Synthesis of a Novel Injectable, ROS-degradable Tissue Engineering Scaffold

John R. Martin, Mukesh K. Gupta, Jonathan M. Page, Scott A. Guelcher, Craig L. Duvall
Department of Biomedical Engineering, Department of Chemical and Biomolecular Engineering – Vanderbilt University

Statement of Purpose: Injectable, biodegradable poly(ester-urethane) scaffolds possess tunable chemical, mechanical, and biological properties and create porous, cell-inductive tissue scaffolds that are desirable for tissue engineering applications[1]. However, hydrolytic degradation of polyester polymer-based scaffolds generates α-hydroxy acid byproducts that decrease local pH. This triggers an autocatalytic degradation mechanism that can lead to rapid, late-stage scaffold mechanical failure[2]. Here, a novel injectable, reactive oxygen species (ROS)-degradable polyurethane-based scaffold has been developed. The scaffold is formed from polythioketal (PTK) prepolymers that are degraded by cell-generated ROS, but not by hydrolysis[3]. Because the PTK-urethane (PTK-UR) formulation selectively degrades by cell-mediated activity, it is predicted to avoid the autocatalytic degradation mechanism and yield better matched rates of cell infiltration and scaffold degradation.

Methods: A family of PTK copolymers were synthesized via the condensation polymerization of 2,2-dimethoxy propane and varying ratios of 1,4-butanedithiol (BDT) and 2-mercaptoethyl ether (MEE). The homobifunctional thiol end-groups were converted to hydroxyl groups by a post-polymerization nucleophilic displacement reaction. Polymer molecular weights were measured by gel permeation chromatography, and copolymer compositions were confirmed by ^{1}H NMR spectroscopy. PTK-UR 3D scaffolds were fabricated using the different PTK copolymers and hexamethylene diisocyanate trimer (HDIt), with the scaffolds' porous architecture being verified with scanning electron microscopy. Mechanical characterization of these scaffolds was accomplished using dynamic mechanical analysis (DMA), with scaffold properties' being quantified under both dry and wet conditions. *In vitro* degradation kinetics of the PTK-UR scaffolds were determined by measuring temporal mass loss of samples incubated in accelerated *in vivo*-mimicking oxidative media (20 wt% H_2O_2 in 0.1M $CoCl_2$) vs. control scaffolds incubated in PBS at 37°C.

Results: Five PTK copolymers were synthesized and fully characterized. These copolymers were named according to the percentage of MEE monomers that comprised the polymer backbone and included 100%, 75%, 50%, 25%, and 0% MEE PTK copolymers. GPC showed that the polymers were all M_n ~ 1000Da with PDI ~1.35, and ^{1}H-NMR analysis verified that the resulting copolymer compositions matched the targeted values (±4% of desired MEE content). DMA characterization of all of the PTK-UR scaffolds showed that they their mechanical properties were all equivalent and were comparable to polyester-based polyurethanes[2]. Unlike polyester-based scaffolds, they the PTK-UR did not have any difference in modulus between wet and dry scaffold conditions. For the degradation studies, the control PTK-UR scaffolds incubated in PBS show no significant degradation over five weeks. Conversely, the scaffold degraded rapidly in oxidative media that mimics accelerated in vivo degradation conditions (**Figure 1).** There was a consistent trend for higher %MEE (the more hydrophilic of the 2 monomers) leading to faster degradation at the earlier time points, but composition did not have a significant effect at the later time points. For simplicity of presentation, data is only shown for 100%, 50%, and 0% MEE-PTK-URs.

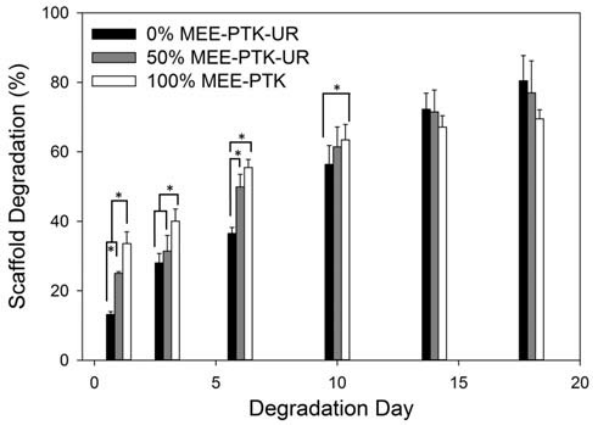

Figure 1. Degradation profiles of PTK-UR scaffolds in accelerated oxidative media.

Conclusions: A new family of PTK copolymers was successfully polymerized and utilized to fabricate PTK-UR scaffolds. There was no clear effect of PTK composition on DMA elastic moduli under either wet or dry conditions. However, unlike hydrogen-bonded polyester-urethane scaffolds, there was also no significant difference between wet and dry properties of these PTK-UR scaffolds. The PTK-UR scaffolds were stable and did not degrade significantly when incubated in PBS over five weeks. However, they showed significant degradation in oxidative conditions as shown in **Fig. 1**. Moreover, the oxidative degradation rates of the PTK-URs followed approximately first-order degradation kinetics, rather than exponential degradation rates typical of autocatalytic degradation of polyesters. PTK-UR scaffolds having more hydrophilic content (100% MEE-PTK-UR > 0% MEE-PTK-UR) degraded faster than the more hydrophobic scaffolds, likely due to increased infiltration of the oxidative media into the more hydrophilic scaffolds. Current studies are exploring cytocompatibility and degradation rate of PTK-UR scaffolds in vivo.

References: **1.** Guelcher et al. Tissue Eng Pt B-Rev 2008, 14, 3-17. **2.** Hafeman et al. Biomaterials 2011, 32, 419-429, **3.**Wilson et al. Nat Mater 2010, 9, 923-928.