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

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ABSTRACT

Introduction: Recent evidence indicates that early removal of eschar by tangential debridement can promote healing. Laser debridement can be used for debridement of areas that prove challenging for debridement using tangential excision. In particular, irradiation with an ArF excimer laser ablates desiccated eschar and is self-terminating, preserving hydrated or viable tissue.

Methods: Thermal burns were created on the flanks of two outbred, female Yorkshire pigs using aluminum bars heated to 70°C and applied for different lengths of time. Three days after injury, burns were debrided using an ArF excimer laser (193 nm). Tissue was harvested immediately after debridement and 7 days after debridement (10 days after burn).

Results: Data from a pilot study demonstrates that ArF excimer laser irradiation removes burn eschar and promotes healing at 10 days after 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.

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1. Introduction

Burns are the most common cause of significant cutaneous tissue loss, with approximately 486,000 civilians seeking medical attention in the United States, claiming the lives of at least 3000 [1,2]. Approximately 10% of burn injuries, or 40,000, undergo acute hospitalization, 60% of which were admitted to 128 burn centers across the country [1,3]. Between

2002–2011, 72% of patients had injury to less than 10% total body surface area (TBSA) [4]. Mortality was higher among patients with greater than 40% TBSA, reaching as high as 56.8% for patients with 70–79.9% TBSA [4].

The current early/immediate standard care for deep-dermal or full-thickness burns is tangential excision of eschar followed by autografting. Tangential excision is often inaccurate, even in expert hands, resulting in removal of viable tissue with consequential excessive scarring and wound contracture

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[5,6]. The first steps in designing a regenerative/restorative solution for burn wounds is the prevention of burn injury progress, followed by precise debridement of necrotic eschars so as not to remove viable tissue. In fact, it has been observed that 41% of debridement specimens contained viable tissue [5]. This fact justifies the need for more precise debridement techniques that conserve viable tissue, while not increasing the burden on the patient. Furthermore, Natesan and colleagues [6] observed that debrided skin contained viable adipose-stem cells, which maintained stemness *in vitro*. When engrafted *in vivo* in mice in Matrigel, they enabled tissue repair, with evidence of blood vessel formation and neocollagenous dermis at 12 days after engraftment. Taken together, these two important studies suggest that more precise debridement could retain viable stem cells in the wound bed, thereby enhancing repair and improving functional outcomes after burns.

Irradiation with short pulses of far ultraviolet (UV) light from an argon fluoride (ArF) excimer laser (193 nm/6.4 eV), approved by the FDA for use in laser-assisted *in situ* keratomileusis (LASIK) and photorefractive keratectomy (PRK), ablates dehydrated protein (eschar) but does not damage hydrated 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. Proteins, on the other hand, degrade when exposed to high-fluence far-UV radiation. 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 ablate eschars, while leaving viable tissue intact. This process has been termed ablative-photodecomposition [7] and was first developed at the IBM Thomas J Watson Research Center, Yorktown Heights, NY. The ArF excimer laser was used to ablate skin [8], producing clean cuts *in vitro* with minimal damage to surrounding tissue. When used *in vivo*, it failed to remove tissue when bleeding began, secondary to the presence of aqueous chloride ions. Thus, irradiation with this laser possesses self-terminating attributes.

2. Methods

2.1. Animal model

The study protocol was conducted at Stony Brook University following Institutional Animal Care and Use Committee (IACUC) Research Review Board approval. Two 3-month old female Yorkshire pigs weighing 25–30 kg were used in this study. Pigs were sedated with a combination of acepromazine 0.1 mg/kg, atropine 0.02 mg/kg, ketamine 20 mg/kg, and xylazine 2.2 mg/kg, administered via intra-muscular injection. Pigs were then intubated and maintained under a surgical plane of 0–5% isoflurane mixed with O₂ USP. Intramuscular injections of buprenex and a trans-dermal patch of fentanyl were used for

pain management after burns, debridement, and harvest of biopsy.

2.2. Burn model

While under general anesthesia, burns were created on the flanks of animals using a 150-g aluminum bar with dimensions 2.5 cm × 2.5 cm × 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 force for 20 or 30 s. Previous work at Stony Brook has shown that burns created with the aluminum bar heated to 70°C and applied for 20 s (denoted as 70/20 burns) injured the skin to a depth of the upper reticular dermis (superficial burns), while those heated to 70°C and applied for 30 s (denoted as 70/30 burns) injured the skin to a depth of deep-reticular dermis at 28 days (mid-dermal burns) [9]. All burns were treated with Vaseline, and protected with Tegaderm, with dressing changes every 3 days.

