Controlled Oxygen Release to Accelerate Diabetic Wound Healing by Simultaneously Promoting Epithelialization and Angiogenesis, and Decreasing Tissue Inflammation

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Statement of Purpose: Non-healing diabetic wound is one of the most common complications for patients with diabetes. Hypoxia represents a key factor in diabetic wounds that delays the healing process. It contributes to persistent inflammation, and compromised angiogenesis and epithelialization. Therefore, sustained oxygenation of the diabetic wounds to alleviate hypoxia is expected to promote wound healing by decreasing tissue inflammation, and stimulating angiogenesis and epithelialization. Yet sustained oxygenation cannot be readily achieved by clinically available hyperbaric oxygen therapy (HBOT) [1]. To address this issue, we developed a long-term oxygenation system consisting of oxygen-release microspheres and a thermosensitive hydrogel. The hydrogel/microspheres construct was able to continuously release oxygen for more than two weeks. In this work, we characterized (1) the effect of released oxygen on the survival and migration of skin cells in vitro; (2) the efficacy of hydrogel/microspheres construct on accelerating diabetic wound healing in a diabetic mouse model, and (3) the therapeutic effect of the oxygenation system on epithelialization, angiogenesis and tissue inflammation in vivo.

Methods: The oxygen-release microspheres were fabricated by double emulsion. The core and shell of the microspheres were polyvinylpyrrolidone (PVP)/hydrogen peroxide (H_2O_2) and poly(N-isopropylacrymide-co-2-hydroxyethyl methacrylate-co-acrylate-oligolactide-co-N-acryloxysuccinimide), respectively (**Fig. 1A**). PVP forms a complex with H_2O_2 to decrease its diffusivity and release rate. Catalase was immobilized on the polymeric shell surface to timely convert the released H_2O_2 into molecular oxygen. A N-isopropylacrymide-based thermosensitive hydrogel was used to deliver the microspheres. Oxygen release kinetics of the hydrogel/microspheres construct was determined following our previously established procedure [2].

To evaluate the effect of oxygen release on cell survival and migration, HaCaT keratinocyte, human dermal fibroblast (HDF) and human arterial endothelial cells (HAEC) were cultured under 1% O_2, serum free, and high glucose conditions to mimic the environment in diabetic wounds. dsDNA content was measured, and scratch assay was performed. Electron paramagnetic resonance was used to determine the intracellular oxygen content in HaCaT cells. For in vivo study, 5-mm wounds were made on the dorsal skin of diabetic mice (db/db) using a Biopsy punch. Hydrogel (Gel group) or hydrogel/oxygen-release microsphere (Gel/ORM) was injected topically into the skin. The wound size was monitored for 16 days. At days 8 and 16, the mice were euthanized and wound tissues were collected for histological analysis. Epithelialization, angiogenesis and tissue inflammation were evaluated by immunohistochemical staining using cytokeratin 10 (K10)/cytokeratin 14 (K14), isolectin/α-smooth muscle actin (αSMA) and CD86, respectively.

Results: The Gel/ORM construct was injectable at 4°C and solidified within 6 seconds at body temperature. The construct released oxygen continuously for two weeks (**Fig. 1B**). The catalase immobilized on the shell allowed the released H_2O_2 to be completely converted into oxygen at the microspheres surface. In vitro, the released oxygen significantly increased the survival and migration rate of keratinocytes (HaCaT), dermal fibroblasts (HDF) and endothelial cells (HAEC) under hypoxia. The enhanced cell survival was due to elevated intracellular oxygen content, confirmed by electron paramagnetic resonance study. In vivo, the Gel/ORM construct significantly increased the wound healing rate in db/db mice (**Fig. 1C**), with only 10% of the wound size at day 16. K10/K14 stainings revealed that the released oxygen accelerated re-epithelialization process in vivo by promoting keratinocyte migration (**Fig. 1D**). Isolectin/αSMA stainings showed that the blood vessel density in Gel/ORM group was twice of that in the Gel group. CD86 staining demonstrated that the Gel/ORM construct did not induce tissue inflammation. Instead, it significantly decreased the density of pro-inflammatory M1 macrophage.

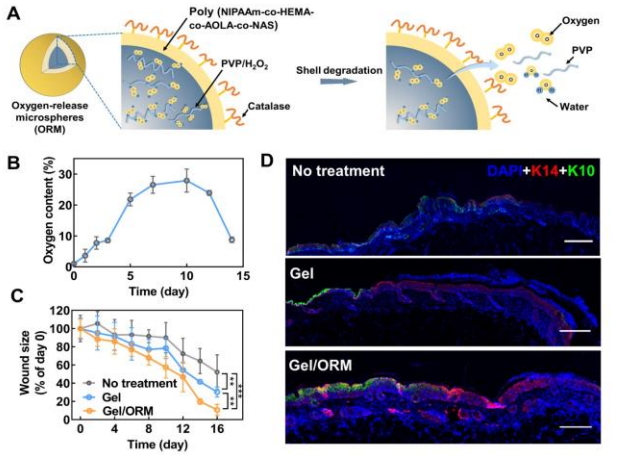

Figure 1. (A) Schematic illustration of the design of oxygen release microspheres; (B) Oxygen release kinetics of hydrogel/microspheres construct over 14 days; (C) Wound closure rate for 16 days post wounding. **p<0.01, ***p<0.001; (D) Immunohistochemical staining of K10 and K14 of the wounds at day 8. Scale bar=200 μm.

Conclusions: A long-term oxygenation system was fabricated to continuously release oxygen in diabetic wounds. The system significantly accelerated wound healing in diabetic mice by promoting epithelialization and angiogenesis, and reducing tissue inflammation.

References: [1] Heyboer III et al. *Advances in wound care* (2017) [2] Fan et al. *Scientific reports* (2018)