Bi-Layered Living Cellular Constructs Mirrored Normal Skin and Accelerated Healing in a Porcine *In Vivo* Diabetic Delayed Wound Healing Model

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Introduction

Dysregulation and impairment in the process of normal wound healing may lead to chronic wounds. A number of skin substitutes are available to cover, support, and treat chronic wounds; however, the mechanism of these products remains largely unknown. In this study, we characterized key extracellular matrix (ECM) components, markers of activated keratinocytes, cellular proliferation, and key cytokines present in a bi-layered living cellular construct (BLCCº) using immunofluorescence (IF) staining. Furthermore, a porcine *in vivo* diabetic delayed wound healing model was utilized to evaluate the impact of single or multiple applications of BLCC treatments using a porcine-derived BLCC.

º Apligraf®, Organogenesis, Canton, MA

Methods

Structural assessments for BLCCs were made using hematoxylin and eosin (H&E) staining. To evaluate components relevant to normal skin, IF staining was completed for key ECM proteins (pro-collagen I, collagen I, collagen III, collagen IV, fibronectin, and laminin), markers of activated keratinocytes (K19), proliferation (Ki67), and key cytokines (VEGF, TGF-β, HGF, IGF-1). To assess the impact of BLCCs on wound healing, a porcine-derived BLCC (pBLCC) was developed and utilized in a diabetic delayed wound healing model. After chemical induction of diabetes, full-thickness wounds were created and then either left untreated (controls) or treated with single or multiple treatments of porcine BLCC.

Extracellular Matrix Proteins in BLCC

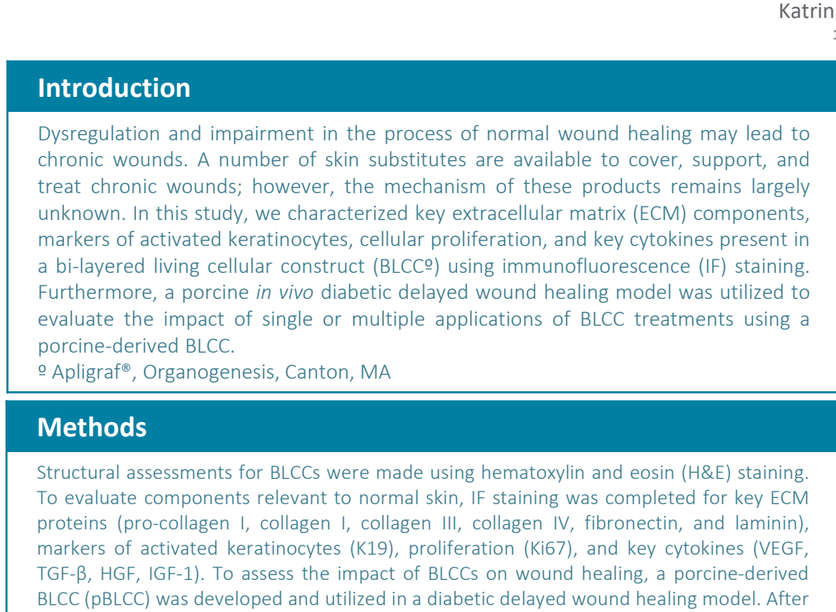

Figure 1: Extracellular matrix proteins present in BLCCs. **A)** Pro-collagen I, **B)** Collagen I, **C)** Collagen III, **D)** Collagen IV, **E)** Fibronectin, and **F)** Laminin. Blue = cell nuclei, orange = IF target. 20x magnification. Scale bar = 50µm for all images.

Activated Keratinocytes, Proliferation, and Cytokines in BLCC

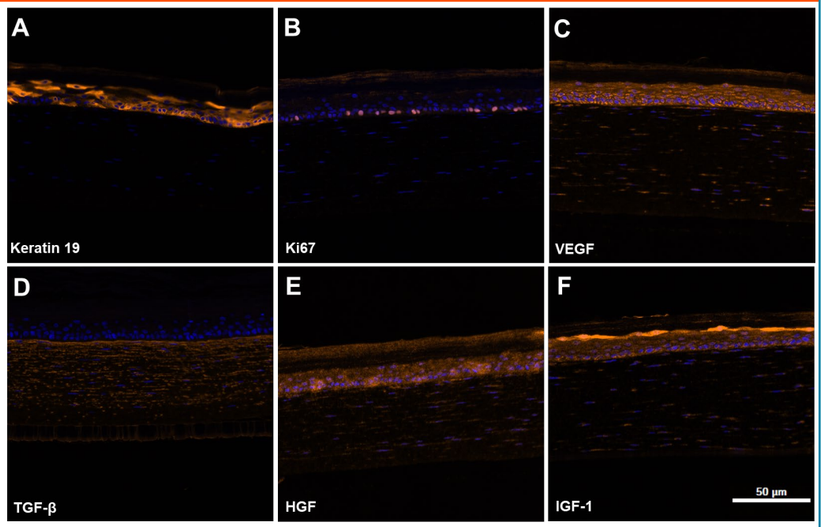

Figure 2: Markers of activated keratinocytes, proliferation, and key cytokines present in BLCCs. **A)** Keratin 19, **B)** Ki67, **C)** VEGF, **D)** TGF-β, **E)** HGF, and **F)** IGF-1. Blue = cell nuclei, orange = IF target. 20x magnification. Scale bar = 50µm for all images.

