Modulation of Cellular Response Using Mechanically Dynamic PDMS Substrates

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Statement of Purpose: Mechanics of the extracellular matrix (ECM) play a pivotal role in governing cell behaviors, such as cell spreading and stem cell differentiation.[1] The stiffness of the ECM has been recapitulated by several biomaterial systems; however, most systems lack the ability to model dynamic phenomena of native ECM during biological processes (e.g., fibrosis). Stiffening hydrogels have been investigated to introduce dynamic processes to cell cultures, but they lack the viscoelastic nature of many tissues.[2] Here, we developed a two-step crosslinking strategy to alter the mechanics of polydimethylsiloxane (PDMS) and demonstrate that cells respond to dynamic mechanics.

Methods: Polydimethylsiloxane (PDMS, Sylgard® 184 silicone elastomer kit) was cured with a base-to-curing agent weight ratio of 65:1 at 37 °C for 48 hr. Photoinitiator (2,2-dimethoxy-2-phenylacetophenone, DMPA) and thiolated crosslinker (4-6 wt% [(mercaptopropyl) methylsiloxane]- dimethylsiloxane copolymer (S-PDMS)) were incorporated into the PDMS substrates through a swelling process using toluene as a swelling solvent, and stiffening was induced with toluene evaporation and light exposure (365 nm, 15 mW/cm^2, 2 min). The viscoelasticity and stiffness of PDMS substrates were measured using atomic force microscopy (AFM) nanoindentation and force relaxation in PBS at the microscale, and dynamic mechanical analysis (DMA) at the macroscale, respectively. NIH 3T3 fibroblasts were used to investigate cell response to the modified PDMS substrates as well as the effect of substrate stiffness on cell behavior.

Results: The stiffness-tunable PDMS substrates were fabricated through a two-step crosslinking process: (1) platinum-catalyzed hydrosilylation to crosslink vinyl-functionalized PDMS base polymer (V-PDMS) and (2) thiol-ene click polymerization between (mercaptopropyl) methylsiloxane-dimethylsiloxane copolymer (S-PDMS) and V-PDMS in the presence of photoinitiator and UV light (Figure 1a). After UV exposure, the increase in crosslink density is signified by the longer relaxation time constants of modified PDMS substrates, as measured by AFM (Figure 1b). The exposure to UV light also resulted in a stiffening of the PDMS substrates from ~3 to 45 kPa (15-fold increase), and the modified PDMS substrates were stable in serum-containing media and could be stiffened for up to 7 days (Figure 1c).

3T3 fibroblasts were cultured on the modified PDMS substrates, where no cytotoxicity was observed even after UV exposure and cells proliferated up to ~ 7-fold increase in cell population after 7 days. Fibroblasts were minimally spread on the PDMS substrates (~ 750 μm^2), but then spread two times more (~ 1500 μm^2) with higher aspect ratios after UV exposure (Figure 1d, e). Spreading was similar to pre-stiffened PDMS substrates (results not shown). These results indicate that the increased PDMS substrate stiffness facilitated the spreading of cells and an elongation in cell morphology.

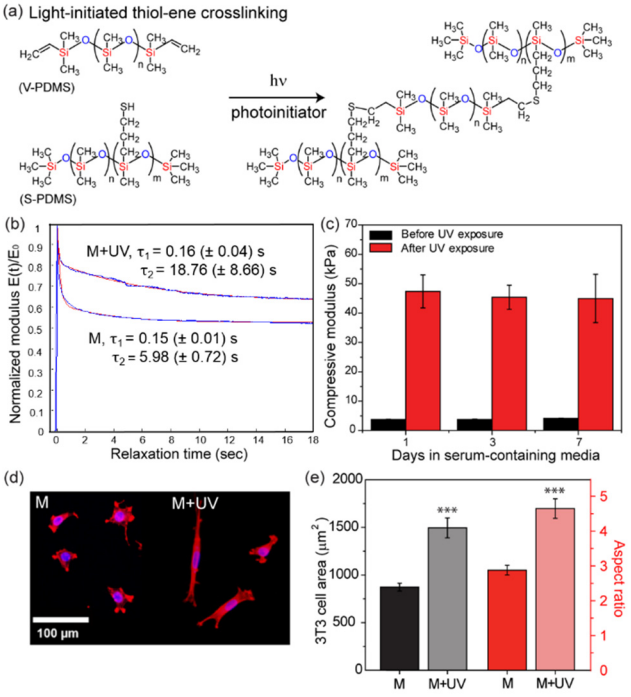

Figure 1. (a) Schematic illustration of the secondary thiol-ene crosslinking of PDMS polymers. (b) Force relaxation curves show different relaxation behaviors of modified PDMS substrates before (M) and after UV exposure (M+UV). E(t), E_0, τ_1 and τ_2 are temporal modulus, initial instantaneous indentation modulus, short and long relaxation time constants, respectively. (c) Stiffness changes of photoresponsive PDMS substrates after an incubation in 10% serum-containing media over time. (d) Fluorescent images, (e) spreading area and aspect ratio of fibroblasts on modified PDMS substrates before and after UV exposure at 24 hr. Data presents mean ± s.e.m. ***$p < 0.001$.

Conclusions: We present an orthogonal crosslinking strategy to fabricate mechanically dynamic PDMS substrates, where the viscoelasticity and stiffness of the PDMS substrates can be temporal and spatial controlled using light. These photoresponsive PDMS substrates are cytocompatible and can be applied to regulate the cell morphology, showing a strong correlation between substrate stiffness and cell spreading and elongation. These results demonstrate the photoresponsive PDMS substrates provides a promising platform to study cell-matrix interactions in a dynamic manner.

References:

1. Higuchi, A. *et al. Chem. Rev.* 2013; 113: 3297.
2. Guvendiren, M. *et al. Nat. Commun*. 2012; 3: 792.