Isolation and Characterization of a Fibroblast Sub-Population Responsible for Cutaneous Scarring in the Ventral Dermis

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PURPOSE: Recent studies have demonstrated the functional heterogeneity of fibroblasts, particularly in terms of their activities during wound healing. Both location within the dermis and embryonic lineages provide a means by which we may now identify the sub-populations of fibroblasts chiefly responsible for connective tissue deposition during scar formation in the dorsal dermis. However, whether these findings translate to the ventral dermis have yet to be elucidated.

METHODS: Prrx1Cre/Rosa26mTmG mice, were used to trace two fibroblast lineages restricted to the ventral dermis. Fibroblasts of different embryonic lineages—based on Prrx1 expression—were isolated from ventral fetal and adult dermis at a series of time points, including the late-gestational transition from scarless to scar-forming wound healing. ATAC-seq (Assay for Transposase-Accessible Chromatin with high throughput sequencing) was also performed in isolated pre- and post-gestational fibroblasts.

RESULTS: Histological analysis revealed that the Prrx1-positive lineage contributed to the majority of connective tissue during scar formation. Flow cytometry demonstrated a shift in fibroblast sub-populations over the course of gestation. Differential patterns of chromatin accessibility shown by ATAC-seq (Assay for Transposase-Accessible Chromatin with high throughput sequencing) was also performed in isolated pre- and post-gestational fibroblasts.

CONCLUSIONS: As in the dorsal dermis, fibroblasts of the ventral dermis demonstrate functional heterogeneity. Further studies may allow targeting of specific sub-populations to improve wound healing.

1. Rinkevich Y, Walmsley GG, Hu MS, et al. Skin fibrosis. Identification and isolation of a dermal lineage with intrinsic fibrogenic potential. Science. 2015;348(6232):aaa2151.
2. Lichtenberger BM, Mastrogiannaki M, Watt FM. Epidermal [beta]-catenin activation remodels the dermis via paracrine signalling to distinct fibroblast lineages. Nat Commun. 2016;7.

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Effects of Topical Hyaluronic Acid Injection in Surgical Site Infection Caused by Staphylococcus Aureus

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PURPOSE: Surgical site infection (SSI) is a common post-operative complication, mainly caused by Staphylococcus aureus (S. aureus). In some cases, antibiotics are insufficient for the treatment of S. aureus infection. S. aureus produces hyaluronidase which degrades hyaluronic acid (HA) and is one of its virulence factors in wound infection. HA prevents bacterial proliferation and has anti-inflammatory effects to promote wound healing. Herein, we studied the effects of HA injection with systemic antibiotics treatment on SSI caused by S. aureus.

MATERIALS AND METHODS: A single 2x1cm² open wound was created on each dorsum of 40 Sprague-Dawley rats. The wound bed was stitched three times with 3-0 vicryl suture inoculated with S. aureus (2x10⁸ CFU/ml) to induce SSI. The test group was treated with 200μg/kg of HA(n=20) and the control group received a subcutaneous injection of normal saline (n=20) in the infected wound. All groups were then treated with intraperitoneal 30mg/kg injections of cefazolin. The stitches were removed two days after the procedure. The gross pathology and bacterial count were assessed at days 2, 4, 6 and 8 post-procedure. The histologic grading and inflammatory cytokines in wound were assessed at day 8 post-procedure. Histologic grading was from 0 to 3 (0: none, 1: minimal, 2: moderate, 3: marked) based on the proportion of each finding within the entire wound.
**RESULTS:** The HA group showed significant reduction in the wound area (wound area of day X/wound area of Day0x100) compared to the control group (day8, 26.54±6.12% vs 50.59±5.50%, respectively; p<0.001), which is an assessment of the gross pathology. The HA group showed also significantly superior wound healing to the control group on histological analysis, including assessment of abscess, necrosis, neutrophil infiltration, edema, fibroplasias (4.2±1.2 vs 11.5±2.1, p<0.001). The HA group was significantly lower than the control group on the levels of TNF-α (324.0±134.0pg/ml vs 433.8±119.9pg/ml, respectively; p=0.01) and IL-1ß (329.8±151.7pg/ml vs 481.7±204.5pg/ml, respectively; p=0.011). In addition, the HA group showed significantly lower bacterial counts compared to the control group (day8, 4.66±0.45logCFU/ml vs 5.35±0.37logCFU/ml, p<0.001).

