GRADIENT HYDROGELS FOR HIGH THROUGHPUT DRUG SCREENING

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ABSTRACT

In this study we demonstrate that hydrogels loaded with a concentration gradient of an active molecule can be used as analytical tools for high throughput screening (HTS) and drug discovery applications. We used a microfluidic mixer to fabricate poly(ethylene glycol) (PEG) hydrogels that contained chemical concentration gradients. Each hydrogel was placed in culture medium in the vicinity of preosteoblast MC3T3-E1 cell layers. The controlled release of the toxin from the hydrogel in a gradient manner was used to study the effect of various chemical concentrations on cell viability. Given its simplicity, ease of storage and portability, this platform is a potentially useful approach to screen a range of drug concentrations simultaneously.

KEYWORDS: Gradient, Hydrogel, Microfluidic, Toxin

INTRODUCTION

Conventional HTS methods can be used to conduct a large number of tests for chemical screening applications [1]. However these methods are carried out in expensive dedicated facilities with limited access. Moreover, the increasing number of target molecules and the high failure rate of drug candidates in the clinic have increased the need for more predictive and cheaper screening capability at early stages of the drug discovery process [2]. Recently, we reported the development of a cross gradient hydrogel to screen materials for cell responses [3]. Here, we expand on these findings to use hydrogels to screen a range of drug concentrations on cells.

EXPERIMENTAL

Microdevice fabrication: The microfluidic device was fabricated by using soft-lithography and replica molding techniques. The SU-8 mold master patterned with a microchannel network (12mm x 10mm x 0.18mm) and a chamber (15mm x 10mm x 0.18mm) was generously provided by Dr Hongkai Wu from Hong Kong University of Science and Technology, Hong Kong. PDMS prepolymer mixed with curing reagent (10:1 mass ratio) was poured in the master mold and cured at 70°C for 1.5 h after degassing in a vacuum chamber. The PDMS microstructured mold was then peeled off from the master and holes were drilled at the inlets and outlets. To allow the gel fixation on its surface, a glass slide was partially treated with 2% (v/v) TMSPMA solution in methanol and baked during 30 min at 100°C before bonding to the PDMS mold. The microfluidic mixer part of the microstructured PDMS layer was permanently bonded to the glass slide after treatment with an oxygen plasma for 10 sec using a plasma cleaner (Harrick Plasma/model PDC-001). Silicone tubes were then fixed at the inlets and outlet with drops of PDMS prepolymer plus curing agent solution cured at 70°C for 1.5 h.

PEG hydrogel and OA gradient formation: FITC-dex was used to visualize the concentration gradients. Two PEGDA 4000 prepolymer solutions with different FITC-dex and okadaic acid (OA) concentrations were injected at the microdevice inlets. Solution injected through the top inlet contained 40%(w/v) PEGDA, 1%(w/v) photoinitiator Irgacure, 1%(w/v) FITC-dex and 2μM OA in 1 ml MilliQ water. Solution injected through the bottom inlet contained only 40%(w/v) PEGDA and 1%(w/v) photoinitiator. Flow rates of 0.5 μl/min for both solutions were controlled by using two syringe pumps (World Precision Instruments/Aladdin syringe pump/ Saratosa, FL/USA). Hydrogel precursor containing FITC and OA concentration gradients was then photopolymerized (UVP/code UVGL-48/Upland, CA/USA) for 5 min at 356 nm. Images were acquired by using an inverted fluorescent microscope (Axio Observer.Z1/ Carl Zeiss Corporation/Tokyo/ Japan) and processed with an Axio Vision Imaging management system.

OA gradient and cell viability: After removing the PDMS layer with scalpel in a clean bench, a coverslip loaded with MC3T3-E1 cell layer was placed on the OA-gradient hydrogel, fixed with four clips and the whole construct was put in 25 ml culture medium for 24 h. The coverslip was then removed and cells were analyzed for viability with a live/dead kit assay (Invitrogen/ code L3224/Tokyo/Japan).

