Hydrolyzed collagen powder dressing has direct favorable effects on wound inflammation, perfusion, and tensile strength

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ABSTRACT

BACKGROUND: Collagen, a key component of the extracellular matrix, play critical roles in the regulation of different phases of wound healing either in its native, fibrillar conformation or as soluble components in the wound milieu. Collagen-based dressings are commonly used in wound care. The objective of this study was to test the effect of a hydrolyzed collagen powder (HCP) on the quality of healing with emphasis on resolution of wound inflammation, perfusion, closure, and tensile strength of the repaired skin. **METHODS:** Murine wound macrophages were isolated using CD11b magnetic beads. Circular sterile PVA sponges (8 mm Θ; soaked in HCP 1g/ml or PBS) were subcutaneously implanted on the back of adult C57BL/6 mice. Sponges were harvested in early (d3) and late (d10) inflammatory phases. Harvested wound macrophages were analyzed for phagocytosis, efferocytosis, PMA-induced ROS production and intracellular cytokine levels. To study wound closure and tensile strength, two 6 mm full-thickness wounds were made on the dorsal skin of mice. The wounds were splinted with a silicon sheet to prevent contraction thereby allowing wounds to heal through granulation and re-epithelialization. HCP was applied topically (50 mg/wound), and the wound was covered with a semi-occlusive dressing (Tegaderm™). Wound planimetry was performed at specified times and blood flow was analyzed using a laser speckle imager. Breaking strength of the healed murine skin was quantified using a tensile tester (TestResources 100R, Shakopee). **RESULTS:** HCP treatment improved phagocytosis in wound macrophages. PMA-inducible ROS was blunted by HCP in late inflammatory wound macrophages (p<0.05; n=8). HCP-treated wound macrophages were more active in efferocytosis (p<0.05; n=7). In d10 of HCP treatment, intracellular pro-inflammatory cytokines were downregulated, and anti-inflammatory cytokines were potentiated in wound macrophages (p<0.05; n=5). Studies on wound closure showed significant improvement in response to topical HCP (p<0.05; n=8). HCP also improved wound perfusion (p<0.05; n=8) and increased the tensile strength of the treated wounds (p<0.05; n=3). **CONCLUSIONS:** This work demonstrates that treating wounds with HCP dressing may reactivate the wound healing process by potently inducing the resolution of inflammation, improved wound perfusion and accelerated closure. Higher tensile strength of HCP treated wounds are likely to minimize wound recurrence.

BACKGROUND

- Wound inflammation is regulated by multiple factors including extracellular matrix (ECM) rich wound environment.
- Macrophages play a key role in wound healing and tissue repair by secretion of cytokines and chemokines that regulates the inflammatory process.
- The timely resolution of acute inflammation is essential to proper healing.
- Such resolution of inflammation is accomplished by the transition of macrophage phenotype from inflammatory type to reparative one.
- Collagen, a vital element of the extracellular matrix, modulates wound healing phases in its fibrillar or soluble forms and has been used as an adjunct therapy to enhance wound healing.
- Native collagen can be denatured and hydrolyzed. The advantages of hydrolyzed collagen (HC) are that it is highly soluble, easily absorbed and distributed in the human body.
- In this work we have explored a specific hydrolyzed collagen powder (HCP) composed of hydrolyzed fragments of Type I Bovine Collagen that are approximately 1/100th the size of native collagen. HCP provides a moist environment for surgical sites and wounds.

OBJECTIVE

Determine the efficacy of HCP on the quality of wound healing

METHODS

- **HCP impact on immune responses:** Murine model: C57bl/6 mice. Polyvinyl alcohol (PVA) sponges were soaked in HCP solution (1g/ml) and were subcutaneously implanted. Wound macrophages (CD 11 b+) were harvested on d3, d7 and d10 post implantation
- **HCP impact on wound closure:** Murine model: C57bl/6 mice. Two 6 mm full thickness splinted wounds were placed on the dorsal skin, equidistant from the midline and adjacent to the 4 limbs. Each of the 2 wounds were treated with HCP 0.8mg/mm^2 on wound topically starting on the day of wounding. Imaging: Digital planimetry (digital imaging for wound size measurement) and wound perfusion (laser speckle imaging LSI). Tensile strength measurement for skin quality: d10 post wounding, wound area was excised for tensile strength measurement

