Wound healing analysis of human and porcine placental membranes in an *in vivo* rat skin defect model

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Statement of Purpose: Skin wounds like bed sores, diabetic ulcers, and burns affect more than 8.2 million patients in the United States, with annual Medicare cost estimated to be $90 billion [1]. Current treatment options include alginates, hydrocolloids, dressings, and foams which often require a secondary dressing for long-term use, have high injury recurrence rates, and ultimately lead to poor healing resolution [2], [3]. Human-derived placental extracellular matrices (ECM) have been identified as alternative skin wound treatments due to their biodegradability, anti-immunogenicity, and delivery of pro-healing growth factors [4], [5]. Though human placental tissue is promising, its usage is still limited by high cost, reliance on donors, and high biochemical variability [6].

Porcine-derived placental ECM are gaining interest to replace human-derived placental wound treatments due to their low cost, abundance in availability, and low variability [7]. This study aims to determine the potential for clinical use of porcine-derived ECM wound treatments compared to their human-derived counterparts in a pre-clinical rat skin defect model. Wound healing was evaluated by macroscopic wound perimeter image analysis on days 3, 7, and 14 and histological analyses.

Methods: 24 adult Sprague Dawley rats (12M + 12F) were used and split into 3 groups of eight rats (4M + 4F). Using a scalpel, each rat had two, 1 cm-diameter defects, that penetrated the panniculus carnosus, made on the upper back. One wound received either the human-derived placental ECM (FlowerAminoPatch™, Triad Life Sciences, USA) or the porcine-derived placental ECM (Innovamatrix™ AC, Triad Life Sciences, USA), while the other received the opposite placental ECM treatment. Silicon rings were used the wound to prevent wound contraction. Wounds were covered with a commercial non-stick hydrocolloid dressing, vet wrap, and rat jacket. Wound dressings were changed every 3 days. Gross images were taken at days 3, 7, and 14. Rats were euthanized at days 3, 7, and 14 to harvest tissue explants for histological analysis.

FIJI ImageJ software was used to quantify the macroscopic change in wound size over time. A ruler was used to set the scale before freehand tracing of the wound perimeter. Software calculated wound surface area values were analyzed in MATLAB to determine statistical differences. ANOVA was used to test for significance ($\alpha = 0.05$).

Microscopic analysis on H&E-stained histology slides was conducted. Day 14 samples were used to visualize new collagen formation in the wound using polarized images.

Results: Wound area analysis results from days 3, 7, and 14 are shown in Figure 1A. A 2-way ANOVA indicated the size of the wounds decreased significantly from day 3 to 14, ($p = 1 \times 10^{-15}$) but there was no difference between groups ($p = 0.82$). There were no differences in H&E-staining between the test membranes. Healing followed typical pattern of polymorphonuclear cell infiltrate at day 3 followed by ingrowth of epithelium, granulation tissue formation and decreased inflammation at day 7 and considerable epithelization and maturing granulation tissue by day 14 (Figure 1B). Polarized images showed maturing dermal collagen organization with more organized collagen are shown in Figure 1C-D.

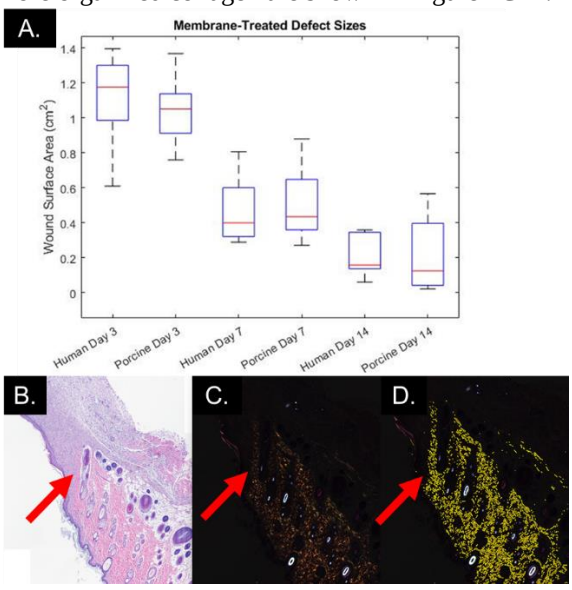

Figure 1. (A) Change in wound area across test groups (n=4) over time. (B-D) Histological collagen deposition analysis, wound edge shown by red arrow.

Conclusions: These results suggest that the potential for clinical use of porcine-derived placental ECM treatments is similar to the human-derived counterpart.

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