Scaffold Optimization and Pre-Vascularization Strategies for Large-Area Bone Regeneration

Amini AR[a,b], Laurencin CT[a,b,c], Nukavarapu SP[a,b,c]
[a] Institute of Regenerative Engineering, [b]Orthopaedic Surgery, [c]Chem, Mat & Biomol Eng, University of Connecticut, CT

Statement of Purpose: Large area bone regeneration aims to repair defects that are unable to heal spontaneously. Repair of large area defects require bone grafts that provides sufficient physical support, and surface area for tissue growth. Current treatment options for large area bone defects include have all been associated with significant challenges and complications. Here, we propose a two-pronged approach for large area bone regeneration, which involves biodegradable matrix optimization, and pre-vascularization of the matrix via seeding with effective progenitor cell populations (i.e., endothelial progenitor cells (EPCs) for enhanced vascularization, and mesenchymal stem cells (MSC) for enhanced bone formation). We demonstrated significantly enhanced vascular and bone formation in our pre-vascularized bone matrices

Methods: PLGA scaffolds with increasing pores were fabricated (1). Accessible pore volume of the matrices was measured via MicroCT. Matrices fabricated with 80 PLGA:20 NaCl were selected as our experimental optimally-porous matrices. $1x10^5$ rabbit MSCs were seeded on each matrix (10 mmx5 mm), and cultured for 21 days in osteogenic media (1). Needle-type fiber optic O_2 microsensors were utilized to analyze O_2 levels in interior of MSC-seeded matrices (1). Rabbit EPCs and MSCs were seeded on optimally-porous matrices and cultured for 2 days *in vitro* (2). Immunostaining of endothelial CD31, and osteogenic marker RunX2 was performed. The pre-vascularized grafts, and acellular grafts were implanted in a rabbit ulnar bone defect (500,000 cells/matrix, 6 animals/grp, 12 week). Histological analysis (Trichrome) was performed to quantify vascularization.

Results:

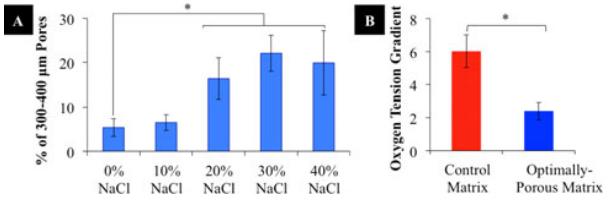

Figure 1. (A) Increasing percent accessible pores in the range of 300-400 µm in matrices fabricated with increasing porogen. (B) O_2 tension gradient in control and optimally-porous matrices.

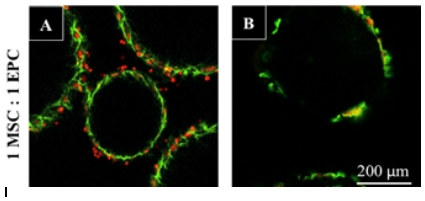

Figure 2. Immunostaining of (A) CD31 and (B) RunX2 in matrices seeded with MSCs and EPCs *in vitro*.

Matrices fabricated with >20% NaCl porogen leaching displayed a significant increase in percentage of pores in the range of 300-400 µm, a previously cited pore size range critical for vascularization of bone constructs. Further, matrices fabricated with >20% NaCl also demonstrated a significant increase O_2 diffusion throughout the construct after long-term culture (21 days) with rabbit MSCs *in vitro* (i.e., decrease in O_2 tension gradient from the exterior to the interior of the matrix). Matrices that displayed significantly enhanced porosity in the critical range for vascularization, decreased O_2 tension gradient *in vitro*, as well as sufficient mechanical strength in the range of human cancellous bone (matrices fabricated with 20% NaCl, 80% PLAGA) were termed as optimally-porous matrices (Fig. 1).

Next, we investigated the combination of MSC and EPCs as potential effective progenitor cells that allow for bone and vascular formation. We seeded MSCs and EPCs at a ratio of 1:1 on optimally porous scaffolds, and demonstrated EPCs display key vascular marker (CD31), and MSCs display key bone marker (RunX2) after 2 days *in vitro* (Fig. 2). These pre-vascularized optimally-porous matrices were then implanted into a rabbit critical-sized ulnar bone defect. After 12 weeks, the pre-vascularized grafts displayed significantly increased bone formation (MicroCT data not shown), and vascularization throughout the construct compared to grafts not initially seeded with any progenitor cell populations (Fig. 3).

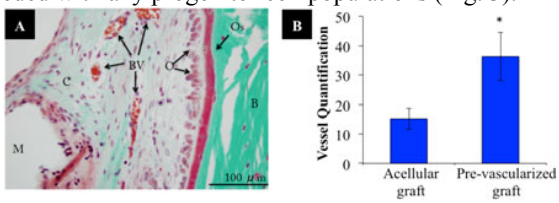

Figure 3. (A) Trichrome staining of pre-vascularized grafts 12 weeks post-implantation (M-microsphere, BV-blood vessel, B-bone). (B) Quantification of vascularization in acellular (control) and pre-vascularized graphs (count/2 mm^2 area).

Conclusions: Although various strategies have been proposed for large area bone regeneration, none have proven clinically successful. Insufficient vascularization of bone defects hinders optimal large area bone regeneration. Here, we have demonstrated a two-pronged approach for successful large area bone regeneration. First, we developed optimally porous constructs that allow for enhanced cell viability, O_2 tension and vascularization throughout the construct. Second, we identified an optimal ratio of clinically relevant progenitor cells for enhanced *in vitro* pre-vascularization, and later, bone and vascular formation *in vivo*. This two-pronged approach has proven to be a successful strategy for large area bone regeneration.

References: 1. Amini *et al.* 2012. Tissue Eng Part A.
2. Amini *et al.* 2012. J Orthop Res.