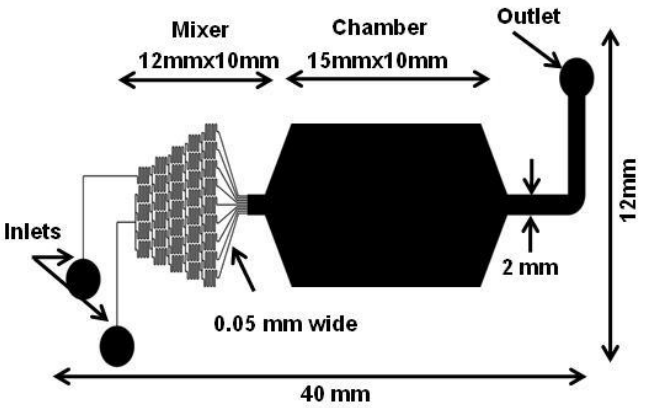


Figure 1: Scheme of the microfluidic device.

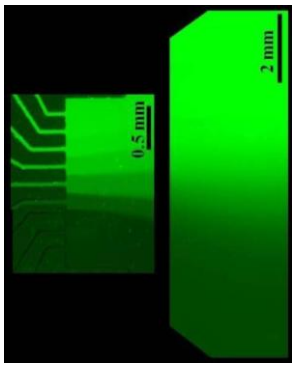


Figure 2: FITC-dex concentration gradient. Top views at the end of the mixer channel network and the chamber entrance.

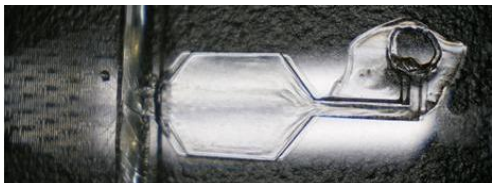
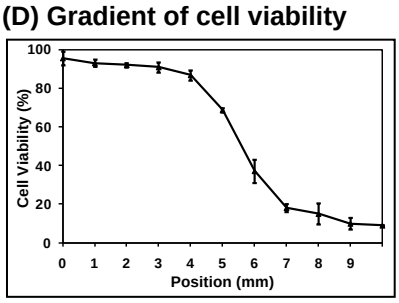


Figure 3: PEG hydrogel after UV polymerization. View with PDMS layer partially removed.



