Electroactive Bandages for Accelerated Wound Healing and Reduce Scarring in a Diabetic Trauma Model

Sama Abdulmalik[1,2], Suranji Wijekoon[1,2], Allen Zennifer[1], Sai Sadhananth[3], Caden Connealy[5], Kenneith W. Bayles[4] and Sangamesh Gurappa Kumbar[1,2,3]

[1]Nebraska Translational Research Center, College of Dentistry, Department of Growth and Development, UNMC, Omaha, NE
[2]College of Engineering, Department of Mechanical and Material Engineering, UNL, Lincon, NE
[3]College of Pharmacy, UNMC, Omaha, NE
[4]Department of Pathology & Microbiology, University of Nebraska Medical Center, Omaha, NE, USA
[5]College of Medicine, UNMC, Omaha, NE

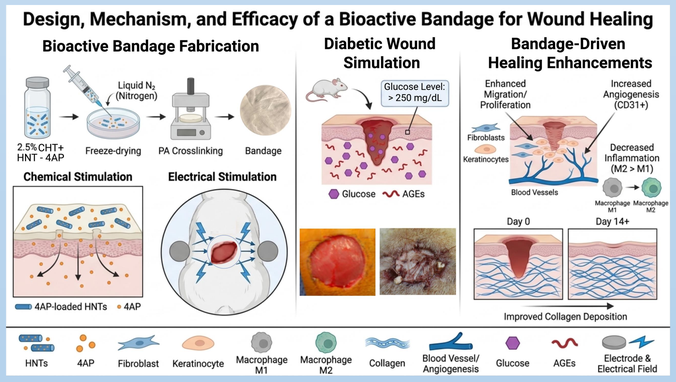
Introduction

- Musculoskeletal injuries of muscle, nerve, vasculature, tendon, and ligament are rising in the US, impacting quality of life, and resulting in pain, loss of function, and significant financial burden[1,2].
- Current practices for repairing musculoskeletal injuries often involve surgical interventions, which lead to scarring.
- Scar tissue is a highly disorganized fibrotic extracellular matrix (ECM) with altered components that present a physical barrier for regeneration[4-6].
- Mitigating scarring and promoting regeneration via pharmacological agents, biomaterials, cells, and other physical forces have varying degrees of success and several difficulties in their implementation[7-10].
- Our prior studies showed that phytate-modified chitosan bandages delivering 4-aminopyridine (4-AP) with ES synergistically enhanced full-thickness wound healing and reduced scarring in healthy rats[10].

Objectives

- We hypothesize that sustained chemical and electrical stimulation enhances drug permeation, modulates immune responses, promotes vascularization, accelerates wound closure, and reduces scarring.
- This electroactive platform is scalable and well-suited for prolonged field care and austere environments.

Study Design



❖ **Evaluation:**

- Immunomodulatory effects of 4-AP with ES were evaluated ***in vitro*** using human dermal fibroblasts and RAW macrophages under **inflammatory (LPS)** and **oxidative stress (H_2O_2)** conditions.
- Diabetes was induced using STZ in 12 week old SD rats
- Full-thickness wounds (4 cm²) were treated with IC scaffolds, with combined treatment **4AP+ES** and compared to PowerHeal® **PwrH (positive cont.)** .

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Results

❖ **The combined treatment of 4-AP and ES modulate immune responses in LPS-stimulated diabetic hDFBs**

