Calcium Phosphate Composite as Stem Cells Delivery Vehicle for Bone Repair

Jerry Chang[1], Michael Strunk[1], Shannon Smith[1,2], Sang-Hyug Park[1,2], Xiaoqin Wang[1,2], David L. Kaplan[2]

[1]ETEX Corporation, 675 Massachusetts Ave, Cambridge, MA 02139

[2]Department of Biomedical Engineering, Tufts University, 4 Colby Street, Medford, MA 02155

Statement of Purpose: This study includes *in-vitro* & *in-vivo* testing to investigate the potential of an injectable & hard-setting biomametic Calcium Phosphate Composite (CPC) to function as a delivery vehicle and scaffold for osteogenic cells. The focus of this study is to prepare and evaluate *in-vitro* & *in-vivo*, a composite CPC based scaffold material that is combined with bone marrow cells in cell culture and rabbit femoral core, critical sized defect model. Cells / scaffold interaction observed *in-vitro* & new bone formation and osteointegration *in-vivo* are analyzed and discussed.

Methods: ***In-vitro*** evaluation including cell culture , Scanning electron microscopy (SEM), Cell spreading from structures, Biochemical analysis, Real-time PCR have been published[1].

***In-vivo* Isolation of Bone Marrow** Animals underwent a bone marrow aspiration (BMA) from the iliac crest immediately prior to surgical exposure of the femurs. 2-4 cc of bone marrow was aspirated from each animal.

Surgical Implantation The lateral femoral condyles of 12 female New Zealand White rabbits were surgically exposed. A 5 mm diameter drill defect approximately 8 mm deep was created in the distal aspect of each lateral femoral condyle. Bone marrow aspirate obtained from iliac crest or saline (control) was combined with test article prior to implantation in rabbits. 1 cc of BMA or sterile saline was aspirated into a syringe containing the CPC scaffold material which was subsequently deployed into the defect.

Results:

***In-vitro* SEM optical image**

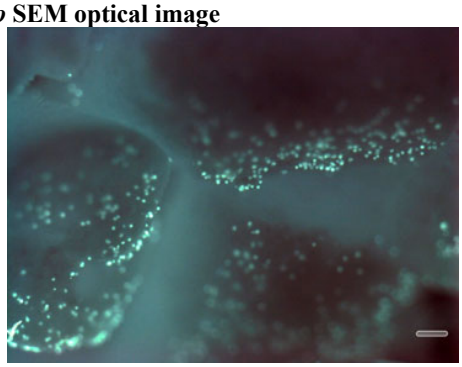

Figure 1. Fluorescently labeled stem cells attached within pore structure; Bar = 100µm

***In-vivo* Gross Observation and Histology**

There was no statistical difference in residual bone graft, bone fill, fibrous tissue, inflammation, GCs, or average cellular response scores (an average of inflammation and GCs) between groups at 6 weeks or 12 weeks. There was also no statistical difference in these scores for either treatment group over time. 12-week CPC-BMA sections had more mature bone marrow between islands of residual implant material compared to CPC-BMA sections at 6 weeks. These results provide some evidence of a accelerated bone maturation rate for the bone marrow group. Bone formed in spaces between the residual CPC scaffold throughout the defects. This was true for both implant types.

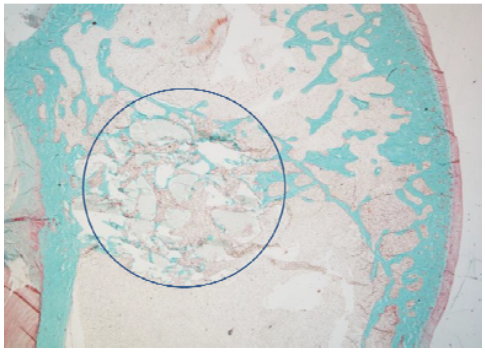

Figure 2. Photomicrograph (7X) of a 12-week section taken from femurs treated with CPC-BMA. A substantial amount of calcified bone and bone marrow, in addition to residual implant material, is seen within the defect (black circle).

Conclusions: Our in-vitro & in-vivo results show good cell protection and lineage-specific differentiation of hMSCs within the Calcium Phosphate matrix. CPC-Saline and CPC-BMA implant groups were similarly well-tolerated and integrated with bone. Although there was evidence of an accelerated bone maturation rate with time for the CPC-BMA group as indicated by increased amounts of mature fatty marrow at 6 weeks which increased between the 6- and 12-week time points. We suggest that in-vitro culture is a good screening tool for formulation development & in-vivo results confirming modified biomimetic CPC's can be successfully used as a delivery vehicles for bone marrow aspirate, resulting in enhanced healing.

References:

1. Park SH. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2011:97B:iss.2:235-244.