Uncovering the role of cell-to-cell communication in tissue response to light-induced mechanical stimulations

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***Abstract* –We present an *in vitro* test bed to explore monolayer tissues as a medium for molecular communication via intra- and intercellular calcium (Ca^{2+}) signaling. The experiment setup employs a light-sensitive azobenzene-based glassy material as cell substrate of *Madin-Darby canine kidney* (MDCK) cells. The material undergoes deformations with visible light and generates mechanical stimulation to single cells or multicellular assemblies. The complete spatiotemporal control of the light stimulation, when combined with live calcium imaging, offers unique opportunities to study not only mechanotransduction in single cells but also the role of cell-to-cell communication in the tissue response.**

Our experiments reveal that Piezo1 is the key mechanosensitive ion channel generating the Ca^{2+} transients after nanoscale mechanical deformation of the cell culture substrate. Computational modeling of the signaling kinetics suggests that the mechanical stimulus triggers multiplexed intercellular signaling that involves Na^+, highlighting the complexity of mechanical signaling in multicellular systems.

Keywords — azobenzene, photopatterning, epithelium, mechanosensitive ion channels, calcium (Ca^{2+}) signaling

I. Introduction

Cells are constantly subjected to mechanical stimuli such as stretch, compression, osmotic stress, and shear from their physical environment. Through specific and highly dynamic protein complexes at the cell membrane, cells can perceive this mechanical information and adapt their form and functions to their niche Extra-Cellular Matrix (ECM) [1]–[3]. One of the first responding and most impactful intracellular responding systems is calcium (Ca^{2+}) signaling: in addition to affecting the cell membrane potential, Ca^{2+} acts as a universal second messenger and participates in numerous cellular signaling pathways, such as contraction, proliferation, secretion, vesicle trafficking, protein synthesis, and apoptosis [4], [5]. Defects and misregulation in Calcium signaling can contribute to pathological conditions.

Over the last 20 years, while the number of techniques to characterize cellular response has exploded, the options to reproducibly introduce mechanical stimulations, especially at sub-tissue levels, have been limited. Early methods such as using compressive, longitudinal stretch or vibrational apparatuses [6] operate at the tissue-wide level, which triggers cellular responses mediated heavily at the ECM level. Meanwhile, local stimulation techniques are mostly point-wise, and therefore samples are obtained with low throughputs [7].

In this work, we have implemented an azobenzene-based molecular glass (disperse red 1 molecular glass – DR1-glass [8]) as a dynamic ECM biomimicking cell substrate for an epithelium monolayer. The microtopography of DR1-glass substrate can be manipulated using basic confocal microscopy (~488nn laser) to generate basal shear stimuli to cells (Fig. 1A). After verifying the light-induced formation of topographical features on the responsive substrate, we showed that the cytosolic Ca^{2+} concentration is perturbed in the epithelial monolayer in response to these local nanoscale modifications of the substrate's surface. Comparing Ca^{2+} responses in directly stimulated cells and their neighbors suggests different Ca^{2+} kinetics and thus highlighting the role of cell-to-cell communication.

II. Experiment setup

The light-responsive thin films were prepared by spin coating DR1-glass on microscopy coverslips (Fig. 1B). MDCK cells constitutively expressing a red Ca^{2+} indicator (jRCaMP1b) were then cultured on fibronectin-coated DR1-glass. The free-form microtopography was inscribed on the thin films by scanning a 488 nm laser with a laser scanning confocal microscope in user-defined regions of interest (ROIs) of the film [9]. We verified with atomic force microscopy (AFM) that the material displacements are mostly lateral and highly reproducible with each iteration of the light stimulation.

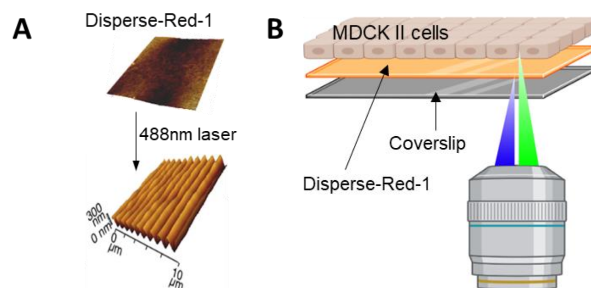

***Figure 1.* A)** The microtopography of DR1-glass substrate can be manipulated with photostimulations (488nn laser). **B)** DR1-glass is coated on the microscope coverslip, on which MDCK II cells are then cultured. Basal mechanical stimulations are applied to cells by displacing DR1-glass material with laser illumination.

III. Induced Calcium responses

The Ca^{2+} surges, observed through the red Ca^{2+} indicator, in response to shear deformation were very fast, occurring immediately (<1s) after the mechanical stimulation (Fig. 2A). The average amplitude of the surges (maximum intensity) depends on the stimulating laser power rather than the number of iterations. Further testing with various drugs strongly supports the role of mechanosensitive Piezo-1 channels as the main triggers of these surges in the photoinscription region.

