Hydrolyzed collagen powder dressing improves wound inflammation, perfusion and tensile strength

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ABSTRACT

BACKGROUND: Collagen, a key component of the extracellular matrix, plays critical roles in the regulation of the phases of wound healing either in its native, fibrillar conformation or as soluble components in the wound milieu. Collagen-based dressings are extensively used in wound care. The objective of this study was to test the effect of 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 (**mϕ**) were isolated using CD11b magnetic beads. Circular sterile PVA sponges (8 mm Θ; soaked in HCP 1g/ml or saline) were subcutaneously implanted on the back of adult C57BL/6 mice. Sponges were harvested in early (day 7) and late (day 10) 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 16 x 8 mm full-thickness excisional wounds were made on the dorsal skin of mice. HCP was applied topically (100 mg per 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, MN). **RESULTS:** HCP treatment improved phagocytosis in wound macrophages. PMA-induced ROS was blunted 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 d7 of HCP treatment, intracellular pro-inflammatory cytokines were down-regulated 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 treatment ($p<0.05$; n=8). HCP treatment improved wound perfusion ($p<0.05$; n=8) and increased the tensile strength of the closed wounds. ($p<0.05$; n=7). **CONCLUSIONS:** In summary, this work demonstrates that treating wounds with HCP dressing reactivates the wound healing process by potently inducing the resolution of inflammation, improved wound perfusion, accelerated closure. Higher tensile strength of HCP treated closed wounds are likely to minimize wound recurrence.

BACKGROUND

- Wound inflammation is regulated by multiple factors including extracellular matrix (ECM) rich wound environment. Post-injury, degradation products of ECM elicit cell signaling which modulates the inflammation and boosts wound healing. 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. The non-resolving persistent inflammation leads to wound chronicity.
- 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 healing due to its central role in regulating several of these processes. Collagen-based wound dressings are biocompatible and are extensively used in wound care.
- Native collagen can be denatured and hydrolyzed with acids, alkali or thermal treatment (with enzymatic digestion) to produce low molecular weight (3–6 KDa) peptides with unique physicochemical and biological properties compared to the native form. The advantages of hydrolyzed collagen (HC) are that it is highly soluble, easily absorbed and distributed in the human body, cost effective, easily emulsified and stabilized for use. It possesses both antioxidant and antimicrobial activities and can stimulate cell proliferation and migration, wound healing, and have biomechanical and antimicrobial characteristics when utilized in nanofibrous scaffolds. HC is available as a power dressing for moderate to heavy exudative wounds.
- 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

- **HCG 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 d7 and d10 post implantation
- **HCG impact on wound closure:** Murine model: C57bl/6 mice. Two 8 X 16 mm full thickness excisional 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 HCG 100 mg/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: d4 post wound closure (d25 post wounding), wound area was excised for tensile strength measurement

Results

HCP induced phagocytosis, efferocytosis and ROS production in *early* (day 7) and *late* (day 10) inflammatory phase murine wound macrophages

