S27: Cell mechanics and cell mechanobiology -2

S27-1 Effect of Local Tensile Stress Field on Bone Matrix and Cell Alignment: an *In Vitro* Study

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Osteocytes are mechano-sensory cells embedded within the bone matrix that align their bodies along the major principal stress direction in the bone and play an important role in regulating functional bone adaptation by remodeling. These osteocytic processes are believed to act as mechanosensors detecting the interstitial fluid flow generated in the pericellular canaliculae and are perpendicularly extended to the trabecular and osteonal micro-surfaces.

To investigate the role of the local mechanical environment on osteocytes during their differentiation from osteoblasts, we developed a novel, *in vitro*, experimental system to control the local mechanical (tensile) stress field produced by cellular contraction and observed the self-organizing, cellular alignment generated via mechano-feedback.

Primary osteoblasts were isolated from mouse calvariae and were seeded onto square, type I collagen gels with different mechanical boundary conditions as follows: (A) all four boundaries fixed and (B) two opposite boundaries fixed along one axis and left freely perpendicular to the axis. After culturing the osteoblasts for 24 h, the cell body alignment was determined by measuring the angle from the fixed axis.

Cells cultured on the gel with the (A) boundary condition were observed to be randomly aligned, whereas those grown under the (B) boundary condition aligned along the fixed axis. This specific alignment resulted from the uniaxial tensile stress field generated by cellular self-contraction in the gel, which further enhanced the alignment of the cells with higher contraction. These results suggest that positive mechano-feedback may cause osteoblasts to align along tensile stress fields.

Considering that this initial alignment will affect the alignment of the osteocytes following their differentiation, further experiments on osteocytes stimulated by differentiation factors will be conducted to investigate the mechanism of osteocytic alignment within the bone matrix.

S27-2 Blood vessel on a chip - 3D vs. 2D

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Inhibiting or normalizing pathological angiogenesis is a therapeutic strategy that has been extensively studied and already brought up clinically with approved drugs. However, most experimental assays for drug development rely on 2D-cell culture models, which fail to mimic sprouting from a parent vessel. We have developed a microvessel-on-a- chip which enables the study of drugs targeting a specific pathway of angiogenesis. Microvessels were prepared using human umbilical vein endothelial cells (HUVEC) within a collagen gel scaffold. Sprouting angiogenesis was induced by VEGF-A, and it was shown to depend on the Notch signaling. 3D structure of microvessel model was non- invasively well

characterized by optical coherence tomography. To examine the efficacy of angiogenic inhibitors, two types of inhibitors: sorafenib and sunitinib which target the VEGF-A/VEGFR-2 pathway were used. A dose dependency of the angiogenesis inhibition could be observed using both inhibitors. Furthermore, the design of the chip enables the study of microvessel permeability by introducing a fluorescent molecule (FITC-dextran; 70 kDa) in the lumen of the parent vessel. It revealed that sorafenib impaired the endothelial barrier function whereas sunitinib did not. Overall this technology should contribute to improve the discovery of promising anti-angiogenic molecules and provide a convenient tool to assess fundamental questions about mechanisms at work at an endothelial-level during VEGF-A-induced angiogenesis.

S27-3 Mechanotargeting of nanoparticles to atherogenic endothelium

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Nanoparticles (NPs) facilitate drug internalization to cells in diseased tissues, which often are characterized by altered mechanical properties (e.g. atherosclerosis). We recently developed a thermodynamic model for nanoparticle uptake that predicted that N(total NP uptake)= $M\varphi \exp[A_0(\mu - \sigma) - 8\kappa\pi]$ where M is cell surface area, φ is NP bulk density, A_0= NP surface area, μ is NP adhesion energy, σ is tension energy, and κ is membrane bending energy. Using patterning of extracellular matrix proteins, we show that NP endocytosis is suppressed when cells convert from a low stress (low aspect ratio) to a high stress (high aspect ratio) state independent of cellular area and time.

Human aortic endothelial cells (HAECs) spread to fill the desired patterns that varied in area and aspect ratio. Larger cells exhibited higher total cellular uptake, due to higher available cell surface area for NP internalization. Cellular uptake was highest on aspect ratios of 1.5 and 2 which is the resting states for cells when they are cultured in 2D culture dishes and decreased as aspect ratio increased. A mechanical model that predicted the internal stress state of cells from focal adhesion location, cell shape, and size, demonstrated that changes in NP uptake from changes in cellular morphology are explained by alteration in internal stress and actin cytoskeletal organization, which was validated using imaging by structured illumination microscopy.
Our findings illustrate the role of cellular mechanics on overall NP uptake in HAECs. Understanding the relationship between changes in cellular mechanical properties and NP endocytosis is essential for designing more effective delivery strategies in tissues affected by mechanobiology-related diseases.

S27-4 The roles of vessel pulsation and dilation in clearing extracellular waste from the brain

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Neuron activity causes the release of metabolites in their extracellular environment. Often these metabolites are neurotoxic and/or detrimental to brain health. For example, increases of extracellular potassium ion concentration in brain can cause spreading depolarization, while buildup of amyloid-β is linked to Alzheimer's disease. The brain lacks a lymphatic vasculature for metabolite clearance, and the clearance pathways remain a major standing question in brain physiology. Recently, it has been

hypothesized that the brain has a circulation system, dubbed the glymphatic system [1], by which clearance has a major convective component in promoting metabolite exchange between interstitial fluid and cerebrospinal fluid along the fluid-filled paravascular spaces around cerebral blood vessels. Understanding the mechanics of said flow would have major implications in all aspects of brain pathophysiology. From a fluid dynamics viewpoint, convective flow needs a pumping mechanism. We consider various convective flows as generated by arterial wall movement induced by cardiac driven pulsations and functional hyperemia. We model fluid flow through the paravascular space, the latter modeled as a poroelastic matrix. Boundary conditions are deduced from two photon-imaging in awake, head-fixed mice as well as from hypotheses formulated in the literature. The equations of motion for the fluid are solved using an arbitrary Lagrangian-Eulerian mixed finite element method [2]. The predicted flow rates are critically reviewed to establish whether or not they are consistent with known physiological conditions.

References

[1] J. J. Iliff et al., Sci. Transl. Med., vol. 4(147), p. 147ra111-147ra111, 2012.

[2] F. Costanzo & S. T. Miller, CMAME, vol. 323, pp. 64–97, 2017.