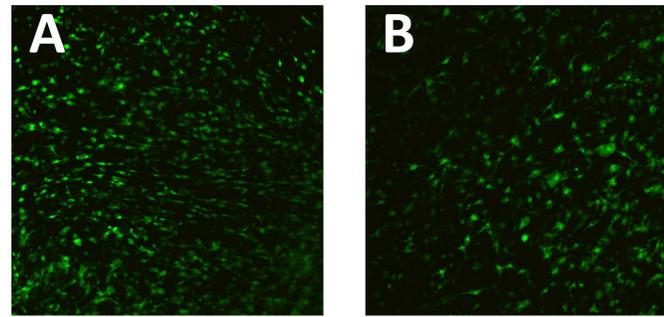


## INTRODUCTION

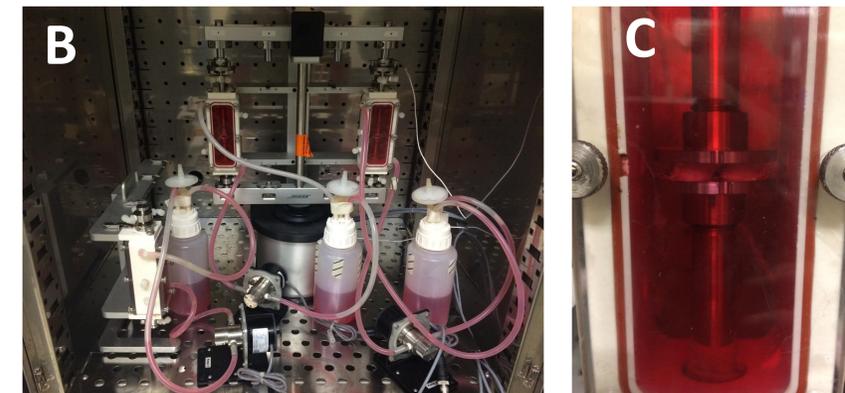
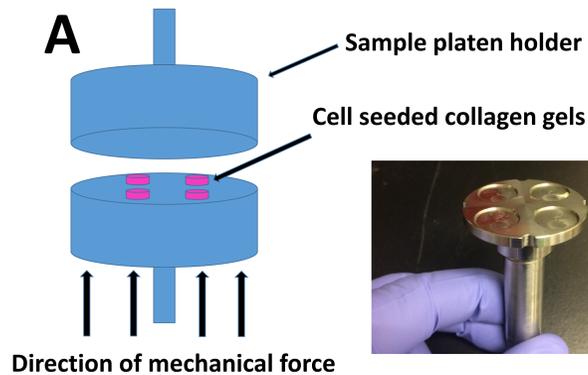
- Skin is the largest organ of the human body that provides a physical barrier to the environment and protects against ultraviolet radiation and toxic agents.
- Aged skin is characterized by a flattening of the dermal-epidermal junction, increased atrophy, and a loss of elasticity of the dermal connective tissue
- This study tested the hypothesis that the application of a unique dynamic mechanical loading regime (change in stimulus) on a human skin analogue (3D collagen gel) consisting of human dermal fibroblasts would result in the production (upregulation) of beneficial molecules associated with healthy skin and possibly, skin repair.

## MATERIALS AND METHODS

- Human dermal fibroblasts (HDF's) from 2 different donors (Female, Caucasian, 50-56 years old, non-smokers) were obtained from ATCC, and grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 7.5% fetal bovine serum at 37°C with 5% CO<sub>2</sub>. The donor sites were the cheek and the temple of the face, for Donor A and Donor B, respectively.
- HDF's were seeded in a collagen gel (4 mg/ml). Viability studies demonstrated that the cells remained viable within in the gels after 7 days (Figure 1). After solidification, cell seeded collagen gels were aseptically loaded into sterile Electroforce 5200 Biodynamic™ chambers (see Figure 2A-C) [1]. Collagen gels were held in place by sample holder platens [2]. Media was supplied to the chamber using a flow rate of 35 ml/min.
- Collagen gels were loaded in compression for 2 minutes at 15Hz (25% strain). After treatment, the gels were allowed to rest for 16 hours and the 2 minute treatment cycle was repeated again followed by a 1 hour rest at 0mm (acute treatment). The acute protocol was a single 16 hr. cycle, while the chronic treatment consisted of an initial treatment for 2 minutes, rest for 16 hours, treat for 2 minutes, rest for 8 hours, repeated over a total of 4 days.
- Control chambers consisted of cell seeded collagen gels not exposed to any mechanical compression. Gels were nourished as per the test gels.
- Upon completion of the experiments, test and control collagen gels were placed in RNeasy™ and stored at 4°C. A miRvana™ RNA extraction kit (Invitrogen, ThermoFisher, USA) was utilized to extract the RNA as per the manufacturer's instructions.
- Reverse transcription was carried out using an Omniscript kit (Qiagen, USA). Resulting cDNA was amplified using a Bio-Rad (USA) iCycler and human specific primer sets for the molecules assessed. Resulting values were normalized against the 18S gene (housekeeping gene). Experimental values were divided by control values to yield fold change.



