GUIDING CARDIAC FIBROBLAST ORGANIZATION BY STIFFNESS PATTERNS OF GELMA HYDROGELS

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Introduction

Cells are greatly influenced by the biomechanical signals of the extracellular matrix (ECM). In particular, ECM stiffness gradients and patterns are critical in cell migration (durotaxis) (1). On the other hand, cell and ECM organization is essential for the function of tissues like the myocardium. Several myocardial diseases, such as myocardial infarction (MI), are associated with a loss of cell organization and change ECM stiffness, with subsequential tissue malfunction. ECM mechanical cues are being used to guide cellular organization in engineered tissues and in vitro environments (2). However, little is known about how ECM stiffness guide cell organization. Therefore, a better understanding of this phenomenon will help to develop novel regenerative strategies. To the best of our knowledge, we report the first experimental evidence on how cardiac fibroblasts (cFb) align on ECM stiffness patterns on gelatin methacryloyl (GelMA) hydrogels.

Methods

10 µL of GelMA solution (10 % w/v) was placed in a bottom glass 6-wellsplate well and allowed for physical gelation. Then, the gels were placed in a fluorescence microscope (Leica, DMi8) stage with a coupled PRIMO (Alvéole, France) device. PRIMO allows for UV-crosslinking of the gels with high-spatial resolution without using photomasks. Stiffness patterns of width 20, 50 and 200 µm separated 50 µm and length 500 µm were created with 10 s of UV illumination at 8.9 mW/cm^2. Nanoindentation (Optics11) was used to measure the stiffness patterns created. Primary cFbs were seeded on top of the stiffness patterned gels and cultured for 24h in high-glucose DMEM medium (supplemented with 10% FBS and 1% P/S). Subsequently, samples were fixed and immunostained for the actin cytoskeleton and nuclei. cFb orientation was calculated using an open-source MatLab code (FOAtool) (3).

Results

Stiffness patterns were created successfully with the stiffness of the UV-exposed area ~5-fold higher than the unexposed (Fig. 1A). cFb cultured on top of the gels aligned along the direction of the patterns (90º) for the 20 and 50 µm width patterns. In contrast, cFb seeded on 200 µm width patterns did not show any preferred aligned direction (Fig. 1B, C).

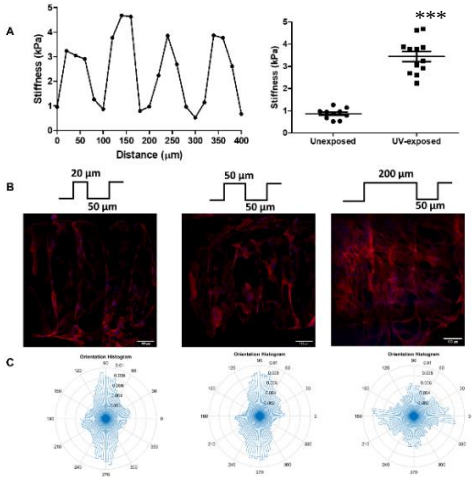

Figure 1: A. Nanoindentation measurements of patterns 20 µm width (left). Stiffness differences between the gel's unexposed and UV-exposed area ($p<0.001$). B. Immunofluorescence images of nuclei (blue) and actin (red) of cFb on top of the 20 µm, 50 µm and 200 µm patterns. C. Angle histograms with the cell fraction alignment. 90º corresponds to the direction of the pattern long axis.

Discussion

We have applied a technique to locally crosslinking GelMA gels with UV light without needing photomasks. This technique can manipulate ECM stiffness in a high resolution in living cultures. Our results show that cFbs align in patterns below 200 µm in width, suggesting that stiffness-guided cell organization is effective at patterns sizes similar to cell dimensions. This phenomenon is similar to contact guidance generated by protein patterns (4). This evidence can serve to improve the understanding of how mechanical cues shape cell and tissue organization after MI.

References

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