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Other

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ArF excimer laser debrides burns without destruction of viable tissue: a pilot study

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Abstract

According to the American Burn Association, each year in the United States, 40,000 cases require hospitalization with an estimated annual expenditure of over $1 billion in associated costs. Burns are dynamic injuries that progress over time, with extension of depth and size of the burn. Recent investigations at Stony Brook University using tangential debridement, demonstrate that early removal of burn eschar can promote healing. However, the central face, ears, hands and feet, enveloped by thin skin, provide a challenging environment for tangential debridement to experienced burn surgeons. Furthermore, tangential debridement often sacrifices viable tissue and impedes optimal engraftment. Here, data from a pilot study demonstrates that ArF excimer laser irradiation removes burn eschar and enhances healing at 10 days post-burn. ArF excimer laser debridement is self-terminating and preserves underlying and adjacent perfused tissue. Potentially, this modality would be ideal for the complex curvilinear structures of the body.

Brief Communication

Burns are the most common cause of significant cutaneous tissue loss.[1] Burn injuries claim the lives of at least 4,000 United States civilians and military personnel each year, and send another 500,000 to seek medical attention.[2] Approximately 40,000 surviving burn victims require hospitalization, and 25,000 of these are admitted to the 125 specialized U.S. burn centers.[2] This accounts for over 900,000 hospital days and more than $1 billion in annual associated costs, including loss of productivity.[3] Of the burn patients admitted to hospitals, 10% have burns that exceed 30% of the total body surface area.[2] In addition, 25% of people with burns covering more than 75% of total body surface area eventually die.[4]

The current standard of care for deep-dermal or full-thickness burns is early cold steel tangential debridement of eschar followed by immediate autografting. Although tangential debridement has been shown to improve wound closure,[5] it often removes necrotic tissue in addition to underlying and adjacent viable tissue,[6] including viable adipose stem cells.[7] Debridement by excision, even in expert hands, often involves this collateral damage, which could result in excessive scarring and wound contracture. Therefore, there is a need for a more precise method of debridement that could minimize or eliminate the risk of over-excision and retain viable stem cells in the wound bed to enhance repair and improve functional outcomes.

The ArF excimer laser can be thought of as a “smart scalpel”, where it emits short pulses of light at 193 nm (6.4 eV) in the far ultraviolet (UV) region of the electromagnetic spectrum to provide a more precise method of debridement. Pulsed UV light of sufficient fluence will ablate protein. However, far UV at 193nm does not damage biological tissue containing sufficient aqueous chloride ions. This is because aqueous chloride ions absorb radiation at 6.4 eV, causing photodetachment of an electron, a non-thermal process that leaves a neutral chlorine atom and an electron solvated in the water. Eventually, on a time scale that is long compared to ablation and thermal diffusion times, the electrons will encounter and recombine with neutral chlorine atoms, giving up the photodetachment energy to heat, with minimal rise in temperature and a corresponding absence of collateral damage to the surrounding tissue. Eschars are desiccated tissue and fibrin clot that are rich in protein with little, if any, aqueous chloride ions. Thus, exposure of tissue to radiation of sufficient fluence from an ArF excimer laser will debride eschars and any other desiccated tissue while sparing bleeding or hydrated adjacent and underlying tissue. This process has been termed ablative-

photodecomposition[8] and was first explored, understood, and explained at the IBM Thomas J Watson Research Center. The ArF excimer laser produced clean cuts *in vitro*[9] with minimal damage to surrounding tissue. When used *in vivo,* bleeding depletes laser fluence sufficiently to suppress further ablation, due to the presence of chloride ions. This modality has been shown to be significantly more effective than tangential and chemical debridement to treat chemical burns.[10]

