A Microdevice for Simultaneous Applications of Topographic Cues and Cyclic Tensile Strains to Live Cells

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Introduction: Cell alignment plays a critical role in tissue development and regeneration, e.g. it is critical for pattern formation during embryogenesis and regulation of contractile strength in musculoskeletal tissues. Therefore, aligning cells *in vitro* is important for mechanobiological studies. Previous studies showed both passive mechanical stimulation (*e.g.* topographic cues) and active mechanical stimulation (*e.g.* cyclic loading) can align the cells. The synergistic effect of these two stimulations is, however, not fully explored, partially due to the lack of appropriate tools for the applications of both stimulations in a controllable manner.

Materials and Methods: Here, surface wrinkles were created on a PDMS substrate by hardening its outmost surface by electrical discharging while stretching it, and releasing the substrate to its original dimensions. The wrinkles formed due to stiffness mismatch were transferred to another PDMS membrane by a double sided replica molding process, were the rectangular membranes were created simultaneously. The wrinkles were perpendicular to the longitudinal direction of the membranes so that the uni-axial strain generated upon membrane deformation aligned with the wrinkles. The winkle depth (peak-to-peak) ranged from 130nm to 500nm. The wavelength-to-depth ratio was about 5 for all the conditions.

Results and Discussion: NIH 3T3 fibroblasts were seeded on the substrate and allowed to adhere. As the culturing proceeds, the cells gradually oriented towards the longitudinal axis of the wrinkles regardless of wrinkle size. After 12 hours, the average cell orientation angles in all the wrinkling groups were about above 70°, suggesting that most cells aligned along the wrinkles. Afterwards, cyclic tensile loadings were applied by pumping the rectangular membranes. Such loading successfully reoriented cells in presence of wrinkling cues. Under the same loading condition, cell reorientation was dependent on wrinkle size, *i.e.* cells on shallower and narrower wrinkles reoriented faster, and vice versa. With the loading frequency of 0.5Hz and the maximal strain magnitude of about 16%, the mean cell orientation angle on 500nm depth wrinkles decreased from 82° at the pre-loading condition to 56° after 2 hours, and further to about 20° after 12 hours (Figure 1a&b). After loading, the cells were kept in culture for 12 hours, during which cells reoriented again towards the longitudinal axis of wrinkles (Figure 1c). Cells on deeper and wider wrinkles reoriented faster, and vice versa. Above results suggested that deeper and wider winkles are stronger topographic cues in regulating cell orientation and resisting the alignment cues from cyclic loading. The loading magnitude varied cell reorientation time in presence of wrinkling cues. Cell reorientation after loading removal, however, was not affected by the loading magnitudes they previously experienced (Figure 1d).

Conclusions: This paper reports the development of a micromechanical stimulator that applies both in-plane mechanical strains and periodical wrinkles towards live cells for studying the synergistic effect of passive and active mechanical stimulations.

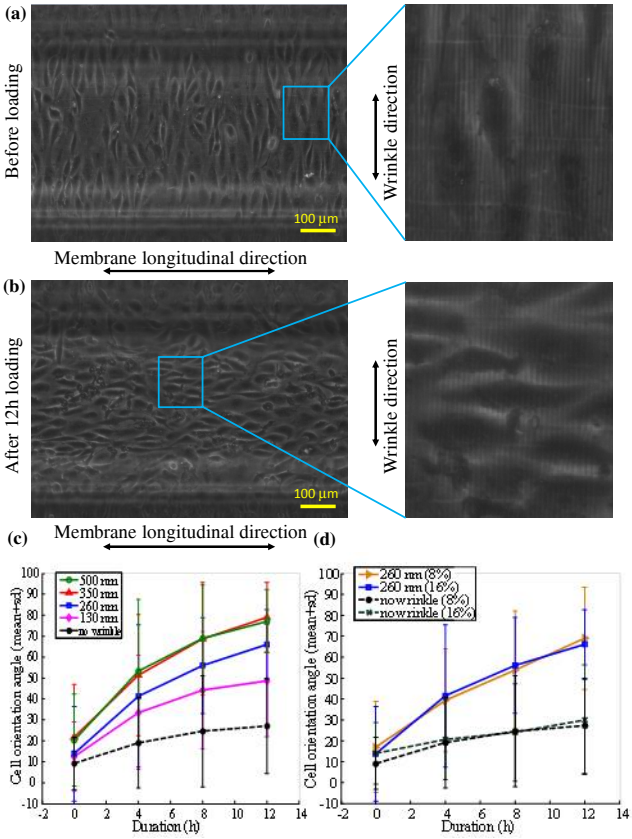

Figure 1: Cell orientation changes: (a) before loading (after 12h seeding) and (b) 12h cyclic loading. The loading frequency was 0.5 Hz. The maximal strain is 16% at the membrane center. The wrinkles were about 500nm in depth and about 2.4μm in wavelength. (c) during the unloading period following cyclic loading with the maximal strain of 16%; and (d) comparison of the orientation changes during the unloading period following the cyclic loading with the maximal strains of 8% and 16%