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Controlled Release of Drugs from Gradient Hydrogels for High-Throughput Analysis of Cell–Drug Interactions

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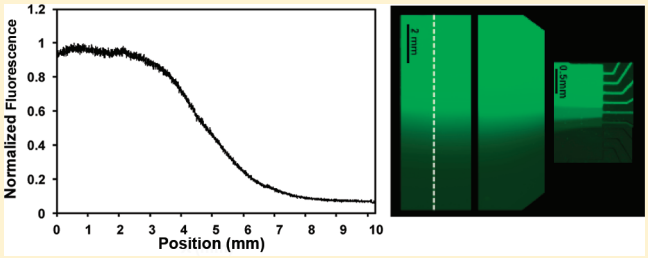
ABSTRACT: In this paper, we report a method to fabricate microengineered hydrogels that contain a concentration gradient of a drug for high-throughput analysis of cell-drug interactions. A microfluidic gradient generator was used to create a concentration gradient of okadaic acid (OA) as a model drug within poly(ethylene glycol) diacrylate hydrogels. These hydrogels were then incubated with MC3T3-E1 cell seeded glass slides to investigate the cell viability through the spatially controlled release of OA. The drug was released from the hydrogel in a gradient manner and induced a gradient of the cell viability. The drug concentration gradient containing hydrogels developed in this study have the potential to be used for drug discovery and diagnostics applications due to their ability to simultaneously test the effects of different concentrations of various chemicals.



The process of drug discovery and development has been limited by a number of scientific and technical challenges such as the need to analyze drug candidates in a more rapid and accurate manner.[1] Conventional high-throughput screening (HTS) methods have revolutionized the process of drug discovery and screening in medicine by performing a large number of tests to assess drug efficacy and determine the effective dose for a desired therapeutic effect on a particular disease.[2] These systems test the effects of multiple concentrations of a drug usually by means of a multiwell plate cell culture system, which has certain limitations such as the necessary processing time, the requirement for large sample volumes, less diagnostic reliability, maximal experimental variability, and the need for expensive equipment and processes.[1−3] Micro- and nanoscale technologies have been studied in various stages of the drug discovery process to overcome these limitations.[4−7] Microfluidics are used in drug discovery studies to perform cell sorting, HTS, protein crystallization, and biosensing.[8] Microfluidic technologies such as multiplexed systems, microwell arrays, plug-based methods, and gradient-generating devices have been used to enable HTS studies for drug discovery applications.[1] The advantages of these techniques include improved sensitivity and lower use of expensive reagents.[8]

Concentration gradients play an important role in drug screening and cell-based studies.[9,10] Gradient-generating microfluidic devices have been used to miniaturize experiments and generate multiple doses simultaneously to investigate cell behavior.[1,8] The use of these devices for generating a gradient of drugs in a three-dimensional (3D) matrix (i.e., hydrogel) will be a powerful addition to the existing HTS processes. The combination of microscale technologies for gradient generation and 3D hydrogels enables the testing of the effects of different concentrations of drug, embedded within the matrix, on the cell behavior.

Hydrogels with gradients of physical and chemical properties have been developed and applied as HTS systems for studying the cell–material interactions[11] and toxicity screening.[12,13] Different approaches have been developed to apply gradient hydrogels to cells and study cell–material interactions.[14] For

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example, chemical gradients can be immobilized in a hydrogel matrix and/or on its surface.[15−17] Gradients can also be embedded inside the matrix as diffusible soluble molecules.[18] Cells can be embedded inside the matrix with the gradient[19] or attached on the matrix surface[20] to study the cell−material interactions. In one such device, Lee et al. fabricated a chip consisting of cell-laden collagen or alginate hydrogels, arrayed on a functionalized glass slide, and used it as a miniaturized 3D cell-culture array for high-throughput toxicity screening of drug candidates and their metabolites.[13] In this method, a microchip was initially prepared by generating 560-spot microarrays of cell-laden collagen spots onto a functionalized glass slide and incubated in cell culture medium for 5 days. An array of drug components were then stamped on the top of the resulting microarrays to evaluate the response of the cells to varying doses of drug.[13] The accuracy of cytoxicity results obtained from this device was confirmed by comparison with cell cultures in 96-well plates.[13] Burdick et al. also developed a microfluidic platform to fabricate poly(ethylene glycol) diacrylate (PEGDA) hydrogels with gradients of arginine-glycine-aspartic acid (RGD) and cross-linking densities.[21] In this study, two distinct macromer/initiator solutions were injected into poly(dimethylsiloxane) (PDMS) channels to obtain prepolymer gradients that were subsequently polymerized to form cross-linked PEGDA hydrogels with gradients of cross-linking densities and RGD within the gel. The PEGDA hydrogels containing an RGD concentration gradient modulated the spatial distribution of adherent endothelial cells, which exhibited preferable adhesion to the regions with higher RGD concentrations.[21] In another study, He et al. employed a microfluidic platform to synthesize composite hydrogels containing cross-gradients of gelatin and chitosan.[20] The resulting gradient hydrogels were used as substrates for culturing smooth muscle cells to screen the cellular response such as morphology, adhesion, and proliferation with respect to the local composition of materials.[20]

