EMERGENCE OF SYNCHRONY IN CARDIAC CELLS THROUGH MECHANICAL COMMUNICATION

Xing Tang[*], Piyush Bajaj[**], Rashid Bashir[**] and Taher Saif[*a)]
[*]*Mechanical Science and Engineering,* [**]*Bioengineering, University of Illinois, Urbana, IL 61801, USA*

Summary It is generally understood that cardiac cells synchronize their beating through electro-chemical signalling. Here we show, theoretically and experimentally, that isolated cardiac cells can communicate with each other through an intervening deformable media. Such communication leads to coupled dynamics and emergence of synchronous beating. The interaction between the cells depends inversely with the elastic modulus of the media, and the distance between them. This finding may explain asynchronous beating of the atrium in patients with atrial fibrillation where the stiffness of the atrial wall becomes significantly harder due to fibrosis [1].

In order to explore the mechanical communication between cells, we culture chicken cardiac cells on soft (1-2KPa) PA (polyacrylamide) gel substrate, functionalized by laminin (Fig 1) [2]. Cells are plated sparsely so that some cells are more than 100 μm apart from their neighbours. We first ask whether an isolated cell can be stimulated mechanically only through the compliant substrate. We bring a probe in contact with the substrate, about 45 μm away from an isolated quiescent (non-beating) cell. The probe is used to apply local shear traction on the substrate. The deformation of the substrate stretches the cell by about 5% (change of size along the stretch direction). The stimulation is applied with about 0.5 Hz frequency. After about seven cycles, the cell begins to beat, and continued to beat long after (about 2 hrs of observation time) the stimulation is withdrawn. The deformation field of the substrate and that of the cell are used to estimate the stiffness of the cell (assuming that the cell is homogeneous), and the traction between the cell and the substrate using finite element analysis. The experiment shows that cardiac cells can be stimulated through the deformation of the substrate alone.

The relative displacement, Δ, between two points at a distance, D, apart on the surface of a half space due to a unit dipole on the surface can be evaluated using Boussinesque solutions. It can be shown that Δ/D varies as $1/G$ and $1/\{x(x+D)(x+2D)\}$, where G is the shear modulus of the half space and x is the distance between the dipole and the nearest of the two points.

One can interpret Δ as a measure of the traction applied on a cell of size D on the substrate due to a unit dipole at a distance x from the cell. The precise value of the traction depends on the stiffness of the cell. Using the stiffness value of the cardiac cell obtained earlier, we determine the stretch Δ of a cell due to a unit dipole as a function of the distance from the dipole using finite element analysis. The stretch decays with distance as $1/\{x(x+D)(x+2D)\}$ and $1/G$. Thus, if the dipole were generated by a cardiac cell, then a neighbouring cardiac cell would be stretched, just as the probe stretched the quiescent cell. A beating cardiac cell would stimulate a quiescent cell to beat, which in turn would stimulate the neighbour, and their dynamics would be coupled. The coupling would lead to synchronize the beating of the cells. Moreover, the cell pair would beat together for longer time compared to a lonely cell, since each would keep the other "awake". The strength of the coupling would decay with the distance between the cells and with increasing stiffness of the substrate.

We test the above theoretical conclusions by culturing cardiac cells at varying distances from each other, and on different stiffness substrates. We find (Fig 2), that the pairs with close by (within 10 μm) partners on 2KPa gel substrate show higher probability of beating together for much longer time synchronously compared to the pairs with far away partners. Moreover, when they are cultured on harder (40 KPa) substrate, this beating-together becomes rare event, consistent with the theoretical predictions. The histogram of the fraction of pairs with both cell beating decays with the distance between the partners of the pair. The qualitative nature of the histogram matches well with theoretical prediction (Fig 2).

References

[1] Dean, R. G., L. C. Balding, R. Candido, W. C. Burns, Z. Cao, Twigg, S. M., and L. M. Burrell. 2005. Journal of Histochemistry Cytochemistry 53:1245-1256..
[2] Xin Tang, Piyush Bajaj, Rashid Bashir and Taher A. Saif *Soft Matter*, 2011, **7**, 6151-6158.

a) Corresponding author. Email: saif@illinois.edu.

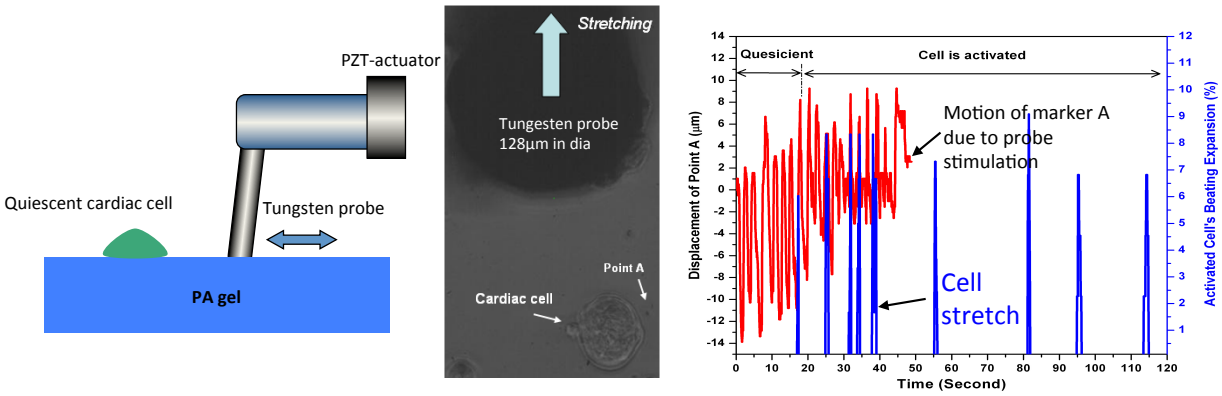

Fig. 1: (left) Schematic of a probe at 45 μm away from a quiescent cardiac cell on a soft substrate. (middle) Phase-contrast image of a chicken cardiomyocyte adhered to a 1KPa gel substrate functionalized by laminin ECM. The probe applies a traction on the substrate dynamically. However, it may slide during motion. The marker, A, on the surface of the substrate moves when the probe is not sliding. Thus the motion of A represents the actual deformation of the substrate. (right) Cell starts to beat after a few cycles of stimulation.

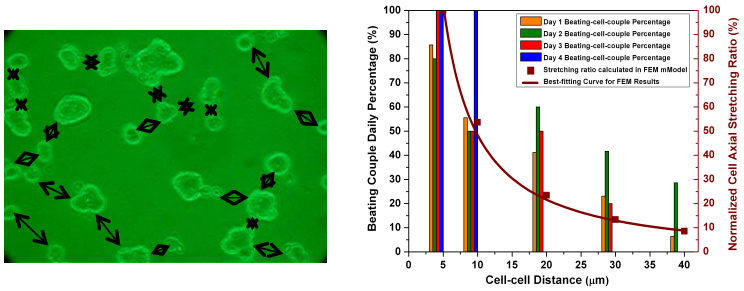

Fig. 2: (left) Cells pairs are grouped by distances between neighbors, such as a group consists of pairs with partners 0-5 μm, 5-10 μm, 10-20 μm apart, etc. (right) Percentages of pairs within each group with both cells beating are plotted for day 1-4. Higher percentages of pairs with close-by cells beat for longer times. The solid line is predicted by theory.