Cell membrane repair protein delivered by a ROS-scavenging hydrogel promotes diabetic wound healing

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Statement of Purpose: Non-healing chronic wound represents a major complication for patients with diabetes[1]. It is characterized by impaired skin tissue homeostasis, prolonged inflammation, and decreased skin cell migration and proliferation. In the diabetic wounds, the elevated content of reactive oxygen species (ROS) leads to lipid peroxidation, which damages cell membrane and causes cell apoptosis. Among those affected cells, hair follicle stem cells (HFSCs) play a key role in initiating wound healing. Ito et al., demonstrated that HFSCs migrate to the epidermal defect, and assist in re-epithelialization, angiogenesis and dermal regeneration following wounding[2]. MG53, a tripartite motif (TRIM) family protein, has been identified as an essential component of the cell membrane repair machinery. However, due to the short half-life of MG53, the therapeutic efficacy of MG53 is largely limited when it is directly administrated into wounds or indirectly delivered by intravenous injection. In the present study, we investigated the function of MG53 in promoting HFSC survival under ROS condition, and develop a novel ROS scavenging hydrogel (AHPPE gel) to encapsulate rhMG53 for the treatment of chronic wounds in diabetic mice.

Methods: The AHPPE gel was synthesized by free radical polymerization of NIPAAm (N-Isopropylacrylamide), (Hydroxyethyl) methacrylate (HEMA) and 4-(acrylato)phenylboronic acid pinacol ester (AHPPE). The chemical structure was confirmed by ^{1}H NMR. RhMG53 was dissolved in 6% hydrogel for the release study. At days 1, 3, 7, 10, 14, and 21, the supernatant was collected, and the fresh release medium was added. The concentration of released rhMG53 in the medium was determined by Bradford protein assay. HFSCs were cultured in the complete media supplemented with 1% penicillin-streptomycin. HFSCs were treated with serum-free medium (control), H_2O_2 (100 μM), MG53 (1 μg/mL) without H_2O_2, and MG53 with H_2O_2. Protein lysates were collected and western blot was then performed. Antibodies used were phosphor-anti-STAT3 (p-STAT3, abcam), anti-STAT3 (STAT3, abcam), and GAPDH (Cell signaling). The chronic wound healing experiment was performed on 10-week old female BKS db/db mice. Two full-thickness wounds were created using a 5 mm biopsy punch on each mouse. Wounds were left open with no dressing and then immediately received 200 μL hydrogel solution, 200 μL rhMG53/hydrogel solution, or rhMG53 (2mg/kg in saline) via subcutaneous injection. The wound size was measured using a digital caliper. Blinded measurement of wound size was performed. Tissue samples were collected at day 8 and immunohistochemistry staining was performed using rabbit anti-cytokeratin 14 (K14).

Results: The AHPPE gel was soluble at 4°C, and can be easily injected through a 27 G needle. The gel exhibited a sol-gel temperature of 18.5°C, and the gelation time at 37°C was 6 seconds. MG53 was gradually released from the AHPPE gel for 21 days (Fig.1a). The release in the first 7 days was relatively faster. After 7 days, the release became slower and relatively linear. The wound closure in the db/db mice without treatment was largely delayed (Fig.1b). The treatment of AHPPE gel resulted in a faster wound closure rate. Administration of rhMG53/AHPPE gel further accelerated the wound closure. K14 staining demonstrated that HFSCs density was significantly higher in the rhMG53/AHPPE gel group compared with no treatment group (Fig.1c). Western blot analysis showed that the p-STAT3 expression was upregulated with rhMG53 treatment under H_2O_2 (Fig.1d).

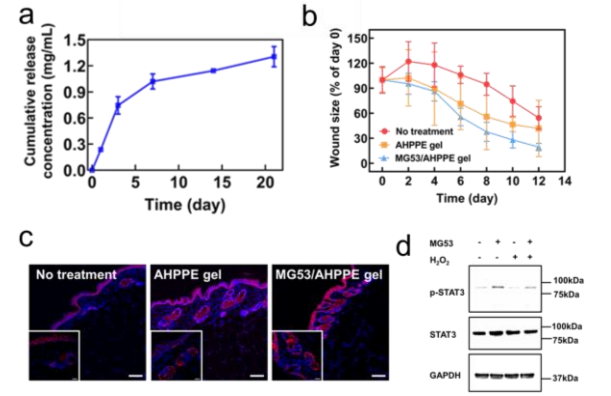

Figure 1. **a.** In vitro release profile of rhMG53 in AHPPE gel. **b.** Wound closure rate for 12 days. Wound size ratios were normalized to day0 (n>6). **c.** K14 staining (10X and 40X) of tissue samples at day8. Scare bar = 100 μm and inset scale bar = 20 μm. **d.** Western blotting of protein expression on HFSCs.

Conclusions: A thermo- and ROS- dual sensitive AHPPE gel was developed to deliver rhMG53. The rhMG53 and AHPPE gel facilitated chronic diabetic wound healing, likely due to increase of HFSCs density. The STAT3 signaling was involved in the MG53-mediated HFSCs protection from oxidative stress.

References:

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2. Ito M., et al. Nature Medicine, 2006, 12(1).