In this study, we present a materials-based approach for miniaturizing HTS technology for drug discovery and diagnostics applications. In particular, concentration gradients of a drug in a hydrogel were used to study the effect of various concentrations of the drug on cell viability. PEGDA hydrogels containing a concentration gradient of okadaic acid (OA), as a model drug, were fabricated by using a microfluidic gradient generator. The effect of OA release from the gradient PEGDA hydrogel on cell viability was assessed by placing a cell seeded glass slide on the gel and performing a cell viability assay. We demonstrate that the miniaturized graded hydrogel could control the release of drug and affect the cell viability in a gradient manner.

MATERIALS AND METHODS

Materials. PDMS prepolymer and curing agent (Silpot 184 kit) were purchased from Dow Corning Toray, Japan. Fluorescence isothiocyanate dextran (FITC-dex, 10 kDa), 3-(trimethoxysilyl)propyl methacrylate (TMSPMA), penicillin−streptomycin, and OA (sodium salt, fw 826.98) were purchased from Sigma, Tokyo, Japan. Photoinitiator Irgacure 295 was purchased from Ciba, Tokyo, Japan. PEGDA (4000) was obtained from Monomer-Polymer and Dajac Laboratories, Trevose, PA. A preosteoblast cell line (MC3T3-E1) was obtained from ATCC, Rockville, MD. Minimal essential medium (α-MEM) and phosphate-buffered saline (PBS) were purchased from Gibco, Tokyo, Japan. Fetal bovine serum (FBS) was obtained from Japan Bioserum. A live/dead assay kit was purchased from Invitrogen. A Lab-Tek chamber slide was purchased from Nunc, New York.

Fabrication of Microfluidic Devices. The microfluidic device was fabricated by using soft lithography as described previously.[22] A microfabricated SU-8 mold master with 180 μm thickness was generously provided by Dr. Hongkai Wu from the Hong Kong University of Science and Technology, Hong Kong. The master mold was patterned with a network of microchannels (12 mm × 10 mm × 0.18 mm) and a chamber (15 mm × 10 mm × 0.18 mm) (Figure 1A). To generate the PDMS microchannel network, PDMS prepolymer was mixed with curing reagent (10:1 mass ratio), poured into 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. The inlets and outlet of the microchannel were created by using a biopsy punch.

Before being bonded to the PDMS mold, a glass slide was partially treated with 2% (v/v) TMSPMA solution in methanol and baked for 30 min at 100 °C to fix the gel onto the treated surface. The microfluidic mixer part of the microstructured PDMS layer was permanently bonded to the glass slide after treatment with an oxygen plasma for 10 s using a plasma cleaner (Harrick Plasma, model PDC-001). During plasma treatment, the chamber part of the microstructured PDMS mold and the corresponding region of the glass slide were masked with plastic films. Silicone tubes were then installed at the inlets and outlet and sealed with PDMS prepolymer and curing agent by baking at 70 °C for 1.5 h.

Generation of Gradient Hydrogels. The fabricated microdevice was used to generate PEGDA gradient hydrogels. The general procedure for the formation of PEGDA gradient hydrogels involved the injection of two distinct polymer solutions through the inlets of a microfluidic mixer, containing a network of microchannels that repeatedly split and mixed the injected solutions. FITC-dex was used as a model molecule to visualize the gradient generation. A solution containing 40% (w/v) PEGDA, 1% (w/v) photoinitiator Irgacure, and 1% (w/v) FITC-dex was injected through one of the inlets, while the same solution but without FITC-dex was injected through the other inlet. The injection flow rates of the solutions were controlled by using two syringe pumps (World Precision Instruments, Aladdin syringe pump, Sarasota, FL). The solutions were injected at a flow rate of 3 or 0.5 μL/min for 15 min to investigate the effect of the injection flow rate on gradient generation. Upon a stable gradient being obtained in the chamber, the hydrogel precursor containing an FITC-dex concentration gradient was photopolymerized for 5 min by exposure to UV light (UVP, code UVGL-48, Upland, CA). The flow was turned off after the first 10 s of UV exposure to prevent distortion of the gradient shape during the polymerization process. The final gradient hydrogel was characterized by using an inverted fluorescent microscope (Axio Observer.Z1, Carl Zeiss Corp., Tokyo, Japan). Images were acquired and processed with an Axio Vision imaging management system.

