Development of a Cerium (III) Nitrate-containing Electrospun Dressing for Mitigating Delayed Eschar Removal

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Introduction

Thermal burns account for 5-10% of casualties sustained in present-day conflicts and are expected to be one of the most common wounds to occur in future conflicts [1]. Timely debridement of dead burn tissue can greatly reduce the chances of mortality and late-stage complications such as burn shock and multiple organ failure [2-5]. However, future conflicts are anticipated to occur in austere environments, where surgical debridement may not be plausible and casualty evacuations could be significantly delayed.

Without access to prompt surgical interventions and standard treatment, burn wounds can progress (become deeper and more extensive) and become highly susceptible to infection [6-8]. Together, these situational complications highlight the significant unmet need for a method to mitigate the negative effects of delayed surgical interventions.

Several studies have demonstrated that topical applications cerium (III) nitrate ($Ce(NO_3)_3$) can be used to delay the need for surgical eschar removal [9-11]. Although the mechanism of how $Ce(NO_3)_3$ hardens the burn eschar is not fully understood, various works suggest that it could be used to prolong the time before surgical intervention is required and/or mitigate late-stage burn patholphysiologies.

Objectives

1. Create an electrospun dressing that can provide both burst and sustained release of $Ce(NO_3)_3$.
2. Determine the impact of the electrospun $Ce(NO_3)_3$ dressing *in vivo* using an established porcine model of deep partial-thickness contact burns.

Methods

Scaffold fabrication and *in vitro* evaluation. Non-woven, randomly aligned meshes were fabricated by coaxially electrospinning onto a grounded mandrel. The sheath was comprised of solubilized $Ce(NO_3)_3$ and polyethylene oxide (PEO), whereas the core was comprised of $Ce(NO_3)_3$ dissolved in a volatile solvent. Dressings were evaluated for topography/morphology and porosity using scanning electron microscopy (SEM) and helium pycnometry, respectively.

***In vivo* scaffold evaluation.** Burns (5 cm x 5 cm) were created on the dorsum of an anesthetized Yorkshire pig using brass blocks heated to 100°C. Burns were cooled for one hour before application of 1) one layer of PEO/$Ce(NO_3)_3$ dressing, 2) four layers of PEO/$Ce(NO_3)_3$ dressing, 3) four layers of an electrospun PEO dressing, 4) Flammacerium® cream (silver sulfadiazine 1%, cerium nitrate 2.2%), or 5) gauze. Wounds were treated twice over four days, surgically debrided, and then allowed to heal for an additional two weeks.

Results

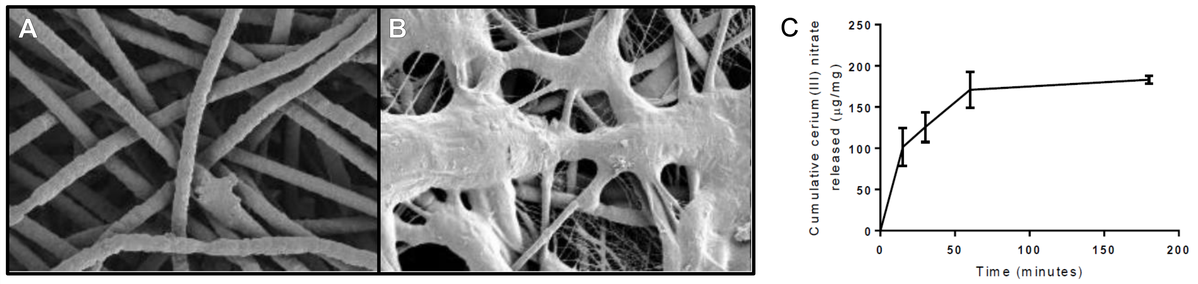

Figure 1. Representative SEM images and release profile of electrospun scaffolds. Scaffolds comprised of (A) PEO (A) or (B) coaxially electrospun PEO/$Ce(NO_3)_3$ were generated. The PEO and PEO/$Ce(NO_3)_3$ scaffolds had average fiber diameters of 1.800 ± 0.100 µm and 1.090 ± 0.250 µm, respectively, and porosities of 56.6 and 36.1%, respectively. C) PEO/$Ce(NO_3)_3$ scaffolds were solubilized in aqueous media for three hours at room temperature. $Ce(NO_3)_3$ concentrations were quantified at 15, 30, 60, and 180 minutes. Data is presented as mean ± standard deviation (SD).

