Stretch Induced Cell Sheet Alignment

E. Thomasson[1], M. Aschwanden[2], R. Ilinca[1], R. Enning[2], A. Stemmer[2], J. Vörös[1], A. Franco-Obregón[1]

[1]*Laboratory of Biosensors and Bioelectronics, ETH Zürich, Switzerland.*

[2] *Nanotechnology Group, ETH Zürich, Switzerland.*

INTRODUCTION: Cell sheets are a promising new strategy in tissue engineering. For instance, primary corneal epithelial cells grown *in vitro* form sheets of tightly adhered cells possessing an ECM matrix that can then be successfully transplanted into hosts without any carrier substrate or sutures. [1] On the other hand, cardiac myocyte cell sheets can fail, since the myocytes often are not oriented properly with reference to cardiac contractility. [2] In order to create a better mimic of *in vivo* conditions the aim of this project is to create aligned cell sheets by applying well defined modalities of stretch. Moreover, cyclic stretch serves as a mitogenic stimulus to promote myoblast proliferation. Lastly, substrate stretch will be used to detach the myoblasts sheets from our actuators. This approach is simple and elegant since no exogenous substrates are required.

METHODS: A two-dimensional electroactive polymer (EAP) bioreactor has been developed. The technique relies on a new methodology to apply well-defined stresses onto differentiating muscle cells with a precision not imaginable with existing technologies. A frequency range of tens of mHz to Hz is possible to achieve. The spatial resolution of stretching, moreover, can approach the single cell level. The substrate facing the cells, Poly(dimethylsiloxane) (PDMS), is turned slightly hydrophilic by UV exposure and subsequently functionalized with weakly cell-adhesive Poly(L-lysine). Proliferating myoblasts from C2C12 culture were plated at different densities onto the surface of the bioreactor and once adhered, different stretch frequencies during different time courses were tested in order to provoke alignment as well as the subsequent detachment.

RESULTS: Myoblast alignment perpendicular to the direction of the stretch vector was achieved reproducibly when cyclic stretch at 0.05 Hz was applied, see figure 1. The effect was visible after a few hours or after stretching over night. Once aligned, the cells remained aligned, regardless of stretching amplitude. Detachment was visible in the cultures plated with high cell density where a sheet left the surface spontaneously within a few hours after plating. As the cell density at the time of plating was decreased, sheets could be mechanically detached in patches preferentially from the region of stretch at the substrate.

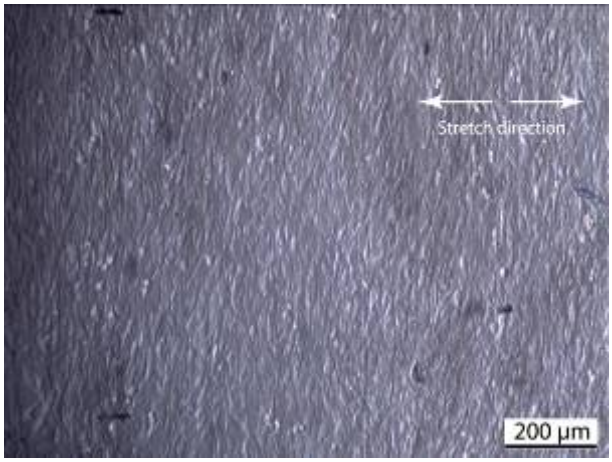

Fig. 1: Myoblasts aligned perpendicularly to the stretch vector.

DISCUSSION & CONCLUSIONS: These results indicate that a platform for the creation of aligned cell sheets is feasible. Alignment has been already achieved. The sheets detaching in patches preferentially on the stretching region indicates that mechanical stimuli can break the cell substrate interaction. Moreover, the cell-cell junctions are preserved in the presence of physiological levels of calcium. Furthermore, it would be interesting to investigate how stretch activated calcium channel blockers might alter the focal adhesions in the direction of detachment. With a functional detachment technique, fusion of layers of sheets would then be the next step towards a functional cell sheet under *in vivo* conditions.

REFERENCES: [1] N. Matsuda, et al (2007) *Advanced Materials* **19**:3089-99. [2] M. N. Giraud et al (2007) *Tissue Engineering* **13**: 1825-36.

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