2.3. Laser debridement

Three days after burn, the ArF excimer laser source (193 nm/6.4 eV), a repurposed NIDEK EC-5000 Corneal Surgery System, was used to debride burns. System parameters were set based on clinical settings in ophthalmology: ablation field diameter = 10.00 mm; fluence = 150 mJ/cm²/pulse; pulse duration = ~10 ns; pulse rate = 40 pps; beam width = 1.0 mm; time to scan the beam across the ablation field = 0.25 s; integrated fluence/s = 600 mJ/cm²/s. The laser source was positioned with its output beam directed perpendicular to the flank of the pig, producing a field of debridement 10 mm or 1 cm in diameter. This field was positioned so that half of it was on the burn and half was on adjacent normal skin (Fig. 1A and B). Two such fields of irradiation, each 10 mm in diameter were positioned on each burn. While the total surface area of each burn was 6.25 cm² area, a total of 0.785 cm² (2 laser field hemispheres, each 0.3925 cm²) was irradiated leaving 5.465 cm² per burn not irradiated/ablated.

A single pig was used to determine the duration of laser irradiation that would effectively debride all necrotic tissue. A cycle of irradiation lasting ~86 s was selected, delivering an integrated fluence of ~52 J/cm²/cycle. Superficial (70/20) burns were laser-debrided three days after burn over several cycles: 2 (172 s); 5 (430 s); or 10 (860 s), followed by harvesting debrided tissue. Between each cycle, the field of debridement was blotted dry using gauze to remove blood and exudate. Immediately after debridement (3 days after burn), 6 mm punch biopsies were used to harvest tissue (Fig. 1C). In a later experiment using a second, female Yorkshire pig, 7 days after debridement (10 days after burn), 8 mm punch biopsies were used to harvest tissue (Fig. 1D). Harvested tissue was bisected and fixed in 2% formaldehyde. After 5 μm sectioning, sections were stained with Haematoxylin and Eosin (H&E) and imaged using an EVOS™ microscope with an internal camera.

3. Results

Depth of debridement was dependent on number of cycles of ArF laser ablation, the integrated fluence of each cycle being

