

The Use of Three-Dimensionally Printed β -Tricalcium Phosphate/Hydroxyapatite to Understand the Regulation of Adenosine Receptors in Osteoclast Formation and Promotion in Bone Regeneration

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Statement of Purpose: Bone defects resulting from trauma or infection need timely and effective treatments to replace damaged bone. Using specialized three-dimensional (3-D) printing technology, combined with bioactive molecules, we can design custom 3-D scaffolds for bone repair. The Hydroxyapatite (HA)/Beta-Tri-Calcium Phosphate (β -TCP) scaffold components provide mechanical strength, conduct bone throughout the scaffold and remodel over time. Adenosine, acting via adenosine receptors (A1, A2A, A2B and A3), plays a critical role in regulating bone metabolism. Dipyridamole (DIPY) increases local adenosine levels by blocking cellular uptake of adenosine and stimulates bone regeneration. We tested the capacity of DIPY, hypothesizing that with a bioactive filler, such as DIPY, these scaffolds may successfully regenerate bone over critical sized bone defects in an *in vivo* model.

Methods: 15% HA:85% β -TCP scaffolds were designed using Robocad software, fabricated using a 3-D Robocasting system, and sintered at 1100°C for 4h. Scanning electron microscopy (SEM) and micro-computed tomography (micro-CT) were used to examine structural aspects on pre/post-sintering, while x-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR) and inductive coupled plasma (ICP) were used to evaluate porosity, crystalline phase quantification, and Ca:P ratio, respectively. Vehicle, BMP-2 and combination drug scaffolds (scaffold + PBS, scaffold + drug, scaffold+ collagen + drug) were implanted in C57B6 mice with 3mm critical size defect for 2, 4 and 8 weeks. DIPY release was assayed *in vitro* spectrophotometrically over time. MicroCT and histological analysis were conducted to determine the degree of new bone formation and remodeling.

Results: Qualitative microstructural evaluation using SEM showed a broader pore/particle size distribution for sintered materials. XRD, FT-IR and ICP results showed substantial deviations in the original 15/85% HA/ β -TCP formulation with the detection of ~10% calcium pyrophosphate. As sintering temperature was increased, lower amounts of the HA (5% HA: ~95% β -TCP) phase was observed. DIPY release assays showed a constant release of the compound in collagen for a period of 10 days. Quantitative and qualitative results from microCT showed similar and significant bone formation and remodeling in HA/ β -TCP- DIPY and HA/ β -TCP-BMP-2 scaffolds when compared to vehicle at 2, 4 and 8 weeks ($P \leq 0.05$, $P \leq 0.05$ and $P \leq 0.01$, respectively). Histological analysis showed increased bone formation and a trend toward increased remodeling in HA/ β -TCP- DIPY and HA/ β -TCP-BMP-2 scaffolds.

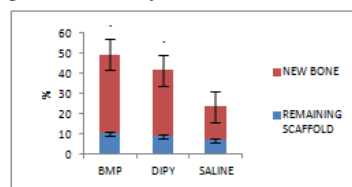


Figure 1. Graph of microCT data showing differences between control and experimental samples at two weeks.

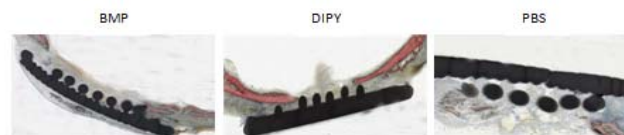


Figure 2. Two weeks histology

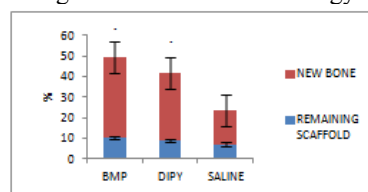


Figure 3. Graph of microCT data showing differences between control and experimental samples at four weeks.



Figure 4. Four weeks histology.

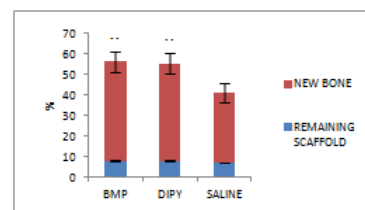


Figure 5. Graph of microCT data showing differences between control and experimental samples at eight weeks.



Figure 6. Eight weeks histology.

Conclusions: Targeting osteoblasts and osteoclasts via appropriate adenosine receptor blockade or stimulation leads to increased bone regeneration in a murine model. Micro-CT and histology results show that the delivery of DIPY in the 3-D ceramic scaffolds promotes bone formation as effectively as BMP-2 *in vivo*. In the future, scaffolds with different HA/TCP ratios and mechanical properties, acting as drug and molecular delivery systems, will have great potential for bone repair in craniofacial and orthopaedic applications.