**Figure 1:** Confocal images of (A) Donor A and (B) Donor B cells sequestered within the collagen gel after 7 days. Images demonstrate that the majority of the cells (green luminosity) within the gels were viable after 7 days.

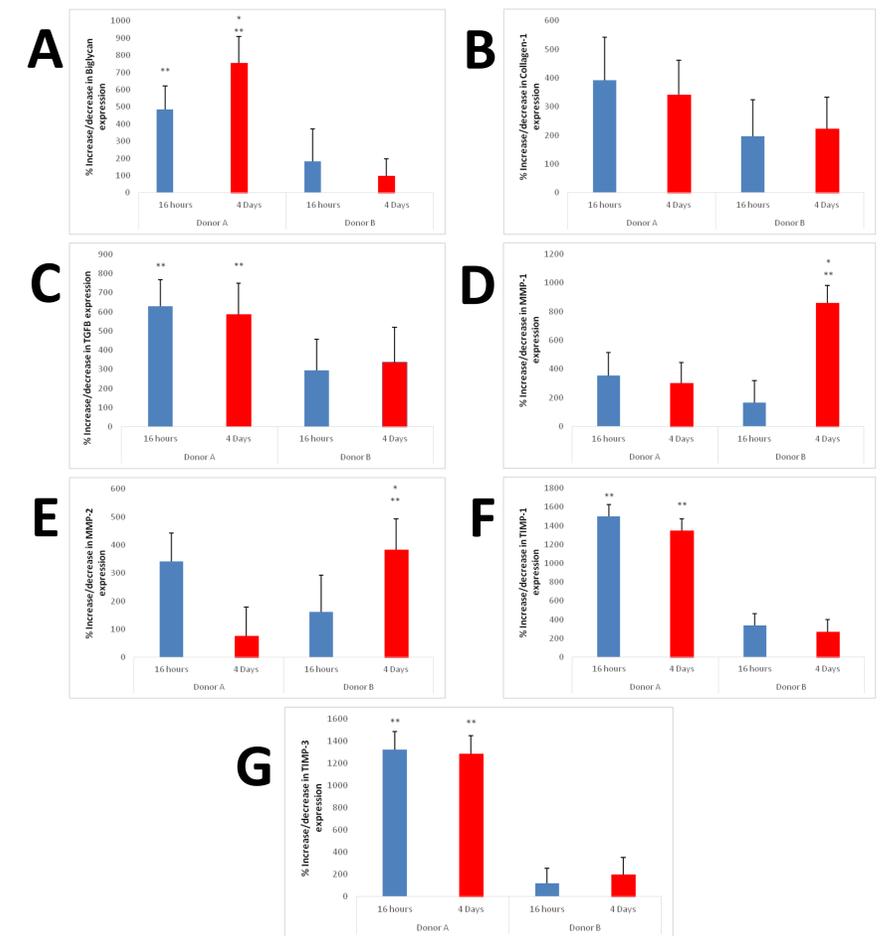


**Figure 2:** (A) Diagram and close up of the testing platens (B) Electroforce 5200 Biodynamic testing system (C) Close up of the Biodynamic testing chamber, samples and sample platen.

## RESULTS

The acute treatment (16 hours) was observed to produce significant upregulation in biglycan, collagen-1, TGFβ, MMP-1, MMP-2, TIMP-1, and TIMP-3 mRNA levels for both donor cell populations (Figure 3)

The chronic treatment (4 days) protocol was observed to continue the trends observed following acute treatment for both donor cell populations. There were some exceptions in that a significant decrease in MMP-2 was observed for Donor A population, whereas significant increases were observed for biglycan for Donor A and MMP-1 and MMP-2 for Donor B (Figure 3).



**Figure 3:** Influence of the novel mechanical loading regimen on the expression of (A) Biglycan (B) Col-1 (C) TGF-β (D) MMP-1 (E) MMP-2 (F) TIMP-1 (G) TIMP-3 after 16 hr [blue] and 4 days [red] for Donor A and B (n = 24 gels, \* = Significant difference (p≤0.05) when compared to 16 hour data of the same donor, \*\* = Significant difference (p≤0.05) when compared to other donor data at the corresponding time point).

## DISCUSSION

- The mechanical loading protocol (optimized in preliminary studies) imposed on the cell seeded collagen gels produced significant upregulation in beneficial molecules associated with hydration, remodelling, and regeneration. Such responses were observed for both the acute and chronic treatment protocols.
- Despite the significant upregulation for both donor cell populations, there were differences in the intensity of the response between Donors A and B. These data suggest that Donor A is a rapid and possibly, high level responder while Donor B is a slower and lower level responder. These differences may be due to genetic or epigenetic differences between the two donors, or possibly differences in environmental exposure, and warrants further investigation.

**References:** [1] Armentano RL, Valdez Jasso D, Cymberknop LJ, Montini Ballarin F, Velez D, Caracciolo PC, Abraham G (2015) High pressure assessment of bilayered electrospun vascular grafts by means of an Electroforce Biodynamic System®. Conf Proc IEEE Eng Med Biol Soc. 2015:3533-6.

[2] Jing Lu, Jacques Laudinet and Sandy Williams (2008) Mechanical stimulation and evaluation of hydrogel biomaterial. 18 (4-5): 335-337.