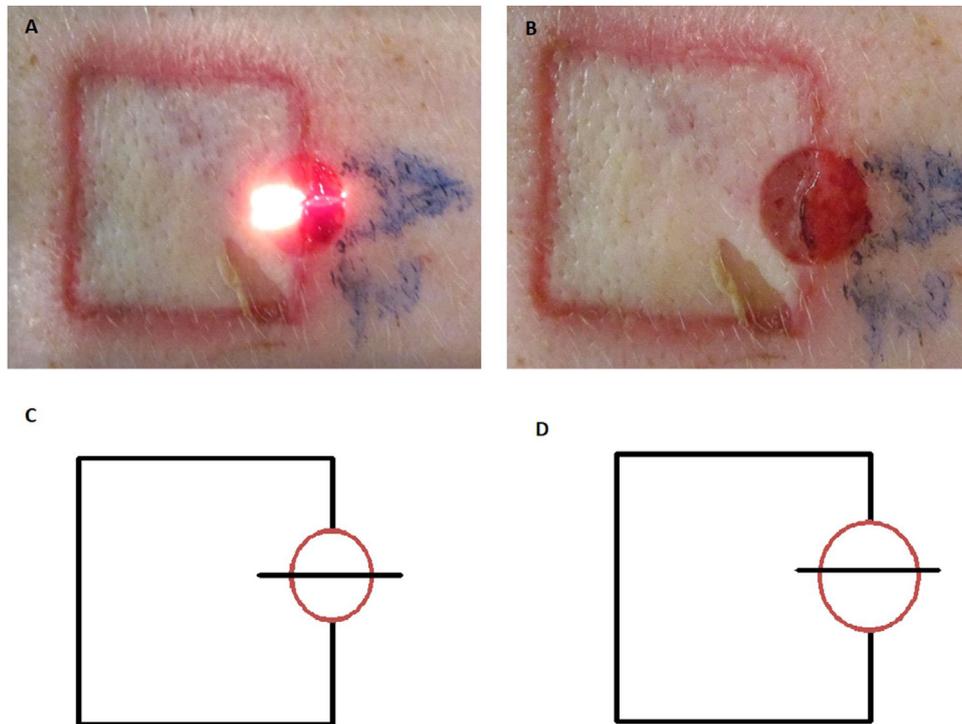


Fig. 1 – 2.5×2.5 cm burns were created on the flanks of pigs using an aluminum bar. Burns and adjacent tissue were debrided via ArF excimer laser irradiation 3 days after injury. (A) The laser source was positioned with its output beam irradiating a 10 mm diameter field of debridement located half on the burn and half on normal skin. (B) This produced erythema after debridement (free blood and exudate had been removed by blotting with gauze). (C) 6 mm punch biopsies were harvested 3 days after 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) 8 mm punch biopsies were harvested 10 days after burn, 7 days after laser debridement. Biopsies were bisected and stored in formalin until sectioning. 5μ sections of bisected wounds were stained using Hematoxylin and Eosin stain.

constant. Debridement for 2 cycles, for a total of ~ 172 s, did not remove the necrotic epidermis (Fig. 2A, left of black vertical line), while leaving normal skin uninjured (Fig. 2A, right of black vertical line). Similarly, 5 cycles (~ 430 s) of debridement did not completely remove the eschar or destroy the necrotic epidermis, with clusters of inflammatory cells in the upper dermis (not shown). However, 10 cycles (~ 860 s) removed necrotic epidermis, sparing underlying

adnexae (Fig. 2B, left of vertical black line). The rete ridges of the normal skin were left intact (Fig. 2B, right of vertical black line). This suggested that 10 cycles, for a cumulative total of ~ 860 s with an integrated fluence of ~ 52 J/cm²/cycle should be efficacious to debride burns.

Having established an optimal duration of efficacy (860s, Fig. 2) using the ~ 86 s cycles, we adjusted irradiation conditions. This increased the length of each cycle to ~ 104 s,

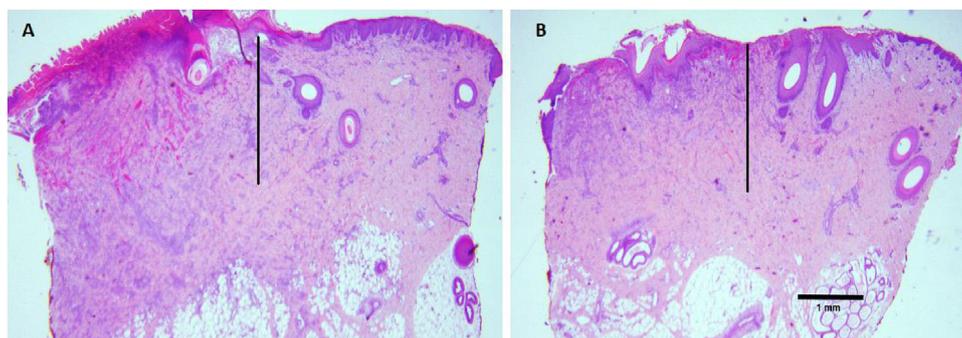


Fig. 2 – Depth of debridement of superficial (70/20) burns increases with increasing number of cycles of ArF excimer ablation. (A) Debridement of burn for 2 cycles (~ 172 s) showed an intact necrotic epidermis (left of vertical black line). Normal skin (right of vertical black line) reveals no destruction of dermis, with rete ridges intact. (B) Debridement of burn for 10 cycles (~ 860 s) showed removal of necrotic epidermis (left of vertical black line) down to rete ridges with clusters of inflammatory cells at the edge of debridement. Normal skin remained intact even with debridement (right of vertical black line). Scale bar = 1 mm (A-B).

with an integrated fluence of $\sim 62\text{J}/\text{cm}^2/\text{cycle}$, reducing the overall number of cycles to 9, for a cumulative total irradiation time of $\sim 936\text{s}$. Under these conditions, the ArF excimer laser was used to debride 70/20 and 70/30 burns created on one female Yorkshire pig, the goal being to determine if laser debridement results in increased healing at 10 days after burn. Again, 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 3 days after burn and harvested immediately after laser irradiation showed complete removal of burn eschar (Fig. 3A, right of vertical black line) while sparing the rete ridges and dermis of normal skin (Fig. 3A, 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 (Fig. 3B, right of vertical black line). Similarly, laser-debrided 70/30 burns debrided 3 days after burn and harvested immediately after laser irradiation showed an absence of necrotic epidermis (Fig. 3C, 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 (Fig. 3D, right of vertical black line). Again, adjacent, normal skin was intact (Fig. 3C and D, left of vertical black lines).

