Trial of the Thrombin-derived Peptide TP508 for Healing a Combined Radiation & Burn Injury

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Introduction / Background

- Combined Radiation Injury (CRI) is a significant threat to both military and civilian health in the event of nuclear conflict. When cutaneous burn injury is combined with radiation exposure there is a synergistic effect with multiplicative impacts on tissue damage creating a complex injury. Animal models of CRI indicate an increased susceptibility to infection, severely dysregulated inflammatory response and wound healing, diffuse vascular injury leading to multiorgan damage, and a significantly reduced threshold for both acute and chronic lethal effects. While severe burns are largely treated with aggressive wound management and surgical interventions, there is a need for non-invasive treatments to address CRI specifically. To meet this need, our study is testing the thrombin-derived peptide TP508 as a treatment for radiation followed by burns using a murine model.
- TP508 is a truncated form of thrombin that works by binding to endothelial, stem, and inflammatory cells to induce signaling cascades which restore vascular function, stimulate progenitor cells, and modulate inflammatory cytokines leading to accelerated tissue repair. Preliminary data in mice shows TP508 increased wound closure and re-epithelialization after full-thickness burns, and in a separate study, TP508 reversed radiation-induced delay of wound healing following a full-thickness dorsal excision paired with radiation exposure.
- Therefore, we hypothesized that TP508 could be applied directly after CRI to decrease burn wound conversion and accelerate wound healing, providing a therapeutic option for CRI which stabilizes the wound, prevents a dysregulated inflammatory response, and accelerates a controlled healing process.

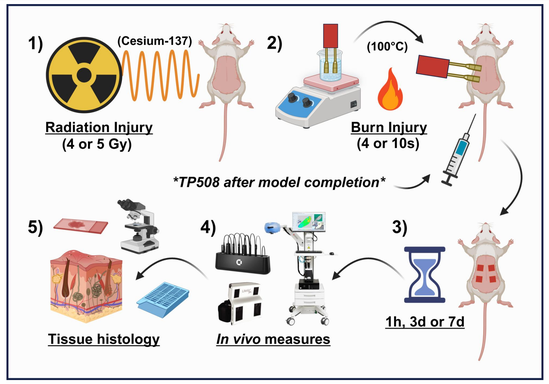
Objective / Purpose

- Currently, there are no FDA-approved treatments for complex trauma caused by nuclear incidents that carry a mass-casualty risk through concurrent delivery of radiation and thermal wounds, which could easily overwhelm healthcare facilities nationwide.
- We sought to develop a clinically relevant combined radiation and burn model to test novel peptide TP508 for its ability to delay burn wound progression and initiate normal healing, which could lead to increased survival, reduce the need for surgical interventions, and lead to better long-term hospital outcomes for patients.

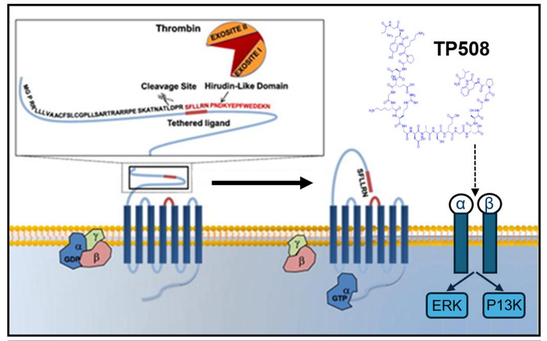
Methods

- **Animal Model Summary**: BALB/c mice, males (age 8w) and females (age 10w), received total body irradiation followed by metal contact burn of <15% total body surface area on the dorsum. Daily Baytril injections were given to prevent infection.
- **Preparation**: Dorsum hair is removed day prior to experiment, buprenorphine ER analgesic is administered prior to treatment to reduce incidence of pain due to burn, isoflurane anesthesia is administered during treatment and for *in vivo* measurements.
- **Combined Radiation-Burn Injury**: Cesium-137 irradiator delivered 4 or 5Gy total body irradiation and shortly after mice were given deep partial-thickness contact burns via heated brass rod (96-100°C) on four sections of dorsum skin while maintaining a fixed 7mm distance between the burns on each flank.
- **TP508 treatment**: Subcutaneous peptide injection is given immediately after combined radiation-burn injury in ongoing work.
- **Endpoints**: 1 hour, 3 days, or 7 days post-injury for both *in vivo* measurements and tissue harvest of skin and other organs.
- **Measurements**: DermaLab Combo II, Perimed Laser Speckle, 3d camera imaging, Weight logs, Skin histology – Hematoxylin & Eosin, Masson's Trichrome, and targeted antibodies. Developed Qualitative Burn Severity Index Scoring system and Burn Healing Index measurement using ImageJ, adapted from prior works.