Validation of Drug Release from Gradient Hydrogels. FITC-dex was used as a model molecule to visualize and characterize the release of drug from the PEGDA gradient hydrogels. The release of FITC-dex from the hydrogel was characterized by soaking the hydrogel, containing an FITC-dex concentration gradient, in 20 mL of PBS solution and measuring the fluorescence intensity values at different time intervals using an inverted fluorescence microscope. The

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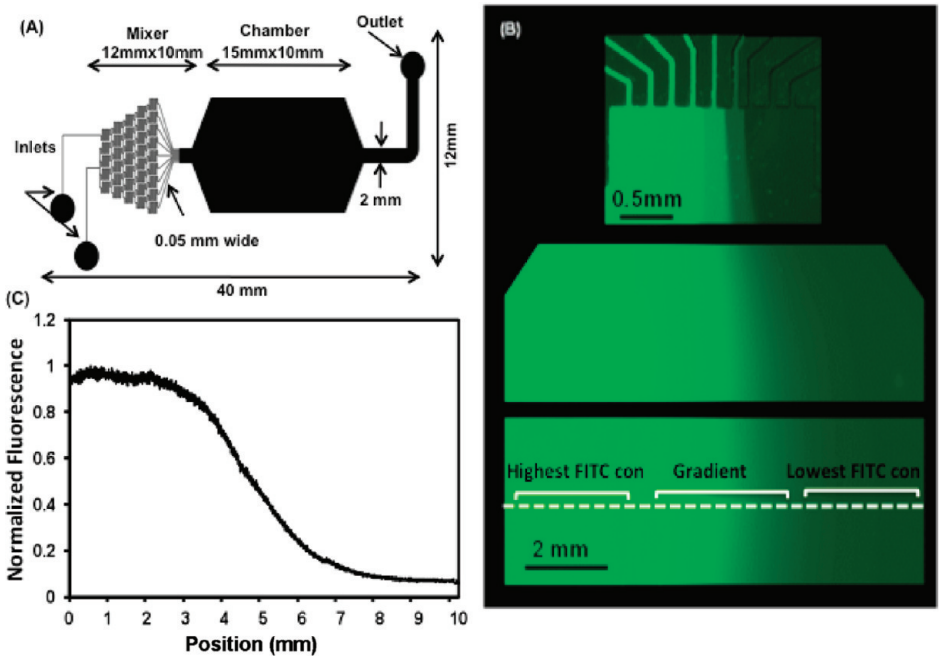

Figure 1. Gradient generation: (A) schematic of the microfluidic device used to generate concentration gradients in a hydrogel with 0.18 mm thickness, (B) fluorescent images of the end of the mixer and the entrance and middle of the chamber where 1 mM FITC-dex solution and water were injected at 0.5 μL/min through the top and bottom inlets, respectively, (C) gradient profile along the dashed line shown in (B).

gradient hydrogel was allowed to swell in PBS for 10 min prior to analysis. The fluorescence intensity values were then obtained at different positions along the gradient in the middle of the chamber. All release experiments were performed at room temperature (25 °C). Three hydrogels were tested for the release study, and at least three values were measured for each position along the gradient hydrogel.

Effect of the Drug Concentration and Treatment Time on Cell Viability. Prior to the fabrication of OA containing hydrogel gradients, a conventional cytotoxicity assay was performed to determine the effect of the OA concentration and treatment time on cell viability. MC3T3-E1 cells were cultured in α-MEM supplemented with 10% FBS and 1% penicillin–streptomycin at 37 °C in a humidified incubator. The culture medium was changed every two days, and cells were passaged weekly. Multiwell toxicity experiments were performed by trypsinizing and seeding the cells into the eight-well Lab Tek chamber slides at a density of 10^5 cells/cm^2. The slides were then incubated at 37 °C for 24 h. Various concentrations of OA (0.02, 0.2, and 2 μM) were then added to the cells, and the resulting cell viability was assessed by using a live/dead kit assay after 2, 6, and 24 h of treatment with the drug. Three wells were treated for each OA concentration and treatment time. A cytotoxicity test was conducted by staining the cells with calcein AM and ethidium homodimer-1 (EthD-1) in a serum-free medium. A 300 μL volume of medium containing calcein AM and EthD-1 was added to each well. Calcein AM is converted to green fluorescent calcein in metabolically active cells through the action of intracellular esterases (excitation ~495, emission ~515 nm). EthD-1 is a DNA-binding dye that enters dead cells through damaged membranes (excitation ~495 nm, emission ~635 nm). Calcein AM and EthD-1 fluorescence was observed by using a fluorescence microscope. At least three images from each well were used for the quantification of cell viability. Image J software was used to count the live and dead cells in each image. The cell viability was then calculated on the basis of the number of live cells divided by the total cell number.

