DERMAL FIBROBLASTS FEEL AND RESPOND TO PHYSIOLOGICAL MECHANICAL CUES IN VITRO

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Introduction

Cells in living tissues such as the skin are constantly exposed to the "mechanome" – a collection of stimuli of mechanical origin that can affect cell behavior and biological functions [1]. *Ex vivo* experiments and related computational models allowed us to quantify secondary chemomechanically coupled stimuli associated with skin stretch, such as local changes in fluid flow (Δμ), osmotic pressure (Δπ) and hydrostatic pressure (ΔP) [2]. Additionally, mechanical stimulation has been shown to improve the maturation of tissue engineered skin and to increase the proliferation of fibroblasts [3]. Tissue expansion – an existing mechanotherapy – stimulates skin growth using stretch, yet the mechanisms behind such profound effects of mechanical stimuli on skin remain unknown. It is likely that fibroblasts play a key role, since they are the main cell type in the dermis capable of remodeling the extracellular matrix, but the magnitude of stimulation needed to cause biological changes in these cells is unknown. We established *in vitro* systems to study the effects of osmolarity, hydrostatic pressure, and fluid flow on dermal fibroblasts in 2D and 3D cultures [4].

Methods

We developed dedicated bioreactors to expose primary adult human dermal fibroblasts cultured on tissue culture plastic (2D) or in collagen hydrogels (3D) to physiological increases in osmotic pressure (10 mOsm), hydrostatic pressure (20 kPa), and flow (10-20 um/sec). As an indicator of initial cell response, we measured intracellular calcium signaling using live-cell imaging. We further checked for downstream signaling pathway activation by western blotting. Lastly, we studied gene expression changes using bulk RNA sequencing.

Results

Live calcium imaging revealed distinct changes in intracellular calcium levels after changes in flow and hydrostatic pressure, but less so in response to osmolarity, suggesting that dermal fibroblasts feel chemomechanical stimuli even at these low levels. Furthermore, western blots revealed increased phosphorylation of AKT after exposure to 20 kPa pressure, suggesting that the initial calcium response to pressure is followed by intracellular signaling (Fig. 1a). Lastly, RNA sequencing showed that cells exposed to hydrostatic or osmotic pressure for 24 hours exhibit significantly altered gene expression in both 2D and 3D cultures, suggesting that the protein signaling cascade triggered by changes in chemical potential leads to transcriptional changes (Fig. 1b). Interestingly, cells in 3D cultures showed a stronger response than cells in 2D.

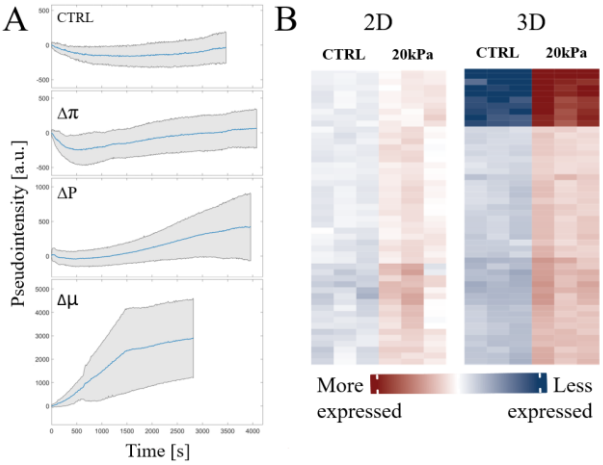

Figure 1: (A) Intracellular calcium levels, measured as pseudointensity, show distinct patterns when fibroblasts are exposed to chemomechanical stimuli. (B) Gene expression profiles of fibroblasts in 2D and 3D culture conditions after 20 kPa hydrostatic pressure for 24 hours compared to controls.

Discussion

Collectively, our results suggest that dermal fibroblasts not only feel, but also actively respond to mechanical stimuli in the physiological range. The response of cells in our 3D system underlines the importance of selecting an appropriate cell environment to study physiological stimuli *in vitro*. Further studies are needed to improve our understanding of human dermal fibroblast response to mechanical cues as this can aid in developing new mechanotherapies and understanding skin pathologies.

References

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