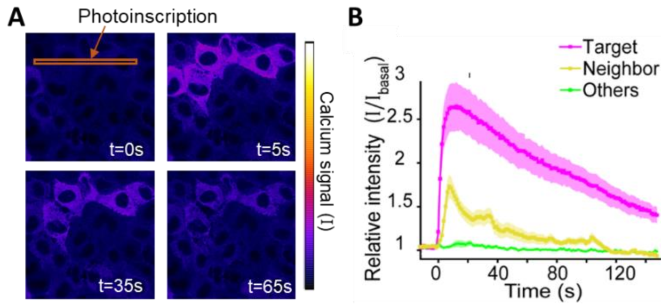

Figure 2. **A)** Tissue's electrophysiological activities in response to local mechanical stimulation (generated with DR1-glass photoinscription). **B)** Analyses of calcium responses in directly targeted cells and in neighboring cells.

Ca^{2+} surges do not occur only in directly targeted cells (purple) but also in neighboring cells (yellow) (Fig. 2A). The speed of Ca^{2+} spreading is fast (<3s between the moments of detections in targeted and neighboring cells). Interestingly, following the initial surges, Ca^{2+} signals in neighboring cells decay faster than those in targeted cells (Fig. 2B), hinting toward distinct Ca^{2+} kinetics in these cells.

IV. A MODEL OF CALCIUM DYNAMICS

Although Piezo1-channels favor Ca^{2+}, they are non-specific cation channels and thus also permit the flow of other cations, including Na^+ [10]. We therefore proposed that in target cells the detected influx of Ca^{2+} is accompanied by Na^+. This increase in cytosolic Na^+ concentration could affect the efficiency of Na^+/Ca^{2+} exchangers (NCX) [11], one of the components responsible for removing cytosolic Ca^{2+} (Fig. 3A). As NCXs use the Na^+ gradient across cell membrane as an energy source, increasing Na^+ concentration would decrease the pumping rate of Ca^{2+} out of the cells. Ultimately, this would result in a longer decay time in target cells. Meanwhile, in neighbor cells no Piezo1-mediated Na^+ influx would occur.

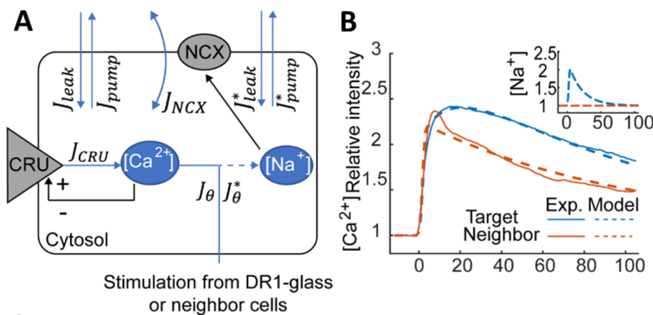

Figure 3. **A)** Model of Ca^{2+} responses following mechanical stimulation. Blue arrows indicate the influxes and outfluxes of Ca^{2+} and N^+ concentrations ($[Ca^{2+}]$ and $[Na^+]$ respectively) in cytosol. **B)** The fitting of the model (dashed) to the data (solid) for target cells (red) and for neighbor cells (blue). The Na+ concentrations used in the model are shown in the inset panel for the respective cell groups.

To test this hypothesis, we extended the simplified model of Ca^{2+} response in Kaouri et al. [12] to account for Na^+ cytosolic concentration (Fig. 3A). In this model, stimulations either from DR1-glass displacements or from adjacent cells trigger an indistinguishable Ca^{2+} influx J_θ in target cells and neighbor cells. However, the influx of Na^+ J^*_θ is set equal to that of Ca^{2+} in target cells and 0 in neighbor cells.

From the data, we select two groups of traces in target cells and neighbor cells respectively with similar Ca^{2+} response amplitude. For each group, we calculated the averaged normalized intensity (Fig. 3B, solid line) and fit our model to these averaged traces. The only free parameters varying between the traces pertain to the influx of Ca^{2+} in cytosol J_θ (i.e., its timing, duration, and intensity). Once the effect of Na^+ was incorporated, the model produced similar Ca^{2+} traces as the experimental data, thus supporting our hypothesis (Fig. 3B).

V. CONCLUSIONS

Here we demonstrated the power of our light-induced mechanical stimulation setup to study Ca^{2+} responses in epithelial monolayer tissues. The spatial flexibility of the system enables stimulation ranging from local subcellular probing to population wide mechanical activation, while its temporal control potential allows to probe the role of several processes of various time scales in Ca^{2+} dynamics. These freedoms will be instrumental in studying how the information about the stimuli propagate within the tissues and how cells collectively process this information. It can also serve as an *in vitro* test bed to explore monolayer tissues as a medium for molecular communication.

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