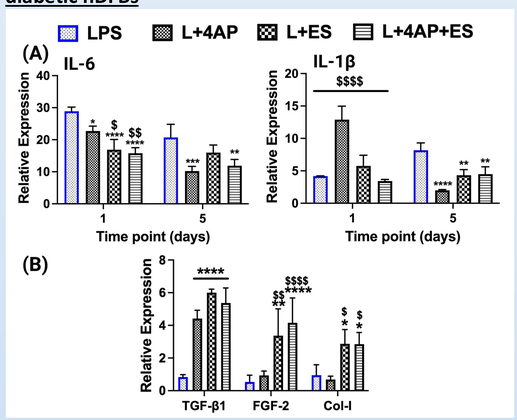

Figure 1: Gene expression of **(A) Proinflammatory genes:** IL-6 and IL-1β **(B) Wound healing related genes:** TGF-β, FGF-2 and Col-I at day 3. Diabetic human skin fibroblast seeded on TCP and treated with 1μg/ml LPS in serum deprived environment 1%FBS. Treatment groups are vehicle control, LPS, L+4AP (100ng/ml), L+ES, and L+4AP+ES. Data (n=4) represented as mean ± SD. A two-way ANOVA with Tukey's post-test. * is p<0.05; ** is p<0.01; *** is p<0.001; and **** is p<0.0001 compared to LPS. $ is p<0.05; $$ is p<0.01; and $$$$ is p<0.0001 compared to L+4AP.

❖ **The combined treatment enhanced cellular protection under H_2O_2-induced oxidative stress by preserving mitochondrial activity**.

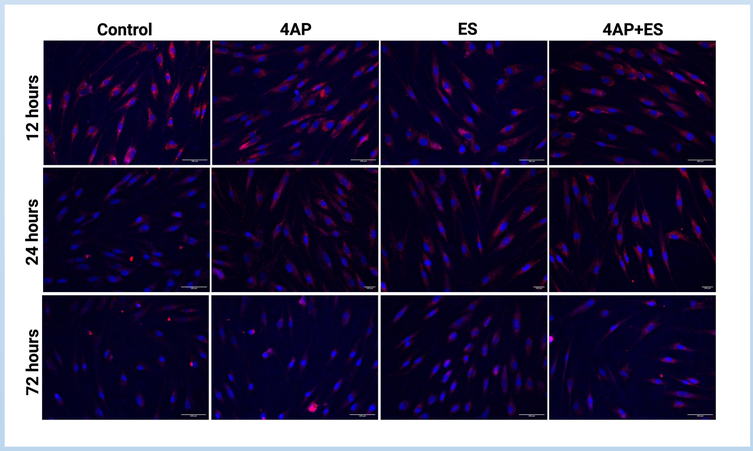

Figure 2: Mitochondrial potential was assessed using MitoTracker Red CMXRos staining in diabetic hDFB cultured with 0.5% FBS and 0.3 mM H_2O_2. Images were taken at 20X magnification. Scale bar is 100 μm.

❖ **The combined treatment of 4-AP and ES modulated immune responses in LPS-stimulated macrophage cells culture**

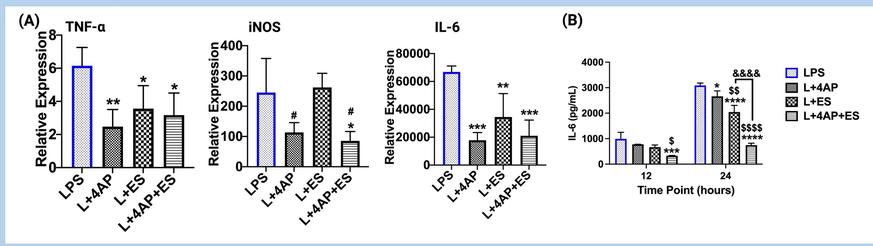

Figure 3: (A) Gene expression of **Proinflammatory genes:** TNF-α, iNOS, and IL-6 **12 hours** post-treatment. **(B) Protein detection of IL-6 from media 12 & 24** hours post-treatment using ELISA. RAW 264.7 cells seeded on TCP and primed with 1μg/ml LPS in serum deprived environment 0%FBS. Treatment groups are LPS, L+4AP (100ng/ml), L+ES, and L+4AP+ES. Data (n=4) represented as mean ± SD. A one-way ANOVA with Tukey's post-test. * is p<0.05; ** is p<0.01; and *** is p<0.001 compared to LPS. # is p<0.05 compared to L+ES. A two-way ANOVA with Tukey's post-test. * is p<0.05; *** is p<0.001; and **** is p<0.0001 compared to LPS. $ is p<0.05; and $$ is p<0.01 compared to L+4AP. &&&& is p<0.0001 compared to 4AP+ES.

