chemically-inducible system to test causal relationships among DNA hydroxymethylation, chromatin accessibility and gene expression in the human genome.

1507-Pos Board B575
Gap Junction Remodeling in a Novel Engineered Heart Tissue System Cultured under Point Stimulation
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Recent advances have shown the importance of electrical stimulation in enhancing maturation and differentiation of engineered heart tissues (EHTs). Conventional electrical maturation protocols pace their EHTs with field stimulation - the tissues are paced simultaneously in a uniform electrical field. This method has been shown to improve cellular alignment, increase contractility, and increase gap junction (Cx) formation. While Cx43 production is increased, the anisotropic ratio is not fully preserved in these culture systems [Trantidou et al, PMID 4459290]. Native cardiac tissue demonstrates an anisotropic propagation of action potential; in adult rat ventricles the conduction velocity (CV) is 3x faster in the longitudinal direction than the transverse direction [Zimmermann et al, PMID 16582915]. We hypothesize that the loss of anisotropy is due to field stimulation not accurately recapitulating the conditions caused by a depolarizing wave traveling through the tissue. In order to better reproduce native myocardium, we designed a bioreactor which applies a point stimulation at one end of our EHT and allows the wave to propagate down the tissue. We hypothesize that this propagating wave will provide the necessary signals to enhance Cx43 production as well as improve localization of gap junction proteins. Maturity was assessed by the measurement of twitch velocity (CV) is 3x faster in the longitudinal direction than the transverse direction. Conventional electrical maturation protocols pace their EHTs with field stimulation - the tissues are paced simultaneously in a uniform electrical field. This method has been shown to improve cellular alignment, increase contractility, and increase gap junction (Cx) formation. While Cx43 production is increased, the anisotropic ratio is not fully preserved in these culture systems [Trantidou et al, PMID 4459290]. Native cardiac tissue demonstrates an anisotropic propagation of action potential; in adult rat ventricles the conduction velocity (CV) is 3x faster in the longitudinal direction than the transverse direction [Zimmermann et al, PMID 16582915]. We hypothesize that the loss of anisotropy is due to field stimulation not accurately recapitulating the conditions caused by a depolarizing wave traveling through the tissue. In order to better reproduce native myocardium, we designed a bioreactor which applies a point stimulation at one end of our EHT and allows the wave to propagate down the tissue. We hypothesize that this propagating wave will provide the necessary signals to enhance Cx43 production as well as improve localization of gap junction proteins. Maturity was assessed by the measurement of twitch velocity (CV) is 3x faster in the longitudinal direction than the transverse direction.

1508-Pos Board B576
Photo-Regulation of Small G Protein RhoA Signal Cascade used by Photochromic Molecules
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Small G protein, known as biomolecule switch protein, has a critical role of intracellular signal transduction using GDP-GTP exchange cycles. RhoA is one of the small G-protein Rho families, which plays important roles of regulation of cell morphology such as cell adhesion, and cytoskeleton. It has been shown that GTP bound active state of RhoA induced enzymatic reaction through downstream cascade such as Rho-associate kinase (ROCK). In this study, we performed to control the function of RhoA using photochromic molecules. We prepared the RhoA mutant Q63L that exhibits constitutive active state. Subsequently cysteine residues were introduced into the functional sites of switch I and 3 regions of the mutant. The mutants were modified with thiol group photoreactive photochromic compound 4,4'-azobenzene-dimaleimide (ABDM). Photo-regulations of the GDP-GTP exchange on the PAM modified RhoA mutants were monitored with fluorescently labeled GTP derivative Mant-GTP. Cis-PAM-mutants showed faster GDP-GTP exchange than Trans-PAM-mutants in the absence Mg. As another way, we designed the photochromic RhoA binding domain peptide, which compete with ROCK in order to control the RhoA function photo-reversibly. The peptide that has a similar amino acid sequence to the RhoA binding coiled-coil region of ROCK and a single cysteine residue at N-terminal was synthesized. The peptides were cross-linked with bifunctional azobenzene derivatives, 4,4'-azobenzene-dimaleimide (ABDM) resulting in dimerization. Interaction of the photochromic peptide with RhoA was examined by the method of pull down assay and fluorescence depolarization. Cis-ABDM peptide showed slightly more effective inhibition of RhoA and ROCK interaction than Trans-ABDM peptide.

