Anisotropically Stiff Micropillars as a Unidirectional Cell Entrapment and Alignment Niche for Cardiomyocytes

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Statement of Purpose: In cardiac tissue engineering, highly aligned and elongated cardiomyocytes are needed to achieve functional cardiac tissues. Even though topographically anisotropic substrates have been utilized to align cardiomyocytes [1, 2], there is still a need for microengineered platforms for highly organized tissue constructs. For the first time in the literature, we have utilized anisotropically stiff micropillar arrays to entrap and align cells within a 3D environment. We hypothesize that in an anisotropically stiff 3D micropillar system will provide the unidirectionally aligned and elongated cellular phenotype essential for cardiac tissue engineering.

Methods: Micropillar arrays were fabricated in both isotropic and anisotropic configurations: (i) 5x5 μm and 7x7 μm (isotropic), and (ii) 5x2.5 μm and 7x2.5 μm (anisotropic). Poly(dimethylsiloxane) (PDMS) replica-molding was used to fabricate negative template by casting PDMS over SU-8 micropillar master. After curing and peeling off, micropillar arrays were obtained by casting PDMS over the negative template. Afterwards, human mesenchymal stem cells (hMSCs) and human cardiomyocytes were seeded on isotropic and anisotropic micropillar arrays. Overnight cultured cells were labeled with cytoskeletal F-Actin and nuclear staining to analyze cell alignment, elongation, and entrapment in 3D anisotropically stiff micropillar environment (Fig. 1a&b). Cellular anisotropy was quantified by taking the ratio of total length of cells in horizontal direction to vertical direction in a field of view.

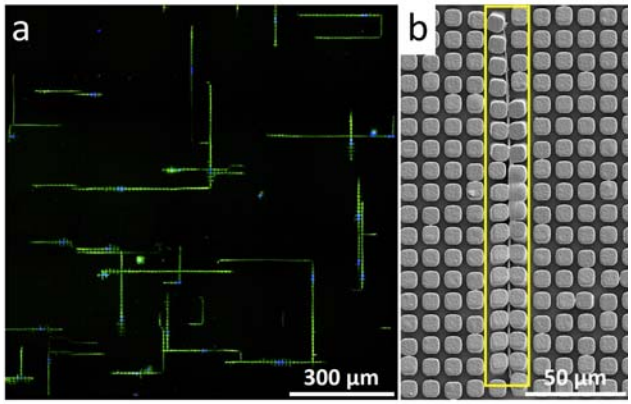

Fig.1. (a) Cells (F-actin, green) and their nuclei (DAPI, blue) display elongation in 3D micropillar environment. (b) Scanning electron microscope image of an elongated cell entrapped within inter-pillar space.

Results: Both hMSCs and cardiomyocytes exhibited binary alignment (i.e. comparable number of cells aligned horizontally and vertically) in isotropically stiff square micropillar arrays (Fig. 2a, c, e, and g). On the other hand, both cell types were elongated consistently parallel to the stiffer axes of anistropically stiff micropillar arrays (Fig. 2b, d, f, and h). Furthermore, both cell types were elongated up to 500 μm in length, filling the gap between two neighboring micropillar arrays.

Conclusions: The microengineered approach presented here induces cells to elongate and align along the stiffer axes of micropillars while confining cells in 3D. Cultivation of highly elongated and aligned cardiomyocyte constructs can potentially lead to a highly structured and functional cardiac tissue. Furthermore, highly organized cell arrays can be utilized in other tissues of elongated cell phenotype such as nerves, tendons and ligaments. Ongoing work is focusing on the effect the presented micropillar system on differentiation of MSCs to cardiomyocytes.

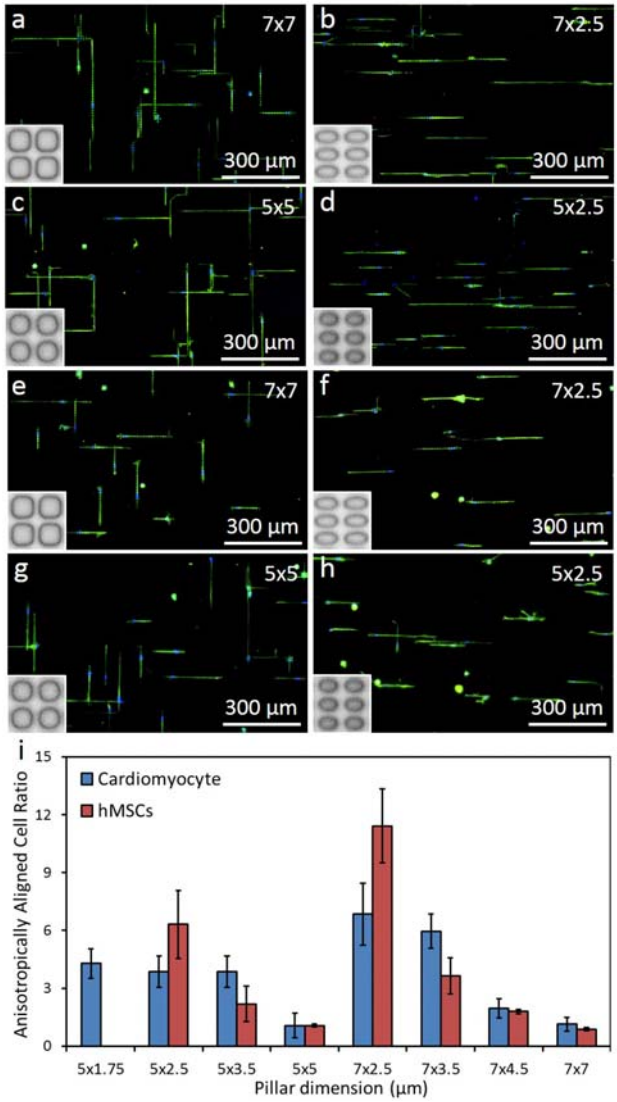

Fig.2. Alignment of hMSCs and cardiomyocytes in micropillar arrays. Alignment and elongation was observed for (a-d) hMCSs and (e-h) cardiomyocytes cultured in micropillar arrays of isotropic and anisotropic geometries, indicated by cytoskeletal F-Actin and nuclear staining. (i) We observed significantly greater alignment of both hMSCs and cardiomyocytes in anisotropically stiff, 7x2.5 μm micropillar array.

References:

[1] Nikkhah M. et al. Biomaterials. 2012;33: 5230-5246.

[2] Rao C. et al. Biomaterials. 2013;34: 2399-2411.