Effect of Toxin Release from the Gradient Hydrogels on Cell Viability. PEGDA hydrogels containing an embedded gradient of OA were fabricated by injecting an aqueous solution of 40% (w/v) PEGDA containing 1% (w/v) photoinitiator, 1% (w/v) FITC-dex, and 2 μM OA through the top inlet and another solution of 40% (w/v) PEGDA and 1% (w/v) photoinitiator from the bottom inlet. The OA gradient was generated according to the procedure previously explained for FITC-dex gradient formation and subsequently stabilized upon photopolymerization. The microfluidic device containing the OA-gradient hydrogel was then transferred under the clean bench, after which the top PDMS mold was removed from the glass slide using a scalpel. The OA-gradient hydrogel fixed on the bottom glass slide was washed twice with PBS and allowed to swell with 100 μL of PBS for 10 min to reach equilibrium.

The effect of the gradient hydrogel on cell viability was investigated by placing an MC3T3-E1 cell seeded glass slide (10^5 cells/cm^2) on the gradient hydrogel and fixing this construct with four sterilized plastic clips. The cell seeded glass slide was incubated with culture medium for 24 h prior to the experiment. The whole assembly was then incubated with 25 mL of culture medium in an incubator at 37 °C for 24 h. The cell viability was determined by using the previously described live/dead assay.

Statistical Analysis. Data were analyzed by using one- and two-way analyses of variance (ANOVAs) followed by Bonferroni's posthoc test. Data are presented as the mean ± standard deviation (SD). All analyses were conducted with GraphPad Prism 5.00 (GraphPad Software, San Diego, CA). The level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Microdevice Fabrication and Concentration Gradient Formation. A microdevice, consisting of a microfluidic

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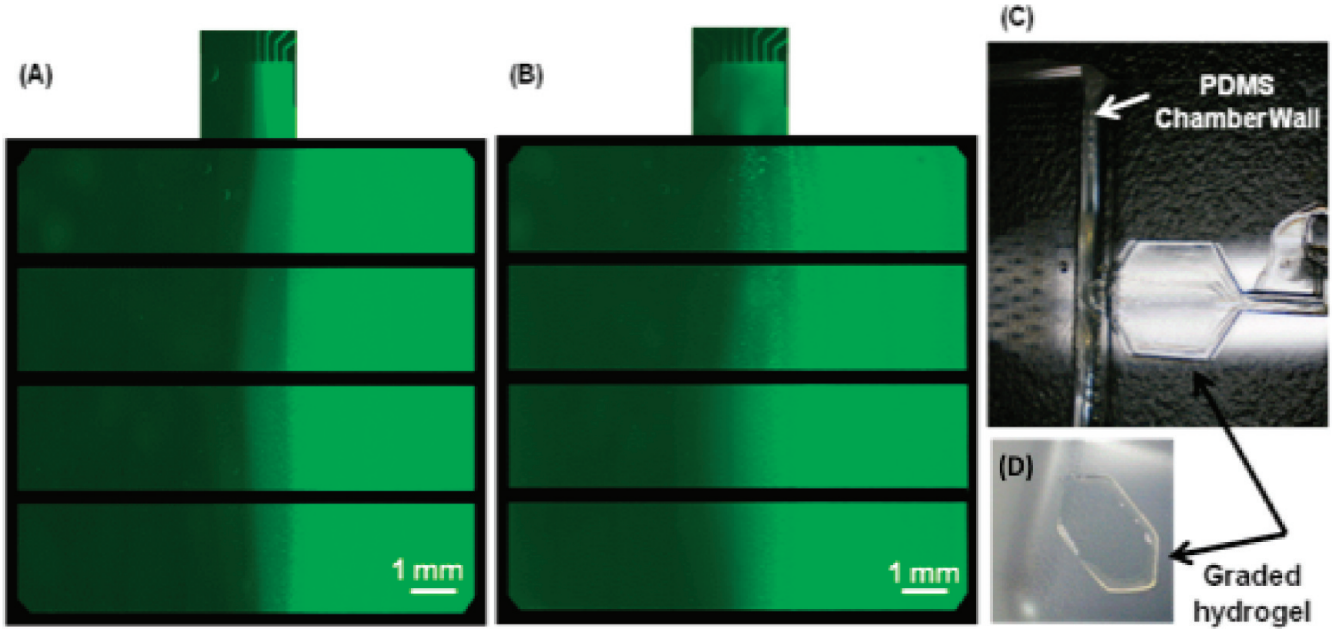

