junctions critical for myocardial function. However, the challenges of generating mechanically strong and synchronously contractile cardiac sheets remain. In this study we characterized substrate-free cardiac cell sheets generated using a partial-lift method, whereby a portion of a cell monolayer is detached from the substrate. These cell sheets are dominated by cell-cell interactions and decoupled from cell-substrate interactions, but remain amenable to biological and chemical perturbations, and more importantly, mechanical conditioning and characterization. We show that lifted cardiac sheets exhibit significant changes in the distribution of cytoskeletal and junctional proteins, and that junctional expression of these proteins is enhanced by mechanical conditioning. Results further demonstrate that the mechanical strength and cohesion of these cell sheets depend on cytoskeletal and cell-cell junctional protein integrity. We also examined the microrheological characteristics, contraction frequency and calcium signaling of the cardiac cell sheets, which are critical for their potential clinical applications. Our results showed that mechanical conditioning the cell sheets alters several of these properties, with potential benefits to their function.
This work represents a first systematic examination of mechanical conditioning on cardiac cells with primarily intercellular interactions. This method offers an unprecedented way to study cell junctions when substrate interactions are no longer dominant. The information gained from this study will help advance our understanding of cell-cell interactions and improve cardiac cell sheet biomechanical properties for tissue regeneration, and particularly heart repair.

3979-Pos Board B707
Nanoindentation Derived Mechanical Properties of the Corneascleral Rim of the Human Eye
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The corneoscleral rim of the eye represents a region with unique anatomical properties due to its location between the cornea and sclera and unique functional properties due to the location of epithelial stem cells in the rim structure (limbus). They guarantee the regeneration of the corneal epithelium and prevent the conjunctival epithelium from growing onto the corneal surface. Survival and self-renewal properties of stem cells depend on specific biological and biomechanical properties of their environment. We therefore measured the local mechanical properties of the human corneoscleral rim using a novel nanoindentation device (Bioindenter CSM Instruments, Neuchatel, Switzerland) developed for soft tissues evaluation. Nanoindentation was performed using a spherical indenter of 0,5mm radius, a maximal load ranging between 20 μN to 30 μN and a penetration depth of several μm to 60μm. The hold period at maximum load was 180 seconds. Young's modulus (E) was calculated using a Hertzian fit to the loading data. E of the central cornea was in the range of 19 kPa, while in the scleral region we found 17 kPa and the limbal rim region 10 kPa. Considerable creep relaxation occurred during the hold period at maximum load, which scaled with the elastic modulus of the different structures. For a better correlation of the indentation results to the morphological characteristics of the corneascleral rim, histological sections of the indented areas were performed. Our results reveal unique biomechanical properties of the corneoscleral rim with distinct mechanical properties for the three anatomical regions. Moreover, the corneoscleral rim appears to provide a very soft biomechanical niche environment for the limbal stem cell. These results may have implications to ex-vivo expansion of limbal stem cells and the development of an artificial limbal stem cell construct for tissue engineering.

3980-Pos Board B708
Light-Modulated Cell Adhesion to Control Cell and Tissue Morphogenesis
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Cell adhesion through integrin receptors to the extracellular matrix is essential for pattern formation during tissue morphogenesis. For example, cell adhesion differences correlate with epithelial branching, and stem cells often reside in microenvironments essential for stem cell maintenance and differentiation. Tissue engineering relies on the ability to replicate in vivo pattern formation to grow complex multicellular tissues in vitro, a largely unresolved challenge. Although cell growth can be patterned by immobilizing matrix proteins by photolithography or microcontact printing, such techniques are not easily adaptable to a physiological 3D environment and most importantly do not allow dynamic control of cell adhesion during in vitro organogenesis. To address this challenge, we started to develop a system by which cell-matrix interactions can be reversibly modulated by patterned 470 nm illumination utilizing a novel short peptide, Zdk, that binds to the dark-state of oat LOV2 with high affinity. Aminosilane-coated coverslips were reacted with NHS-PEG-biotin conjugates, and a biotinylated LOV2 domain attached through neutravidin. As proof-of-principle, we bound mCherry-Zdk-RGD fusion proteins to biotin-LOV2-coated coverslips, and demonstrate rapid, reversible and patterned light-induced release of mCherry-Zdk-RGD from the surface. We are investigating how light-triggered release of cell matrix attachment controls focal adhesion dynamics and cell migration. Rapid rebinding of mCherry-Zdk-RGD is due to the photochemical properties of LOV2, which rapidly reverts to the dark state after photoactivation. Because this may be too fast to manipulate cell-matrix adhesions that turn over at a substantially slower timescale, we carried out a mutagenesis screen of LOV2 variants generated by error-prone PCR. Using fluorescence recovery of the LOV2-bound flavin molecule as readout, we identified numerous novel mutations near the flavin-binding site that revert to the dark state at much slower rates, presumably by stabilizing the flavin-cysteinyl adduct.