In order to demonstrate efficacy of the ArF excimer laser, at Stony Brook University a porcine vertical burn injury progression model[11] was used on two 3-month old female Yorkshire pigs weighing 25-30 kg. The study protocol was conducted following Institutional Animal Care and Use Committee (IACUC) Research Review Board approval. While under general anesthesia, burns were created on the flanks of animals using a 150 gram aluminum bar with dimensions 2.5 cm x 2.5 cm x 7.5 cm, heated in a water bath to 70°C. After blotting the bar dry, it was applied perpendicular to the flank with 2 kg of pressure for 20 or 30 sec. Previous work at Stony Brook has shown that burns created with the aluminum bar heated to 70°C and applied for 20 sec (denoted as 70/20) injured the skin to a depth of the upper reticular dermis, while those heated to 70°C and applied for 30s (denoted as 70/30 burns) injured the skin to a depth of deep-reticular dermis at 28 days.[11] All burns were treated with Vaseline, and protected with Tegaderm. Three days post-burn, the ArF excimer laser source, a repurposed Nidek EC-5000 Corneal Surgery System, was positioned with its output beam emerging 17 cm perpendicularly above the flank of the pig, such that the aiming cross-hairs overlapped on the skin (**Figure 1A**), producing a field of debridement 10 mm in diameter, half on the burn and half on adjacent normal skin (**Figure 1B**). Each laser pulse delivered a fluence of ~150 mJ/cm^2 to the irradiated skin.

For the initial ablation study (N=1 pig, n=1 burn/duration of ablation, **Figure 1**), an ablation cycle of irradiation, set automatically by the laser system for corneal tissue removal to a depth of 200 µm, lasted for ~86 sec, delivering an integrated fluence of ~53 J/cm^2. A second study was performed (N=2 pigs, n=1 burn per condition) to demonstrate the ability of the ArF excimer laser to accelerate burn wound healing (**Figure 2**). For this study, the instrument was set for corneal tissue removal to a depth of 240 µm, hence the ablation cycle lasted for ~104 sec. After each cycle, the field of debridement was blotted dry with gauze, as normal skin bled, while burns did not. At the end of laser debridement, wounds were treated with Vaseline, and protected with Tegaderm. Wound tissue was harvested using 6-8 mm punch biopsies immediately post-debridement (3 days post-burn, **Figure 1C**), or using 8-10 mm punch biopsies 7 days post-

debridement (10 days post-burn, **Figure 1D**). Biopsied tissue was harvested, bisected and fixed in 2% formaldehyde. After 5 µm sectioning, specimens were stained with Haematoxylin and Eosin (H&E) and imaged using an EVOS microscope with an internal camera.

For the initial ablation study, 70/20 burns were debrided three days post-injury for different lengths of time (173, 431, 863, or 1035 sec), followed by harvesting debrided tissue. Debridement for a total of ~173 sec (2 cycles) did not remove the necrotic epidermis (**Figure 1E**, left of black vertical line), while leaving normal skin uninjured (**Figure 1E**, right of black vertical line). Likewise, ~431 sec of debridement (5 cycles) did not completely remove the eschar or destroy the necrotic epidermis (data not shown). However, debridement for a total of ~863 sec (10 cycles) removed necrotic epidermis, sparing underlying adnexae (**Figure 1F**, left of vertical black line). The rete ridges of the normal skin were left intact (**Figure 1F**, right of vertical black line). Debridement for a total of ~1035 sec (12 cycles) resulted in complete removal of necrotic epidermis, considerable inflammation in the upper dermis and loss of adnexal structures (data not shown). This suggested that a cumulative total of 863 sec – 1035 sec for an integrated fluence of ~53 J/cm^2/cycle should be efficacious to debride both superficial and deep-dermal burns.

To demonstrate that laser debridement results in increased healing at 10 days post-burn, 70/20 and 70/30 burns were debrided for a total of ~936 sec (9 cycles of ~104 sec each). The field of debridement was positioned half on normal skin and half on the burn. Debrided burns were compared to non-debrided burns and debrided normal skin as controls. 70/20 burns debrided three days post-burn showed complete removal of burn eschar (**Figure 2A**, right of vertical black line) while sparing the rete ridges and dermis of normal skin (**Figure 2A**, left of vertical black line). The laser-debrided burn showed marked reduction of plugged vasculature in the dermis, as opposed to a non-debrided burn (**Figure 2B**, right of vertical black line). Similarly, laser-debrided 70/30 burn showed an absence of necrotic epidermis (**Figure 2C**, right of vertical black line), while non-debrided burns showed plugged vasculature in the upper dermis and infiltrating clusters of inflammatory cells in the lower dermis (**Figure 2D**, right of vertical black line). Again, adjacent, normal skin was intact (**Figure 2C and D**, left of vertical black lines).