Graphical Abstract



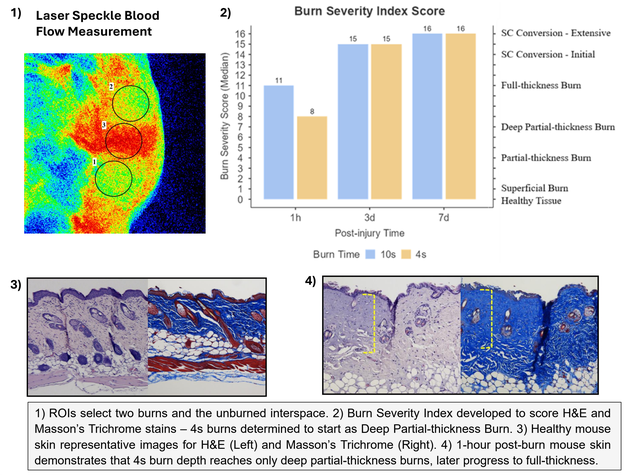
Experimental Flowchart: Mice are first shaved 24h prior to experiments and then subjected to Total Body Irradiation via Cs-137. Within 1 hour, they are given contact burns with a dual-probe brass rod heated to 100ºC via water bath. Mice receive daily antibiotics and wellness checks until 1h, 3d, or 7d post-injury and then undergo *in vivo* measures & tissue harvesting.



TP508 is a novel 23-aa thrombin-derived peptide that bypasses G-protein coupled protease-activated receptor PAR and instead induces alternate signaling pathway without proteolytic functions or coagulation activation:

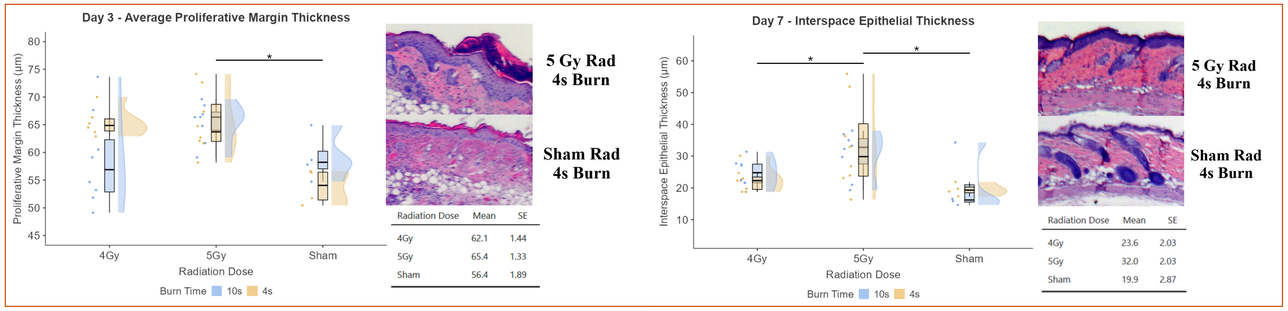
- Restores vascular function in endothelial cells (NO), stimulates progenitor stem cells, optimizes inflammation, and accelerates tissue repair
- Integrin $\alpha_v\beta_3$ implicated, mechanism not fully understood
- ERK & P13K → proliferation, survival, migration, angiogenesis

Model Development



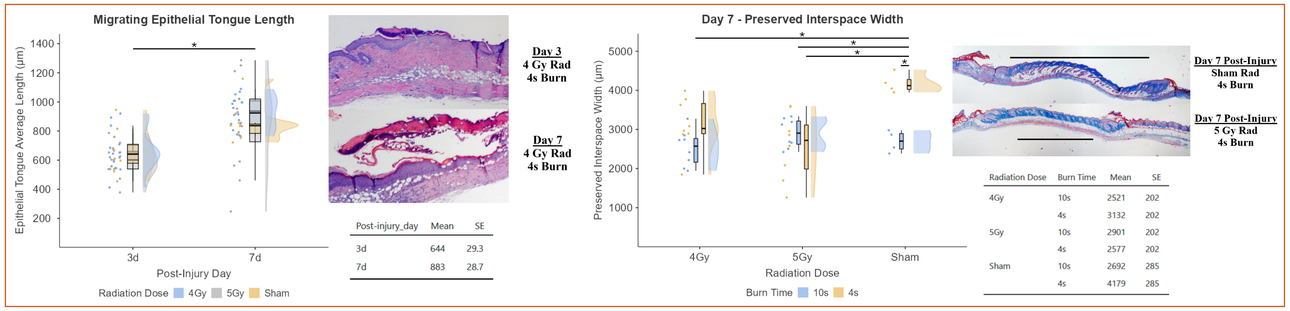
1) ROIs select two burns and the unburned interspace. 2) Burn Severity Index developed to score H&E and Masson's Trichrome stains – 4s burns determined to start as Deep Partial-thickness Burn. 3) Healthy mouse skin representative images for H&E (Left) and Masson's Trichrome (Right). 4) 1-hour post-burn mouse skin demonstrates that 4s burn depth reaches only deep partial-thickness burns, later progress to full-thickness.

