PSE, ESPCI Paris: Bio generator using muscle cells

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Material and methods

Electrical stimulation device:

A simple RC circuit is used with two copper wires immersed in the culture media of the Petri dish that contains the muscle cells. The signal is created with an arbitrary waveform generator (Agilent), and its propagation is monitored with an oscilloscope and an electrical probe that is moved at the resistance, the capacity and the Petri Dish, to ensure that the Petri Dish's content allows the current to go through. Square signals are used with the greatest available voltage (20vpp). The frequency of the signal must be under 3Hz in order to avoid cells blocking (permanent contraction).

Preparation of the stamps:

First, the patterns were created on a computer-aided design (AutoCAD), with basic shapes based on previous literature: 2000um-long straight lines and hybrid lines, formed by a linear element and a circular belly; with width varying from 100um to 800um. The experimenter can choose to make the patterns longer or put more lines per pattern to obtain a larger muscle in the end. The width of the lines should not go beyond 500um, as the muscle cells will differentiate with random orientation, resulting in inefficient muscular contraction. A master is then created on a silicon wafer by standard photolithography. The stamps are obtained by casting PDMS (Sylgard184) on the masters and baking them at 70°C for 90 minutes. Recasting non-baked PDMS on baked PDMS can form both positive and negative sides of the patterns, as it will not alter or damage the finished design. Each pattern is then carefully isolated from the others using a cutter blade, providing the individual stamps. The stamps should be about one cm thick, to allow an acceptable resilience when manipulated. Thicker stamps are not recommended: because PDMS is lightly porous, it will retain ethanol when sterilized, which will take time to evaporate. Remaining ethanol in the stamps could lead to pressing it out on the Petri dish when stamping, and killing the myoblast cells upon seeding.

Ink preparation:

All biological solutions must be prepared under sterile conditions, otherwise cellular death will occur rapidly. The fibronectin solutions are obtained by dissolving the appropriate (corresponding to the desired concentration) amount of solid fibronectin from bovine plasma (Sigma F4759) in freshly 22um filtered PBS (can be bought both prepared and in tablets to be dissolved), filling 10ml tubes. We advise using 10ml tubes as fibronectin is a costly item and several solutions must be prepared, with different concentrations. The collagen solutions are prepared from a 3mg/ml type I collagen solution, that is diluted in freshly 22um filtered PBS to obtain the desired concentrations. Adhesive protein solutions are stored under refrigeration.

Stamp coating and stamping protocol:

As previously pointed out, the following experiments must be carried out under sterile conditions to avoid early cellular death. Each stamp is covered with the ink using a p-1000ml micropipette. A drop of ink is laid on the stamp, and carefully spread on the entire surface of the stamp with a pipet cone. The surface tension between the PDMS and the protein solution is strong enough to confine the liquid to the stamp's surface when the ink is stirred with the cone. If the initial drop is not enough to cover the entire surface, it is possible to add drops on the stamp. The coated stamps are then incubated at 37°C, 5% CO_2 atmosphere in Petri dishes for 30 minutes. When the incubation is finished, each stamp is dried with pressurized nitrogen and stamped on its Petri Dish. This task should be carried out both carefully and quickly: the nitrogen is located in a non-sterile zone, but the drying is crucial to the success of the experiment. Pressurized air can be used but is not itself sterile, whereas pressurized nitrogen is. The Petri dishes are then incubated for another 20 minutes. At this point, the adhesive proteins are being transferred on the Petri Dishes by physisorption and are polymerizing, forming a cellular-adhesive pattern. The stamps are then carefully detached from the Petri Dishes. The quality of the pattern can be directly observed with the naked eye. The first ones can also be observed under a microscope but we recommend keeping the stamps and Petri dishes in the sterile working station at all times.

Petri dish blocking:

Although not strictly necessary to obtain patterned muscle cells, this step allows a near-perfect confinement of the cells in the pattern. 2% Pluronic F-127 is used to block all non-patterned regions of the Petri dishes. Approximately 1ml of Pluronic F-127 is dropped in the Petri dishes, uniformly spread by slowly leaning the dish in the required directions, and set still for three minutes. The amount of Pluronic per Petri dish is not important, as long as the entire bottom is covered. However, we recommend that a minimum is used, for economy reasons, but most importantly because Pluronic is a surfactant, and acts as a very effective soap. If it splashes out of the Petri Dish, everything will become highly slippery, including the experimenter's hands. The Petri dishes are then rinced three times with sterile distilled water.

Cell seeding, culture and differentiation protocol:

C2C12 murine myoblast cells are seeded on the Petri dish with around 2000 cells/cm2. This number is not crucial as the next step is to have the cells reach confluence. The cells are cultivated in 8ml C2C12 culture media, which is changed every two days. The cells are rinsed with PBS every time the media is changed. At this point, it is possible to observe the cellular confinement on the patterns with a microscope. Upon reaching confluence on the patterns, the culture media is replaced by differentiation media, which is changed every two days. Differentiation into myotubes typically takes 2-6 days. Differentiated muscle cells are long and have several nuclei.

Closer observation of the cells:

Fluorescent microscopy is carried out using Phalloidin-Atto 488 for actin labelling and SYTO Orange Fluorescent Dye for nucleus labelling. To fix the cells for observations a solution of 4% of paraformaldehyde and 4% of sucrose is used.