3981-Pos Board B709
Neutrophil Rolling on Patches of Selectin
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For a neutrophil to reach an infection site, it circulates through the blood, adheres to the blood vessel wall, initially rolling, then adhering firmly; finally, the neutrophil actively passes through the wall. The initial rolling of a neutrophil is primarily mediated by adhesion of receptors on the neutrophil to selectins on the blood vessel wall. A neutrophil has hundreds of surface protrusions called microvilli, "little fingers"; most of the adhesion receptors involved in initial rolling are situated on the tips of microvilli. Here, I present theoretical analysis showing that the dynamics of a rolling neutrophil are qualitatively different depending on whether its microvilli are typically bound with single or multiple receptors. The role of multiple binding on a single microvillus increases with the surface concentration of selectin, up to a limit. P-selectin is ordinarily stored in granules in an endothelial cell, and released in patches on the cell membrane during inflammation. There is some experimental evidence suggesting additionally that patches of P-selectin are concentrated near the boundaries of endothelial cells. Here, a numerical model of neutrophil rolling which accounts for discrete receptor-ligand interactions is used to show how patchy distribution of P-selectin can promote neutrophil arrest and direct neutrophils to boundaries of endothelial cells, facilitating extravasation.

Membrane Pumps, Transporters, and Exchangers III

3982-Pos Board B710
Characterizing Conformational Ensemble and Free Energy Landscape of ABC Exporters using a Novel System-Specific Sampling Approach
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During their transport cycle, ATP-binding cassette (ABC) exporters undergo large-scale conformational changes between inward-facing (IF) and outward-facing (OF) states. Using a novel approach based on designing system-specific reaction coordinates and employing nonequilibrium simulations, we have recently characterized the IF-OF conformational transition pathway of MsbA, a bacterial ABC exporter whose structure has been solved in multiple functional states including an OF conformation and two IF conformations (termed IF-closed and IF-open based on the extent to which the cytoplasmic side is open). Due to low resolution of MsbA crystal structures in IF conformation, only C^{α} atoms have been reported; however our unique system-specific sampling strategy results in a stable all-atom model of MsbA in the IF conformation in the presence of explicit membrane and water. In order to more accurately characterize the conformational ensemble of nucleotide-free *apo* MsbA, we also employ a free energy calculation technique based on a combination of umbrella sampling and replica exchange methods, using a reaction coordinate defined based on the orientation of transmembrane helices. The results indicate that the IF-closed conformation is associated with a relatively deep minimum and the IF-open-like conformations are also thermally accessible. However, the deepest free energy basin is associated with an IF conformation, more open than IF-closed and less open than IF-open, resembling the crystal structures of P-glycoprotein (a MsbA homolog) which are obtained at higher resolutions. The overall picture emerging from the reconstructed free energies is that MsbA is fairly flexible in its resting state in the absence of nucleotides and substrates. The approach proposed here provides a framework to study large-scale conformational changes of membrane transporters whose computational investigation at an atomic resolution may not be currently feasible using conventional methods.