Hyperbaric oxygen-generating hydrogels for enhanced wound healing

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Statement of Purpose: Oxygen plays a critical role as a substrate for metabolism and as a signaling molecule regulating cellular activities. In particular, hyperbaric oxygen has been demonstrated to facilitate wound healing processes *via* transient oxidative stress in surrounding tissues. Herein, we report a new type of oxygen-generating biomaterials, hyperbaric oxygen-generating (HOG) hydrogels, that can serve as a bioactive acellular matrix generating hyperbaric oxygen. We designed hydrogel networks *via* calcium peroxide (CaO_2)-mediated oxidative crosslinking reaction with oxygen generation. We characterized its controllable physicochemical properties, including oxygen-releasing behavior. We demonstrate that the HOG hydrogel enhances the proliferative activities of human dermal fibroblasts (HDFs) and endothelial cells (ECs) *in vitro* and promotes wound healing and repair with enhanced tissue ingrowth and neovascularization from the host tissues *in vivo*.

Methods: We synthesized thiolated-gelatin (GtnSH) by conjugating Traut's reagent (TR). The HOG hydrogels were fabricated by simply mixing GtnSH and CaO_2 solutions. The phase transition was determined by the vial tilting methods. We assessed elastic modulus (G') of hydrogels using a rheometric fluid spectrometer. To determine the oxygen levels, we monitored dissolved oxygen (DO) levels using a commercially available oxygen sensor. We evaluated the cytocompatibility of the functionalized polymers and HOG hydrogels using HDFs. To investigate the effect of HOG hydrogels on cellular activities, we cultured human umbilical vein endothelial cells (HUVECs) with different types of hydrogels (normoxic, NG *vs.* hyperoxic, HG) for up to three days. To confirm the *in vivo* angiogenic effect of the HOG hydrogels, we transplanted the two types of hydrogels (NG *vs.* HG) subcutaneously into mice and examined them for up to 7 days. We also investigated the therapeutic efficacy of the HOG hydrogels for wound healing using the mouse skin defect models.

Results: We fabricated HOG hydrogels *via* the CaO_2-mediated oxidative crosslinking reaction to form disulfide bonds, which induce hydrogel networks. (Fig. 1a). To evaluate hydrogel formation and gelation kinetics, we determined phase-transition time depending on degree of substitution (DS) of TR (12.9-79.0 μmol/g of polymer), polymers (3-7 wt%) and CaO_2 concentrations (0-0.75 wt%), demonstrating higher concentrations of TR, GtnSH polymer and CaO_2 induced faster hydrogel formation (ranged from 49 secs to 34 min). We monitored the time-course elastic modulus of the hydrogels depending on TR, polymers, and CaO_2 concentrations, resulting in controllable mechanical properties (30-3700 Pa). We next investigated oxygen-generating kinetics and sustained oxygen release depending on CaO_2 concentrations. We noticed that higher CaO_2 contents induced higher DO_{max} levels (G5C0.25, 47.3% pO_2; G5C0.5, 62.0% pO_2; G5C0.75, 70.6% pO_2; G5C1 83.1% pO_2) and prolonged oxygen release for 12 days (Fig. 1b). We next evaluated the cytotoxicity of HOG hydrogels using HDFs, showing excellent cell viability in optimal conditions. Also, we investigated the effect of HOG hydrogels on the cell proliferation, demonstrating that the HG group exhibited enhanced cell proliferative activity compared to the control group (at day 1: HDFs, 121.4%; HUVECs, 128.5%; at day 3: HDFs, 327.3%; HUVECs, 126.4%). We examined the therapeutic efficacy of the HOG hydrogels for wound healing. Interestingly, we found that the HG matrices facilitated re-epithelialization and wound remodeling with enhanced tissue infiltration and neovascularization compared to the NG matrices (Fig. 1c).

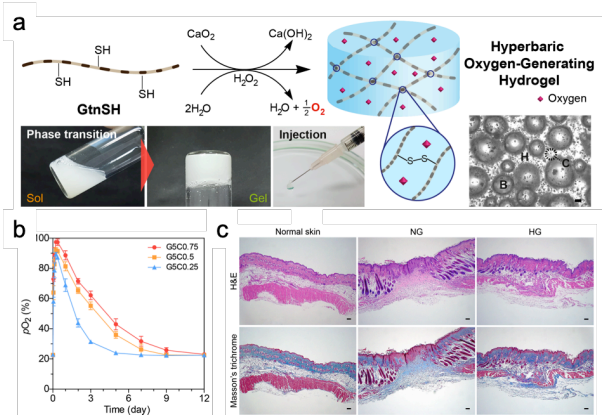

Figure 1. Schematic representation of gel formation and digital images of sol-to-gel phase transition and hydrogel injection **(a)**. Oxygen-releasing kinetics **(b)** depending on CaO_2 concentrations **(c)**. Histological sections of hydrogels stained with H&E and Masson's trichrome. Scale bars, 100 μm.

Conclusions: We developed a new class of biomaterials that can generate hyperbaric oxygen and act as an injectable and dynamic matrix. The HOG hydrogel has controllable physicochemical properties and oxygen generation by varying the CaO_2 content. The HDFs and HUVECs treated with HOG hydrogels showed enhanced cell proliferative activities. Moreover, the HOG hydrogel promoted wound healing with tissue ingrowth and vascular cell recruitment *in vivo*. To the best of our knowledge, this is a new type of *in situ* crosslinkable HOG hydrogel material. We propose that our HOG hydrogels have great potential as an advanced oxygen-generating biomaterial for a wide range of possible applications, including treatment of wound and vascular disorders as well as tissue regenerative medicine applications.

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