Results



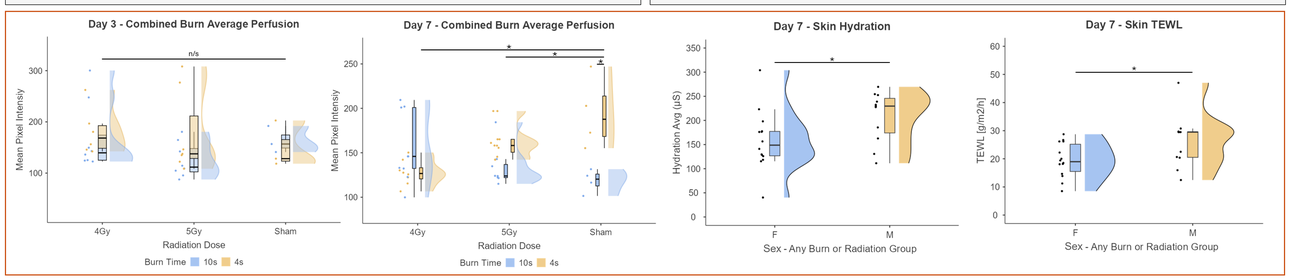
Average Proliferative Margin Thickness ANOVA – Radiation Dose: F = 7.62, p = 0.002, η^2= 0.282.
Post Hoc Tukey's Test – Radiation Dose: 5Gy – Sham: p = 0.001, Mean Difference = 9.02.

Interspace Epithelial Thickness ANOVA – Radiation Dose: F = 7.40, p = 0.002, η^2= 0.298.
Post Hoc Tukey's Test – Radiation Dose: 5Gy – Sham: p = 0.004, 5Gy – 4Gy: p = 0.016.



Migrating Epithelial Tongue Length ANOVA – Post-injury Day: F = 33.73, p < 0.001, η^2= 0.311.
Post Hoc Tukey's Test did not yield any significant comparisons between other factors.

Preserved Interspace Width ANOVA – Radiation: F = 4.29, p = 0.022, η^2= 0.311. Burn Time: F = 9.68, p = 0.004, η^2 = 0.146. Radiation Dose x Burn Time: F = 7.12, p = 0.003, η^2 = 0.214. *Significant Tukey Contrasts.



Perimed Laser Speckle – Day 7 Burn Perfusion ANOVA – Burn Time: F = 9.18, p = 0.004, η^2 = 0.128.
Day 3 Burn Perfusion ANOVA did not yield significance. *Significant Day 7 Tukey Contrasts

DermaLab Combo – Day 7 Skin Hydration ANOVA – Sex: F = 4.72, p = 0.049, η^2= 0.311.
Day 7 Skin Transepidermal Water Loss (TEWL) – Sex: F = 5.51, p = 0.035, η^2= 0.179.

Conclusions

- Our murine burn model has established that 4s contact burns achieve deep-partial thickness burns which convert to full-thickness within 3 days and by 7 days have extensive subcutaneous damage.
- Histological scoring systems using semi-quantitative analysis in ImageJ were able to discern additional damage caused to the skin by radiation alongside burns, most notably 5 Gy causing thickening of the epidermis between burns and along the proliferative margin.
- Notably, the addition of 5 Gy radiation to 4s burns resulted in the "healthy" interspace between two burns being significantly smaller.
- *In vivo* measurements found changes in blood flow near burns due to the addition of radiation injury, and Male mice were found to have higher skin hydration yet lose more water due to TEWL.

Future Directions

- With both the burn mouse model and combined radiation-burn injury dosing experiments completed we have determined the optimal combination of injuries to begin testing novel peptide TP508.
- We have recently begun using 5 Gy of total body irradiation alongside 4s contact burns to produce combined radiation-burn injury and are testing doses of 5, 10, and 20mg/kg of TP508 injected subcutaneously directly after application of burns.
- We also plan on using targeted antibodies in our samples to examine cell-specific responses in the skin during wound healing.

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