cell contraction is the result of the cellular anchorage loss in focal adhesions. This excitation-contraction coupling of ASM cells on TGT coated surface was monitored with calcium indicators, and the time delays between histamine injection, intracellular Calcium concentration increase, and morphological contraction was analyzed to offer an insight into the kinetic process of the ASM contraction.

2054-Pos

Red Blood Cell Membrane Oxidation/Aging Toward Cell Death: Photosensitizer Stress of Cis-Porphyrin

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For cancer and skin infection/damaging treatment, photosensitizer drugs, such as photofrin and foscan, of photodynamic therapy (PDT) have been developed for last decades. In general, the strategy of curing these diseases is how PDT triggers cell death, apoptosis, and necrosis, under photosensitizer mediated oxidative stress. The PDT shows a certain success for this aim, but not everything, especially for metastatic cancer cells. We assume that this is because the fundamental study of the photosensitizer effect at the cell membrane, especially stress signaling through cell membrane with cytoskeletal network proteins complex, is not well understood. Therefore, since last year, we have been investigating the mechanical property and cytoskeletal network formation/reformation under photosensitizer mediated oxidative stress with cis-porphyrin (CisDiMPyP) by using red blood cell (RBC) membrane.

In the last meeting (San Francisco, 2018), we reported continuous RBC membrane shear modulus increasing and morphological change before hemolysis under the oxidative stress. Since then, with further experiments, we found a specified three distinguishable phenomena by the oxidation degree, i.e. 1) resistance of membrane mechanical property for a mild oxidative stress, 2) dynamical/statistical irreversible shear modulus increasing, from 6.7 x 10^{-6} to 11 x 10^{-6} N/m, for a moderate oxidative stress, and 3) a plastic-like behavior, Heinz body, and lysis for a severe oxidative stress. The same/similar phenomenon is also reported by RBC aging (storage) study as a reference. We consider that there is a significant correlation between photosensitizer-mediated oxidative stress, aging stress for cell membrane, and cell death. Therefore, understanding the mechanism of cell membrane mechanical property change with cytoskeletal network reformation under oxidative stress will not only give a therapeutic development of PDT in future but also a comprehensive concept for the correlation of cell oxidation, aging, and death.

2055-Pos

Biophysical Model Reveals the Role of CCM Proteins in Collective Behavior of Endothelial Cells

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A number of vascular diseases are associated with abnormal development of blood vessels. Dysregulated formation of blood vessels in the brain leading to Cerebral Cavernous Malformation (CCM) may predispose affected individuals to stroke even in the early years of their lives. Although coordinated behavior of endothelial cells is of central importance to embryonic development and maintenance of vascular homeostasis after birth, the underlying mechanisms of this biomechanical process remain poorly understood. In this work, we use a biophysical simulation model to dissect the mechanisms contributing to an emergent behavior of the multicellular system in the context of endothelial tube formation.Our cell model explicitly includes the dynamics of protrusions, by which cells interact with each other and the extracellular matrix, and an elastic cell body that moves and changes its shape in response to the mechanical forces resulting from these interactions. We inform and validate the model by imaging the collective dynamics of endothelial cells during tube formation both under wild-type conditions and with the knockdown of each of three CCM genes (*krit1*, *ccm2*, and *pdcd10*). This approach allowed us to bridge the single-cell and multi-cell scales of the system's description and dissect the differential effects of CCM proteins on the biomechanics of the coordinated cell behavior. Specifically, we showed that an imbalance of cell-cell (upon PDCD10 loss) or cell-ECM (upon KRIT1 loss) adhesion explains the distinct defects in the tubular structures of the CCM1 and CCM3 phenotypes. Furthermore, we showed that the incomplete rescue of the CCM phenotypes by the Rho kinase inhibition is explained by additional perturbations of the cell biomechanics unrelated to the deficiency of CCM proteins but involving the modulation of cell spreading, protrusion contractility, and the efficiency of long-range cell-cell sensing.

2056-Pos

Nanonet Inter-Fiber Spacing Controls Plasticity in Cell Migration

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Biomechanical cues within tissue microenvironments are critical for maintaining homeostasis, and their disruption can contribute to malignant transformation and metastasis. Once transformed, metastatic cancer cells can migrate persistently by adapting (plasticity) to changes in the local fibrous extracellular matrix (ECM). Current *in vitro* strategies to recapitulate persistent migration rely exclusively on the use of aligned geometries. However, if other fiber configurations can achieve persistent migration remains unclear. Here, by controlling inter-fiber spacing in crosshatch networks (3,6,18,36 and 54 μm), we regulate plasticity of the migratory phenotype. At dense inter-fiber spacing (3 and 6 μm), unexpectedly, cells migrate persistently at high speeds in elongated 3D shapes featuring thick nuclei and short focal adhesion cluster (FAC) lengths. With increasing spacing (18-36 μm), cells display 2D diffusive random-walk migration with flattened nuclei, broad leading edges, and narrow trailing edges. Cells form longer FACs and migrate by rapid detachment (recoil) of the trailing edge, which causes the nucleus to undergo significant deformation (35% strain). Finally, at large spacing (54 μm), cells attain stable non-migratory kite shapes. Interestingly, cells on 3-μm spacing take longer times to spread post-seeding, lack well-developed f-actin stress fibers, display the highest nucleus-cell body alignment during migration, and have the shortest nucleus relaxation times post-recoil. In addition, gene expression profiling of cells on fibers show a decrease in transcriptional potential and a differential upregulation of metabolic pathways. Notably, for the first time, we demonstrate that anisotropic substrates are not the only requisite configuration for achieving persistent cell migration, and we envision the ability to regulate plasticity in cell shapes and migratory phenotype will afford new opportunities in studying metastatic invasion, regenerative wound healing, and stem cell migrational homing.

2057-Pos

Mechanical Contraction of Blood Clots Impaired Due to Platelet Dysfunction and Disintegration

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Platelets are the smallest blood cells that play a pivotal role in generation of mechanical forces to induce blood clot contraction. By pulling on fibrin fibers platelets cause volumetric shrinkage of blood clots and thrombi that makes them stiff and impermeable. Despite pathophysiological importance of the platelet contractile function, the relationship between platelet contractility and their metabolic and structural states is largely unknown. We hypothesized that contraction of blood clots attenuates with time due to progressive changes in the metabolic activity and structural integrity of platelets and platelet aggregates. By applying a combination of time-laps confocal microscopy, biomechanical measurements of the contractile forces, and rotational rheometry to characterize clot viscoelasticity we examined the relation between the contractile function and alterations of structural and metabolic characteristics of viable human platelets in plasma clots over the course of contraction. Our results show that in contracting thrombin-induced plasma clots a significant portion of platelets breaks up into submicron-size organelle containing fragments including mitochondria. Remarkably, some mitochondria in activated platelets localize in filopodia and get released in the extracellular milieu. Cessation of clot contraction after about half an hour of incubation coincided with platelet fragmentation. Concurrently, both the mitochondrial membrane potential ($\Delta\psi_m$) in platelets and ATP revealed significant decrease correlated with the termination of platelet-driven clot contraction. To conclude,loss of contractility in thrombin-induced plasma clots is attributed to gradual dysfunction and fragmentation of platelets concurrent with their adverse metabolic and structural alterations. Disintegration of platelets in contracting clots may be an underappreciated important biophysical mechanism for platelet consumption and/or elimination in thrombotic conditions, such as disseminated intravascular coagulation. Supported by the Program for Competitive Growth at KFU, AHA grant 17SDG33680177.