Reconfigurable Substrate Topography for Tracking Cell Dynamics

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Statement of Purpose: Microtopography in both natural biological systems and synthetic materials can guide cell expressions, including cell morphology, migration, and differentiation. However, the interactions and mechanisms between cells and topography are still unclear. Microtopographic substrates with on-demand reversibility are promising in revealing such mechanisms because it can correspond to cellular responses. Here, we present a lithography-free reconfigurable substrate that is controllable using small strain stimulation. The microtopography with a consistent microwavy pattern is generated due to two-layer instability of a coating film and a substrate. This method advantages in measuring cellular dynamics because it is rapid and easy to manipulate the forms of topography, and also provides minimized interrupting external stimuli.

Methods: Reconfigurable PDMS substrates are switched in two states, flat and microwavy, to activate the cell-topography responses. Briefly, the PDMS substrate is coated with a thin layer of silicon oxide, 100 nm in thickness, applied under a small percentage of strain. Due to instability of the two layers, the substrate can generate buckling patterns in responses to applied strain. Before seeding NIH 3T3 fibroblast cells, the substrate is sterilized and coated with small RGD peptides to increase the cell attachment. The cells are cultured on the substrate with a flat surface for 24 hours. The substrate is either non-switched, as a negative control, or switched in two manners: flat-to-wavy and flat-to-wavy-to-flat to activate the cell responses. The cells are fixed and stained using phalloidin and DAPI. Both live and fixed cell morphodynamics is recorded at different time points up to six hours.

Results: The PDMS substrate with a thin layer of silicon oxide on the surface can generate the microtopography at very low pre-strain percentage. The minimum pre-strain that provides consistent buckling pattern is 3%, as showed in the **Figure 1**. This feature can be repeatedly switched on and off according to the strain applied. The distinct three states of the substrate features include: flat substrates (A = 1.53 ± 0.55 nm and Rms = 0.317 ± 0.048 nm); parallel grating arrays ($A_{\parallel}$ = 483.6 ± 7.8 nm and $\lambda_{\parallel}$ = 4.78 ± 0.16 mm); perpendicular grating arrays ($A_{\perp}$ = 429.3 ± 5.8 nm and $\lambda_{\perp}$ = 4.95 ± 0.36 mm). For cell-topography responses, the morphology of 3T3 fibroblasts is significantly different after 24 hours of cell seeding between flat and wavy substrates. This illustrates that the cells can sense and response to the generated features. We further investigate the cell morphodynamics after that time point by switching the feature from flat to wavy and from flat to wavy to flat. Comparing to the static flat and wavy substrate, both of the switching patterns stimulate the cell responses to the topographical changes. Briefly, switching from flat to wavy yields continuously gradual changes of morphodynamics toward the cell morphology on the static wavy surface over six hours. Switching from flat to wavy to flat activates the similar change as the previous switching pattern in the first two hours, approximately. The morphodynamics then perpetually change back to the beginning flat response. The live cell imaging of a single cell also confirms the morphological change in the similar manner.

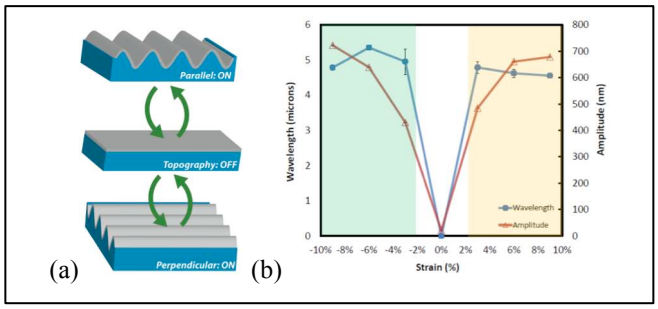

Figure 1. Reconfigurable topographic substrate: (a) On-demand switchable microwavy substrate. (b) Amplitude and wavelength in response to applied strain.

Conclusions: Recognizable substrate is a tool for elucidating cell-topography interactions. Here, we fabricate the topographic substrate that can be controlled using small strain for limiting external stimuli. The substrate presents consistent buckling patterns that benefit in measuring cell responses, such as cell morphodynamics. This technique can also measure or control cell migration and differentiation that could be used in various biomedical applications.

References: (H Jiang. Proc Natl Acad Sci USA. 2007;104:15607–12.) (H Wu. Org Electron. 2013;14:1636–42)