Porcine Wound Healing Testing of ROS-degradable Poly(thioketal) Urethane Scaffold Variants

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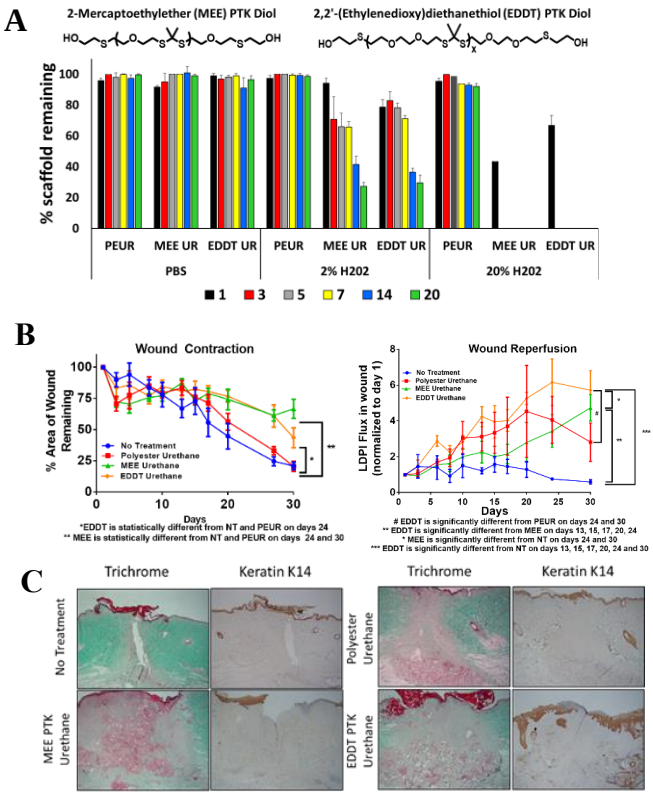
Statement of Purpose: We have recently developed and tested reactive oxygen species (ROS) responsive 2-mercaptoethyl ether poly(thiol ketal) urethane (MEE-PTK-UR) scaffolds in rats and pigs. PTK scaffolds more effectively stent wounds and promote filling of the wound bed with healthy, non-fibrotic granulation tissue relative to more conventional, hydrolytically-degradable scaffolds which suffer from autocatalytic degradation and are prone to contracture and formation of poor quality tissue[1,2,3]. Here, we hypothesize that by integration of more hydrophilic PTK diols into the PTK-UR scaffolds, we can increase rapid resorption of scaffold degradation products in order to reduce the in vivo inflammatory response and promote more effective tissue repair.

Methods: A novel 2,2′-(Ethylenedioxy) diethanethiol EDDT PTK diol (shown in Fig A) was synthesized using a condensation polymerization method similar to the MEE PTK diols (described previously[2,3]). Urethane scaffolds were synthesized using lysine triisocyanate (LTI) via liquid reactive modeling. Oxidative degradation of MEE and EDDT PTK-UR scaffolds along with hydrolytically-degradable polyester urethane (PE-UR) scaffolds was assessed in PBS and H_2O_2 (2% and 20%). In vivo testing of PE-UR, MEE-PTK-UR, and EDDT-PTK-UR scaffolds was carried out in 2 x 1 cm full-thickness excisional skin wounds in an 8-week-old female pig. The wounds were either left empty or filled with the 3 candidate scaffold types. Blood perfusion was quantified using laser Doppler perfusion imaging. The pigs were sacrificed 30 days post treatment, and wounds were histologically evaluated with trichrome staining and Keratin K14 IHC.

Results: We successfully synthesized EDDT-PTK-UR scaffolds with a more hydrophilic PTK diol. MEE- and EDDT- PTK-UR scaffolds both showed selective dose dependent degradation in oxidative media at 37°C. After 20 days of incubation in 2% H_2O_2 (which we have found closely predicts in vivo degradation rate), 35% of both PTK-based scaffolds remained. No significant degradation differences were seen between MEE and EDDT PTK UR scaffolds. Histological analyses showed closure of wounds treated with all three UR scaffold types 30 days after wound creation. PTK-UR treated wounds showed enhanced stenting compared to PE-UR treated and untreated wounds (Fig B). No significant difference in rate of wound closure was observed between the two different PTK-UR scaffolds. Both MEE- and EDDT- PTK-UR had better wound stenting properties compared to PE-UR and empty wounds allowing for more isotropic collagen organization and decreased formation of aligned collagen and myofibroblasts (to be quantified). Blood perfusion



studies demonstrated no significant change in blood perfusion in untreated wounds relative to day 1 of wounding. However, PE, EDDT and MEE PTK treatments showed increasing levels of blood flow during a 30 day period. The more hydrophilic EDDT-PTK-UR treated wounds had significantly higher blood perfusion compared to MEE PTK treatments between days 13-24 (Fig B). Histological assessment of wounds showed robust granulation tissue in MEE- and EDDT- PTK-UR scaffold-treated wounds. The more hydrophilic EDDT-PTK-UR treated wounds showed qualitatively higher re-epithelization compared to MEE PTK UR scaffold treated wounds (Figure C).

Conclusions: A new PTK diol was used to create a novel PTK-UR foam. EDDT- and MEE- PTK-UR scaffolds outperformed unfilled and PE-UR scaffolds in terms of wound stenting. The new EDDT-PTK-UR scaffolds promoted robust granulation tissue formation / re-epithelialization. This experimental formulation outperformed all other groups in terms of wound perfusion, potentially driven by an ideal combination of cell-mediated degradation, wound stenting, and rapid resorption of hydrophilic degradation products.

References: [1] Patil et al. Tissue Eng. Part C 2017 [2] Martin et al. Adv Healthcare Mater 2016 [3] Martin et al. Biomaterials 2014