A Collagen-Anorganic Bone Composite for Bone Repair: Part I. *In Vitro* Characterization Studies

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Introduction:

Bone is composed of an organic phase and an inorganic phase. The organic phase is mostly type I collagen and the inorganic phase is apatite-based crystals that are aligned along the collagen fibers to provide mechanical properties for function. In trauma or disease resulting in the loss of bone beyond the critical size, the bone defect must be filled with a grafting material that can guide and conduct new bone formation. Anorganic bone, a deproteinated bone, has been used clinically as a bone conductive scaffold for decades. In order to preserve the osteoconductive property and yet improving clinical usage, we have engineered a porous composite of type I collagen fibers and anorganic bone for bone repair applications. Due to its high liquid absorption capability, the composite should function well when in combination with autologous bone marrow to provide osteoconductive as well as osteoinductive and osteogenic properties. In part I, we present results of *in vitro* characterization studies of the engineered composite. *In vivo* studies of repairing critical size defect of rabbit radial bone using this composite in combination with autologous bone marrow will be presented in the part II of this series.

Materials / Methods:

Type I collagen fibers and anorganic bone were the same materials used in Collagen Matrix's other implantable devices. Composite of type I collagen fibers and anorganic bone (OssiMend™) was prepared by dispersing anorganic bone particles (< 75µm) in 1.5% (w/v) collagen fiber dispersion such that the final weight ratio of collagen to anorganic bone was 50:50. The mixture was freeze dried and crosslinked to control its *in vivo* stability. SEM micrograph, X-ray diffraction and FTIR spectroscopy analyzes were conducted at New York University. Pore sizes were measured from SEM micrographs. Thermal stability (T_s) was measured using a Mettler Toledo 822^e/700 DSC differential scanning calorimeter. *In vitro* mineral dissolution study was carried out at pH 5.6 to simulate more closely the local osteoclast environment during bone graft resorption. Calcium ion release as a function of time was measured as an indicator for the dissolution of the mineral phase. The study was followed for 60 days to gather data points for extrapolation to the total dissolution time. The calcium concentration was then converted to the percent of mineral dissolution as a function of time.

Results:

Figure 1 is an SEM micrograph of OssiMend. Mineral particles are dispersed throughout the porous matrix of collagen and can be seen at 100X. The pore sizes of OssiMend are in the range of 100µm to 500µm. The high porosity of OssiMend indicates that the device has a high liquid absorption capacity for the delivery of functionally active osteoinductive factors and osteogenic cells from the bone marrow. The solution absorption capacity for OssiMend is 21.9 ± 0.6ml/g.

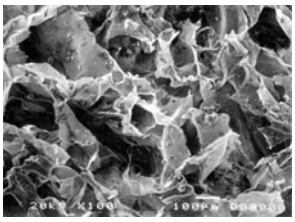

Figure 1: SEM Image

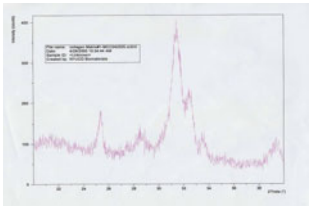

Figure 2: X-Ray Diffraction

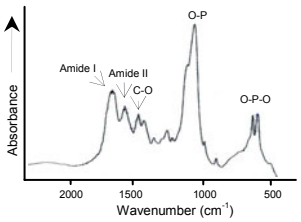

Figure 3: FTIR Spectrum

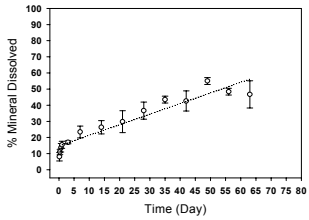

Figure 4: Dissolution Profile

Figure 2 is a powder X-ray diffraction pattern of OssiMend. The diffraction pattern of the anorganic bone in the composite is consistent with apatite-like structure with broader reflections, indicating that the crystal size of the mineral in OssiMend is smaller than the sintered synthetic apatite. The domain size calculated along the 002 reflection plane of the mineral was 29nm. Figure 3 is an FTIR spectrum of OssiMend. The major IR absorption peaks for OssiMend are consistent with mature bone structure consisting of mineral phase and protein phase, including various phosphate ion bands and amide I and amide II bands that are typical for protein materials. Figure 4 summarizes the results of dissolution studies. A linear regression curve fitting shows that total mineral dissolution *in vitro* is about 130 days, which is in line with *in vivo* resorption of OssiMend (Part II). T_s of OssiMend indicates that collagen will also resorbed in about 12 weeks.

Discussion / Conclusion:

Results from this study indicate that the collagen-anorganic bone composite we engineered has the characteristics that are useful for bone repair applications. The mineral particles distributed in a three dimensional porous collagen scaffold with adequate stability can provide a suitable biological environment for osteoblast cell migration, conduction and new bone deposition while the implant is slowly resorbed. The accompanying presentation verifies that the porous collagen-anorganic bone composite in combination with autologous bone marrow has the ability to repair a critical size defect of radial bone in the rabbit model.