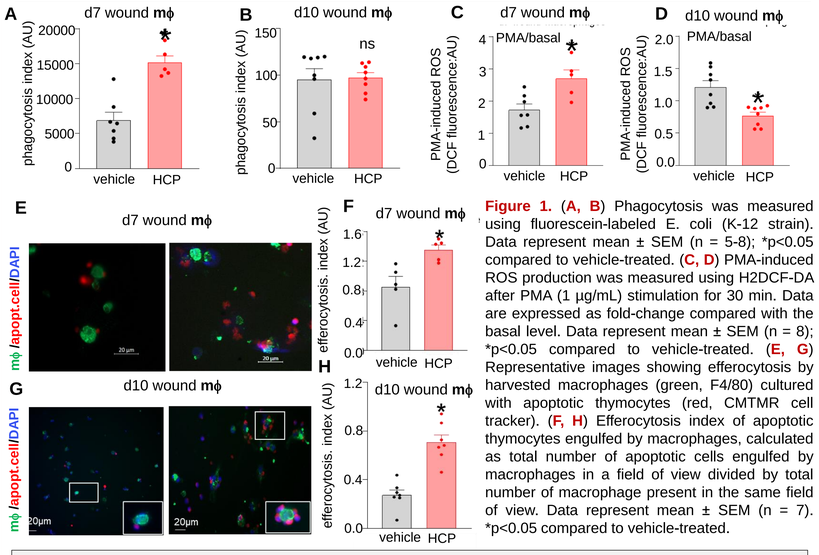

Figure 1. (**A, B**) Phagocytosis was measured using fluorescein-labeled E. coli (K-12 strain). Data represent mean ± SEM (n = 5-8); *p<0.05 compared to vehicle-treated. (**C, D**) PMA-induced ROS production was measured using H2DCF-DA after PMA (1 μg/mL) stimulation for 30 min. Data are expressed as fold-change compared with the basal level. Data represent mean ± SEM (n = 8); *p<0.05 compared to vehicle-treated. (**E, G**) Representative images showing efferocytosis by harvested macrophages (green, F4/80) cultured with apoptotic thymocytes (red, CMTMR cell tracker). (**F, H**) 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.

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

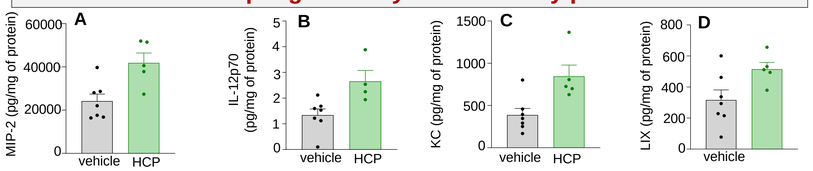

Figure 2. PVA sponges were treated with HCP (1 g/ml), implanted subcutaneously following incisional wounding in C57BL/6 mice. Day 7 wound macrophages were harvested from the sponges. Intracellular cytokines were measured from the macrophage cell lysate using cytokine array and normalized to total protein of lysate. Shown are data for **A.** Macrophage inflammatory protein (MIP-2), **B.** Interleukin 12 (IL-12p70), **C.** Keratinocyte chemoattractant (KC), **D.** LIX/CXCL-5 Data represent mean ± SEM (n = 4-7). *p<0.05 compared to vehicle-treated.

HCP significantly attenuated pro-inflammatory cytokines in wound macrophage of *late* inflammatory phase.

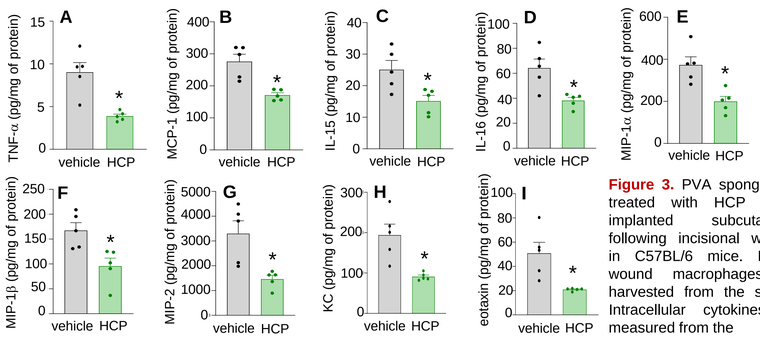

Figure 3. PVA sponges were treated with HCP (1 g/ml), implanted subcutaneously following incisional wounding in C57BL/6 mice. Day 10 wound macrophages were harvested from the sponges. Intracellular cytokines were measured from the macrophage cell lysate using cytokine array and normalized to total protein of lysate. Data represent mean ± SEM (n = 5). *p<0.05 compared to vehicle-treated. Shown are data for **A.** Tumor necrosis factor – alpha (TNF-α), **B.** Monocyte chemoattractant protein 1 (MCP-1), **C.** Interleukin 15 (IL-15), **D.** Interleukin 15 (IL-16), E. Macrophage inflammatory protein 1 alpha (MIP-1α), F. Macrophage inflammatory protein 1 beta (MIP-1β), , **G.** Macrophage inflammatory protein 2 (MIP-2), **H.** Keratinocyte chemoattractant (KC), **I.** Eotaxin. Data represent mean ± SEM (n = 4-7). *p<0.05 compared to vehicle-treated.