Acknowledgments

- The study was supported by the National Institute of Biomedical Imaging and Bioengineering of the National Institutes of Health (#R01NS134604, #R01EB034202, #R01AR078908 and #R01EB030060); U.S. Army Medical Research Acquisition Activity (USAMRAA) through the CDMRP Peer-Reviewed Medical Research Program (Award No. W81XWH2010321, PR230581, and HT94252410137); the DoD support from BA230234 (Award # HT94252410944).

❖ **The combination of electrical stimulation and 4AP accelerated wound closure in a diabetic rat model**

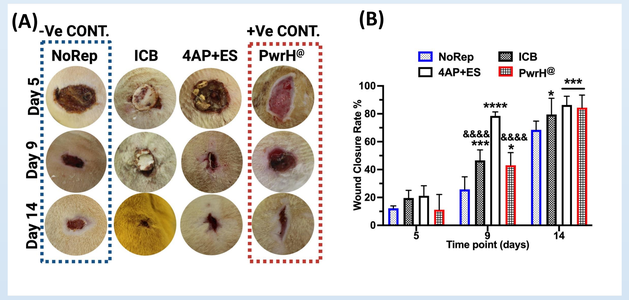

Figure 4: (A) Time course skin wound closure in a diabetic rat model treated with ionically conductive bandage (ICB), 4AP+ES and compared to no repair (negative control) or PowerHeal® (positive control) **(B)** Quantification of the wound closure rate: 4AP+ES treatment was significantly higher than the other groups. All samples (n=6) were evaluated using ImageJ. Data are presented as mean ± SD. A two-way ANOVA with Tukey's post-test. * is p< 0.05; *** is p< 0.001; and **** is p< 0.0001 compared to negative control. &&&& is p<0.0001 compared to 4AP+ES.

❖ **The combined treatment accelerated wound healing and reduced SEI**

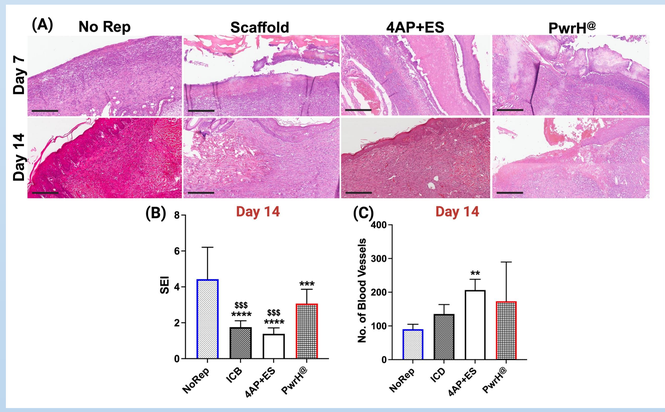

Figure 5: (A) Representative images of Hematoxylin & Eosin staining 14 days post-surgery. Images were taken at 10X magnification. Scale bar is 300 μm. **(B)** Scar elevation index (SEI) analysis of the skin. **(C)** Number of blood vessels. Data is presented as mean ± SD. A one-way ANOVA followed by Tukey's post-test. ** is p<0.01; *** is p<0.001; **** is p<0.0001 compared to negative control. $$$ is p<0.001 compared to positive control

❖ **The combined treatment 4AP+ES promoted collagen deposition and fiber organization comparable to those of native tissue.**

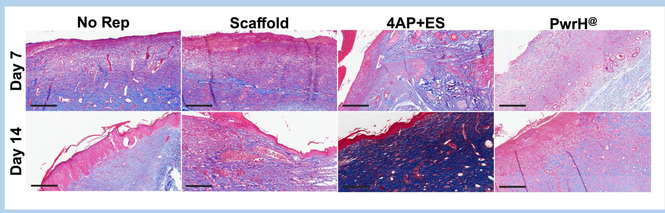

Figure 6: Masson Trichrome staining where red stain indicates cytoplasm and keratin deposition and blue stain represents collagen deposition. Images were taken at 10X magnification. Scale bar is 300 μm.

Conclusion and Future Work

- The combined treatment demonstrated superior efficacy in promoting the healing of full-thickness skin defects in diabetic rats.
- Future studies will investigate the signaling pathways underlying the observed therapeutic effects and enhanced wound-healing response.