Particularly noteworthy was the observation of dermal regeneration and re-epithelialization in 70/30 burns debrided 3 days after burn and harvested 7 days after debridement (10 days after burn) (Fig. 3E, right of vertical black line). In comparison, non-debrided burns harvested 7 days after debridement, showed necrotic tissue in the upper wound and considerable granulation tissue formation down to the hypodermis (Fig. 3F, right of vertical black line).

4. Discussion

Current surgical standard-of-care for burn management is tangential excision of the wound at early time-points (2-7 days after burn). Clinical outcomes largely depend on the surgeon's ability to accurately excise all the necrotic tissue while minimally affecting the surrounding viable tissue. Laser irradiation presents an attractive alternative to tangential excision in the removal of necrotic eschar. In this proof-of-concept study, we re-purposed a LASIK instrument with an ArF excimer laser to debride superficial- and mid-dermal burns in pigs. Our evidence suggests that debridement of burns using an ArF excimer laser removes eschar while retaining normal viable tissue with enhanced preservation of dermal architecture. This meets the safety, selectivity, and efficacy criteria for debridement [10].

A critical concern in burn management is timing of debridement. In the clinic, burns are typically excised and grafted between 2-7 days after injury, and evidence suggests this early excision is associated with positive clinical outcomes at later time-points [11-14]. In our model of mid-dermal burns in pigs, injury progresses over the first 48 h after burn [9,15]. In the first stage of progression immediately after injury, endothelial necrosis in the dermis is apparent at 1 h after burn, and is predictive of interstitial cell necrosis at 24 h and tissue necrosis at 7 days after burn [15]. Damage continues to

progress over 24 h after burn when tissue damage is secondary to erythrocyte plugging of blood vessels in the deep dermis [16]. This loss of blood supply in the dermis and the progressive desiccation of the eschar on the surface can be detected using infra-red thermography as a reduction in temperature at 48 h after burn [17,18]. Together, these studies provided us with a time window of 0-48 h within which debridement could prove useful to arrest injury progression, enhance wound closure (7-14 days after burn), and reduce scar depth (28 days after burn). Therefore, we tested different times of tangential excision of mid-dermal burns *without* grafting: 0.5 h [19], 24 h, and 48 h (24 and 48 h unpublished observations, Clark et al.) after burn. Excision at 0.5 h after burn does not improve depth of injury 24 h after burn or scar depth at 28 days post-burn, but does improve wound closure at 7 and 10 days post-burn [19]. Excision at 24 versus 48 h after burn revealed no difference in wound closure (7, 10, and 14 days after burn) or scar depth (28 days after burn). In the context of an ArF excimer laser selective for dehydrated tissue, a desiccated eschar is available at or beyond 72 h after burn (unpublished observations). In the burn clinic, while immediate (within 24 h) excision and grafting has been reported for small burns (less than 15% TBSA) [20], more extensive burns, which have greater clinical complications, the first feasible, operable day is generally considered to be 72 h after burn [21-23]. Therefore, debridement using the ArF excimer laser 72 h after burn was consonant with our current understanding of burn pathophysiology and the clinical standard-of-care for debridement.

As observed by us in the porcine model and documented previously [12], tangential excision at early time points (2-7 days post-burn) can result in significant blood loss. One method that has been suggested to minimize loss of blood is a subcutaneous or subeschar injection of diluted epinephrine [24,25]. The benefits of this method remain unclear [24,26], and epinephrine can confound visual confirmation of arteriolar bleeding during tangential excision [27]. Since the ArF excimer laser radiation is absorbed non-thermally by aqueous chloride ions, ablation self-terminates when it encounters viable, hydrated tissue [8]. In our study, ArF laser ablation did not cause any systemic or local adverse effects and resulted in minimal blood loss. Blotting the field of ablation using gauze pads was sufficient to remove blood.