In vivo Full-Thickness Porcine Wound Model

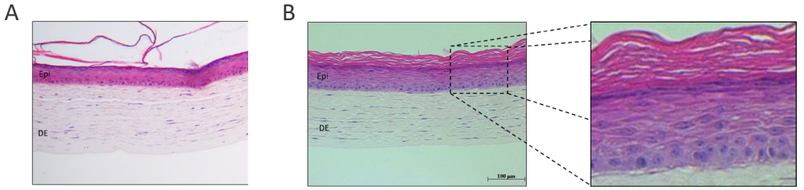

Figure 3: Porcine BLCC showed comparable morphology as human analogs using H&E staining. **A)** Human BLCC is composed with intact epidermis (Epi) and dermal equivalent (DE). **B)** Porcine BLCC is comparable to human with stratified epidermal layers clearly visible (expansion).

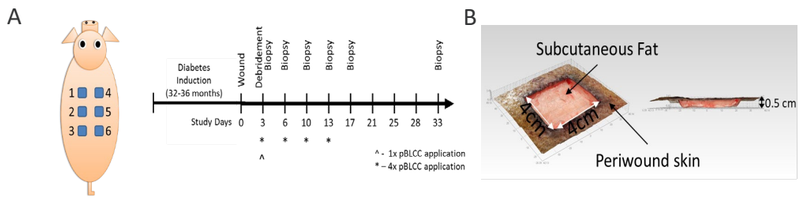

Figure 4: *In vivo* full-thickness model. **A)** Study design and timeline. **B)** 3D rendering of the full-thickness wounds extending 0.5cm to the subcutaneous fat layer.

Treatment with pBLCC Improves Wound Healing

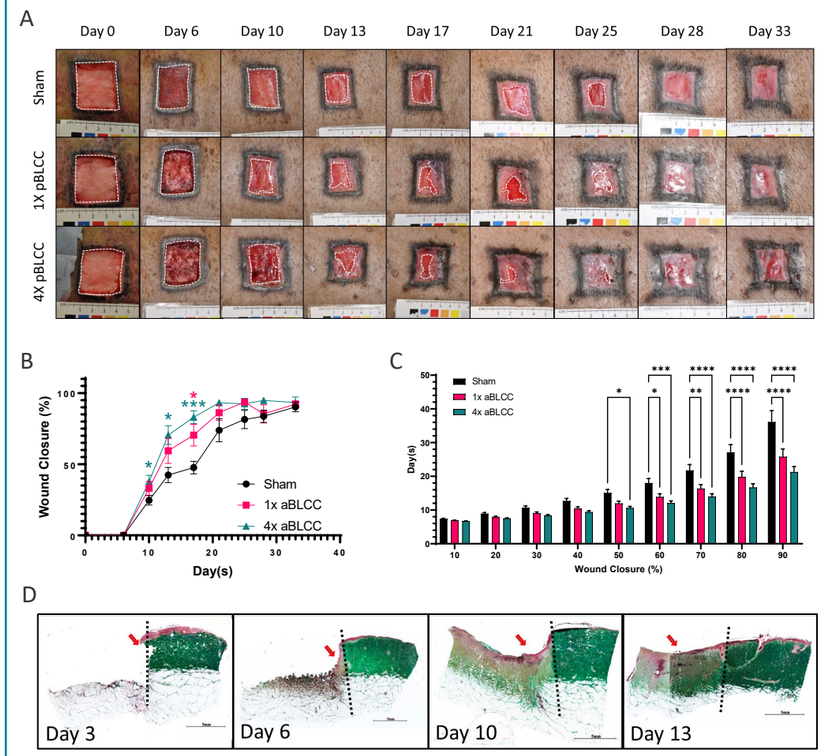

Figure 5: Single and multiple treatments of pBLCC improves wound healing in a full-thickness wound. **A)** Representative photographic analysis of wounds over time per group. **B)** Wound closure over time. **C)** Exponential plateau interpolation of wound closure based on *in vivo* data acquisition. **D)** Representative images of re-epithelialization after multiple treatments of pBLCC. Mean ± standard error reported. *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001.

Conclusions

- BLCCs contain complex components and extracellular matrix proteins that are consistent with normal skin and previous immunohistochemistry of BLCC.
- Single and multiple (4x) treatments with pBLCC resulted in significant improvements in wound healing in an *in vivo* full-thickness porcine wound model; however, 4x BLCC resulted in significant differences earlier and at more time points, suggesting a greater impact from multiple treatments.
- Using predictive methods, 4x pBLCC treated animals are predicted to achieve ≥50% wound closure quicker than sham. 1x pBLCC treated animals are predicted to achieve ≥60% wound closure quicker than sham.
- Increased wound closure was primarily driven by increased rate of re-epithelization in response to pBLCC treatment *in vivo*.

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