP.

Surface modifications of CaP biomaterials can be performed using P-15. Modifications can be rapidly detected using Confocal Raman microscopy. Raman spectra show that the proposed macromolecule, P-15, is interacting with the surface of ABM beyond physisorption and resting on the surface of CaP. This allows for P-15 to be available for cellular adhesion after washing any un-coordinated peptide from the surface.

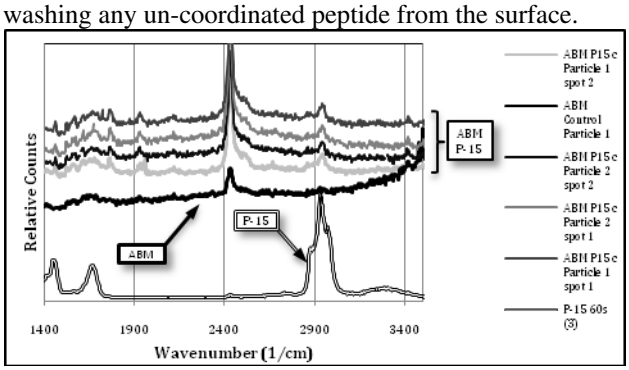

Figure 2. Staggered plot of Raman spectra for four samples of ABM.P-15 showing P-15 coating with peaks appearing at ~2950 cm^{-1} and 1450 cm^{-1}. P-15 is on a secondary axis.

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