

are able to form extraordinarily stable vesicular membranes against a number of chemical, physical and mechanical stressors. In this study, we demonstrated that PLFE can also form free-standing “planar” membranes on micro-pores (~100 micrometer) of polydimethylsiloxane (PDMS) thin films embedded in printed circuit board (PCB)-based fluidics. Using electrochemical impedance spectroscopy (EIS), we found that the dielectric properties of PLFE planar membranes suspended on the PDMS films are distinctly different from those obtained from diester lipid and triblock copolymer membranes. In addition to resistance (R) and capacitance (C) that were seen in all the membranes examined, PLFE planar membranes showed an inductance (L) component. Furthermore, PLFE planar membranes displayed a relatively large membrane resistance, suggesting that, among the membranes examined, PLFE planar membrane would be a better matrix for studying channel proteins and transmembrane events. PLFE planar membranes also exhibited a sharp decrease in phase angle with the frequency of the input AC signal at ~1 MHz, which could be utilized to develop sensors for monitoring PLFE membrane integrity in fluidics. Since the stability of free-standing planar lipid membranes increases with increasing membrane packing tightness and PLFE lipid membranes are more tightly packed than those made of diester lipids, PLFE free-standing planar membranes are expected to be considerably stable. All these salient features make PLFE planar membranes particularly attractive for model studies of channel proteins and transmembrane events and for high-throughput drug screening.

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Deducing the Macromolecular Organization of *Arabidopsis Thaliana* Leaf Cuticles by Solid-State NMR

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Terrestrial plants regulate environmental interactions via insoluble polymers assembled in their epidermal and/or peridermal cell walls. The plant cuticles with waterproofing and antimicrobial capabilities represent a unique class of biological assemblies composed of ester-linked and insoluble constituents such as biopolyester cutins, lipid waxes, and chemically recalcitrant cutans. Solid-state nuclear magnetic resonance (NMR) offers a powerful technique to probe the structural and dynamical properties of these geochemically important and structurally amorphous biological systems that can also motivate the engineering of water-resistant bioinspired materials from renewable sources. Based on key genes and biosynthetic pathways identified in *Arabidopsis thaliana* leaf cuticles, our current solid-state NMR study delineates compositional variations and multiple-timescale (μ s-ns) dynamics for several genetically tailored and insoluble plant cuticle systems, linking macromolecular organization with protective performance and operational design.

2454-Pos Board B591

Injectable Reverse Thermal Gel Biopolymers may Act as an Extracellular Matrix and Cell Vehicle for Cardiac Tissue Engineering

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Background: Recent investigations demonstrated that tissue engineering represents a promising strategy to repair diseased hearts. We hypothesized that temperature-responsive materials could be developed as extracellular matrix scaffolds and cell delivery vehicles could be used in cardiac tissue engineering.

Methods and results: We developed injectable reverse thermal gel (RTG) biopolymers that are designed to transition from low viscous liquid to a solid gel by exposure to body temperature. This allows deployment through a small gauge needle to the target area with minimal surgical intervention. For this study we tested different RTG biopolymers with and without chemical incorporation of laminin. In vitro 3D culture experiments were performed with adult rat ventricular myocytes (ARVM) by mixing 3x10³ cells with 50 μ l of liquid-phase polymeric solution (25°C) and permitting transition to a gel at 37°C. These cultured cells were incubated 8 days in the gel matrix. As controls, ARVMs were plated on 2D traditional laminin coated dishes. Compared to control groups, we found that the 3D matrix improved ARVM viability: after 3

days of culture, ARVM viability increased by 27% and 17% in the RTG-laminin and in the RTG, respectively. At the 8th day of culture, 63% of the ARVMs in the RTG-laminin group were rod shaped and viable, while in RTG and control groups, most of the ARVMs were round shaped and non-viable.

Conclusion: These preliminary proof-of-concept results demonstrate excellent cell viability in the RTG-laminin biopolymer for up to 8 days and show feasibility of a novel cell delivery system that permits reversible liquid-to-gel transition from room to body temperature. This holds tremendous potential for cardiac tissue engineering.

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Influence of Chemical Conjugation Strategies on Fibronectin's Bioactivity

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Since thirty years, efforts were put forward to engineer materials that could be used to build up biomedical devices that have to come in contact with human tissues. The first devices that were developed were designed to reproduce the basic functions of the tissue or organ that they were intended to replace. For example, the first designs of arterial prostheses were made from materials that can be formed as tubes with sufficient mechanical properties and chemical stability, to withstand the pulsatile blood pressure for many years without failure. Only few concerns were raised regarding the interaction between the material and the physiological environment. Nowadays, the common strategy consists in developing materials that are likely to proactively interact with their environment.

For instance, one idea is to coat the surface of biomaterials with proteins of the extracellular matrix, therefore promoting cell adhesion and proliferation. Fibronectin is one of these proteins and has been the subject of several investigations as to its potential to be used to promote cell/material interaction once conjugated to the surface of biomaterials. In this context, the aim of this study was to compare two strategies of FN immobilization in regards to the amount of bound FN and its biological activity. Two heterobifunctional cross-linkers were used to conjugate FN to ammonia plasma-treated PTFE: glutaric anhydride (GA) and Sulfo-succinimidyl 4-[p-maleimidophenyl] butyrate (sulfo-SMPB). In addition, we have also investigated the influence of adding either a hydrophilic or hydrophobic spacer between the protein and the surface on the protein bioactivity. On one hand, our results demonstrated that fibronectin RGD sequences are more available when the protein is conjugated through its lysine moieties. On the other hand, an hydrophobic spacer between fibronectin and the biomaterial surface is also likely to promote the protein bioactivity.

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Deconstructing the Role of the Microenvironment on Drug Efficacy in a Brain-Mimetic Platform for Cutaneous Metastatic Melanoma

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Although survival in patients with malignant melanoma has significantly improved due to therapeutic interventions based on the molecular basis of tumor etiology, durable responses in the face of metastatic disease are rarely realized. A “systems pharmacological” approach to uncover drug potency at the physically distinct stages of the metastatic cascade is required. We modeled disparate microenvironments in the brain; the perivascular niche and hyaluronic acid (HA) rich parenchyma, to assess contextual drug efficacy. These two microenvironments are not only differ in composition, but in dimensionality, with the perivascular niche inducing a 2D morphology in cells, while the HA-rich parenchyma leads to 3D cellular clusters. These in vitro models recapitulated in vivo morphology and motility for an isogenic, human model of melanoma metastatic progression. By independently modulating adhesion strength and ECM composition, we found that ERK inhibition decreased cell adhesion, whereas BRAF inhibition was only effective when combined with an ERK inhibitor. BRAF and ERK inhibition individually reduced cell motility in the less metastatic clone, with a lesser effect on the more metastatic clone. We observed that cells are resistant to BRAFV600E inhibition when cultured in 3D Fibronectin rich HA hydrogels, but Laminin rich HA-gels offered no protection. The opposite held true for ERK inhibition. These data reinforce that a dynamic microenvironment not only contributes to systemic metastasis, but also significantly modifies drug efficacy.