**A low-cost, high throughput and time-efficient method for generating single cells /cell cluster array by polymer on polymer stamping**

Peipei Zhang, Yong Qiao and Ming Su
Department of Biomedical Engineering, Worcester Polytechnic Institute, Worcester, MA, 01605.

**Statement of Purpose:** Well-controlled cell positioning and adhesion on surfaces attracted considerable interests in fundamental cell biology, as well as biomedical engineering area including tissue engineering, cell-based biosensor and drug screening. The basic principle of generating well-defined cell arrays is to create cell-favorable patterns and unfavorable background so as to arrange cells spatially. The cell-favorable patterns can be generated by various methods which usually requires complicated fabrication procedures. On the other hand, the cell unfavorable background mainly relies poly(ethylene glycol) based chemistry. However, PEG based chemistry is usually limited by requiring a long reaction time, forming island-like domains and heterogeneous coatings, and oxidizing under ambient conditions. We aims to develop a low-cost, high throughput method for generating single cell and cell cluster arrays with well-defined number of cells.

**Methods:** Figure 1A shows the schematic procedures of generating single cell or cell cluster arrays by polymer-on-polymer stamping. (Jiang, Zheng et al. 2002) Briefly, polyelectrolyte multilayer are formed on a PDMS stamp and a glass substrate by layer-by-layer assembly, respectively. The PDMS stamp is then printed onto the glass slide and peeled off after 45 sec.. Multilayer on the protruding micropillars of PDMS stamp is transferred onto the glass slide, generating isolated multilayer patterns. The first layer on the PDMS stamp and final layer on the glass substrate should be oppositely charged so as to generate electrostatic interactions between the pattern and background. The first layer on the PDMS stamp should be positively charged for the purpose of cell attachment.

**Results:** Florescence images of multilayer patterns are observed under florescence microscope (Figure 1B).Single cell or cell cluster arrays are generated on the multilayer patterns. Live/dead assay demonstrated that cells are still alive 3 hours after being patterned. Flow cytometry test shows that patterning does not affect cell cycle (data not included). By generating multilayer pattern with diameters of 15, 30 and 60 μm, cell clusters with, 2±1, 4±1 and 8±2 cells, respectively (Figure1B to G).

**Conclusions:** A low-cost, time efficient and high throughput method is developed to generate living cell arrays with well-controlled number of cells. In principle, this method allows generation of cell clusters with any number of cells by using PDMS micropillar stamps with different diameters. Further studies will include patterning two or more than two types of cells by this method. Other future studies will also involve using insulin-secreting cell and studying how normal cell functions are affected by cell-cell interactions and components of the multilayer thin film.

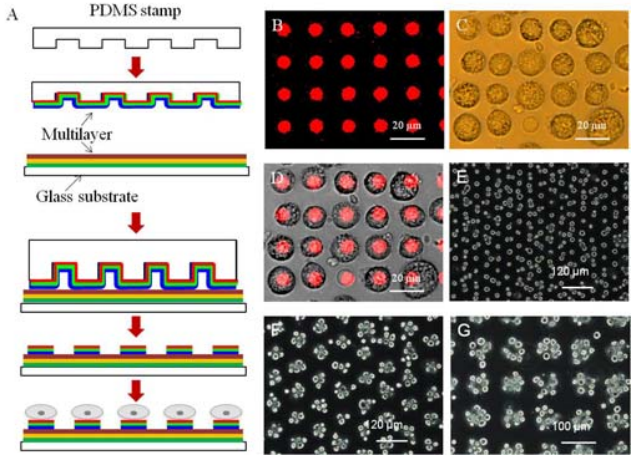


Figure 1. A). Schematic illustration of generating single cells arrays by polymer-on-polymer stamping. B) Polyelectrolyte multilayer pattern on a glass substrate coated with multilayer. C) XX cells attached on the multilayer pattern. D) Merged image of A and B. cell cluster arrays generated with multilayer patterns with a diameter of E) 15, F) 30, G) 60 μm

**References:**

Jiang, X. P., H. P. Zheng, S. Gourdin and P. T. Hammond (2002). Langmuir **18**(7): 2607-2615.