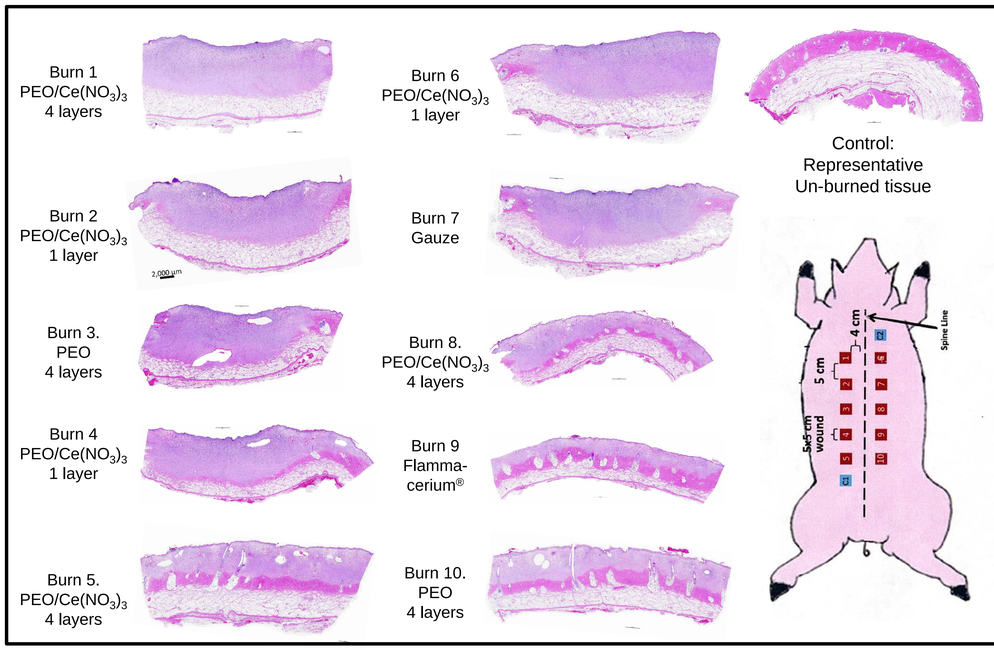

Figure 2. H&E staining of deep-partial thickness burn wounds, 14 days after debridement. Burns were made on the dorsum of an anesthetized Yorkshire pig (lower right). Wounds were treated every two days for four days, then surgically debrided to punctate bleeding. Two weeks later, biopsy strips were harvested. Images are ordered with Burns 1 and 6 (cranial) at the top, and Burns 5 and 10 (caudal) at the bottom). Scale bars = 2,000 µm.

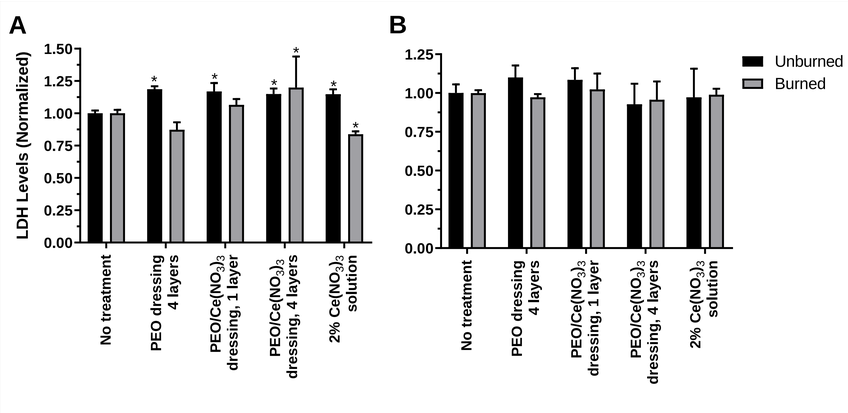

Figure 3. *Ex vivo* treatment with PEO/$Ce(NO_3)_3$ scaffolds does not significantly reduce cell viability. Treatments were applied to the epidermal surface of isolated pig tissue (black) or isolated pig tissue that had been burned *ex vivo* (grey). Tissues were incubated at 32 ℃ 5% CO_2 for 24 (A) or 72 (B) hours. *p ≤ 0.05 compared to respective no treatment control. Data are representative of three independent experiments with technical triplicates; mean ± SD.

Conclusions

- $Ce(NO_3)_3$ was successfully incorporated into a lightweight, pliable electrospun dressing. The co-axially spun fibers contained 80-175 µg $Ce(NO_3)_3$ per mg of scaffold material.
- After two application, burn eschars treated with either the PEO/$Ce(NO_3)_3$ dressing or the Flammacerium® cream had a distinctive leathery feel.
- Histology of the post-debridement wounds suggests that two application of the PEO/$Ce(NO_3)_3$ dressing (four layers per application) may reduce the debridement depth necessary to remove necrotic tissue from deep-partial thickness bun wounds. Additional studies are necessary to confirm this observation.
- The PEO/$Ce(NO_3)_3$ dressing displayed minor viability effects on porcine skin that was burned *ex* vivo. LDH levels were examined 24 or 72 hours after application.

Acknowledgements

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Statements

- Research was conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare Regulations, and the principles of the Guide for the Care and Use of Laboratory Animals, National Research Council. The facility's Institutional Animal Care and Use Committee approved all research conducted in this study. The facility where this research was conducted is fully accredited by the AAALAC.
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