RESULTS

HCP induced phagocytosis, efferocytosis and ROS production in early and late inflammatory phase murine wound macrophages

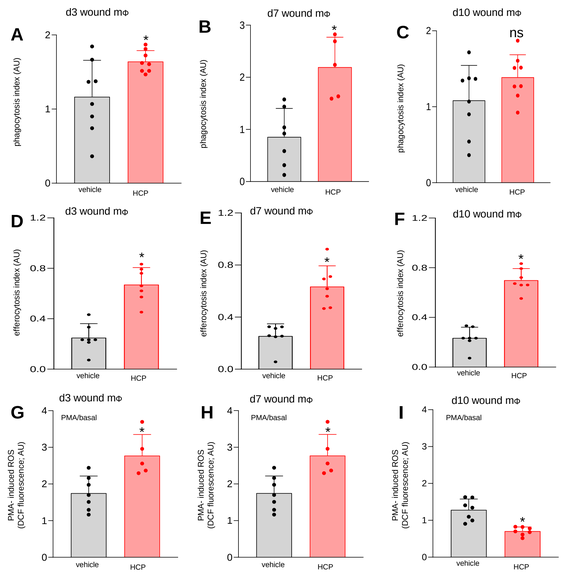


Figure 1. (**A-C**) Phagocytosis was measured using fluorescein-labeled E. coli (K-12 strain). Data represent mean ± SEM (n = 5-8); *p<0.05 compared to vehicle-treated.
(**D-F**) Efferocytosis index of apoptotic thymocytes engulfed by macrophages, calculated as total number of apoptotic cells engulfed by macrophages in a field of view divided by total number of macrophage present in the same field of view. Data represent mean ± SEM (n = 7). *p<0.05 compared to vehicle-treated.
(**G-I**) PMA-induced ROS production was measured using H2DCF-DA after PMA (1 µg/mL) stimulation for 30 min. Data are expressed as fold-change compared with the basal level. Data represent mean ± SEM (n = 8); *p<0.05 compared to vehicle-treated.

HCP significantly potentiated pro-inflammatory cytokines in wound macrophage of early inflammatory phase

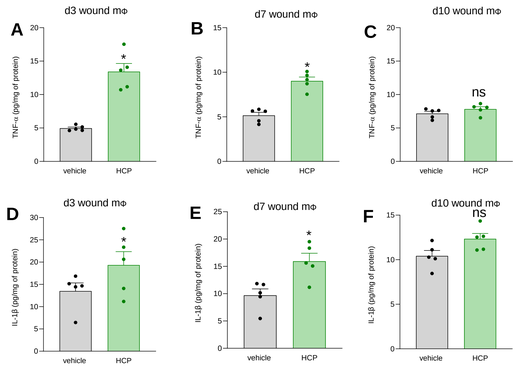


Figure 2. PVA sponges were treated with HCP (1 g/ml), implanted subcutaneously following incisional wounding in C57BL/6 mice. Day 3, 7 and 10 wound macrophages were harvested from the sponges. Intracellular cytokines were measured from the macrophage cell lysate using cytokine array and normalized to total protein of lysate. Shown are data for TNF-α (**A-C**) and IL-1β (**D-F**). Data represent mean ± SEM (n = 5). *p<0.05 compared to vehicle-treated.

HCP induced anti-inflammatory cytokines in wound macrophage of late inflammatory phase

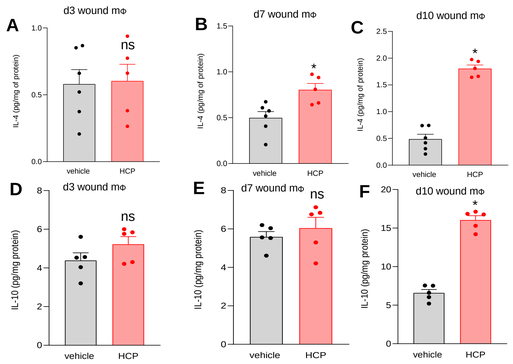


Figure 3. PVA sponges were treated with HCP (1 g/ml), implanted subcutaneously following incisional wounding in C57BL/6 mice. Day 3, 7 and 10 wound macrophages were harvested from the sponges. Intracellular cytokines were measured from the macrophage cell lysate using cytokine array and normalized to total protein of lysate. Shown are data for IL-4 (**A-C**) and IL-10 (**D-F**). Data represent mean ± SEM (n = 5). *p<0.05 compared to vehicle-treated.

