Topical Hyaluronic Acid- Metformin Conjugate Hydrogel for Frostbite injury

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INTRODUCTION

Military personnel operating in extreme cold environments are at risk for frostbite, which can cause tissue necrosis, fibrosis, and permanent disability [1]. Current field-deployable treatments do not adequately prevent chronic tissue damage.

Hydrogels can provide a moist wound environment and serve as efficient drug-delivery platforms. Hyaluronic acid (HA), a natural polymer and key extracellular matrix component, promotes cell proliferation, migration, and angiogenesis. Metformin (Met), commonly used for diabetes, also exhibits pro-angiogenic, anti-inflammatory, and autophagy-modulating effects when applied topically [2, 3].

We have developed a HA–Met conjugate hydrogel designed to provide both a protective wound environment and biologically active modulation of inflammation, angiogenesis, and extracellular matrix remodeling. HA supports cell migration and structural repair, while Met activates pro-angiogenic and anti-inflammatory pathways and modulates tissue remodeling responses, thus combining beneficial properties of both into a single sustained-delivery topical gel. We hypothesized that the HA–Met conjugate would enhance structural and functional skin regeneration following cold-induced injury.

AIM OF THE STUDY

To determine whether topical HA–Met conjugate hydrogel improves skin regeneration and reduces fibrosis following frostbite injury in a rat model.

MATERIALS AND METHODS

- A frostbite model was established in 10-week old female rats by applying a 1 cm-diameter dry ice pellet to dorsal skin for 1 minute under anesthesia (Fig. 1A).
- Four lesions were created per animal.
- Rats were randomly assigned to three groups (n = 4 rats/group) and treated as follows, 1: Untreated control; 2: HA hydrogel (0.2 mL/day); 3: HA-Met hydrogel (0.2 mL/day) applied topically for 14 days (Fig. 1B, C).
- At 2 weeks post-treatment, wound healing was assessed histologically using H&E, Masson's Trichrome, (MT), and Picrosirius Red under polarized light (PSR PL).
- Pathological changes in the epidermis and dermis were semi-quantified using ImageJ software.
- The data were analyzed using Mann-Whitney test. A p-value below 0.05 denoted significant differences between the two groups.

RESULTS

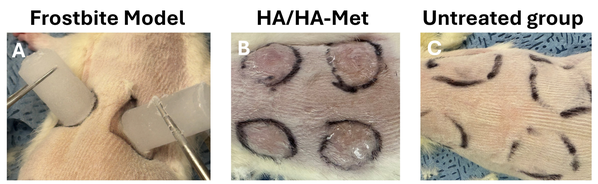

Fig. 1 Experimental frostbite model and treatment groups. (A) Induction of frostbite by applying a dry ice pellet to the rat dorsal skin for 1 minute. (**B**) Daily topical application of HA or HA–Met hydrogel for 14 days. (**C**) Untreated control group.

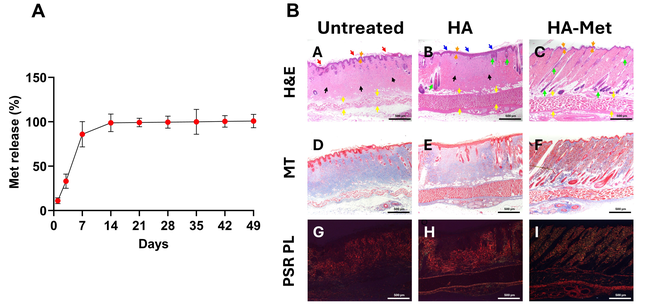

Fig. 2 Met is released from HA-Met conjugate in a time-dependent manner (A). HA–Met treatment results in enhanced tissue repair and regeneration compared with HA and control treatments assessed by H&E, MT, and PSR PL **(B).** Black scale bars 500 μm; white scale bars: 500 μm.

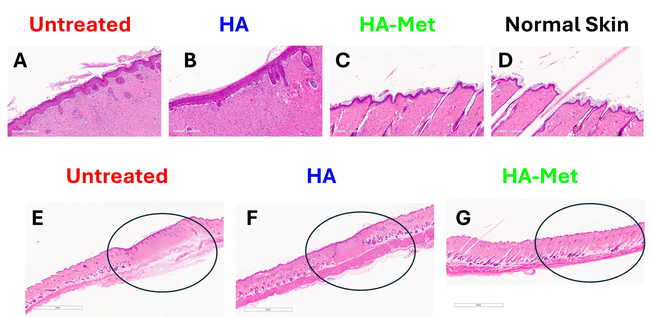

Fig. 3 HA–Met treatment more effectively reduces pathological tissue changes than HA treatment alone. HA-Met group has thinner epidermis and less scar tissue. Epidermis thickness measured in 10X magnification slides (**A-D**). Pathological changes were measured in the 1X slides (black oval, **E–G**).

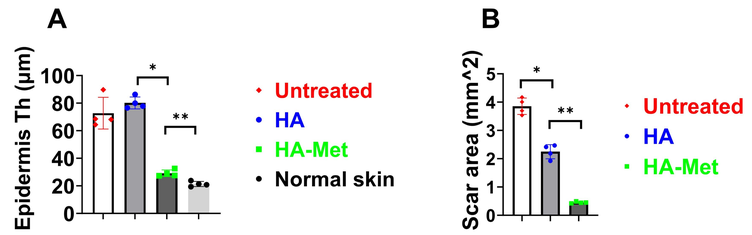

Fig. 4 HA–Met treatment significantly reduces both epidermal thickness (A) and scar tissue area (B) relative to comparison groups, whereas HA alone reduces scar tissue formation without affecting epidermal thickness. n = 4. *p; **p < 0.05.

DISCUSSION

- HA–Met treatment improved epidermal organization and reduced abnormal epidermal thickening after frostbite injury.
- HA–Met reduced scar area and promoted more organized collagen remodeling compared with HA alone or no treatment.
- These findings suggest that conjugating metformin to HA provides biological benefits beyond those of the HA hydrogel vehicle alone.
- Larger studies are needed to confirm efficacy, optimize dosing, and evaluate functional recovery and safety.

SIGNIFICANCE

A locally applied HA–Met hydrogel may provide a practical treatment for reducing tissue damage and fibrotic healing after frostbite in military operational settings.

ACKNOWLEDGEMENTS

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