Particularly noteworthy was the observation of dermal regeneration and re-epithelialization in debrided 70/30 burns (**Figure 2E**, right of vertical black line) at 7 days post-debridement (10 days post-burn). In comparison, non-debrided burns (**Figure 2F**, right of vertical black line), showed necrotic tissue in the upper wound and considerable granulation

tissue formation down to the hypodermis. This suggests enhanced healing of 70/30 burns at 10 days post-burn, with early debridement using the ArF excimer laser. Others have shown that enhanced healing at early time-points correlates with reduced scarring.[12] This suggests that debridement of mid-dermal burns using laser irradiation would reduce the depth of scar at 28 days, compared to non-debrided burns

This study presents evidence that irradiation of a thermal burn with an ArF excimer laser can promote healing by selectively debriding necrotic tissue and preserving viable tissue. Future experiments with the ArF excimer laser will determine the optimal time for debridement, the appropriate range of laser fluence for effective debridement, and most importantly determine whether ArF excimer laser debridement has the ability to reduce scar depth at 28 days post-burn.

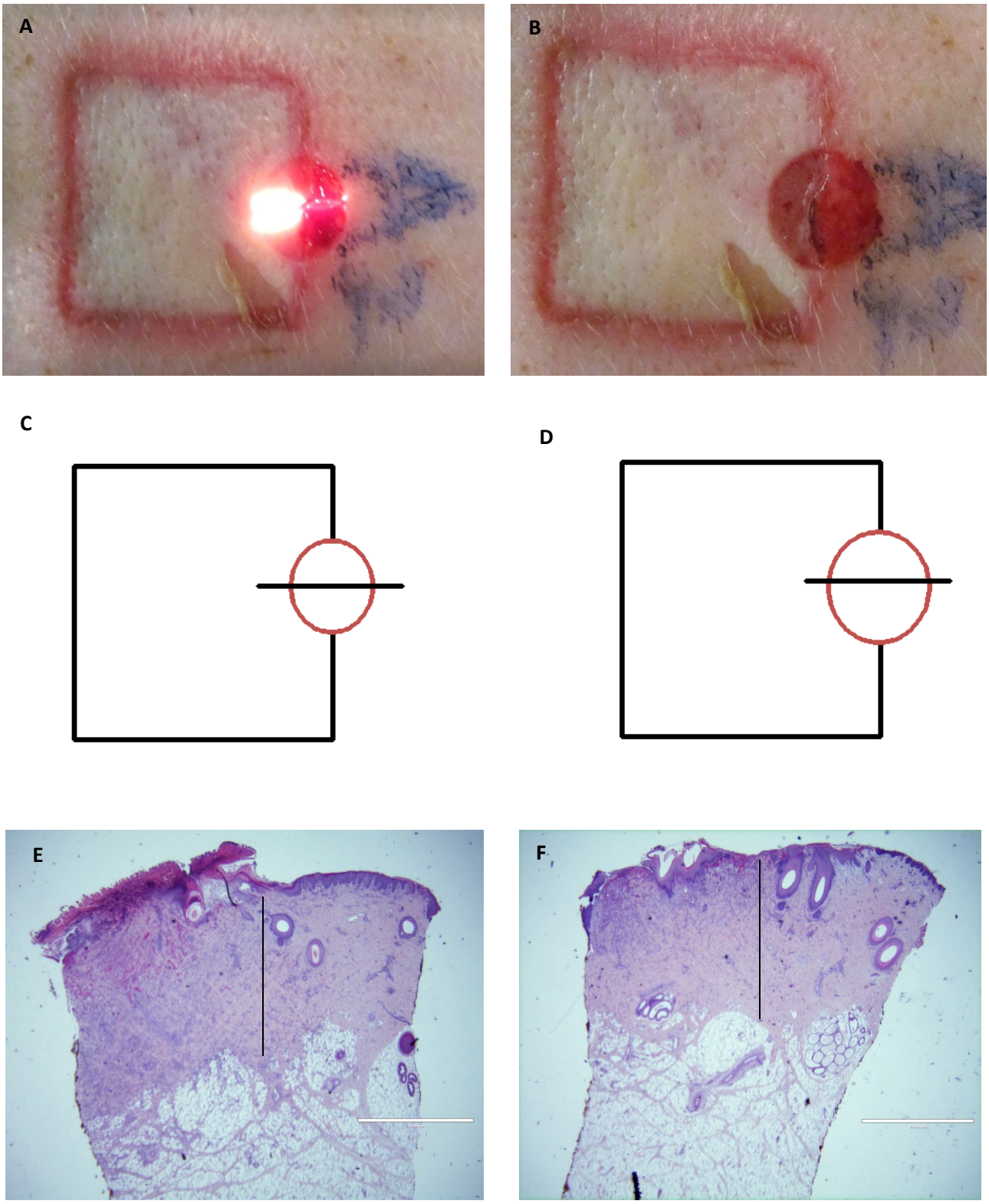