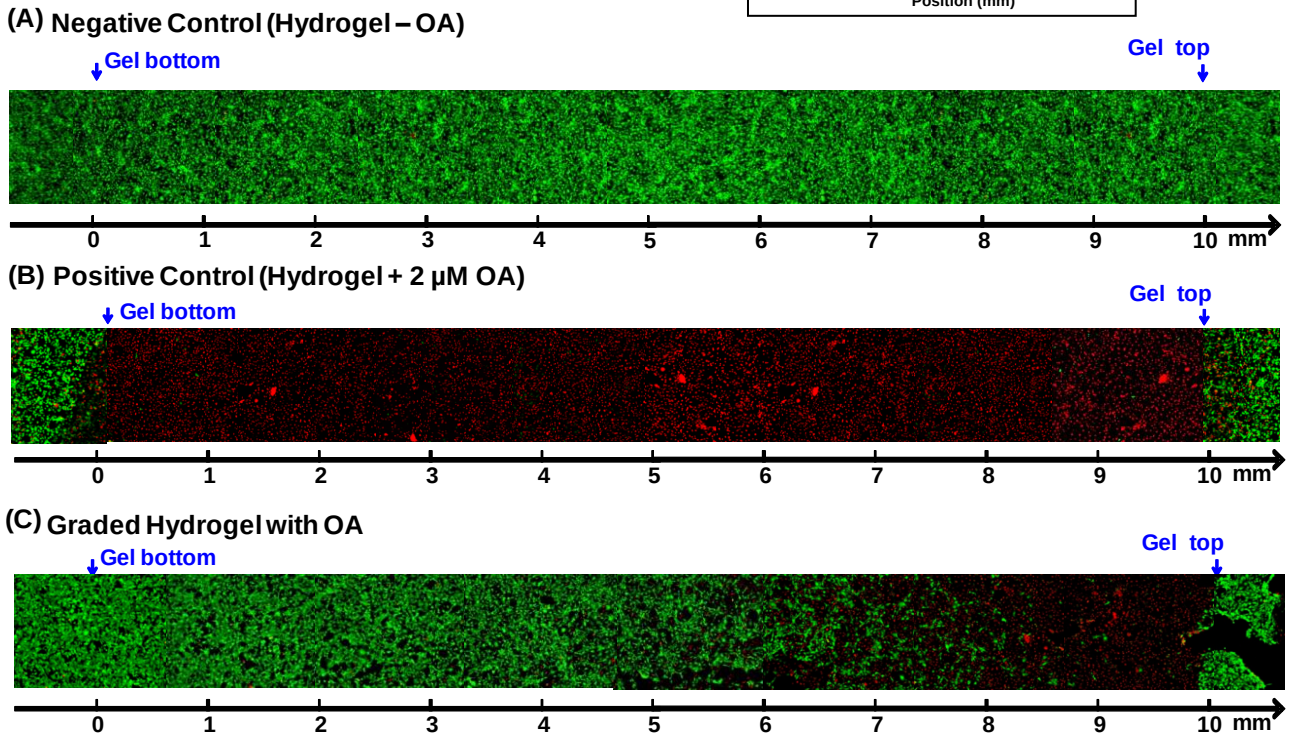


Figure 4: Cell viability after culture in contact with PEG hydrogel (A) without OA, (B) with 2 μM OA, (C) with 2μM-0M OA concentration gradient. Living and dead cells are marked with green and red fluorescence, ly. (D)Quantification of cell viability after 24 h culture with hydrogel loaded with OA gradient. (Note that gel top and bottom correspond to the sides on different edges of the channel).

RESULTS AND DISCUSSION

We used a microfabricated PDMS device to fabricate PEG hydrogels loaded with a model drug concentration gradient. This device consisted of a microfluidic mixer coupled to a chamber (Fig.1). Jeon et al. previously showed that such microfluidic system can be used to obtain spatially and temporally stable dynamic gradients with excellent control over the gradient characteristics [4]. To visualize the gradient generation in the microdevice we used the fluorescent dye fluorescein isothyocyanate-dextran (FITC-dex). We generated a FITC-dex concentration gradients by injecting through the inlets two flows of PEGDA prepolymer plus photoinitiator solution with different concentrations in FITC-dex. The resulting gradient was formed in the chamber part of the device (Fig. 2). As in such gradient generator the solutions flow rates affect greatly the gradient formation [5], we set up the flow rates of the injected solutions at 0.5 μl/min for both flows to obtain a smooth gradient. PEG gels were then photopolymerized under UV exposure during 5 minutes (Fig. 3) while flows were turn off within the first 10 s of UV exposition.

Similarly, by injecting through the microdevice inlets two flows of PEGDA prepolymer plus photoinitiator solution with different concentrations of OA, we fabricated PEG hydrogels with embedded gradient of OA [0-2μM] as a model drug to investigate the effects of the toxin release on cell viability. After removing the PDMS layer with scalpel in a clean bench, a coverslip loaded with MC3T3-E1 cell layer was placed on the hydrogel, fixed with four clips and the whole construct was put in 25 ml culture medium for 24 h. The coverslip was then removed and cells were analyzed for viability by calcein-AM (green/live cells) and ethidium homodimer (red/dead cells). Fig. 4A shows that for gels that did not contain OA, 95±1.2% of cells remained viable. Alternatively, only 8±0.1% of cells that were exposed to the hydrogels that contained 2 μM OA remained viable (Fig 4B). Finally, when PEG hydrogels were loaded with a [2μM-0M] OA gradient, the toxin was released in a gradient manner and cell viability also followed this gradient profile from 92.5±1.2% to 9±0.5% when facing the hydrogel regions which contained the lowest and highest OA concentrations, respectively (Fig 4C and 4D). Hydrogels are often used as drug delivery systems and different strategies for drug delivery have been applied [6]. Our experiments showed that our gradient hydrogel provides a spatially controlled delivery of OA and therefore allows to test a whole range of toxin or drug concentrations in the same time.

CONCLUSION

Thus, we have developed a potentially useful system for various drug discovery applications by using the controlled release properties of gels. A variety of molecules in whole range concentrations can be delivered easily thought our platform enable HTS studies.

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