

Bone Tissue Engineering: Effect of Dynamic Perfusion

Successful healing of critical-sized bone defects requires the implantation of bioactive materials that are capable of stimulating vascular infiltration, tissue integration, and normal bone remodeling. Our overarching hypothesis is that these materials can be synthesized by *in vitro* culture of osteoprogenitor cells within porous scaffolds under stimuli that induce deposition of osteogenic and angiogenic bioactive factors. We have shown that fluid flow is a critical component of this strategy because 1) it delivers oxygen and nutrients to maintain the viability of cells within biomaterial scaffolds, and 2) it activates mechanotransductive signaling pathways, which in turn induce expression of vascular endothelial growth factor (VEGF)-A and bone morphogenetic protein (BMP)-2. We have recently discovered that different dynamic perfusion regimens (e.g., steady, intermittent, pulsatile flow) modulate expression of these bioactive factors and late markers of osteoblastic differentiation, and propose that dynamic perfusion can be used to 1) probe the molecular signaling pathways by which shearing flow stimulates expression of bioactive factors, and 2) identify promising perfusion regimens for achieving bioactive materials suitable for repair of osseous defects. In this project, we will focus on cell signaling through mitogen-activated protein kinases (MAPKs) ERK and p38 and transcription factors AP-1 and Osterix, and induction of the bioactive factors BMP-2, 4 and 7, and VEGF-A. However, translation of these studies to the manufacture of materials suitable for *in vivo* testing requires non-destructive imaging modalities that are capable of monitoring the deposition of bioactive factors *in situ*. Therefore, a novel molecular imaging system using bioluminescence computed tomography (BLT) will be developed and used to visualize BMP-2 expression within perfused porous scaffolds. The specific aims of this project are as follows:

Aim 1: Determine the effect of pulsatile flow strategies on cell signaling and expression of bioactive factors. A family of pulsatile flow regimens will be applied to planar cultures to modulate cell signaling and gene expression. Markers of cell signaling will include phosphorylation of ERK and p38, and DNA-binding and transactivation activity of transcription factors AP-1 and Osterix. Expression of target genes BMP-2, 4, and 7 and VEGF-A will be measured.

Aim 2: Determine the critical role of p38 signaling in pulsatile flow-induced expression of bioactive factors. RNAi gene silencing and pharmacologic inhibition will be used to probe the role of p38 activity in response to pulsatile flow in planar cultures. DNA-binding and transactivation activity of transcription factors AP-1 and Osterix, and gene expression of BMP-2, 4, and 7 and VEGF-A will be measured.

Aim 3: Implement bioluminescence computed tomography to monitor BMP-2 induction in perfused porous scaffolds. Osteoprogenitor cells will be transfected with an p(BMP-2)Luc reporter gene, seeded within porous scaffolds, and cultured under pulsatile perfusion to induce expression of BMP-2. BLT will be used to reconstruct the point source distribution and quantitatively determine the bioluminescence power of the luciferase reporter gene. Validation of the reporter gene induction and BLT reconstruction will also be performed.

The outcomes of this project will be 1) determination of the effect of dynamic perfusion strategies on expression BMP-2, 4, 7 and VEGF-A, 2) insight into the molecular mechanism by which flow induces these genes, and 3) establishment of a non-destructive imaging modality to monitor BMP-2 expression. More importantly, this research project represents three facets of a multi-disciplinary effort by the investigators, that is aimed at achieving a clinically effective engineered bone tissue. Other facets of this project include design of novel degradable biomaterial scaffolds, determination of the role of scaffold modulus on osteoblastic differentiation of progenitor cells, characterization of convective mass transport in perfused scaffolds.