Figure 1. 2.5 x 2.5 cm burns were created on the flanks of pigs using an aluminum bar. Burns and adjacent tissue were debrided via ArF excimer laser irradiation 3 days post-injury. **A.** The aiming cross-hairs of the laser, overlapping at the surface of the skin, were positioned such that a 10mm diameter field of debridement was located half on the burn and half on normal skin. **B.** This produced erythema post debridement (free blood and exudate had been removed). **C.** 6mm

punch biopsies were harvested 3 days post-burn, immediately after laser debridement. Biopsies were bisected and stored in formalin till sectioning. 5µ sections of bisected wounds were stained using Hematoxylin and Eosin stain. **D.** 8mm punch biopsies were harvested 10 days post-burn, 7 days after laser debridement. Biopsies were bisected and stored in formalin till sectioning. 5µ sections of bisected wounds were stained using Hematoxylin and Eosin stain. **E.** 70/20 burn debrided for ~173 sec showed a necrotic epidermis (left of vertical black line. Normal skin (right of vertical black line) reveals no destruction of dermis, with rete ridges intact. **F.** Debridement of burn for ~863 sec showed removal of necrotic epidermis (left of vertical black line) with clusters of inflammatory cells at the edge of debridement. Normal skin remained intact even with debridement (right of vertical black line). Scale bar=2mm.