Figure 2. Gradient hydrogel formation: fluorescent images of the FITC-dex concentration gradient in the PEGDA precursor solution (A) before and (B) after UV photopolymerization (the gradients are shown at different regions of the chamber such as the entrance, top, middle, and bottom), (C, D) images of the photopolymerized PEGDA hydrogel loaded with the FITC-dex concentration gradient.

gradient generator coupled with a chamber, was fabricated for generating hydrogel gradients (Figure 1A). The microfluidic mixer unit consisted of two inlets connected to a serpentine microchannel (50 μm wide) network, allowing for mixing different drug concentration streams by diffusion. At the end of this network, all streams converged in a broad channel (2 mm wide) and a concentration gradient was generated perpendicular to the flow. This device uses the laminar property of the flow, for which there is only lateral mixing by diffusion. FITC-dex, which emits in the green wavelength (λ_{ex} = 495 nm, λ_{em} = 521 nm), was used as a model molecule to visualize the concentration gradient generated by using the fabricated microdevice. At the inlets of the chamber, the injected solutions merged and a concentration gradient of fluorescence was generated as a result of the incorporation of FITC-dex in the inlet solutions. As shown in Figure 1B, the chamber is comprised of three different regions: (i) a region with the highest FITC-dex concentration (top of the chamber), (ii) a gradient region, and (iii) a region with the lowest concentration of FITC-dex (bottom of the chamber). This gradient was observed in various locations of the chamber (such as the entrance and middle section) as indicated in Figure 1B.

The flow rate of solutions at the inlets can significantly affect the gradient formation. Generally, at high flow rates, there is not enough time for mixing and the gradient reaches a plateau near the edge of the channels; while at slow flow rates, due to the significant mixing, the extremes of the gradient are minimized.[21] Burdick et al. controlled the gradient of rhodamine in PEGDA solution by the injection flow rate and obtained a linear gradient when using 0.3 μL/min per inlet.[21] In our study, a step gradient was observed when an injection rate of 3 μL/min was used in both inlets. However, a slow flow rate of 0.5 μL/min per inlet produced a smooth gradient profile which was stabilized in the middle of the chamber as shown in Figure 1C. Consequently, a 0.5 μL/min injection rate was used to generate the gradient concentration in PEGDA hydrogel.

Effect of Photopolymerization on a Gradient Hydrogel. The effect of UV photopolymerization on a gradient hydrogel was investigated by preparing a PEGDA hydrogel containing an FITC-dex concentration gradient and comparing the fluorescent images of the gel before and after photopolymerization (Figure 2A,B). The presence of an FITC-dex concentration gradient in the photopolymerized hydrogel (Figure 2B) indicated that UV photopolymerization did not destroy the gradient concentration within the hydrogel. The resulting hydrogel was obtained after removal of the PDMS mold from the glass slide and was mechanically stable (Figure 2C and D).

Characterization of a Model Drug Release from Gradient Hydrogels. The release of drugs from a hydrogel can be controlled through different mechanisms such as diffusion, chemical methods, swelling, and environmentally responsive systems.[23] The drug release mechanism for the fabricated gradient hydrogel in this study was based on diffusion. The release of encapsulated drug from the hydrogel depends on the solute molecular size and the cross-linking density.[24] Several mathematical models have been developed to correlate the drug diffusion coefficient to the hydrogel characteristics and predict the drug release from the hydrogel.[25,26] In general, as the cross-linking density increases, the pore size of the hydrogel decreases, which leads to a reduction in the swelling ratio, as well as the rate of drug release from the hydrogel.[27] We performed preliminary experiments to investigate the effect of the FITC-dex molecular mass (10, 20, and 40 kDa) and PEGDA concentration (40%, 50%, 60%, and 70%) on the chemical release from a nongradient hydrogel (data not shown). Maximum fluorescence release was observed for the 40% PEGDA hydrogel loaded with 10 kDa FITC-dex. As we were interested in rapid release of the chemical from our gels, we used a PEGDA solution of 40% and 10 kDa FITC-dex to fabricate gradient hydrogels.

