# Effect of physical stimulus for cell alignment and differentiation

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The cell is the smallest unite of life, which can provide us with important structural and functional information. Usually chemical and biological methods are applied to modify cell behaviors, while recent years have witnessed considerable growth in stimulating cells by physical techniques. Plenty of researches shed light on mimicking internal elctrophysiological phenomena for the sake of nerve regeneration and cardiac differentiation in vitro, but there are rarely talking about applying two or more physical stimulations together. For revealing the mysteries of physical stimulations to cells, a cell-based physical stimulating and bioelectric signal sensing system consisted of **t**ransparent **o**xide ITO (Indium Tin Oxide) interdigitated **e**lectrodes (TOEs) that acquired superior bioaffinity than metal materials and realized real-time detection directly by optical microscopy was demonstrated.

In this work, ITO comb microelectrodes were fabricated on a 2.5cm*2.5cm glass substrate by the standard lithography procedures. This kind of structure can work as both cell sensing components after welding with external copper wires and microgroove offering mechanical stimulation to cells. ST2, the mouse bone marrow stromal cells, was chosen for the study due to its comprehensive research value of cell differentiation and orientation in the field of regenerative medicine. Although individual mechanisms of mechanical and magnetic stimulations to cells are seems well established, whether these influences brought by two stimulations can be directly superposed or not is still uncertain. In our previous work, we have already obtained the optimal electrodes with (3μm) for enhancing the alignment of ST2 so that 3μm width comb electrodes array was fabricated to act as a mechanical stimulator. The static magnetic field ranged at 20mT, 50mT, 200mT and 500mT were externally applied parallel and perpendicular to the direction of electrodes. Moreover, besides the function of microgroove, ITO comb electrodes can also be used to measure the electrical characteristics at different cell states, which provide us the possibility to obtain information when physical stimulations affects cells. To test the performance of our device, ITO interdigitated electrodes were firstly utilized to the impedance changes of cell attachment, where two branches of comb electrodes worked as counter electrodes (CE) and working electrodes (WE) separately.

After applying two kinds of physical stimulations together, Figure 1(c) illustrates that the alignment of cells coming from microgroove would be broken by the low intensity and y direction magnetic field while the opposite results would occur if under stronger intensity. Exactly reason has not been discussed yet, but it maybe from the torque between collagen and fibrin in extracellular matrix because they will rotate towards vertical and parallel orientation respectively to magnetic field direction. In addition, by using this simple device we were able to successfully detect the change of impedance after cell attached, so there is also an opportunity to measure the electrical condition after physical factors stimulates cells.

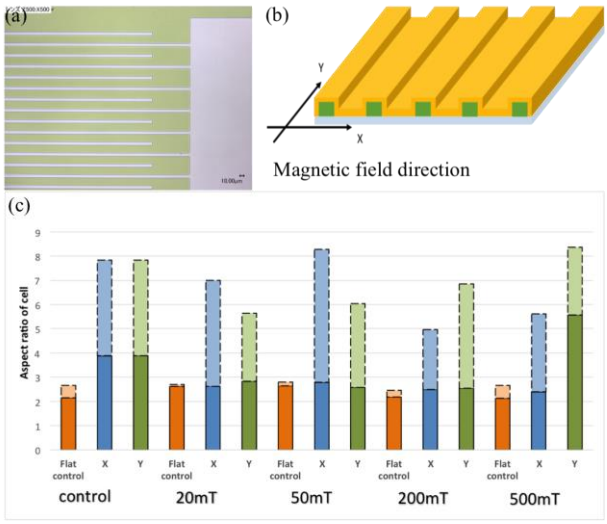


Figure 1. The joint effect of mechanical and magnetic stimulations to ST2 cells (a) Micrography of ITO comb electrodes structure (Dark regions are glass while bright parts are ITO; electrode width is 10μm); (b) Schematic of applying external magentic field at parallel (y) and perpendicular (x) to ITO electrodes; (c) Aspect ratio of ST2 cells after cultured on the surface of microgroove (ITO elelctrodes) for 48h and 1h weak static magentic field

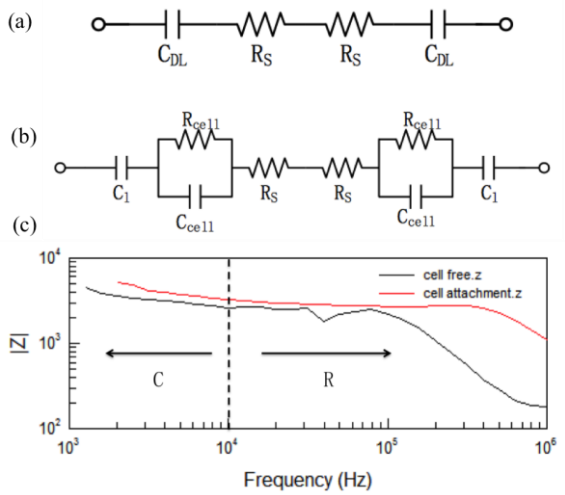


Figure 2. (a) The equivalent circuit model for the two-branch comb electrodes without any cells, where $C_{DL}$ stands for the interfacial capacitance of Helmholtz double layer and $R_S$ is the resistance of culture solution; (b) The equivalent circuit after cells attach on the electrodes surface and then start their proliferation, $R_{CELL}$ is the resistance from the culture medium between growing cells and $C_{CELL}$ directly reflects the information of those cells attaching on the electrodes, (c) the impedance varying against frequency of cell free and cell attachment