Osteoclast Cell Proliferation, Differentiation and Resorption on Polyurethane/Bone Composite Implants for Reconstruction of Craniofacial Defects

Pedro Alvarez[1], Abiraman Srinivasan[1], Jerald Dumas[2], Anuradha Karunanidhi[1], Jin Ku Kim[1], Scott Guelcher[2] and Jeffrey Hollinger[1].

[1]Bone Tissue Engineering Centre, Carnegie Mellon University, Pittsburgh, PA

[2]Department of Chemical Engineering, Vanderbilt University, Nashville, TN

Statement of Purpose: Massive craniofacial defects are currently reconstructed using nonresorbable plastics (e.g., poly (methyl methacrylate): PMMA) that transiently may restore patient form. However, currently available materials do not remodel and integrate with host tissue, and can become infected. Revision surgeries are often necessary. We propose a physiological alternative that will overcome the constraining limitations of contemporary bone substitutes. *Mineralized bone particle (MBP)/poly (ester urethane) urea (PUR) composite* implants incorporating osteo-angiogenic growth factors will be developed to overcome the technical barrier to regenerate bone loss in the cranio-orbital complex. The regenerated bone will be enduring through the sustainable restoration of form and function as a consequence of the physiological process of remodeling. The clinical goal is to develop MBP/PUR material with osteo-angiogenic growth factors that will induce new bone formation and facilitate the osseointegration process and resorbed over a period of time.

Materials and Methods. Different ratios of MBP/PUR composite materials were synthesized to study the biocompatibility and biodegradation using RAW 264.7 murine macrophage cells. Polymer disks were sectioned 200-400µm using Isomet (Buhler, low speed saw). The material was cleaned thoroughly in sonicator and ethanol sterilized and air dried. The *in vitro* biodegradation experiments with cells were carried out as per the ASTM standard No. F1903. Briefly, 5 x 10^4 RAW 264.7 murine monocyte macrophage cells were seeded directly onto the disk in a 24-well tissue culture plate and were treated with 20 ng/ml of Receptor Activated by Nuclear Factor κ- B Ligand (RANKL) in DMEM containing 10% FBS and 1% penicillin/streptomycin for 4, 7, 14 and 28 days. Osteoclast differentiation was determined using CD14 immunofluorescence and Tartrate Resistant Acid Phosphatase enzyme (TRAP) staining, osteoclast cell proliferation by PCNA immunostaining and resorption was determined by Cathepsin K and SEM- Focused Ion Beam Milling (FIB). RANKL ELISA was performed to measure osteoclast differentiation. Actin phalloidin staining was performed to visualize actin ring formation to confirm MBP/PUR surface resorption. The MBP/PUR rods after 28 days were embedded in methyl methacrylate and processed for histological analysis and Transmission Electron Microscopy to study cell-material interaction at micro and ultrastructural level.

Results: Live/Dead staining was performed to test the viability of these cells and found more than 80% of cells were viable suggesting that the MBP/PUR is biocompatible and non-toxic. Actin staining and SEM showed good cell attachment and anchorage to the MBP/PUR surface as early as 24 hours (Fig.1 A and B). Cell proliferation was markedly high at Day 7 for groups incubated with and without RANKL.

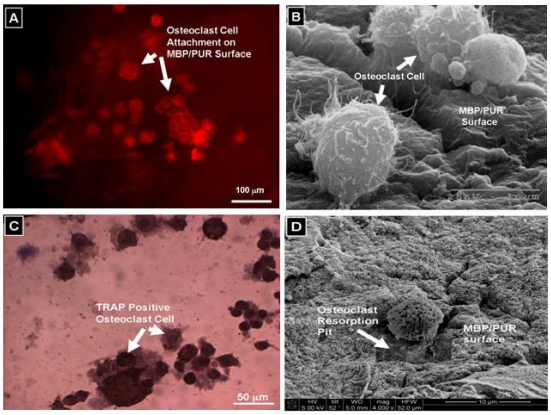

Figure 1 A and B show osteoclast cell attachment, 1C show TRAP positive cells and 1D show MBP/PUR surface resorption as determined by SEM-FIB.

CD14 and TRAP staining (Fig.1C) confirmed RAW 264.7 murine monocyte macrophage differentiation to osteoclast cell lineage. CD14 and TRAP staining were positive on cells exposed to RANKL when compared to cells that were not treated with RANKL and the osteoclast differentiation was observed as early as Day 7. Osteoclast resorption was more distinct for Days 14 and 28 when compared to early stages (days 4 and 7) as confirmed by SEM-FIB (Fig.1D). Cathepsin K and Actin staining further confirmed RANKL induced resorption. MBP/PUR surface was intact even after 28 days and showed moderate surface resorption by osteoclast cells suggesting the material to be stable and not actively being degraded.

Conclusion: MBP-PUR material are non-toxic and promote cell attachment, proliferation and differentiation of osteoclast cells and showed moderate surface resorption over 28 days of culture suggesting the material will provide adequate mechanical stability to the cranio-orbital complex.

Acknowledgement: This work was funded by the Center for Military Biomaterials Research (DOD -2110 - PO5-694362) and Bone Tissue Engineering Center.