The release profiles of FITC-dex from the PEGDA hydrogel containing a concentration gradient of FITCdex is shown in

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Figure 3. On the basis of the chemistry of our entrapped molecules, we do not anticipate a significant amount of cross-

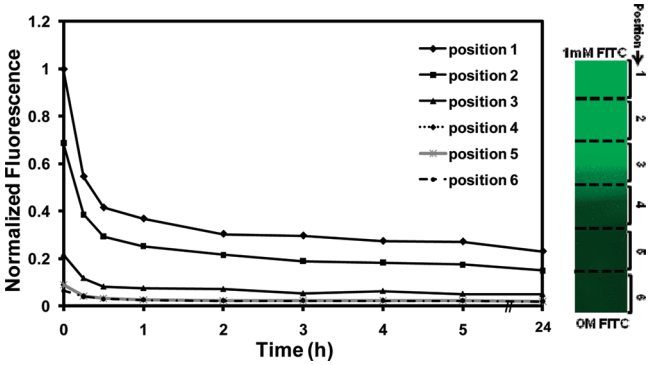

Figure 3. Release profiles from the gradient hydrogels: FITC-dex fluorescence release in PBS from the PEGDA hydrogel loaded with an FITC-dex concentration gradient at different time intervals. The fluorescence intensity values were obtained at different positions along the gradient in the middle of the chamber. Three hydrogels were tested for the release study, and at least three values were measured for each position along the gradient hydrogel. The release profiles were shifted down from position 1 to position 6, confirming that FITC-dex was released from the hydrogel in a gradient manner.

linking. Thus, we anticipate that FITC-dex molecules were trapped within the pores of the gels. The release of these molecules was, therefore, controlled by the pore sizes of the gel. The fluorescence intensity values were obtained at different positions along the gradient region of the hydrogel. As shown in Figure 3, at least 60% of the FITC-dex release occurred in the first 1 h at position 1 with the highest concentration of FITC-dex. This result was in agreement with the previous studies in which the release profile of a small molecular weight drug from a hydrogel was studied.[28] For example, Peppas et al. fabricated a PEGDA hydrogel containing a small molecular weight protein drug (diltiazem) and controlled the release of drug from the hydrogel by the 3D structure of the gel;[28] they reported that 90% of drug release occurred in the first 90 min.[28] In our experiments, the release of FITC-dex from the gradient hydrogel at position 1 increased to 73% and 77% after 4 and 24 h, respectively (Figure 3). This release profile was characteristic of a burst release,[29] indicating that the solute was not restricted by the polymer mesh size. The release profile after this initial burst release was followed by a slower and sustained fluorescence release phase. The maximum release (after 24 h) was decreased approximately 19-fold from 77% in position 1 to 4% in position 6, confirming that FITC-dex released from the hydrogel in a gradient manner.

Effects of Drug on Cell Viability. A conventional cytotoxicity assay in a multiwell system was performed to determine the effects of the OA concentration and treatment time on cell viability. MC3T3-E1 cells, cultured in eight-well plate lab-Tek chamber slides, were incubated with various concentrations of OA, and the cell viability was tested at different time intervals after drug treatment. OA is a phycotoxin produced by dinoflagellates; this phycotoxin is a tumor promoter and an inducer of cytotoxicity and apoptosis in cultured mammalian cells.[30−33] We have previously shown that this drug induced cellular barrier disruption in human colon carcinoma cells (Caco-2) after 4 h of treatment.[33] In this study, OA was selected as a model drug to study the effect of drug release from the gradient hydrogel on cell viability. As indicated in Figure 4A,, cell viability was decreased by increasing the drug concentration and treatment time. For example, at a drug concentration of 2 μM, cell viability was significantly decreased from 81.5% ± 11.6% to 64.75% ± 7.8% and 27.51% ± 5.1% when the treatment time was increased from 2 to 6 h and to 24 h, respectively ($p < 0.05$ and $p < 0.001$, respectively). Increasing the drug concentrations from 0 to 2 μM led to a 3.5-fold decrease in cell viability after 24 h of drug treatment ($p < 0.001$). The lowest cell viability was obtained when the cells were treated with the highest concentration of OA (2 μM) for 24 h at 37 °C. These results were in agreement with previous cytotoxicity studies where the loss of cellular barrier activity was observed after treatment by 2 μM OA.[34] In our study, we used an upper drug concentration of 2 μM and a 24 h treatment time to study the effect of OA on cell viability.

