Differentiation of Adipose-Derived Stem Cells on Bone Tissue Engineering Scaffolds

Anthony J. Wirtel[1], Michelle D. Kofron[1], Addisu Mesfin[2], Joshua Li[3], Cato T. Laurencin[1,3,4]
[1]University of Virginia Department of Biomedical Engineering
[2]Drexel University College of Medicine
[3] University of Virginia Department of Orthopaedic Surgery
[4] University of Virginia Department of Chemical Engineering

Statement of Purpose: Approximately 800,000 bone graft procedures are conducted annually in the United States. While the gold standard of care is use of autograft bone, alternatives are needed. Bone graft substitutes have been developed but are limited by osteogenicity, osteoinductivity or osteoconductivity. Use of scaffolds in conjunction with marrow-derived mesenchymal stem cells has been shown effective but requires harvest of cells from bone marrow cavities and is limited by supply and need for painful surgery. Zuk [1] demonstrated that adipose tissue is a source of multipotent stem cells inducible through media supplementation into lineages for osteoblasts, fibroblast and adipose cells. We hypothesize that cells derived from a population of human adipose stromal cells can be driven to differentiate toward an osteoblast lineage while seeded on sintered microsphere scaffolds of bioerodible polymers.

Methods:

Manufacture of scaffolds: Microspheres were prepared from 85:15 poly(lactide-*co*-glycolide, PLAGA) *through* a solvent evaporation technique described previously [2], and selected for a size range of 600-710μm. Scaffolds were fabricated by sintering solid microspheres above the glass transition temperature in a 10mm mold.

Derivation of tissue: Adipose tissue was harvested from the human infrapatellar fat pad during total knee replacement and conducted with approval by the University of Virginia's Institutional Review Board. Tissue was rinsed, manually dissected into sections less than $2mm^3$ and digested in 1% collagenase. The cell suspension was centrifuged and supernatant removed. Cells were resuspended and counted with a hemocytometer and plated at a density of $7{,}000/cm^2$ in control media (DMEM + 10% FBS and 1% antibiotics). Cells were expanded through 4 passages and plated onto PLAGA scaffolds at a density of 50,000 cells per scaffold. Plates were fed with either control media or induction media [control media + 0.2μM dexamethasone (Sigma) + 50.0μM ascorbic acid (Fisher Scientific, Inc.) + 10 mM ß–glycerophosphate (Sigma, Inc.)]. Unseeded scaffolds served as controls throughout experiments. Cells were evaluated for proliferation, morphology and phenotypic expression after 3, 7, 14, ands 21 days and additionally at 28 days specifically for mineralization.

Proliferation and Alkaline phosphatase Expression: Proliferation was measured by cellular DNA analysis with a Pico Green assay (Invitrogen, Inc.) and compared to a standard curve using a Tecan spectrophotometer. Alkaline phosphatase was measured through colorimetric assay (BioRad, Inc.) and compared to a standard curve.

SEM Imaging: Cells on scaffolds were fixed in gluteraldehyde and dehydrated with an ethanol series and imaged with a JEOL JSM 6400 at 15kV.

Mineralized Matrix Measurement: Scaffolds were stained with Alizarin Red S (Sigma, Inc.). Stain was released with cetylpyridinium chloride and analyzed for optical density at 550nm.

Results and Discussion: Proliferation as determined by DNA quantification indicated that cell growth between control and induction media groups was not statistically different. Levels of alkaline phosphatase increased over time but differences between control and induction groups were not statistically significant (*figure 1*), matching results previously reported [1]. Measurement of alizarin red indicated a significant increase in mineralized matrix at day 14, 21 and 28 for scaffolds exposed to induction media (*figure 2*). SEM suggested the presence of mineralized matrix on scaffolds.

Our results indicate that adipose-derived stromal cells can be driven towards an osteoblastic phenotype and will proliferate when seeded on PLAGA scaffolds developed for musculoskeletal tissue engineering applications. To our knowledge this is the first reported use of adipose-derived cells driven to an osteoblast lineage on sintered PLAGA microsphere scaffolds. This is an exciting result and will potentially allow use of easily harvested tissue as an adjunct to bone tissue engineering scaffolds capable of supporting cell attachment, proliferation as well as phenotypic differentiation.

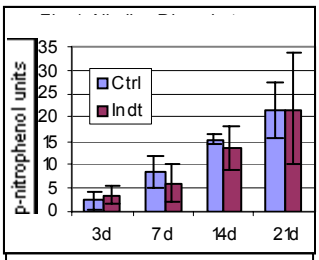

Figure 1: Alkaline phosphatase expression by cells seeded on microsphere-based scaffolds

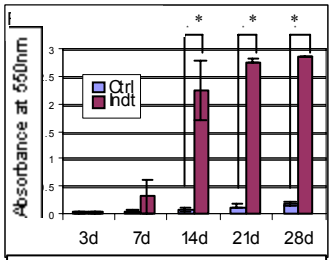

Figure 2: Scaffold mineralized matrix with quantitative Alizarin Red S

Conclusion: This work demonstrates that cells derived from readily accessible adipose tissue might offer a greater range of treatment options when combined with bone graft substitutes. Given the current availability of PLAGA implants approved for various indications by the U.S. FDA, the ability of these scaffolds to support cell differentiation is an appealing development for potential clinical use.

References: ***[1] Zuk PA Mol Biol Cell 2002 Dec;13(12):4279-95. [2] Borden M J Biomed Mater Res. 2002 Sep 5;61(3):421-9***