1509-Pos Board B577
Connectosomes for Direct Molecular Delivery to the Cellular Cytosplasm
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Passive diffusion of drugs and reagents across the cell’s plasma membrane barrier is an inefficient and poorly controlled process, despite its fundamental importance to biotechnology, cell biology, and pharmaceutics. In particular, the fundamental requirement for membrane permeability frequently limits the accumulation of drugs in the cytoplasm, undermining drug efficacy and over-constraining drug design. In contrast, gap junctions, transmembrane protein channels that physically connect the cytoplasm of adjacent cells, bypass the plasma membrane, permitting a diverse range of molecules to move rapidly from the cytoplasm of one cell to the next, from ions and metabolites to siRNA and chemotherapeutics. Our work aims to address the challenge of crossing the plasma membrane barrier by using the cellular gap junction network to deliver drugs directly to the cytoplasm of tumor cells. Specifically, we have developed Connectosomes, cell-derived vesicle materials that contain functional gap junction channels and can form gap junction interfaces with cells. We formed Connectosomes by harvesting plasma membrane vesicles from donor cells that were engineered to overexpress gap junction channels. These novel materials transferred small molecules, including fluorescent dyes and chemotherapeutics, directly to the cellular cytoplasm. Remarkably, using Connectosomes to deliver the chemotherapeutic doxorubicin reduced the therapeutically effective dose of the drug by more than 10 fold in comparison to the free drug and 100 fold in comparison to conventional liposomal doxorubicin. These results demonstrate the ability of Connectosomes to substantially increase the efficiency of molecular transport into the cytoplasm. This increase in efficiency has the potential to boost the effectiveness of existing drugs, such as chemotherapeutics, helping to address long-standing problems such as dose-limiting toxicity and multidrug resistance. Further, in bypassing the plasma membrane barrier, Connectosomes remove a key constraint on therapeutic design, enabling the development and delivery of membrane-impermeable drugs and reagents.

1510-Pos Board B578
Engineering the Extracellular Loops of Outermembrane Protein G in Creating a Nanopore Sensing Platform
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Outermembrane protein G (OmpG) is a 14-stranded beta barrel proin naturally found in the outermembrane of E. coli. It contains seven flexible extracellular loops that create a dynamic behavior in single channel recording termed gating. OmpG gating and its 8 Angstrom diameter make it unsuitable for sensing protein analytes that cannot enter its lumen, a process called translocation. Thus, we have taken advantage of its extracellular loops to facilitate binding of target analytes. We have seen that upon binding to loop 6, proteins can be detected via the changes in OmpG gating. The changes in gating can be mediated by electrostatic interactions between the OmpG loops and the binding pocket of the protein analyte. We therefore extended our study to create OmpG nanopores with altered charged properties to understand the mechanism of detection and to enhance the binding signal of a specific target in a mixture. We also found that detection of proteins using OmpG can be achieved not only by using loop 6 but also with four other loops. With this knowledge we can engineer multiple binding sites into one nanopore to lower the limit of detection creating a more sensitive nanopore sensor. By gaining a fundamental understanding of OmpG and how it can interact with analytes will help in the building of a robust OmpG library for a plethora of target analytes.

1511-Pos Board B579
Analysis of Conformational Change with the Multimerization of Small GTPase Ras Induced by Chemical Modification at HVR Domain
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The lipid-anchored small G protein Ras is a central regulator of cellular signal transduction processes and functions as a molecular switch. Switching mechanisms based on conformational changes in the nucleotide-binding motifs have been well studied at the molecular level. H-Ras has the hypervariable region (HVR) at the C-terminal. The cysteine residues in HVR are known important roles. Therefore, the phenomenon of multimerization may reflect the physiological function of Ras. In this study, we analyzed the conformational change of small GTPase Ras induced by chemical modification at HVR Domain. Small angle X-ray scattering data suggested that Ras modified with bulky hydrophobic SH reactive reagents induced multimerization of Ras. The HVR domain is believed to perform physiologically important roles. Therefore, the phenomenon of multimerization may reflect the physiological function of Ras. In this study, we analyzed the conformational change of small GTPase Ras induced by chemical modification at HVR Domain.