Lasers have been used to treat cutaneous conditions, and ablation/irradiation has been shown to have effects on the dermal matrix with increased cytokine and collagen production [28]. Modalities used are: continuous and pulsed infrared CO_2 (10.6 μm) and Er:YAG (2940 nm) lasers for skin resurfacing and ablation [29]. The first of these, continuous CO_2 lasers, could ablate tissue but left a region of residual thermal damage [30-32]. Later improvements to this technology used pulsed CO_2 lasers that produced high fluence (0-20 J/cm^2), allowing for greater ablation depths with lower residual thermal damage [31,33-36]. Glatter et al. [33] used a CO_2 laser to debride eschar 48 h after creating full-thickness burns. Burn and underlying necrotic tissue was debrided until underlying adipose tissue was visible, followed by grafting over. No significant differences were noted histologically or macroscopically between burns debrided using CO_2 laser ablation and cold-steel excision [33]. The second technology, Er:YAG lasers, have been demonstrated to induce re-epithelialization in patients

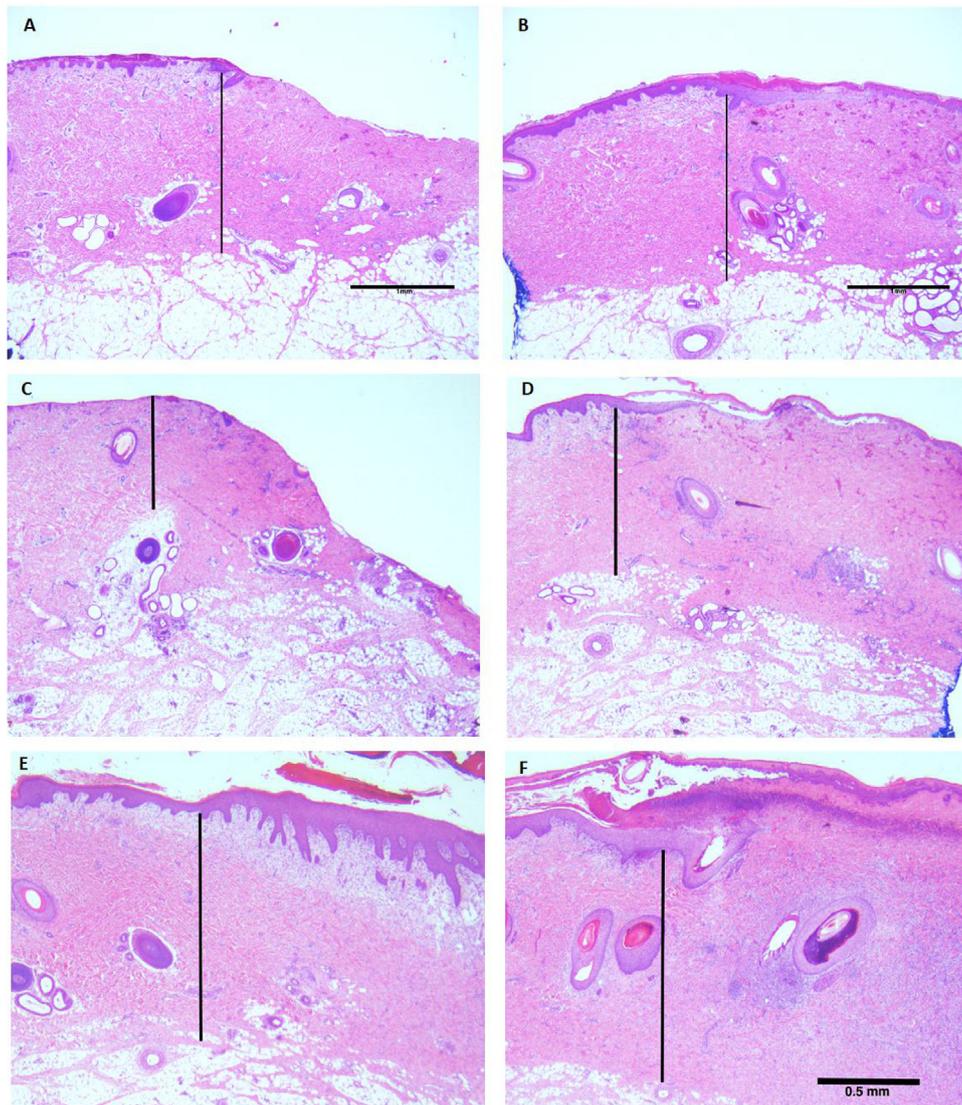


Fig. 3 – Burns and adjacent tissue, debrided via ArF excimer laser irradiation for ~936s, 3 days after burn injury, demonstrated increased healing. (A) 70/20 burn harvested immediately after laser irradiation (right side of vertical black line) was completely debrided as judged by absence of necrotic 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 after 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 after burn injury (7 days after laser debridement) was re-epithelialized and showed mature neodermis. (F) Non-debrided edge of burn (right of vertical black line) harvested at 10 days after burn injury 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 = 1 mm (A-B). Scale bar = 0.5 mm (C-F).

with atrophic scars and photodamage but have been associated with the undesirable side effect of hyperpigmentation [37].

