Chitosan Based Hydrogels for Delivering Oxygen and Sequestering Reactive Oxygen Species in Wound Healing

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Statement of Purpose: Oxygen treatment is known to promote chronic wound healing; however, current oxygen therapies are inadequate for broad patient care. Our recent invention of crosslinked chitosan hydrogels with immobilized perfluorocarbons (MACF) allows for the creation of hydrogel dressings that harness perfluoro-carbon (PFC) oxygenation abilities long-term while also sequestering reactive oxygen species (ROS). The overall goal of this work is to test a unique inductive hydrogel scaffold containing specifically tethered PFCs to chitosan. We hypothesize that MACF hydrogels will deliver beneficial levels of oxygen, sequester ROS and when applied in vivo on wounds, will promote wound healing responses while mitigating inflammatory responses.

Methods

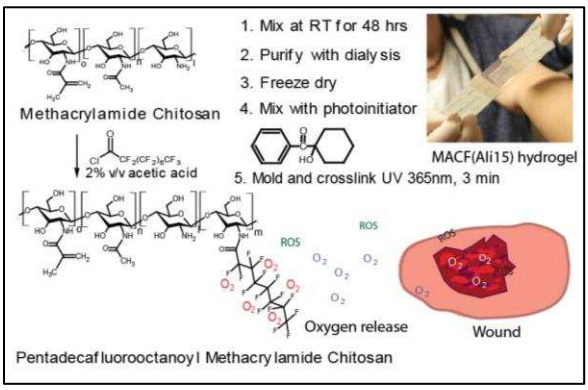

Figure 1. Scheme for creating MACF oxygenating scaffolds.

Materials synthesis, analyses and in vivo testing: MACF was synthesized by methacrylation of chitosan (MAC) followed by fluorination (short chain Ali5, long chain aliphatic Ali15 and aromatic Ar5 PFCs). Hydrogels were prepared from these materials and we tested oxygen uptake and release of these materials. MACFAli15 hydrogels were chosen for further studies as they showed the highest oxygen delivering capacity. We first tested the degradation (in lysozyme solution), oxygen flux (in custom chamber) and ROS sequestering capacity (CellRox assay, nitric oxide radicals, hydroxyl radicals) of MAC and MACF(Ali15) gels via several in vitro assays. For animal testing, full thickness wounds were created, MAC and MACFAli15 hydrogel bandages were applied on excisional wounds in Wistar rats with or without oxygenation and compared to no treatment and healthy skin controls. All hydrogel treatments were placed on wounds and replaced every 2 d for 8 d total. At day 8, animals were sacrificed and wound tissue was taken for metabolomics analysis or fixed for histology. For metabolomics, tissue was digested and extracts were run through TripleTOF mass spectrometer (AB SCIEX) to characterize the secretome at day 8.

Histology and assessment: After fixation, samples were paraffin embedded and sectioned. H&E and Masson's trichrome were performed to quantify host response.

Results and Discussion: We have successfully shown that covalent immobilization of PFCs to chitosan based hydrogels imparts oxygen delivering capacity to the material and MACFAli15 shows sustained delivery of oxygen up to 5 days in vitro[1].

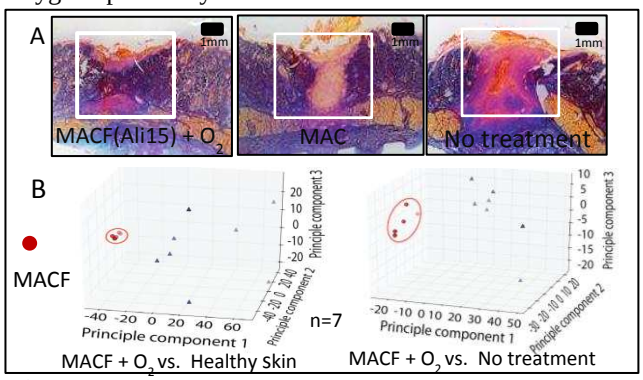

Figure 2. A) Masson's trichrome results from tissue sections show that oxygen saturated MACF (+ O_2) treatment results in superior collagen generation (blue) and regeneration. B) Principle component (PCA) analysis results for metabolomics study shows clear differences between MACF+O_2 treatments vs. healthy skin and no treatment; MACF clustering circled in red.

Results from in vitro oxygen flux measurements revealed that MACFAli15 oxygenated gels sustained a higher O_2 diffusive flux as compared to atmospheric gels (-log(flux) values of 15.68 vs. 14.61 –log(mol/cm^2/s), p = 0.0001). Degradation studies showed that MACF gels degraded 50% by mass after day 3. The ROS studies revealed that MACFAli15 sequesters ROS and reduces oxidative stress better than other hydrogel treatments. Histology analysis results from the animal study revealed the benefits of oxygen delivery to the wound from our saturated MACF hydrogels with enhanced re-epithelialization, neovascularization, and collagen synthesis (Fig. 2A). Analyses of metabolites revealed significantly different metabolic profiles for our treatment versus controls ($p<0.01$, Fig. 2b). This included upregulation /downregulation in key metabolites by up to several units of fold change belonging to wound healing specific pathways such as fatty acid metabolism, steroid hormone synthesis, and arachidonic acid metabolism, etc.

Conclusions: These results demonstrate that oxygen delivery to the wound through MACF hydrogels benefits the wound healing process. Excitingly we show for the first time that the tethered PFCs generate free binding sites enabling ROS to be sequestered away from wound. Now that safety has been demonstrated we are now applying our treatments in full thickness excisional wound pig model, and are especially interested efficacy of our MACF hydrogels and its developmental potential for oxygen delivery in chronic/diabetic wound healing.

[1] Asanka Wijekoon et al., Acta Biomaterialia 9 (2013) 5653–5664