In Vitro Mineralization of PEUUR and PEUUR/DBM Composite Foams

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Statement of Purpose: Porous poly(esterurethane urea) (PEUUR) scaffolds degrade to non-toxic by-products and support the migration of cells and ingrowth of new tissue *in vivo*.[1,2] These two-component materials can be synthesized by reactive liquid molding, thereby making them potentially suitable for injectable applications. The objective of this study is to investigate *in vitro* differentiation and matrix mineralization of three different cell types in the presence of PEUUR and PEUUR/demineralized bone matrix (DBM) composite foams prepared from lysine triisocyanate (LTI). The clinical goal is to develop injectable PEUUR scaffolds with demineralized bone particles that deliver biologically active components to enhance healing of bone fractures.

Methods: PEUUR foams were prepared by reactive liquid molding of LTI and a hardener comprising polyester polyol, water, catalyst, stabilizer, and pore opener.[2] PEUUR/DBM composite foams (18 and 38 wt-% DBM) were prepared by adding DBM to the hardener prior to adding the LTI. The polyester polyol was a 70/30 w/w poly(ε-caprolactone-*co*-glycolide) triol. The water content in the hardener was 1.5 parts per hundred parts polyol (pphp). Water reacts with LTI to form gaseous carbon dioxide, which functions as a blowing agent. The reactions of LTI with water and polyol were catalyzed by 3 pphp triethylenediamine. Three distinct cell lines (a) Mouse embryonic osteoblast-like cells (MC3T3)–subclone E4, (b) human osteosarcoma cells (MG63), and (c) human mesenchymal stem cells (HMSC) were used to evaluate osteoblast differentiation and matrix mineralization in response to PEUUR and PEUUR-DBM composites. Polymer scaffolds were seeded with $5x10^4$ cell per 10 mg foam and cultured in a 24-well tissue culture plate for 1, 7, and 28 days. For osteoblast differentiation and mineralization, MC3T3 and MG63 cells were cultured in osteogenic media (OS+) containing 50 mg/mL of ascorbic acid, 10 mM β-glycerophosphate. MG63 cells received 10^5 M dexamethasone, while HMSC cells were treated with hMSC Osteogenic Differentiation BulletKit® (Cambrex). Controls were treated with plain media (OS-). To detect osteoblast mineralization, 8 µg/mL of Tetracycline HCl was added 2 days prior to harvest. Media samples and cell lysate were collected and assayed for calcium, phosphorous, alkaline phosphatase, and osteocalcin by ELISA after 1, 7, and 28 days. Polymer foams seeded with cells were fixed in 2% paraformaldehyde for histology or 2.5% glutaraldehyde for SEM-EDAX and FT-IR analysis. Immunofluorescent staining was performed on the foams seeded with cells for OSX, Runx2, ALP osteoblast differentiation markers. Mineralization staining for phosphorous (Von Kossa) and calcium (alizarin red) was performed on the polymer samples at 28 days.

Results/Discussion: SEM and histology showed good cell attachment and proliferation on LTI and LTI-DBM composite foams, suggesting the material is biocompatible and promotes osteoblast cell attachment and proliferation (**Figure 1**). MC3T3, MG63, and HMSC cells showed increased expression of ALP with LTI-DBM composites by Day 7 when compared to LTI alone, suggesting LTI-DBM favored early differentiation of osteoblast cells. Immunofluorescence staining for ALP, Type-I collagen, OSX, and RUNX-2 showed increased expression with LTI-DBM in the presence of OS+ media when compared to OS- controls. Tetracycline labeling of HMSC (**Figure 2**), MC3T3, and MG63 cells seeded on LTI and LTI-DBM foams showed mineralized extracellular matrix after 28 days in the presence of osteogenic media, while no mineralization was observed in controls. This suggests that LTI and LTI-DBM composites favored cell proliferation, differentiation, maturation, and matrix mineralization for each of the three cell types. Furthermore, Von Kossa and Alizarin red staining, FT-IR, and SEM-EDAX confirmed greater cell/matrix mineralization with LTI-DBM composites than with LTI alone.

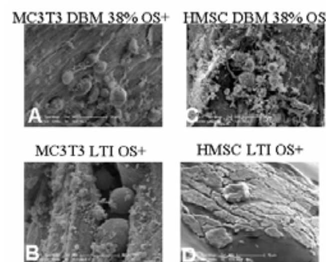

Figure 1. SEM of LTI-DBM -38% (A & C) and LTI (B & C) show MC3T3 and HMSC cells after 28 days.

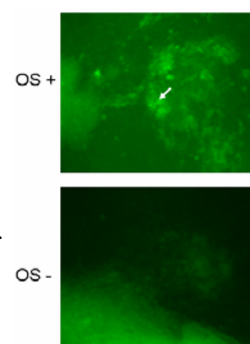

Fig 2. LTI foams seeded with HMSC labeled with tetracycline after 28 days.

Conclusion: All the materials promoted osteoblast mineralization in the presence of osteogenic media when compared to non-osteogenic media. However, the extent of mineralization and the expression of osteoblast differentiation factors varied between cell lines and polymers.

References:
1.Zhang, J.-Y.*et al.*, *Biomaterials* **2000,** 21, 1247-1258.
2.Guelcher, S. A *et al.*,, *Tissue Eng 2006*,12:.

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