A PEGDA hydrogel containing an OA (0−2 μM) concentration gradient was used to investigate the effect of drug release on cell viability. A glass slide seeded with MC3T3-E1 cells was placed on the fabricated gradient hydrogel and fixed on the gel by using plastic clips. Cells were incubated with the hydrogel for 24 h at 37 °C. Although the fluorescence release study from the gradient PEGDA hydrogel (Figure 3) showed that most of the FITC-dex was released from the hydrogel within 4 h, the OA-gradient gel was kept in contact with cells for 24 h to obtain a maximum toxicity effect; these data were confirmed by a conventional cytotoxicity assay in a multiwell system after 24 h of incubation with 2 μM OA. A PEGDA hydrogel without OA was used as the negative control and showed 95% ± 1.2% cell viability in various locations of the hydrogel (Figure 4B). The positive control in this study was a PEGDA hydrogel embedded with 2 μM OA that exhibited 8% ± 0.1% cell viability (Figure 4C). The effect of drug release from the different positions of the OA gradient hydrogel on cell viability is shown in Figure 4D. In this experiment, the cell viability was significantly decreased from position 4 to position 10 (top of the gel) along the gradient hydrogel ($p < 0.001$). However, there was no significant drop in cell viability from position 1 (bottom of the gel) to position 4. As shown in Figure 4F, the cell viability significantly decreased from 92.5% ± 1.2% at the bottom of the hydrogel containing the lowest concentration of OA to 9% ± 0.5% at the top of the hydrogel with the highest OA concentration ($p < 0.001$). These data confirmed that OA was released from the hydrogel in a gradient manner to influence cell viability. The gradient hydrogel could provide a spatially controlled delivery of drug since the positions of the cell-seeded glass slide that were not in contact with the gradient hydrogel presented only living cells (Figure 4D).

Several studies have been performed to investigate the effect of gradient concentrations of drugs on cell viability using microfluidic devices.[35−38] For example, in one study, a microfluidic gradient maker was utilized to test the effect of bupivacaine and lidocaine anesthetic concentrations on myoblasts.[35] The cells were exposed to a continuous concentration gradient of toxin by using a microfluidic device with laminar flow within the channels.[35] Although the designed device can be used to increase the efficiency of drug testing, the cell viabilities were affected by the shear stress. In our recent study, we fabricated a microfluidic-based concentration gradient of neourotoxin for the creation of an in vitro model of Parkinson's disease.[36] We showed that a neurotoxin concentration gradient induced a gradient of cell viability in neural

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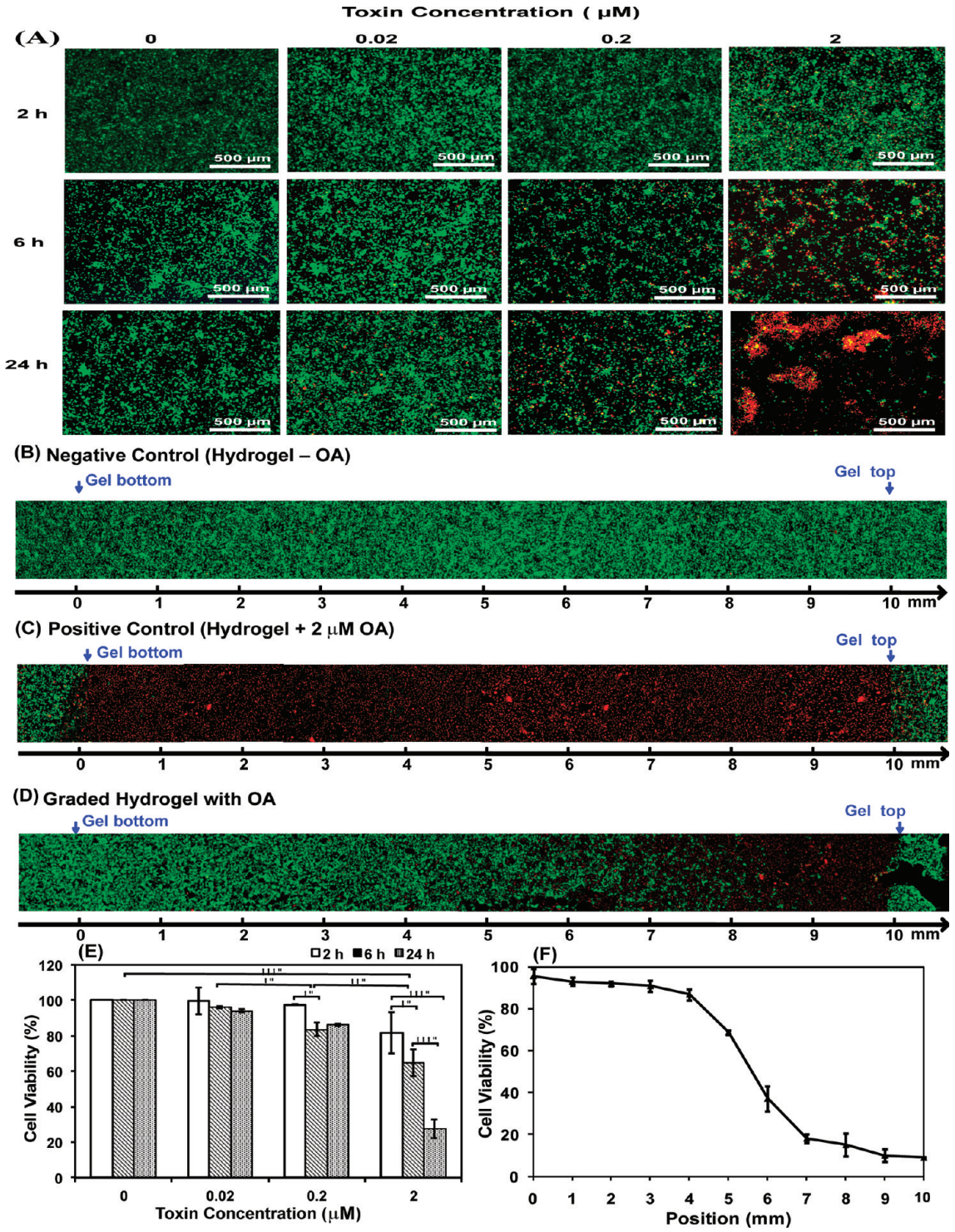

