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# Inkjet Printing-Facilitated Micropatterned Multicellular Structure Generation

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*Micropatterned multicellular structures (e.g., cell microarrays, strips and spheroids) are important in vitro systems for high-throughput biological and biomedical assays in a well-controlled manner, such as automated gene editing and drug screening, cardiomyocyte maturation assay, cell migration assay, etc. To form micropatterned multicellular structures, special substrate surface chemistry treatments and/or geometric designs are needed in order to confine cells within the desired patterns. Existing approaches mainly rely on either contact-printing of extracellular matrix (ECM) proteins on non-cell-adhesive substrates or confining cells into pre-formed microwell arrays. Both approaches involve tedious and time-consuming microfabrication processes. In this study, we take advantage of inkjet printing technology to generate cell microarrays on hydrogel substrates. The evolution of the multicellular structures is systematically characterized using optical microscopy. Our new strategy significantly reduces the turnaround time and provides great flexibility to generate micropatterned multicellular structures, which will greatly benefit biological and biomedical communities.*

## 1. Introduction

Animal models have been historically used to test biological hypotheses and test the pharmacologic activity and acute toxicity for new drugs prior to human clinical trials. However, animal tests are expensive, time-consuming and, more concerningly, tend to produce unreliable results due to species differences between human and other animals[1]. The drawbacks of animal models have largely motivated the development of in vitro models in biological, biomedical and biomedicine research. With the growing demand for *in vitro* testing, micropatterned multicellular structures (MPMCSs) have gained widespread attention in recent years due to their advantages over traditional cell culture systems, such as more standardized culture, higher throughput, and lower consumption of cells and reagents. MPMCS-based *in vitro* systems have enabled high-throughput quantitative analyses in various applications, including drug screening[2], cardiomyocyte maturation assay[3], cell migration assay[4], tissue engineering[5], etc.

To form MPMCSs, special substrate surface chemistry treatments and/or geometric designs are often needed in order to confine cells to form desired patterns. One of the most commonly used approaches is to micro-pattern extracellular matrix (ECM) proteins on a non-cell-adhesive substrate (e.g., hydrogel, PDMS, etc.) using contact printing technique[6]. Since cells can only adhere to the region coated with ECM，they grow into MPMSs as specified by the physical mold used for contact printing. Another common approach is to allow MPMCSs to form within microwells which prevent cells from migrating away[7]. However, these existing techniques not only require sophisticated and expensive machines, but also involve complex fabrication processes which are tedious and time-consuming. In addition, one needs to go through all the fabrication processes with a new mask when a new pattern is needed. To overcome these issues, we propose a new method to produce MPMCSs by generating ECM patterns on non-cell-adhesive substrates using inkjet printing technique. Our new strategy significantly reduces the turnaround time and provides great flexibility to generate MPMCSs, which will greatly benefit biological and biomedical research communities.



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## 2. MPMCS formation on hydrogel substrates

Hydrogels have been widely used in biological and biomedical research as their softness well mimics *in vivo* cellular microenvironment. Polyacrylamide (PA) hydrogel is biocompatible but non-cell-adhesive, so cells can not adhere on PA hydrogel without coating ECM. To generate MPMCSs on PA hydrogel substrates, we use a prototype HP D100 dispenser to dispense ECM droplets onto a glass coverslip with a prototype T1 cassette (Fig. 1A) and then transfer the ECM pattern from the glass coverslip onto the hydrogel substrate (Fig. 1B). To transfer ECM pattern, we make PA hydrogel by sandwiching PA gel precursor solution between the glass coverslip with ECM pattern and a chemically activated glass coverslip (Fig. 1B). The activated coverslip is activated with 2% 3-Aminopropyl-trimethoxysilane (APTMS) in isopropanol solution, followed with 1% glutaraldehyde solution in water. A layer of active aldehyde groups that can chemically bond with the amino groups in PA gel are formed on the activated coverslip surface, which makes the PA hydrogel remain bounded with the activated coverslip after peeling off the regular coverslip. In the meanwhile, the ECM pattern on the regular coverslip is transferred into the PA hydrogel substrate.

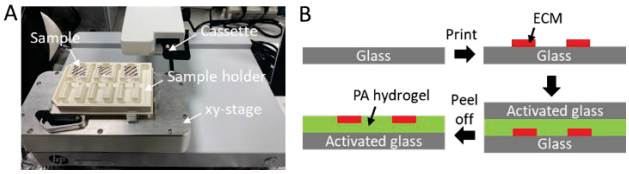


Fig. 1 (A) A photo of the prototype HP D100 dispenser with glass coverslip samples placed in a 3D-printed sample holder. (B) Schematic illustration of the workflow of ECM pattern generation on PA hydrogel substrates.

A desired ECM pattern is specified in the HP D100 prototype patterning software which controls the movement of samples within x-y plane. The separation distance between neighboring spots and the arrangement of these can be easily controlled by modifying the pattern design in the software (Fig. 2A), while the size of the ECM spots can be easily controlled by varying the volume of the dispensed ECM solution (Fig. 2B).

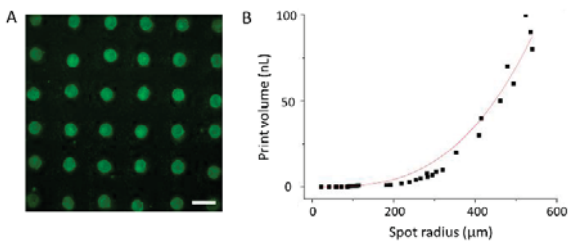


Fig. 2 (A) A representative optical microscopy image of ECM spot array printed at a droplet volume of 1 nL. Scale bar: 400 μm. (B) The relationship between print volume and ECM spot radius.

As a demonstration, we seed C2C12 myoblast cells on the hydrogel substrate with fibronectin patterns generated with the proposed approach and find that cells successfully attach onto the patterned ECM spot and grow into a confluent monolayer after one day (Fig. 3A). The two-dimensional (2D) cell monolayer starts with a spiral arrangement and gradually transform into a n aster arrangement (Fig. 3A) and eventually grow into a 3D structure (Fig. 3B). Our observations are consistent with the recent report on the swirling protrusion by Guillamat *et al*[8].

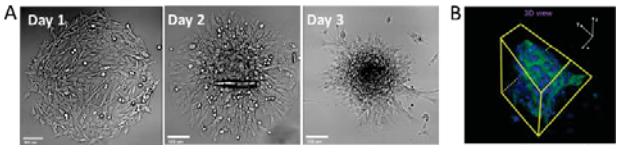


Fig. 3 (A) Formation and temporal evolution of a C2C12 multicellular structure on PA hydrogel substrate. (B) 3D laser confocal image of a C2C12 cell cluster.

## 3. Conclusions

We have demonstrated that inkjet printing of ECM solution facilitated by the prototype HP D100 dispenser enables a simple and fast way to pattern ECM on PA hydrogel substrate. The ECM pattern on such a non-cell-adhesive substrate allows the formation of C2C12 MPMCSs by enabling cells to adhere onto and grow within the patterned ECM spots. Compared to the existing approaches enabled by contact printing and microwell fabrication, the workflow of our method is more efficient and does not involve any microfabrication process. Such an innovative method greatly reduces the turnaround time and provides great flexibility to generate MPMCSs, which can be readily adopted by other researchers in biological and biomedical research communities for many other applications.

## ACKNOWLEDGEMENT

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