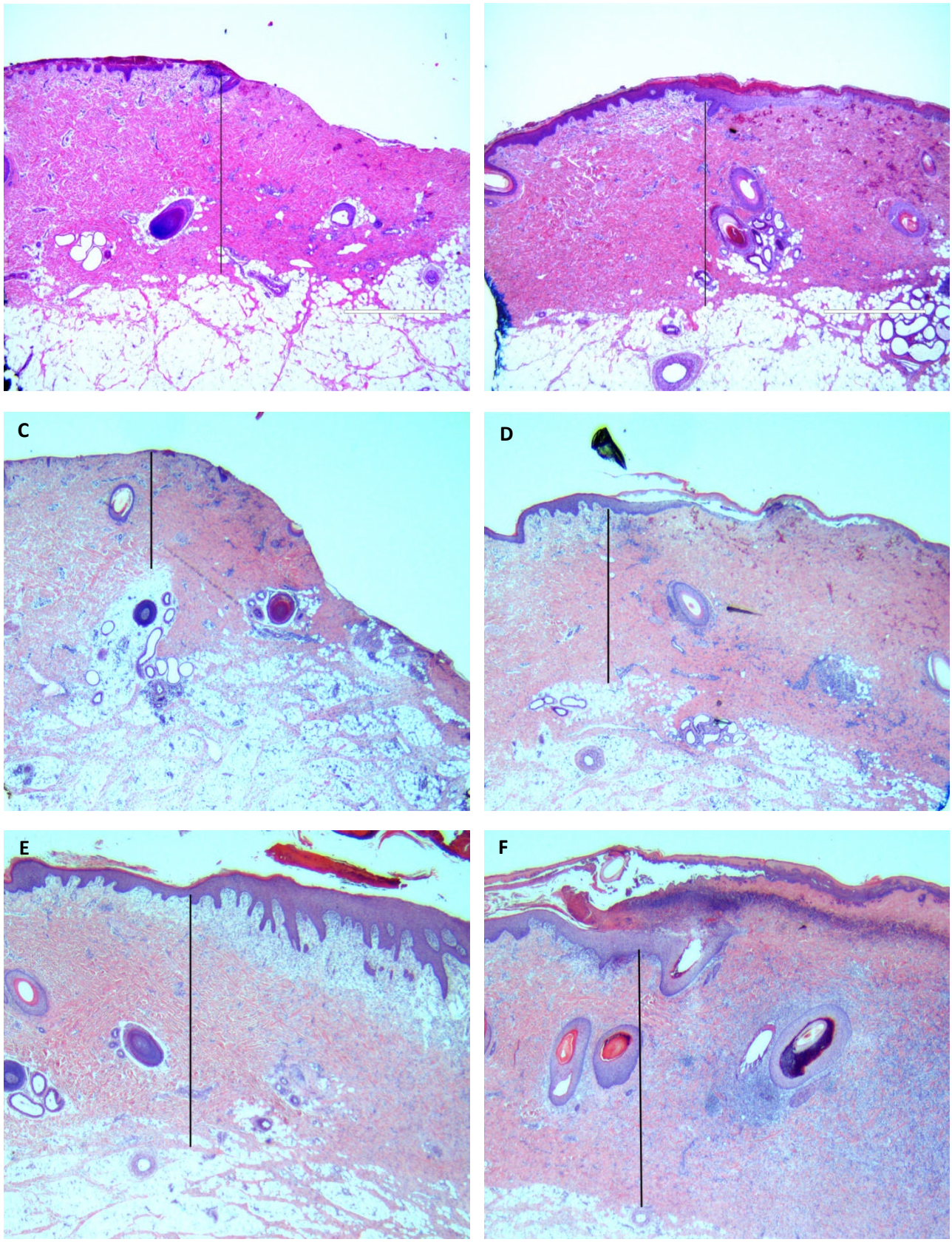

Figure 2. Burns and adjacent tissue, debrided via ArF excimer laser irradiation for ~936 sec, 3 days post-injury, demonstrated increased healing. **A.** 70/20 burn harvested immediately after laser irradiation (right side of vertical black line) was completely debrided as judged by absence of necrotic epidermis and marked reduction of plugged

microvasculature. Adjacent normal skin (left side of vertical black line) reveals little dermal destruction with retention of epidermal rete ridges. **B.** Non-debrided 70/20 burn (right of vertical black line), 3 days post-burn retains necrotic epidermis and dermis. Dark red clusters in upper dermis of burned tissue delineate plugged microvasculature. **C.** 70/30 burn harvested immediately after irradiation (right of vertical black line) was completely debrided as judged by absence of necrotic epidermis. Adjacent normal tissue (left of vertical black line) reveals little dermal destruction. **D.** Non-debrided 70/30 burn (right of vertical black line) retains necrotic epidermis and dermis. Dark red clusters in upper dermis delineate plugged microvasculature and blue clusters in deeper dermis delineate clusters of inflammatory cells. Adjacent normal tissue (left of vertical black line) reveals no destruction. **E.** Debrided 70/30 burn (right of vertical black line) and adjacent normal tissue (left of vertical black line) harvested at 10 days post burn injury (7 days post laser debridement) was re-epithelialized and showed mature neodermis. **F.** Non debrided edge of burn (right of vertical black line) failed to completely re-epithelialize and showed necrosis around a hair follicle and exuberant granulation tissue down to the fat. Adjacent normal tissue is left of line. Scale bar=2mm.

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