Secretome-loaded Plasma-alginate Composite (PAC) Gels to Treat of Deep Partial-thickness Burn Injuries in a Porcine Burn Injury Model

Marc Thompson, Kameel Zuniga, Lucy Shaffer, Michelle Holik, Carole Nail, Logan Leatherman, Robert Christy, Shanmugasundaram Natesan
US Army Institute of Surgical Research, JBSA Fort Sam Houston, TX

Statement of Purpose: As injured warfighters are moved to higher echelons of care, the resources available increase and care approaches, but doesn't reach, the standards of a civilian or definitive care military hospital. Transport to care facilities can be delayed hours or days; therefore, it is critical to render appropriate initial treatments since evacuation can occur in austere environments with limited supplies administered by untrained medical personnel. Plasma-based materials have recently attracted significant attention in regenerative medicine, both as a xeno-free source for unique growth factors as well as a supporting scaffold for therapeutic cell delivery. Alginate-based biomaterials have similarly proven to be biocompatible and successful in a number of tissue regeneration and wound healing applications, with advantageous mechanical properties. Extracellular vesicles (secretomes/EVs) are recently gaining attention as a cell-free alternative to treat various pathophysiological conditions like inflammation and other tissue injury. The proposed secretomes are nanometer size particles secreted from multi-vesicular bodies (MVB) originating from umbilical cord stem cells that contain lipids, proteins, and RNAs which contribute to paracrine signaling following a physiological insult. The current efforts aim to recapitulate the immunomodulatory benefits of secretomes as anti-inflammatory agents to mitigate the pro-inflammatory response seen in deep partial-thickness burn injuries, when delivered by a PAC gel dressing to improve wound healing.

Methods: To create partial-thickness burns, a 100°C heated 5x5 cm diameter brass block was applied to the back of an anesthetized swine to create twelve burns situated 2-3 cm from the spine. Three wounds were treated with sterile gauze (SOC), three wounds were treated with an unloaded PAC gel (vehicle control), three wounds were treated with a low dose of the Axolotl PAC gel (PAC/Ax 0.1X), and three other wounds were treated with a higher dose of the Axolotl PAC gel (PAC/Ax 1X). Two unburned regions served as physiological controls. Before the burn injury at day -4 and at pre-assigned time points post-burn (days -3, 0, 4, 7, 14, 21, and 28), wounds were imaged (Digital, Silhouette, MolecuLight, and Moor Laser Doppler Imaging) and biopsied harvested. Once the burns were allowed to cool and after all biopsy samples and images have been obtained, the assigned treatment was applied carefully on their respective wound at each time point to sufficiently cover the entire wound. All burns were debrided at day 0. Half of the biopsy was evaluated histologically by H&E and immunohistochemistry (IHC) to determine the depth (mean ± standard error of mean) of damaged tissue and wound healing over the experimental period. Remaining biopsy samples were preserved using AllProtect for proteomic analysis. At days -4, -3, 0, and 7, blood was drawn from the biopsy sites of unburned controls, SOC-treated burns, and PAC/Ax 1X-treated burns and analyzed for cytokine and chemokine concentration through Luminex.

Results: No significant infection was observed through MolecuLight in any of the burn wounds. Histological scoring is currently under review with a veterinary pathologist and caspase-3 apoptosis and TUNEL staining is currently being measured and analyzed. Moor Laser Doppler Imaging showed no differences between treatments but showed increased blood flow (flux) after day 0 (debridement day) which peaked at day 4 or 7 and decreased to similar levels as the unburned control group. Silhouette imaging was conducted to measure wound area over time, which showed wound area decreased over time with all treatment groups, indicating contraction. Interestingly at day 14, the PAC gel vehicle control had a larger wound area than the SOC-treated control, although not significant. IL-2 and IL-4 levels in blood remained at the same levels at each time point measured for all treatments with no significance observed between treatments. IL-6 levels in blood peaked at day 0 and decreased at day 7. IL-1ra concentration, an anti-inflammatory cytokine receptor, was lower in blood from PAC/Ax 1X-treated burns than SOC-treated burns at each time point for all pigs with significance observed ($p=0.056$) at day 0 for one pig. Proteomic analyses quantified cytokine/chemokine levels through WES and Luminex-based systems.

Disclosure: 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. The views expressed in this abstract are those of the author(s) and do not reflect the official policy or position of the U.S. Army Medical Department, Department of the Army, DoD, or the U.S. Government.