HCP induced anti-inflammatory cytokine IL-4 in *early* inflammatory phase and potentiated pro-resolution cytokine IL-10 at *late* inflammatory phase

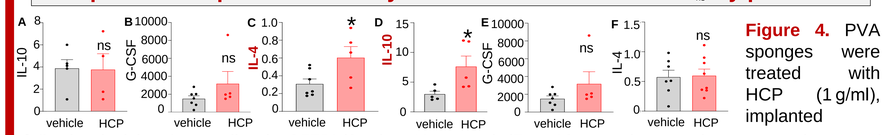

Figure 4. PVA sponges were treated with HCP (1 g/ml), implanted following incisional wounding in C57BL/6 mice. Harvested d7 or 10 wound macrophages were harvested and tested for levels of IL-10 (**A,D**), G-CSF (**B,E**) and IL-4 (**C,F**). Values are shown as pg/mg of protein. Data represent mean ± SEM (n = 4-7). *p<0.05 compared to vehicle-treated.

HCP potentiated the expression of VEGF in murine wound macrophage of late inflammatory phase

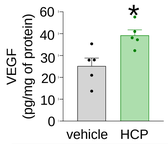

Figure 5. PVA sponges were treated with HCP (1 g/ml), implanted subcutaneously following incisional wounding in C57BL/6 mice. Day 10 wound macrophages were harvested from the sponges. VEGF level was measured from the macrophage cell lysate using cytokine array and normalized to total protein of lysate. Data represent mean ± SEM (n = 5). *p<0.05 compared to vehicle-treated.

HCP significantly improved blood flow and wound closure

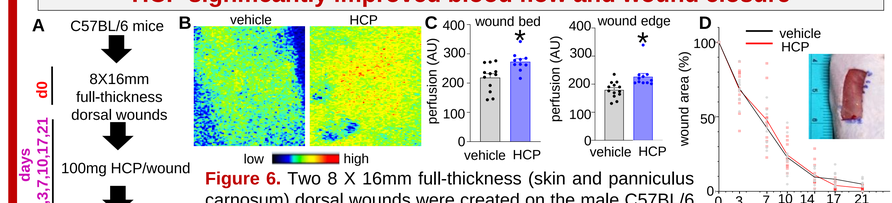

Figure 6. Two 8 X 16mm full-thickness (skin and panniculus carnosum) dorsal wounds were created on the male C57BL/6 mice. Wounds were treated with 100mg HCP/wound. (**A-C**) Wound perfusion was measured on day 7 post-wounding using laser speckle imaging Data represent mean ± SEM (n = 10-12). *p<0.05 compared to control wounds. (**D**) Wounds were imaged on days 0, 3, 7, 10, 14, 17 and 21 post-wounding. Wound areas were calculated using digital planimetry. Data represent mean ± SEM (n = 8). *p<0.05 compared to control wounds.

HCP significantly improved the tensile strength of the repaired skin

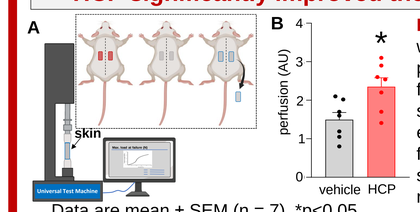

Figure 7. (**A**) Two 8 X 16mm full-thickness dorsal wounds were created on the male C57BL/6 mice. Inset shows paired 8 X 16mm wound (red) in a mouse (left). Following four days wound closure (right), 12 X 20mm strip of the skin containing the closed wound at the center was excised (bottom) and mantled 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 d25 post-wounding. Data are mean ± SEM (n = 7). *p<0.05

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.

Acknowledgments

- Product Information: Hydrolyzed Collagen Powder (HCP); Trademark: *CellerateRX®
- Conflict: CK Sen is paid consultant of Sanara MedTech, TX. Current studies were supported with funds from Sanara MedTech. Study design and conduct of experiments were not influenced by input from the company.