HCP significantly improved blood flow and wound closure

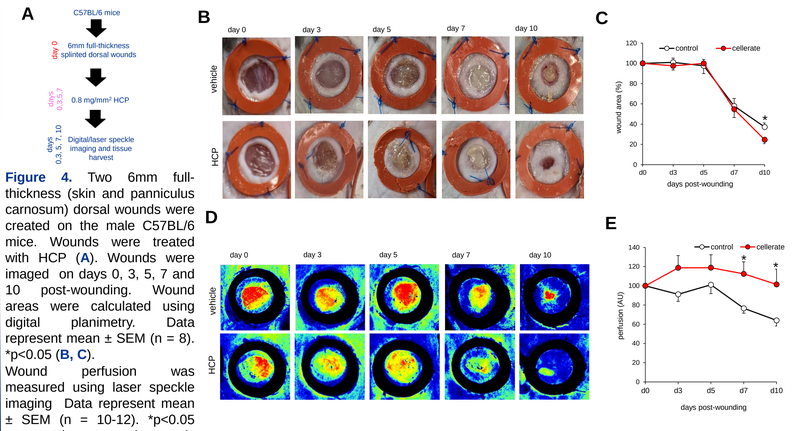


Figure 4. Two 6mm full-thickness (skin and panniculus carnosum) dorsal wounds were created on the male C57BL/6 mice. Wounds were treated with HCP (**A**). Wounds were imaged on days 0, 3, 5, 7 and 10 post-wounding. Wound areas were calculated using digital planimetry. Data represent mean ± SEM (n = 8). *p<0.05 (**B, C**).
Wound perfusion was measured using laser speckle imaging Data represent mean ± SEM (n = 10-12). *p<0.05 compared to control wounds (**D, E**).

HCP potentiated the expression of VFGF in murine wound of late inflammatory phase

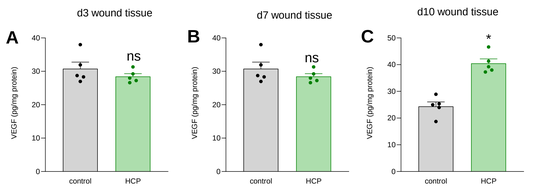
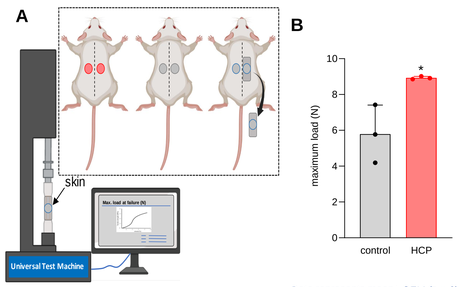


Figure 5. Day 3, 7 and 10 wound tissue were harvested, and cytokines were measured from the tissue lysate and normalized to total protein of lysate. Shown are data for VEGF (**A-C**). Data represent mean ± SEM (n = 5). *p<0.05 compared to control (untreated).

HCP significantly improved the tensile strength of the repaired skin



Data represents mean± SEM (n = 3)
p<0.05 compared to control (untreated)

Figure 6. (**A**) Two 6mm full-thickness (skin and panniculus carnosum) dorsal wounds were created on the male C57BL/6 mice. Inset shows paired 6mm wound (red) in a mouse (left). Following 10 days post-wounding (right), 12 X 20mm strip of the skin containing the wound at the center was excised (bottom) and mantled on Universal Test Machine for the measurement of load required as breaking strength.
(**B**) Tensile strength was measured on repaired skin of closed wounds on d10 post-wounding.

SUMMARY OF OBSERVATIONS

1. **HCP induced phagocytosis, efferocytosis and ROS production in murine wound macrophages**
2. **HCP potentiated pro and anti-inflammatory cytokines at appropriate stages of wound healing**
3. **HCP improved wound perfusion and closure**
4. **HCP improved the tensile strength of closed wounds**

CONCLUSION

This work demonstrates that the HCP-based wound dressing induces production of anti-inflammatory and pro-angiogenic cytokines and facilitates polarization of wound-site macrophages towards pro-healing phenotype which helps in quality healing.

ACKNOWLEDGEMENTS

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