Figure 4. Effect of drug on cell viability. Fluorescent micrographs of (A) MC3T3 cells cultured in a multiwell plate and treated with various concentrations of toxin at different exposure times; cells incubated with PEGDA hydrogel (B) without OA (negative control), (C) with 2 μMOA (positive control), and (D) with an OA concentration gradient for 24 h. Dead cells are marked in red by ethidium bromide, while living cells are marked in green by calcein-AM. (E) Quantification of cell viability in a multiwell plate system. The cell viability was significantly reduced by increasing the toxin concentration and treatment time (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). (F) Cell viability quantifications in a hydrogel embedded with a concentration gradient of OA. The cell viabilities from position 4 to position 10 along the hydrogel show statistically significant differences ($p < 0.001$). The lowest cell viability was observed at position 10 with the highest OA concentration. Error bars represent the SD of averages obtained on three images from each of three independent samples per condition.

cells cultured within the microchannels. The lowest cell viability was observed in the region of the microchannel with the highest toxin concentration.[36] The developed technique can be used as an efficient platform for drug discovery and drug screening techniques. However, in all these studies, the cells were directly exposed to a concentration gradient of drug without any control of drug release. The combination of PEGDA hydrogels with the microfluidic device in our study

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could provide a powerful tool to control the drug release from the gel and eliminate the direct exposure of cells to the tested drug.

The multiwell plate system was used to assess the correlation between cell viability and toxin concentration in a gradient hydrogel. Generally, lower cell viability was obtained when using a gradient system compared to a multiwell plate system. For example, at the highest concentration of OA (2 μM), the cell viability was 27.51% ± 5.1% and 9% ± 0.5% in the multiwell plate system and gradient hydrogel, respectively. This effect may be attributed to the differences in culture conditions in these systems. Therefore, the multiwell plate system cannot be used to determine the OA concentration that cells are experiencing at different positions in the OA gradient hydrogel. The concentration of drug affecting cell viability along the gradient hydrogel can be determined through the conjugation of the drug with a fluorescent molecule and correlation of its gradient profile to cell viability at different locations of the hydrogel.

The hydrogel containing a drug concentration gradient in our study can be used to improve cell-based assays in drug discovery and diagnostics applications by simultaneously testing the effects of different concentrations of drug on the cells. The developed technique in this study will overcome the problems associated with currently used cell-based in vitro assays, which are performed by seeding the cells within multiwell dishes. This miniaturized microfluidic platform may enable medical diagnosis in a more rapid and accurate manner and is of great potential for drug discovery and HTS cell-based assays.

CONCLUSIONS

In this study we developed a new technique to fabricate hydrogels containing a concentration gradient of drugs for HTS applications. A microfluidic device was used to generate concentration gradients of FITC-dex and OA in PEGDA hydrogels. The results indicated that more than 70% of the FITC-dex loaded into the gel was released in PBS within 4 h in a gradient manner. The gradient hydrogel spatially delivered drug to the cells and induced a gradient of cell viability along the concentration gradient of OA within the gel. The designed process can be useful in minimizing the high costs associated with finding and validating new drugs. It is also possible to miniaturize experiments and reduce the amount of drug consumption compared to cell-based in vitro assays, which required the use of a high volume of drug solution. The microfluidic platform in this study may be used as a powerful technique for delivery of a variety of molecules and growth factors to control various aspects of the cellular microenvironment and enable HTS studies.

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