Biodegradation of Polyurethane Tissue Engineering Scaffolds

Andrea E. Hafeman,[1,2] Katarzyna J. Zienkiewicz,[1,2] Lillian M. Nanney,[3] Jeffrey M. Davidson,[4,5] Scott A. Guelcher[1,2]
[1]Dept. of Chemical Engineering, [2]Center for Bone Biology; [3]Dept. of Plastic Surgery, Dept. of Cell Biology,
[4]Dept. of Pathology; Vanderbilt University; [5]Dept. of Veterans Affairs, TVHS; Nashville, TN, USA

Statement of Purpose: Biodegradable polyurethanes (PUR) hold much promise for application in wound healing and tissue engineering. They have demonstrated biocompatibility and support of new tissue formation in skin [1] and bone [2], as well as nerve and cardiac regeneration. However, understanding the material degradation, as well as the cellular response, is pivotal to the success of such biomaterials *in vivo*. These materials ideally degrade and clear from the body by natural processes, with a limited inflammatory response. Aliphatic polyester PURs typically degrade by hydrolysis of the ester linkages, but they undergo significantly accelerated bulk degradation *in vivo*, suggesting cell-mediated effects. It has been reported that enzymatic activity and oxidative stress from reactive oxygen species secreted from macrophages contribute to *in vivo* degradation [3,4]. In this study, we investigated the *in vivo* cellular response and degradation mechanisms of biodegradable, porous PUR scaffolds.

Methods: Two-component PUR scaffolds were synthesized by gas foaming of hexamethylene diisocyanate trimer (HDIt) or lysine triisocyanate (LTI) with 900-Da polyester triols [1]. Scaffold discs (10x2mm) were implanted into dorsal excisional wounds of Sprague-Dawley rats. Material degradation and dermal healing were assessed up to 28 days by histological sections and staining with CD68 and PGP9.5 antibodies to identify cell populations responsible for the material degradation. Scaffolds were also cultured *in vitro* with RAW264.5 murine macrophages for 2 to 8 weeks, then fixed and vacuum-dried for SEM imaging to evaluate cell morphology and material degradation. To investigate cell-mediated degradation mechanisms, triplicate 25-mg samples were incubated *in vitro* with PBS buffer control, hydrolytic enzymes (cholesterol esterase, carboxyl esterase, and lipase), and 20-wt% (H_2O_2) with cobalt chloride (generates oxygen radicals). Each week, for up to 8 weeks, the materials were vacuum-dried and weighed to assess mass loss. Degradation products were analyzed with HPLC and colorimetric-based assays specifically for α-hydroxy and isocyanuric acids, lysine, & ethanolamine.

Results: In all cases, the rates of PUR degradation and new tissue formation correlated directly. The materials consistently degraded faster *in vivo* than *in vitro*, although they followed similar trends as *in vitro* (ie: LTI materials degrade at a faster rate than HDIt materials). Histology and antibody staining showed that materials were transiently surrounded by macrophages and foreign body giant cells (Fig. 1). After the material remnants were completely resorbed, giant cells were no longer evident. SEM images of PUR scaffolds cultured with RAW264.5 macrophages revealed adherent cells (Fig. 1) and some giant cell formation, along with material surface pitting.

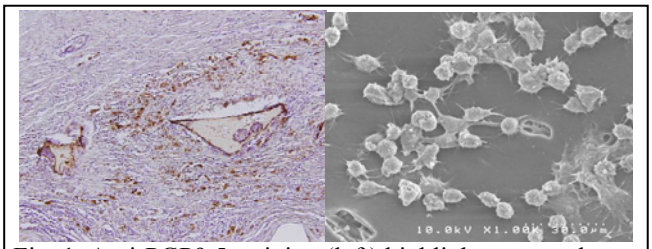

Fig. 1. Anti-PGP9.5 staining (left) highlights macrophages surrounding material remnants. SEM image of RAW264.5 cells with PUR scaffolds after 2w *in vitro* culture (right).

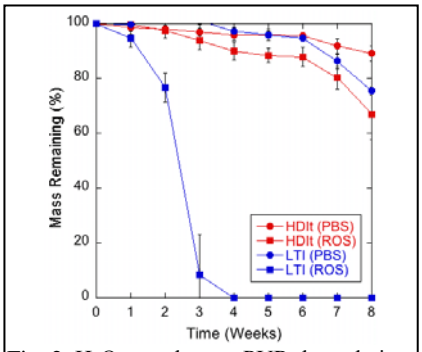

Fig. 2. H_2O_2 accelerates PUR degradation.

LTI materials generated more α-hydroxy acid fragments than the HDIt materials at any given time point. Incubation with hydrolytic enzymes accelerated material degradation slightly, with little difference among the three candidate enzymes. The H_2O_2 solution had a significantly greater effect on PUR degradation, especially for LTI-based materials (Fig. 2). Preliminary analysis of the degradation products suggests that the reactive oxygen species (ROS) may degrade the isocyanate hard segments as well as the soft segments, with greater production of lysine fragments in the ROS media.

Conclusions: These biodegradable PUR materials demonstrate potential as a template for tissue regeneration. They degrade completely to soluble products, which is important for their biocompatibility and clinical performance. The surface pitting was consistent with chain scission and loss of low-MW segments, which was not seen until much later in buffer. These pits likely form directly under the adhered macrophages, in pockets of high ROS concentration. These PUR materials lack hydrogen-bonded hard segments, which may allow ROS to access otherwise-protected labile bonds. Furthermore, ester hydrolysis in the lysine residue and isocyanate backbone may create a locally low pH to further accelerate degradation (as likely also occurs in the soft segment). Such extensive hard segment oxidation has not been documented before in biodegradable polyurethanes, and appears to be specific to lysine-derived polyurethanes.

References: 1. Hafeman et al. *Pharm Res* 2008, 2. Li et al. *Biomaterials* 2009. 3. Christenson et al. JBMRA 2004. 4. Santerre et al. *Biomaterials* 2005.

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