**CONCLUSION:** Immediate local injection of HA in the wound can reduce the occurrence of SSI and can promote wound healing. This suggests that HA injection with antibiotics at the surgical site is effective for preventing or treating SSI caused by *S. aureus*.

**REFERENCES**

1. C.D. Owens, K. Stoessel. Surgical site infections: epidemiology, microbiology and prevention. *Journal of Hospital Infection.* 2008;70(S2):3–10
2. George Makris, John D. Wright, Eileen Ingham, et al. The hyaluronate lyase of *Staphylococcus aureus* – a virulence factor?. *Microbiology.* 2004;15:2005–2013.
3. Manuela G. Neuman, Radu M. Nanau, Loida Oruña, et al. In vitro Anti-Inflammatory Effects of Hyaluronic Acid in Ethanol-Induced Damage in Skin Cells. *J Pharm Pharmaceut Sci.* 2011;14(3):425–437.
4. Naoki Fujimura, Hideaki Obara, Koichi Suda, et al. A novel rat model of incisional surgical site infection model developed using absorbable multifilament thread inoculated with *Escherichia coli*. *J Infect Chemother.* 2015;21(4):312–315.
5. Prasun H Mehta, Kathleen A Dunn, John F Bradfield, et al. Contaminated wounds: infection rates with subcutaneous sutures. *Ann Emerg Med.* 1996;27:43–48.

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**Non-Thermal Plasma Treatment Safely and Rapidly Eradicates MRSA from Infected Wounds**

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**INTRODUCTION:** *Steri-lysis™* technology is a novel, non-thermal plasma (NTP)/free radical, portable device that delivers a highly active reactive oxygen and nitrogen species (RONS) mixture within a closed loop system. Previously, we demonstrated that *Steri-lysis™* technology rapidly and reliably sterilized cell phones, which are well known to harbor microorganisms, including pathogenic *Staphylococcus aureus*. Given the device’s remarkable disinfection efficacy, we then investigated its potential applicability to living tissue, and established that NTP is safe for use on wounded and unwounded skin. Herein, we investigate the effectiveness of NTP treatment of infected wounds in an *in vivo* murine model.

**MATERIALS AND METHODS:** Two 6 mm, full-thickness, splinted excisional wounds were created on both sides of the dorsal midline of C57bl/6 mice, and inoculated with 25 microliters of 1.5 × 10⁸ colony forming units (CFU)/mL methicillin-resistant *Staphylococcus aureus* (MRSA) per wound. Mice were treated with NTP for 10 minutes or 20 minutes, respectively, for a total of 3 treatments every other day for 7 days. Wounds were aseptically swabbed and plated onto trypticase soy agar pre- and post-NTP treatment to monitor interim bacterial reduction during the study period. On day 7, wound tissue was harvested and homogenized for quantitative analysis of CFU/gram tissue remaining.

**RESULTS:** After NTP treatment, there was no gross or histological evidence of residue, aberrant dermal architecture, or edema in any of the wounds or intact skin. Interim agar growth from pre- and post-NTP aseptic swabbing revealed one logarithmic reduction in bacterial load for the 20-minute group, which was subsequently confirmed with serial dilutions of full-thickness, homogenized wound tissue. Wounds inoculated with MRSA and treated with NTP for 20 minutes had a 90% reduction in MRSA CFUs/gram of homogenized tissue (p = 0.0441) compared to untreated wounds. Efficacy of 20 minutes NTP treatment was confirmed by lack of anti-staph ab staining on sectioned wounds.

**CONCLUSION:** Our novel non thermal plasma device effectively disinfects clinically infected wounds in an *in vivo* murine wound healing model, without any evidence of damage to the wound or surrounding tissues. Effective against pathogenic MRSA, *Steri-lysis™* is an innovative, low cost, portable technology that could potentially...