Healing Capacity of Bilayered Living Cellular Constructs: Gene Expression in an *In Vitro* Outgrowth Model

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ABSTRACT

Introduction: Bilayered living cellular construct (BLCC*) is the only commercially available tissue engineered cellular skin substitute with both an epidermal layer containing keratinocytes and dermal layer consisting of fibroblasts. When clinically required, fenestration of the product is performed to allow excessive wound exudate drainage; interestingly, fenestration of the BLCC results in a wound healing response of the fenestration *in vitro*[1,2], suggesting BLCC retains advanced capacity for healing. To further evaluate this capacity for wound healing, an *in vitro* outgrowth model was designed, and genetic regulation was assessed.

Methods: 4mm biopsy punches of BLCC were placed onto a tissue engineered dermal layer (DE). Units were raised to the liquid/air interface and cultured with a media designed to promote differentiation and migration of cells. Samples were assessed at days 3, 6, and 9 for outgrowth using Nile Blue staining. For genetic assessment, punches and outgrowth were excised on days 3, 6, and 9 and assessed using a RT^2 Wound Healing Profiler Array.

Results: An epithelial tongue outgrowth from BLCC punches was observed, with a total outgrowth area of approximately 32mm^2 by day 3 which doubled by day 9. Gene expression determined differential regulation of cytokines/chemokines, growth factors, cell-cell interacting proteins, and collagen synthesis/catabolic genes over time. Gene Ontology (GO) terms included those associated with cell-matrix adhesion, cell population proliferation, regulation of cellular migration, and response to wounding.

Discussion: Elevated cytokine and chemokine expression in wounded BLCC promotes the migration of epithelial cells and recruitment/maturation of immune cells. Differential regulation of proteinases is related to remodeling the extracellular matrix and support cellular trafficking. GO terms highlighted the BLCC's response to wounding and how the living cells promoted healing within this *in vitro* model.

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EXPERIMENTAL MODEL

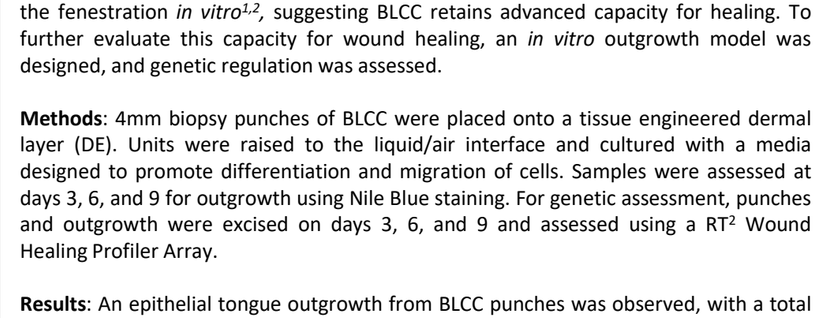

Figure 1. Experimental model. Biopsy punches of BLCC were placed upon a dermal equivalent (DE) layer and allowed to grow for 3, 6 or 9 days. At termination, samples were either assessed with hematoxylin and eosin (H&E) staining, stained with Nile Blue, or processed for RT-PCR assessment.

BLCC HEALS ITSELF THROUGH RE-EPITHELIALIZATION

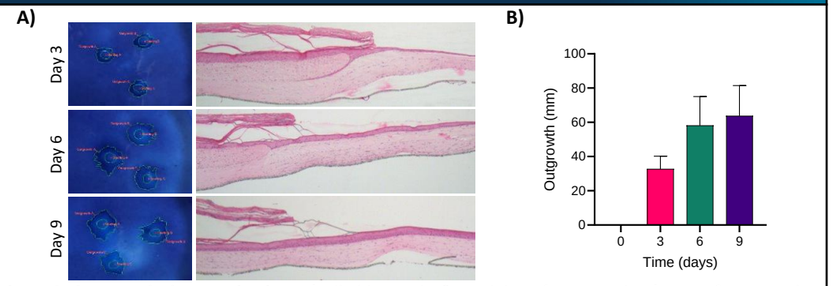

Figure 2. Outgrowth characterization. A) Nile blue and H&E staining of outgrowth. **B)** Area of outgrowth expressed as means ± StDev.

