

## BIOENGINEERED SUBSTRATA TO PROBE SUBSTRATE COMPLIANCE EFFECTS ON VASCULAR SMOOTH MUSCLE CELL BEHAVIOR

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**INTRODUCTION:** During the development of vascular occlusive disease, stiffening of the vessel is often observed. Recent studies have shown that changes in substrate compliance affect cell adhesion, migration, and differentiation. Vascular smooth muscle cell (VSMC) proliferation is altered during vascular injury and contributes to restenosis. However, the effect of substrate compliance on VSMC behavior is unclear. We hypothesize that the biomechanical properties of the substrate can modulate VSMC behavior. In order to test this hypothesis, we investigated the behavior of VSMCs on polydimethylsiloxane (PDMS) substrata with mechanical properties in the range of human aortas.

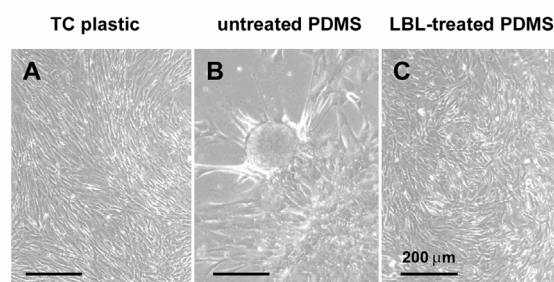
**METHODS: *Substrate preparation and characterization.*** Polydimethylsiloxane (PDMS) substrata are prepared using SYLGARD 184 (Dow Corning). Different ratios of silicone elastomer base and curing agent are mixed to obtain PDMS substrata with different elastic moduli. The surfaces of the PDMS substrata are modified with polyelectrolyte layers using the layer-by-layer (LBL) technique. Briefly, layers of polyethyleneimine (PEI, 0.1%) and polystyrenesulfonate (PSS, 0.3%) are alternatively assembled onto the PDMS surface. The substrata are coated with a total of 6 layers and were washed with sterile PBS. The elastic moduli are determined using a standard tensile test.

**Cell culture.** Bovine vascular smooth muscle cells are maintained in Dulbecco's modified Eagle's medium supplemented with 10% calf serum, L-glutamine and penicillin-streptomycin. Cells are grown to confluence and then serum-starved for 2 days. Cells are re-suspended in either serum-free media or complete media (10% serum) for experiments.

**Cell attachment.** The number of cells attached after 2 hours is determined by acid phosphatase assay.

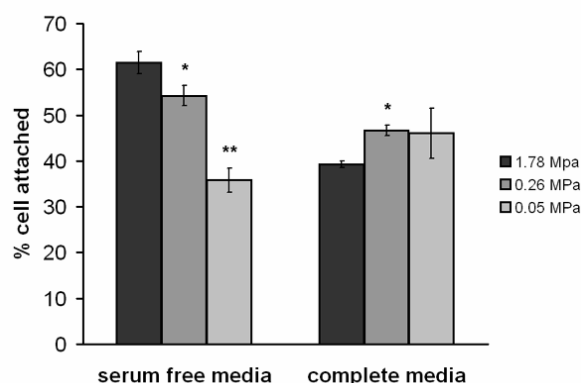
**Cell proliferation.** Serum-stimulated rates of cell proliferation are determined by acid phosphatase assay.

**RESULTS:** Using three different ratios of silicone elastomer base and curing agent, we were able to generate substrata with elastic modulus ranging from 0.05 to 1.78 MPa. Layer-by-layer treatment of PDMS significantly improved VSMC adhesion (Fig 1).



**Fig. 1: Surface treatment of PDMS is required to maintain VSMC adhesion.** Phase contrast images of VSMCs after 5 days culture on (A) tissue culture plastic, (B) untreated PDMS, and (C) layer-by-layer-treated PDMS.

We found that percent cell attachment increases with substrate elastic modulus (Fig 2). However, this effect is diminished in the presence of serum. Moreover, the amount of attached cells is reduced when 10% serum is added on the substrata with the highest elastic modulus.



**Fig. 2: VSMC attachment to LBL-treated PDMS substrata in the presence or absence of serum.** Values are reported as averages and S.E.M.

We found that the rate of serum-stimulated VSMC proliferation increases when the elastic modulus of PDMS decreases (Fig 3).

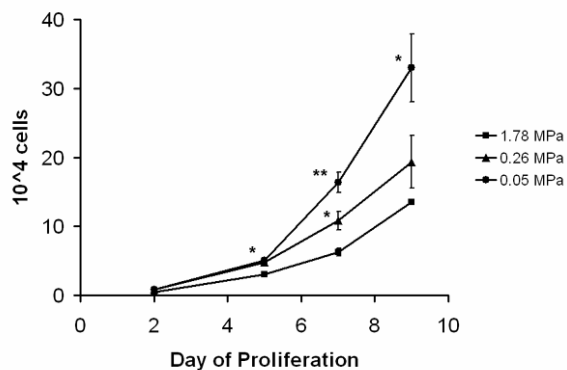


Fig. 3: Serum-stimulated VSMC proliferation on PDMS substrata with different elastic moduli. Values are reported as averages and S.E.M.

**DISCUSSION & CONCLUSIONS:** The elastic modulus of human aortas has been reported to range from 0.1 to 3 MPa [1]. The elastic moduli of the PDMS substrata in our study fall in this range. Others have used PDMS to study cell-substrate interactions, but a major problem has been to maintain cell adhesion for extended periods of time [2]. Recently, Ai *et al.* [3] showed that layer-by-layer treatment of PDMS improves significantly cell adhesion to PDMS. Applying their method, we find that VSMC adhesion can be maintained for at least 2 weeks (data not shown).

We observe an effect of substrate compliance on cell attachment in the *absence of serum*. It is not

surprising that we observe different effects in the presence and absence of serum. Cells are known to respond to soluble growth factors or ECM molecules in serum. While the presence of serum appears to mask the effects of substrate compliance on VSMC attachment, we clearly observe an effect of substrate compliance on serum-stimulated VSMC proliferation.

In conclusion, PDMS is a useful tool for studying the effects of substrate compliance on VSMC behavior. More important, our results show that VSMCs are capable of sensing and responding to changes in substrate compliance in a range of elastic moduli that is physiologically relevant.

**REFERENCES:** <sup>1</sup>H. Abe, K. Hayashi, and M. Sato (1996) *Data book on mechanical properties of living cells, tissues, and organs*, Springer-Verlag. <sup>2</sup>J.J. Cunningham, J. Nikolovski, J.J. Linderman, and D.J. Mooney (2002) *Biotechniques* **32**: 876. <sup>3</sup>H. Ai, Y. Lvov, D. Mills, M. Jennings, J. Alexander, and S. Jones (2003) *Cell Biochem. Biophys.* **38**: 103.

**ACKNOWLEDGEMENTS:** This work was supported by the Whitaker Foundation, NSF CAREER Award, and the Henry Luce Foundation. We thank Keiko Ookawa-Chinzei for helpful discussions.