Previous reports [34,38,39] and anecdotal evidence in the literature acknowledge that both infrared CO₂ and Er:YAG lasers are associated with side-effects including scarring, patient discomfort, and longer recovery [29]. Compared to CO₂

(10.6 μm) or Er:YAG lasers (2940 nm) that emit infrared light absorbed by water in the skin, the ArF excimer laser (193 nm) emits pulses of far ultraviolet light, which are absorbed by a much thinner (~0.3 μm) layer of dehydrated skin and can ablate that layer of skin with minimal residual thermal damage to surrounding tissue. Importantly, Eldad et al. [40]

reported that of several debridement modalities including tangential excision, chemical debridement, and ablation with an “Excimer laser (308nm in xenon gas)” (according to the published paper), the latter accelerated healing of partial thickness chemical burns in a guinea pig model. However, according to D. Palanker, the laser physicist co-author of the Eldad et al. paper (private communication), a 193nm ArF excimer laser, not an “Excimer laser (308nm in xenon gas)”, was used in that study. In addition, the laser ablation enabled the controlled debridement with minimal blood loss [40]. Here, we have made a similar observation to the Eldad et al. study [40] with the 193nm ArF excimer laser in a Yorkshire thermal burn injury model, as it produced total debridement of necrotic tissue with little, if any damage to the underlying dermis, minimal blood loss, and accelerated healing. Presumably, the lack of residual thermal damage to underlying viable tissue when irradiated with the laser contributes to enhanced healing at 10days post injury seen by Eldad et al. [40] and in this study by us (Fig. 3E and F), which has been correlated with reduced scarring at later time points [41].

5. Limitations and future work

The goal of this study was to establish proof-of-concept evidence for a novel debridement modality. Our chief limitation in the study was the small sample size. Future experiments will enlarge the sample size to achieve statistically significant results. Larger sample sizes will allow us to determine a time window of laser debridement within a 0-7 day after burn period which can enhance wound closure (7, 10, 14, and 17 days after burn) and perhaps reduce scar depth (28 days post-burn). Direct comparisons will also be made between tangential excision and ArF excimer laser debridement. While this study used only superficial (70/20)- and mid-dermal (70/30) burns, future studies will use the porcine model of burn injury [9] to determine if the ArF excimer laser can debride deep-dermal burns (80°C/30s and 90°C/20s).

In this study, we repurposed an instrument previously used for ophthalmologic applications, but that is suboptimal for burn debridement. We propose to re-engineer the instrument to optimize performance in burn surgery. Slits inside the instrument scan a narrow beam of 1mm width 10 times to achieve an ablation field of diameter 10mm. By re-engineering the instrument to eliminate the scanning mechanism while keeping the fluence unchanged at 150mJ/cm²/pulse, each pulse can irradiate the entire ablation field, increasing the rate of debridement by a factor of 10. The instrument used in this study ran at a pulse repetition rate of 40pps. Increasing the pulse rate from 40 to 50pps will increase the rate of debridement by a factor of 1.25. These two modifications alone improve the rate of debridement by a factor of 12.5 (10×1.25). Additionally, the maximum energy output of the ArF excimer laser in the Nidek EC-5000 system used here is 0.5J/pulse. Modifying the optics of the system to utilize all the energy per pulse, including increasing the fluence per pulse, can further increase the rate of debridement. However, the caveat with increased fluence is the possibility of photoacoustic damage. Previous studies in rodents irradiated by an ArF excimer laser at a fluence of 156mJ/cm²/pulse on uninjured, hydrated skin, reported photoacoustic

damage distal to the site of ablation [42-44]. The re-engineering efforts described above will take into account the possibility for secondary tissue damage.

6. Conclusion

In this pilot study, our goal was to determine if an ArF excimer laser operating at 193nm can selectively and accurately debride eschar. Using a porcine burn injury model developed in our lab, we demonstrate that irradiating superficial- and mid-dermal burns with an ArF laser for ~936s selectively debrides desiccated eschar, while preserving underlying viable tissue and adjoining normal dermal architecture, with minimal loss of blood.

Conflict of interest

Dr. Clark is the President of NeoMatrix Therapeutics Inc., a startup company working on novel therapies for acute and cutaneous trauma, including burns. The authors declare no conflicts of interest in the study.

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