COORDINATED REGULATION OF GENES PROMOTING OUTGROWTH IN BLCCs

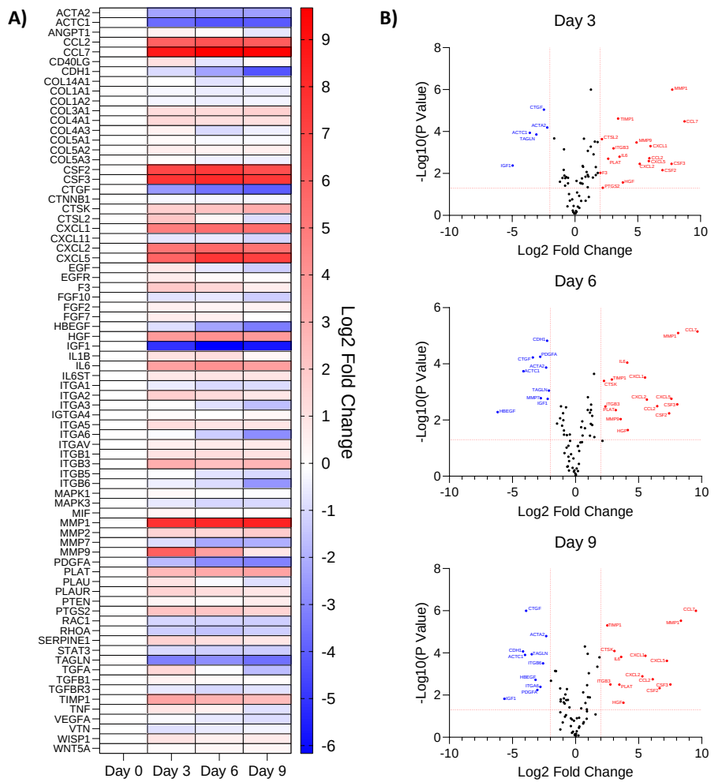

Figure 3. Genes up/down regulated as BLCC extends onto DE layer. A) Heatmap showing genes up/downregulated; Log2 Fold change expression. **B)** Volcano plots with threshold cutoffs at P value ≤ 0.05 and ± 2 Log2 Fold Changes. Upregulated genes shown in Red, downregulated genes shown in Blue.

GENES OF INTEREST: RECRUITMENT, ACTIVATION, BINDING, REMODELING

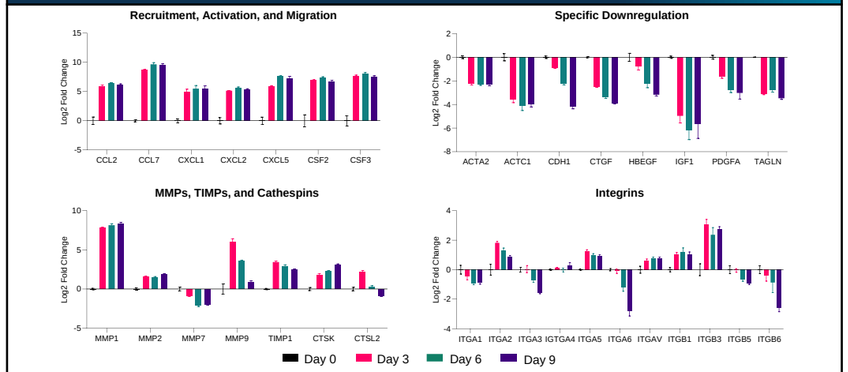

Figure 4. Target genes regulated during outgrowth. Highlights of some genes of interest regarding recruitment, activation, and migration of cells, matrix remodeling, integrins, and specifically downregulated genes. Together, these genes impact the outgrowth process of the BLCC onto the DE.

GENE ONTOLOGY AND REACTOME PATHWAY ASSESSMENT

Figure 5. GO term and reactome pathway highlight pathways associated with healing and recruitment of immune cells. While the BLCC does not contain immune cells, chemokines and cytokines generated by the BLCC could recruit host cells to the site of injury and promote a wound healing phenotype.

CONCLUSIONS

1) Biopsy punches of BLCCs extend onto the DE for the duration of the experiment.
2) Specific genetic regulation was observed that promotes migration of keratinocytes and immune/inflammatory cells.
3) Gene ontology and reactome pathway analyses determined several pathways that could promote patient healing:
   a) Cell chemotaxis, myeloid leukocyte recruitment, IL-10 signaling, IL-4/IL-13 signaling
   b) ECM organization, activation of MMPs, collagen catabolic process

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