

S14: Cell mechanics and cell mechanobiology-1

S14-1 Effect of Physical Environment on Cell Migration Using Microchannel Device

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Cell migration plays an important role in many physiological and pathological processes such as morphogenesis, wound healing, and tumor metastasis. Although the majority of such events occur with cells moving as a group, called collective cell migration, the mechanism of collective cell migration has not been well understood. Since it is known that mechanical environment may affect cell behavior the aim of this study is to focus on the effect of substrate rigidity and anisotropy on cell migration behavior.

A PDMS-based microfluidic device was fabricated, which consists of microchannels with micropillars of circular cross-section to serve different substrate stiffness and micropillars of ellipsoidal cross-section to serve anisotropic substrate stiffness. Cell migration was initiated when the microchannels were backfilled with the medium. A set of microscopic images of the top of micropillars including cells and the bottom of the same micropillars was obtained every 10 min up to 24 h. Cellular traction forces were calculated from the deflection of micropillars and the spring constant of micropillars through an image analysis.

After the onset of cell migration experiment, the cells migrated into microchannels with micropillar substrates throughout a 24 h experimental period. It was demonstrated that cells consisting of collective cell migration generated traction forces with different magnitudes and directions depending on their relative positions, possibly reflecting positional differences in mechanical roles within a moving cell group. It was also found that the cells migrated faster with increasing the stiffness of substrate and that the cells migrated faster in the direction with being stiffer than another direction, indicating the cells could mon

S14-2 Protein Kinase C α Translocation in Endothelial Cells in Response to Mechanical Stimulus

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Mechanical wounding of an endothelial monolayer induces an immediate Ca²⁺ wave. Several hours after mechanical wounding, the denuded area is covered by endothelial cells (ECs) that migrate to the wound. This migration process is closely related to protein kinase C α (PKC α), a Ca²⁺-dependent protein that translocates from the cytosol to the cell membrane. Because the cells adjacent to the wounded area are the first to migrate into the wound, we investigated whether mechanical wound induces PKC α translocation in cells adjacent to mechanically

wounded ECs. We monitored Ca^{2+} dynamics and PKC α translocation simultaneously using fluorescent microscopy. For this simultaneous observation, we used Fura-2–acetoxymethyl ester to visualize Ca^{2+} and constructed a Green fluorescent protein-tagged fusion protein to visualize PKC α . Mechanical wounding induced an immediate Ca^{2+} wave in the endothelial monolayer that emanated from the mechanically wounded cells to neighboring cells. Almost concurrently, PKC α in cells adjacent to the wounded cells translocates to the cell membrane then accumulates at the periphery of cells near the mechanically wounded cells. We hypothesize that this PKC α translocation is induced (1) by intercellular communication, such as paracrine signaling and gap junction, or (2) by mechanical stress, such as unloading of cell-cell tension. When intercellular communication was inhibited, the directional translocation occurs. On the other hand, it did not occur when the mechanosensitive channel was inhibited. Our results indicated that the implication of PKC α translocation in the Ca^{2+} signaling pathway in response to mechanical stress in ECs.

S14-3 Hydrostatic pressure-induced DNA breaks in chondrocytes and its relationship with chromatin architecture

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Introduction:Hydrostatic pressure (HP) promotes chondrogenesis in development by accelerating differentiation from stem cells to chondrocytes [1]. On the other hand, HP is suggested to induce harmful responses such as DNA breaks and apoptosis [2]. In this study, we hypothesized that HP-induced DNA break/repair plays as a critical biomechanical process to screen specific genes related to chondrocytic differentiation. Especially, we focused on chromatin dynamics under mechanical force that characterizes gene stabilities [3].
Methods:Mice-derived cell line for chondrocyte progenitor cells (ATDC5), were seeded on glass bottom dishes, which were packed in plastic bags and were loaded under cyclic HP with a peak value of 1 MPa and with a frequency of 0.3 Hz. After pressurization, immunostaining for gamma-H2AX, a histone H2AX phosphorylated near double-strand DNA breaks, was performed. Images for DAPI were binarized and the intensity for gamma-H2AX in the corresponding area was analyzed.
Results:We observed significant DNA breaks in more than 20 % of ATDC5 cells. Remarkably, we found that cyclic HP (Peak value: 1 MPa) induced more DNA breaks than constant HP (10 MPa). By employing confocal microscopy, we also found that regions of compacted DNAs with high intensity for DAPI, avoided from DNA breaks under cyclic HP.

Discussion: Here we showed that cyclic HP induced DNA breaks in ATDC5 cells. Chromatin dynamics would determine which genes are damaged and conserved under HP. We are going to analyze chromatin dynamics by employing chromatin conformation capture method and gene expression.

References:

- [1] Thomas, E. M. et al., J. Tissue Eng. Regen. Med. 2015; 9: 889–902.
- [2] Montagne, K. et al., PLoS ONE. 2017; 12: e0183226.
- [3] Le, H. Q. et al., Nat. Cell Biol. 2016; 18: 864-875.

S14-4 In situ, fluorescence lifetime-based measurements of cell membrane micromechanics

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The mechanical moduli of lipid bilayers govern cell adhesion, endocytosis, ion channel conductivity, and organization of signaling complexes. We have developed a new technique to measure bilayer moduli using area per lipid and its variance from the stretched exponential distribution of fluorescence lifetimes of 1'-dioctadecyl-3,3,3'-tetramethylindocarbocyanine perchlorate (DiI). The mean and variance of lifetime and corresponding areal distributions were calculated and related to bilayer compressibility (K_A) and bending (K_C) moduli according to $K_A = k_B T \langle A \rangle / \text{Var}(A)$ and $K_C = K_A h^2 / 24$, where h is membrane thickness.

For saturated lipids, area-per-lipid was on the order of 60 nm^2 , nearly equivalent to literature values obtained using NMR or x-ray scattering, and were shown to be inversely proportional to the chain length of the lipid. Further, area-per-lipid increased with increasing temperature and swelling of the nanoliposomes in ways related to chain length and saturation state, further validating the technique. Compressibility and bending moduli decreased with increasing temperature, and increased with increasing chain length. Unsaturated lipids had lower moduli than their saturated counterparts.

Finally, we provide the first in situ measurements of area-per-lipid and bilayer moduli for intact cells. For human red blood cells, the area-per-lipid was 68 \AA^2 and compressibility and bending moduli of the lipid bilayer were 6.9 mN/m and $4.4 k_B T$, respectively. For human aortic endothelial cells plated onto fibronectin-coated glass dishes K_A and K_C were 165.4 mN/m and $105.7 k_B T$, respectively, highlighting differences between red blood cell and endothelial cell moduli that are important for their respective mechanobiological functions.