Biomimetic Mineralization of Dense Collagen Substrates Derived from Demineralized Manatee Bone

Taili Thula-Mata[1], Douglas E. Rodriguez[1], Myong Hwa Lee[1], Jacob Podschun[2], Laura Pendi[2], Laurie B. Gower[1].
[1]Materials Science & Engineering Department, University of Florida, Gainesville, FL, USA.
[2]Chemistry Department, University of Ulm, Ulm, Germany.

Statement of Purpose: Bone is a multifunctional organ with a complex structure consisting of organic and inorganic substances which are interpenetrated and arranged to accomplish specific functions. Therefore, the development of bone substitutes has become a challenge. Our group has introduced a novel technique called the polymer-induced liquid-precursor (PILP) process, consisting on the addition of acidic polymers to a mineralization solution inducing a liquid-amorphous mineral precursor [1, 2]. Via the PILP process, we have been able to develop a collagen-hydroxyapatite composite with the fundamental structure of bone, wherein nano-hydroxyapatite crystals are embedded in the collagen fibril [3, 4].

The objective of the present study was to investigate the use of this polymer-directed crystallization process to mineralize dense collagen substrates. To examine collagen scaffolds that truly represent the dense-packed matrix of bone, manatee bone was demineralized to isolate its collagen matrix, consisting of a dense, lamellar osteonal microstructure. This biogenic collagen scaffold was then remineralized with calcium phosphate using polyaspartate to direct the mineralization process through an amorphous precursor pathway in an attempt to restore mineral into the dense substrate.

Methods: Prior to demineralization, bone samples were cut into rectangular strips of 40 x 3 x 0.5 mm using a wet diamond saw (Exakt Technologies, Hamburg, Germany). Demineralization of bone pieces was carried out in a 0.5 M EDTA solution (Acros Organics, Morris Plains, NJ, pH adjusted to 8.0 with NaOH) containing 0.02% (w/v) sodium azide (Sigma, St. Louis, MO) to avoid bacterial contamination.

Demineralized bone samples were remineralized with calcium phosphate (CaP) via the polymer-induced liquid-precursor (PILP) process. In this study, the mineralization solution was prepared by mixing equal volumes of 9 mM $CaCl_2 \cdot 2H_2O$ (Sigma, St. Louis, MO) and 4.2 mM K_2HPO_4 (Sigma, St. Louis, MO) solutions. To maintain the pH of the mineralization solution at 7.4, calcium and potassium solutions were made in Tris-buffered saline (TBS) supplemented with 0.02% (w/v) sodium azide.

Poly-L-aspartic acid sodium salt (Mw: 10,500 or 27,000 Da; Sigma or Alamanda Polymers, Huntsville, AL) was added to the calcium solution before mixing an equal volume of the phosphate counterion solution. Samples were incubated at 37°C under continuous stirring for 7-14 days. A fully characterization of the remineralized bone specimens was performed, including XRD, SEM, TEM, and TG/DTA analyses.

Results: The morphology of manatee rib bone appeared very rough with highly mineralized collagen fibrils showing some degree of orientation, depending on the sample area (Fig 1a). This initial morphology changed after the demineralization process with the calcium-chelating agent, where a smoother surface was observed from Fig. 1b. The morphology of the PILP-remineralized samples (Fig. 1c) appeared very similar to that of the original bone where the collagen fibrils appeared to be covered by a smooth extrafibrillar mineral coating, likely caused by hypermineralization. The anisotropic structure of the underlying collagen fibrils could still be seen under this extrafibrillar coating, as was the case for the native bone.

To further analyze mineral distribution across the bulk specimen, SEM in backscattered electron (BSE) mode and elemental mapping analysis of the PILP-remineralized samples was performed (Fig. 2). Regions of electron-dense material were found at the edges while a pronounced enhancement of osteonal structures was observed in the BSE images of PILP-remineralized bone cross-sections (Figs. 2b and 2f). This was particularly apparent in the elemental mapping results. High levels of calcium and phosphorous were localized at the surfaces of the bulk sample (Fig. 2c-d), and in the oval structures of osteons (Fig. 2g-h).

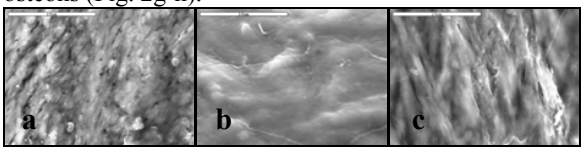

Figure 1. SEM of manatee samples treated under different conditions: (a) untreated, native bone, (b) EDTA demineralized bone, (c) PILP-remineralized bone.

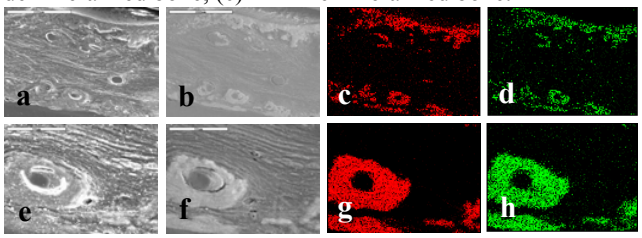

Figure 2. SEM and element mapping of PILP-remineralized manatee bone. (a, e) SEM and (b, f) backscattered electron mode images. Backscattered electron mapping showing calcium (c, g) and phosphorus (d, h) distribution across the sample.

Conclusions: Using a biogenic collagen scaffold obtained from demineralized manatee bone, it was demonstrated that a bone-like nanostructure (intrafibrillar mineral) and microstructure (lamellar osteons) could be restored using the PILP mineralization process. The results of this work lead us closer to the development of bone-like composites that could become the next generation of synthetic bone grafts.

References: [1]L Gower, J Crys Grow. 2000;210:719-734. [2]L Dai, J of Non-Crys Sol. 2008;354:1845–1854. [3]M Olszta, Mat Sci & Eng R-Rep. 2007;58:77-116. [4]S. Jee, Acta Biom. 2010;6:3676-3686.