Integrins bind specifically ECM proteins and induce in-out and out-in signaling. The actin cytoskeleton, when linked to these adhesive sites, exerts forces onto the substrate leading to stabilize the cell shape and/or to migrate. Signaling crosstalks between these two major compounds: adhesive sites and actin bundles, are still unclear because organizations of both complexes can be highly different from one cell to another one during cell spreading.
Micropatterning techniques are efficiently used to constraint cells to exhibit well reproducible cytoskeleton and adhesive site organizations. This reduces biological dispersion and allows statistical spatiotemporal analysis. Investigations on relevant cell adhesion actors through experimental measurements should point out links among them. The outcome of this work is to study relationships between cell spreading behaviors and extracellular matrix properties. We choose to simplify the cell environment by reducing the extracellular matrix to microfabricated substrates covered by a specified type of adhesive proteins. We thus use the geometry controlled by the adhesive pattern and the extracellular matrix biochemistry controlled by the type of protein adsorbed on the substrate to quantify the resulting cytoskeleton and adhesion sites organizations.

2864-Pos Board B634
Lymphocyte Dynamics on Aligned Endothelial Cells
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Immune cells circulating blood vessels recognize the signatures of tissue inflammation in blood vessels and undergo multiple cascades of interactions with inflamed endothelium to eventually leave out the vessels. Parallel plate flow chamber assay has been extensively used to investigate detailed mechanisms of lymphocyte-endothelial cell (ECs) interactions. However, orientation of ECs, which may play significant roles in lymphocyte dynamics on inflamed endothelium, has not been considered in these assays yet. Indeed, healthy blood vessels are composed of endothelium aligned in parallel with the direction of blood flow. To address the importance of ECs alignment on lymphocyte dynamics, we firstly aligned ECs on the substrates containing nanoscale grooves and ridges. Then, dynamics of T cells on the aligned endothelial layers (either parallel or perpendicular to the direction of flow) and randomly oriented ECs were monitored by video microscopy. Firstly, we quantitatively analyzed the direction of T cell crawling under the conditions stated above. Interestingly, the direction of T cell crawling was not significantly affected by the flow direction, but rather biased toward the direction of EC alignment. Indeed, when junctions and nuclei of ECs were visualized, T cells preferentially migrated along the EC the junctions, while avoided crawling on top of EC nucleus. Based on this observation, we hypothesized that topographical structures and mechanical properties of ECs guided crawling of T cells and performed a number of experiments to test the hypothesis. Secondly, we investigated the effect of EC alignment on transendothelial migration (TEM) of T cells. T cells on poorly-aligned ECs underwent TEM more quickly and frequently than T cells on well-aligned ECs. Further analysis revealed that local alignment of EC junctions surrounded by more than three ECs form preferential sites for TEM of T cells.

2865-Pos Board B635
Universal Rheological Properties of Cells in Intermediate States Observed in Stress Relaxation of Cell Sheets
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Rheological properties of living cells are related to the cytoskeletal structures such as actin filaments, microtubules and intermediate filaments. Previous studies revealed that single cells in steady states followed a power-law rheology in time and frequency domains and the power-law curves intersected at a single point at a high time and frequency region. However, it is little known how the rheological property of cells varies in their intermediate states, in which the cytoskeletal structures are drastically and artificially being modified. In this study, we measured the ensemble-averaged relaxation modulus $G(t)$ of cells in the intermediate states by successive stretching and relaxing of cell sheets, which were fabricated by peeling confluent cells from a microfabricated substrate. We succeeded to observe the change in $G(t)$, which followed the power-law rheology, just after modifying actin filaments with cytochalasin-D and Jasplakinolide. Interestingly, the power-law curves of cells in intermediate states also intersected at the single point, which was consistent with that of cells in steady state. The results suggested that the universal features of power-law rheology of cells resulted in the rheological properties of cells as a material, not any active regulation of cells. The time dependent Poisson's ratio of cells in intermediate states will be characterized.

2866-Pos Board B636
Viscoelastic Properties of Vascular Endothelial Cells Exposed to Uniaxial Stretch
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Vascular endothelial cells (VECs) that line the interior of blood vessels are continually stretched as the vessel walls expand and contract. It is widely accepted that VECs will remodel themselves in response to this mechanical stimuli, leading to changes in cell function. Few studies, however, have analyzed the mechanical properties of these cells under stretch. We hypothesize that uniaxial stretch will cause an anisotropic realignment of actin filaments, and a change in the viscoelastic properties of the cell. To test this hypothesis, VECs were grown on a thin, transparent membrane mounted on a microscope. The membrane was stretched, consequently stretching the cells. Time-lapse sequences of the cells were taken every hour with a time resolution of 10 Hz. The random trajectories of intracellular endogenous particles were tracked using in-house algorithms. Using a novel particle tracking microrheology formulation, that takes into account the anisotropic nature of the cytoplasm, these trajectories were analyzed and the mechanical properties of VECs subjected to various stretching conditions were calculated and compared.

2867-Pos Board B637
Extracellular Matrix Presentation Modulates Vascular Smooth Muscle Cell Mechanotransduction
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The development of atherosclerotic lesions involves phenotypic changes among resident vascular smooth muscle cells (VSMCs) that often contribute to inflammation at the site of injury and are correlated with stiffening of the vessel wall. Studies have shown that there are major alterations in extracellular matrix (ECM) composition and mechanical properties during atherosclerosis that likely contribute to VSMC pathology, yet precisely how such changes lead to regulation of VSMC behavior remains poorly understood. In this study, we used substrates with tunable mechanics to investigate the combined influence of ECM stiffness and composition on VSMC adhesion, spreading, proliferation, and traction force generation. To model the stiffening ECM, we synthesized 25kPa and 135kPa polyacrylamide substrates functionalized with equal mass quantities of fibronectin, laminin, type I collagen, or a combination of fibronectin and laminin. On fibronectin and collagen substrates, we observed that increasing stiffness stimulates VSMC adhesion, spreading, and proliferation, whereas on laminin substrates, the effect is reversed, with 135kPa substrates supporting 35% less attachment ($p<0.05$), 25% smaller cell area ($p<0.05$), and 10% less proliferation than 25kPa substrates. We also examined attachment on gels containing varying ratios of fibronectin and laminin, and found that cell number on 135kPa versus 25kPa substrates was a direct function of the proportion of each ligand, i.e., gels with more fibronectin supported higher attachment at 135kPa, while gels with more laminin supported higher attachment at 25kPa. We then quantified single cell traction forces on 10kPa substrates containing fibronectin or laminin and found that total force per area on laminin was 55% less than on fibronectin ($p<0.05$). Collectively, our results demonstrate that VSMC response to substrate stiffness is critically dependent on ligand biochemistry, and have broad implications for the study of VSMC physiology and mechanotransduction in other cell types.

2868-Pos Board B638
HUVEC Chemotaxis and Force Generation Depend on Substrate Mechanics and Chemical Gradient
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Directed migration of endothelial cells is crucial for angiogenesis and vascular remodeling. This migration is known to depend on both chemical and mechanical interactions. It has been shown that HUVECs migrate towards VEGF, but individual cell tracking in a stable and quantifiable gradient has not been done. In order to control for both chemical and mechanical interactions, we have used a microfluidic device that can generate a chemical gradient of VEGF in a spatially and temporally stable manner. This microfluidic device is placed over a polyacrylamide gel so that a range of physiological substrates, on which HUVECs migrate, could be tested. HUVECs were individually tracked and observed to chemotax towards higher VEGF concentrations on a variety of