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1954-Pos

Metabolic fingerprinting of HUVEC cells during phenotypic shifts using fluorescence lifetime image microscopy (FLIM)

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Adult human vascular endothelial cells (ECs) change their phenotype in response to changes in vascular tumor microenvironments, oxygen concentration and during wound healing. During angiogenesis, the physiological process of creating new blood vessels, hundreds of genes are activated with one of the most important being VEGF (vascular endothelial growth factor). VEGF, the signaling protein, stimulates EC migration, proliferation, and vascularization. During angiogenesis, ECs undergo an 'angiogenic switch'; they change from a resting (quiescent) phenotype to a more elongated and mobile (tip cells) or proliferative and mobile (stalk cells) phenotype. Differences between phenotypes are not easy to assess. Currently, the most common approaches include Seahorse assays, fluorescent labeling or transfections in an attempt to categorize the HUVEC phenotypes by their metabolic signature. Although these methods have shown the importance of metabolic profiling to separate the cells, they are invasive and may ultimately alter the cellular metabolism. Additionally, a non-invasive metabolic imaging technique would extend to applicability to animal models. We propose to classify HUVECS phenotypes by their metabolic signature by characterizing NADH, a metabolic molecule that is naturally autofluorescent and indicative of the metabolic state (glycolysis or oxidative phosphorylation) depending on the ratio between its free and enzyme-bound forms. In particular, by analyzing the lifetime of NADH it is possible to directly obtain the free/bound ratio and therefore learn whether a cell is more/less glycolytic, respectively. Here, we categorized changes in NADH lifetime using FLIM to obtain a spatial distribution of lifetimes to understand the EC metabolic shifts after pro- and anti-angiogenic treatments in 2D and 3D cultures. These results will provide an important insight on EC phenotype, metabolism, and migration patterns which will benefit studies regarding endothelial dysfunction and cancer metastasis.

1955-Pos

Probing the relationship between functional and structural networks in the pancreatic islet using computational modeling of the islet electrophysiology and calcium microscopy

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The pancreatic Islet is composed of hundreds of highly heterogeneous, insulin-secreting beta cells. Gap-junction protein channels form links between this complex network of dynamic cells, allowing them to synchronize and secrete insulin in a pulsatile fashion. This synchronization is essential for healthy glucose homeostasis and is lost preceding Type I and Type II Diabetes. Therefore, understanding the beta-cell network and how it reacts to perturbations holds significant promise to new diabetes diagnostics and therapies. In this talk, we present data derived from computational modeling of islet electrophysiology and microscopy tools to analyze the relationship between the functional synchronization network and the structural gap junction network. We investigate the characteristics of highly synchronized cells and their influence over Islet dynamics and the influence of the structural network topology on the functional network. Our data show that a cell's metabolic rate, not gap junction conductivity is the primary indicator of functional synchronization on a cellular level. On an islet-wide level, we show that the functional network structure is highly organized, cannot be explained by stochasticity, and the gap junction structural network has a significant impact on this functional network structure. These results suggest an alternative interpretation of the relationship between structure and function within the pancreatic Islet and thus how Islet coordination may break down in diabetes.

1956-Pos

Coupling indirect correlative light and electron microscopy (*iCLEM*) with computational modeling to expand the physiologist's reach into the nanoscale

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Correlative light and electron microscopy (CLEM) is emerging as a powerful tool for nanoscale structural studies. Challenges arise when obtaining precisely registered images of nanoscale neighborhoods within the same sample with multiple microscopes. This severely limits experimental throughput and precludes quantitative assessment of heterogeneous samples. We have developed indirect CLEM [*iCLEM*] as a low-cost, high throughput option with extensive quantitative capabilities. *iCLEM* leverages the high imaging throughput of confocal microscopy, STORM single molecule localization microscopy, and transmission electron microscopy (TEM) and exploits structural fiducials / landmarks identifiable via both light and electron microscopy to correlate measurements. This approach is enabling us to systematically investigate the structural underpinnings of electrical propagation in normal and diseased hearts. Specifically, we are obtaining the first-ever quantitative picture of the cardiac intercalated disk, which is responsible for electrically and mechanical coupling cardiomyocytes. *iCLEM* quantitatively captures the complexity and heterogeneity of the ID within and between hearts. ID ultrastructure from micro- through nano-scales quantified from TEM are used to construct populations of finite element models of IDs, capturing both intra- and inter- individual variability. These meshes are populated with electrogenic proteins based on STORM-based Relative Localization Analysis (STORM-RLA) of single molecule localization data. The resulting 3D finite element models of IDs contrast with previous approaches which ignored the ID or oversimplified it to a homogenous 2D circle. By incorporating such structural detail into computational models of electrical signal propagation, we are uncovering previously unappreciated structure-function relationships that determine the regularity of the heart's rhythm. These predictions, along with functional imaging studies of electrical signal spread in the heart, are providing the basis for the development of novel classes of anti-arrhythmic drugs.

1957-Pos

Mechanical processing effects on the secondary plant cell wall arrangement in *Sorghum bicolor*

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Unlocking one third of plant biomass as a renewable feedstock for fuels and materials depends on the effective deconstruction of the secondary plant cell wall. Over 90% of the native secondary plant cell wall is composed of cellulose and hemicellulose polysaccharides and the lignin aromatic polymers. Deconstruction refers to the processes which digest polymers into desired subunits. Present deconstruction processes are centered on lignin-first extraction to overcome the recalcitrance of biomass: the accumulation of indigestible plant polymers during deconstruction. Although recalcitrance is broadly correlated with lignin and hemicellulose, *how* the secondary plant cell wall polymers rearrange on a molecular level to cause recalcitrance during deconstruction remains in question. Monitoring where recalcitrance is occurring during deconstruction is challenging partially because the plant cell wall is inherently insoluble and often requires additional extraction for analysis. Recent availability of ^{13}C-enriched plant biomass has enabled the use of solid-state Nuclear Magnetic Resonance (NMR) experiments to refine the model of native cell wall structure in plant tissue without extraction or solubilization of plant material. Solid-state NMR has the advantageous ability to non-invasively probe the structure of the secondary plant cell wall throughout deconstruction pathways. Could the mechanical processing used to overcome insolubility and increase chemical penetration of the plant cell wall structure be a common cause of recalcitrance during deconstruction? Here, we present results from solid state NMR and electron microscopy measurements that report on changes in the secondary plant cell wall of ^{13}C labeled *Sorghum bicolor* stems after lab scale mechanical processing using vibratory ball-milling.

1958-Pos

Growth- and nutrient-dependent G1/S transcription factor upregulation is controlled at the transcriptional level and is critical for proliferation in poor nutrient conditions

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Ensuring size homeostasis in cell populations requires individual cells to pass a critical size threshold at the G1/S transition of the cell cycle where they commit to division. This threshold gates the onset of a massive transcription program that drives division. How is the G1/S program activated? Using sensitive quantitative imaging methods, we discovered that the key G1/S transcription complexes (SBF: Swi4-Swi6, MBF: Mbp1-Swi6, G1/S TFs) are limiting with respect to their DNA target sites in small daughter cells, and gradually accumulate in G1 phase to levels permitting saturation of target promoters. The same