Analysis of Alkaline Phosphatase Expression and Tissue Mineralization in a Woven Tissue Engineering Test System

Gilmore, JA[1,2], Yin, F[1,3], Burg, KJL[1,2,4]

Institute for Biological Interfaces of Engineering[1], Department of Bioengineering[2], Department of Biological Sciences[3], Clemson University, Clemson, SC

[4]Department of Chemical Engineering, Kansas State University, Manhattan, KS

Statement of Purpose:

Researchers have used many different tissue engineering strategies to treat traumatic bone fractures and pathological defects, for example, implantation of allograft materials in combination with biologics such as bone morphogenic proteins (BMPs)[1], calcium phosphate-based materials[2], or absorbable polymers seeded with stem cells.[3]

Regardless of the scaffold, the goal of most bone tissue engineering designs is the direction of native or implanted stem-like cells toward an osteogenic phenotype. Meshes have long been considered viable as soft-tissue scaffolds due to their attractive tensile and torsional properties. This purpose of this work was to analyze the expression of the early-stage osteogenic (OG) marker alkaline phosphatase (ALP) as well as the mineralization of cells implanted on variably woven polymeric meshes. That is, the efficacy of woven mesh scaffolds as viable bone tissue engineering scaffolds capable of hosting differentiating bone cells was evaluated. Two types of mesh chemistry and two weave configurations were considered.

Methods:

The Plain weave and the Crowfoot weave configurations were tested, with varying fiber placement in an over/under pattern. Woven meshes consisted of the following two synthetic polymers: polylactide (PL; Natureworks LLC, 2003d biopolymer, ~ 228,000 Da), poly-l-lactide-co-caprolactone (PLCL; Purac, Purasorb PLC 7015, ~ 154,500 Da). Meshes were woven on a novel bio-loom[4] and stored under vacuum until cell studies were conducted. Prior to cell culture, meshes were cleaned in 75% ethanol, then treated with ultraviolet radiation for 6 hr. Meshes were then soaked in Dulbecco's Modified Eagle's Medium (DMEM; Atlanta Biologics) supplemented with 50% fetal bovine serum (FBS; Life Technologies) for 1 hr. D1 murine mesenchymal stem cells (ATCC, passage 5-8) were cultured on each mesh in non-treated 24-well plates (Corning). Cells were cultured in DMEM supplemented with 10% FBS, antibiotic-antimycotic (Life Technologies), and fungizone (Life Technologies). Each mesh was cultured with D1 cells for 7 days, with the first 24 hours of culture on an orbital shaker at 100 rpm. A control group of cells cultured with no mesh present was also added for analysis. After day 7 of culture, medium was changed to an OG differentiation cocktail containing DMEM, FBS, fungizone, antibiotic-antimycotic, beta-glycerophosphate (β-GP) and ascorbic acid. The introduction of OG cocktail marked day 1 of the 28 day study period; medium was changed every 3 days. ALP expression was measured with a colorimetric ALP absorbance assay (405 nm). Cell mineralization was qualitatively analyzed with Alizarin Red (ARS) staining and subsequent light microscopy over the 28-day period.

Results:

ALP expression results for this work were statistically analyzed via a 2-tailed, paired t-test across all time points. Total sample size for each mesh type was 15 (3 per time point). Statistical analysis revealed no significant difference ($p < 0.05$) in ALP production in the PL mesh groups versus the PLCL mesh groups overall, or the Plain configuration versus the Crowfoot configuration ($p=0.167$ and 0.196, respectively). However, analysis of the combination of variables revealed significantly more ALP expression for PL-Crowfoot meshes over PL-Plain meshes ($p=0.033$) and PLCL-Plain meshes over PLCL-Crowfoot meshes ($p < .001$). ALP results demonstrated early production (between days 14-21) across all mesh groups, as shown below in Figure 1. ARS results showed a general increase in positive red staining for mineralization as time progressed. However these results were difficult to interpret due to retention of ARS dye in the fibers comprising the meshes.

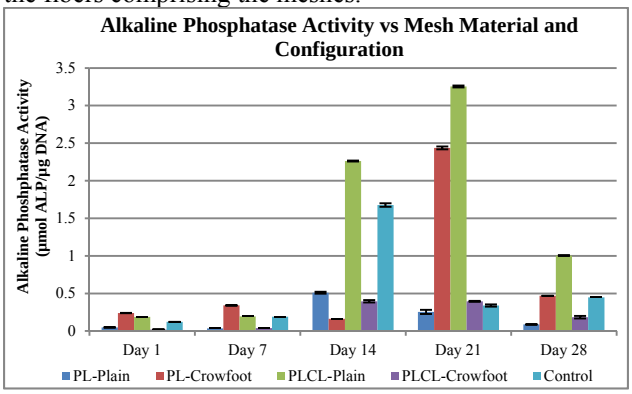

Figure 1: ALP activity as measured by colorimetric absorbance at 405 nm for various mesh types and configurations over a 28-day study.

Conclusions:

ALP production and mineralization in the cultures suggest that the woven meshes incorporated in this study are viable bone tissue engineering scaffold candidates. The ability to vary mesh material and configuration may lead to tunable tissue regeneration. Future studies will focus on the *in vitro* development of these meshes for bone tissue engineering applications.

Acknowledgements

NSF EFRI REM CBE0736007; Hunter Endowment

References

1. Hidaka C et al., *Spine*, 2003; 28:2049-57
2. Vallet-Regi M, Gonzalez-Calbet JM, *Prog in Solid State Chem*, 2004; 32:1-31
3. Kretlow JD, Mikos AG, *Tissue Eng*, 2007; 13(5):927-38
4. Mersereau B et al., *TERM*, 2012; 9(3):116-27