Influence of Polymer Scaffold Architecture on the Shear Stress Distribution and Dynamic Culture of Preosteoblastic Cells

S.B. VanGordon[1], R.S. Voronov[2], T.B. Blue[2], R.L. Shambaugh[2], D.V. Papavassiliou[2] and V.I. Sikavitsas[1,2]
[1]Bioengineering and [2]Chemical, Biological, and Materials Engineering, University of Oklahoma, Norman, OK

Statement of Purpose: Porous polymer scaffolds of different architectures are used in tissue engineering to investigate novel bone graft alternatives. In some cases these polymer scaffolds are used in conjunction with mechanical stimuli from fluid flow using perfusion bioreactors. The fluid flow creates shear stress along the material surface where cells are seeded promoting proliferation and differentiation. General analysis of the fluid shear stress within simple scaffold structures has been investigated but the examination of the actual shear stresses on real polymer scaffolds has not been fully examined yet.

In this study the fluid dynamic environments of scaffolds of different architectures, nonwoven fiber meshes and solvent cast/particulate leached porous foams, made of poly(L-lactic acid) with similar porosities and surface to total volume ratios were examined. The influence of shear forces and scaffold architecture on the proliferation and differentiation along the osteoblastic lineage of mesenchymal stems cells was studied under static and dynamic conditions using a perfusion bioreactor on these scaffolds.

Methods: Poly(L-lactic acid) (PLLA, 114,500 MW, 1.87 PDI, Birmingham Polymers) polymer foams were produced using the solvent casting/particulate leaching technique with sodium chloride crystals (250-350μm, Sigma) as the porogen and PLLA (grade 6251D, 108,500 MW, 1.90 PDI, NatureWorks LLC) fiber meshes were made from melt blowing. Average fiber diameter was ~35μm and both scaffold types had porosities of ~85%.

Each type of scaffold was scanned using micro-computed tomography (μCT, VivaCT40, ScanCo) at a resolution of 10μm and reconstructed 3-dimensionaly using a custom Matlab® code. The fluid dynamics in each scaffold type where then analyzed using lattice Boltzmann method (LBM), allowing the measurement of the shear stress on the scaffold walls.

Scaffolds were also seeded with 500,000 mesenchymal stem cells (MSC) per scaffold and cultured statically and dynamically in a perfusion bioreactor under 0.5mL/min/scaffold flow using osteogenic media (α-minimum essential media, (Invitrogen) supplemented with 10% fetal bovine serum (Atlanta Biologicals), 50g/mL ascorbic acid , 10mM β-glycerophosphate, and 10^{-8}M dexamethasone (Sigma)) for periods of 4 and 8 days. After the culture period scaffolds were analyzed using an alkaline phosphatase assay (ALP) and a DNA assay for scaffold cellularity. Long term cultures were also imaged using scanning electron microscopy (SEM).

Results: Numerical analysis found that the average surface shear stresses were comparable for scaffolds of both architectures with nonwoven fiber meshes 0.27g/cm·s^{2}and porous foams 0.25g/cm·s^2 for a culturing flow rate. The distribution of shear stresses was also found to be similar for both scaffold geometries following a lognormal distribution with a tapered skewing towards higher shear stresses and a standard deviation of 0.21g/cm s^2 (fibers) and 0.18g/cm s^2 (foams).

Cell growth studies showed higher cellularity and ALP activity on scaffold cultures grown dynamically in a flow perfusion bioreactor over statically grown scaffolds. Dynamically cultured fiber meshes had higher ALP activity at 4days but similar activity to porous foams cultured dynamically at 8 days (Figure 1).

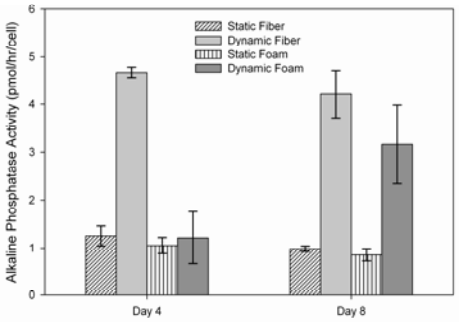

Figure 1. ALP activity of cell/polymer nonwoven fiber meshes and porous foams for 4 and 8 day dynamic cultures under flow perfusion and static conditions (n = 3, P<0.05)

SEM imaging displayed increased amounts of cells and extracellular matix on dynamically cultured scaffolds with cells showing cuboidal morphologies. Dynamic foams showed clogging of pores from cells and deposited tissue in long term cultured scaffolds.

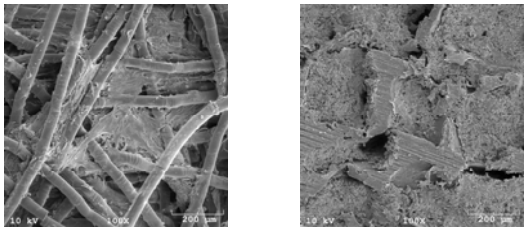

Figure 2. SEM images of the inlet surfaces of long term cultured scaffolds. A)Statically cultured foam scaffold, b)dynamically cultured foam scaffold, (100x magnification and scale bar is 200 μm.)

Conclusions: Architectures of polymer scaffolds with similar porosities and surface area to total volume ratios have comparable average shear stresses and shear stress distribution caused by flow perfusion.
Independent of scaffold architecture, dynamically cultured MSCs had improved growth and differentiation over statically cultured MSCs within the first 8 days. Cells seeded on fiber scaffolds appeared to be able to grow to neighboring fibers allowing for higher initial cell proliferation and differentiation. Cells seeded upon porous foams needed to lay down extracellular matrix and migrate to make cellular connections before cellular proliferation and differentiation could occur more rapidly. Continuous growth of cells and tissue in dynamic culture causes the clogging of pores in foam scaffolds while nonwoven fiber mesh scaffold pores appear to be open indicating the ability for longer term perfusion cultures.