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Hydrogel patches on live cells through surface mediated polymerization

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Abstract

Many naturally-occurring cells possess an intrinsic ability to cross biological barriers that block conventional drug delivery, and these cells offer a possible mode of active transport across the blood brain barrier or into the core of tumor masses. While many technologies exist to form complete, nanoparticle loaded coatings on cells, a complete coating on the cell surface would disrupt the interaction of cells with their environments. To address this issue, cell surface patches that partially cover cell surfaces might provide a superior approach for cell mediated therapeutic delivery. The goal of this study is to establish a simplified approach to produce polymeric patches of arbitrary shapes on a live cell via surface-mediated photopolymerization. Cell surfaces were nonspecifically labeled with eosin, and polyethylene (glycol) diacrylate (PEGDA) coatings were directed to specific sites using a 530 nm irradiation through a chrome-coated photomask. These coatings may entrap drug loaded or imaging particles. The nonspecific formation of PEGDA hydrogel coatings increased with irradiation time, light intensity, and initiating species. 40 mW/cm² irradiation for 5 min delivered high-resolution patterns on the surface of A549 cells, and these cells remained viable for 48 h post-patterning with fluorescent nanoparticle loaded coatings. This work first demonstrated the feasibility to photo-pattern polymer patches directly onto the surface of cells.

Graphical abstract

Keywords

Cell patch; photolithographic polymerization; PEGDA hydrogel

INTRODUCTION

Targeted delivery seeks to minimize off target effects by localizing the therapeutic agent to the site of intended action.^{1, 2} Much of the prior work focuses on modifying a therapeutic particle's surface chemistry, targeting ligands, shape, and size.^{3–5} Recently, groups have begun loading a therapeutic cargo on the surface of a cell for a targeted effect.^{6, 7} For example, stem cell-binding silica nanoparticles have been utilized for delivering doxorubicin to glioma tumor cells.³ These cell carriers have also delivered encapsulated drugs through the blood-brain barrier and poorly accessible tumor tissues.^{8, 9} A significant obstacle in these approaches is the adhesion of drugs to the surface of the transporter cell while not precluding the cell-directed transport throughout the body or interfering with nutrient transportation through the cell membrane.^{10, 11} A coating that only covers part of a cell's surface would be capable of drug delivery while still allowing the cells to interact normally with the body. Swiston and Rubner attached polyelectrolyte multilayer patches on lymphocytes without altering their survival, proliferation rate, and normal cell functions.¹⁰ This is the first evidence that cellular patches can confine a drug cargo to a region of a cell's surface. Since then, additional applications of cell patches have been explored using stem cells for delivery of drug-loaded polymers to tumor tissue⁸ or monocytes to deliver payloads to inflamed tissues.⁹

A variety of techniques have been developed to produce drug loaded cellular patches. The PEM patches made by Rubner et al. were manufactured as pre-patterned films with a top layer conjugated with a cell binding ligand.¹⁰ A similar concept was applied by Mitragotri to generate multilayered “backpack” on monocytes composed of poly (methacrylic acid), poly (vinyl-pyrrolidone), poly (allylamine hydrochloride) and anionic iron oxide magnetic nanoparticles for delivery to inflamed tissues.^{9, 12} The production of cellular patches through these techniques requires multiple time consuming steps of polymer film formation and conjugation. In an alternative approach, Anderson et al. used sulfosuccinimidyl-6-(biotinamido) hexanoate (sulfo-NHS-LC-biotin) to induce global biotinylation of cell surface protein on hMSCs, followed by high affinity of biotinylated cell surface with NeutrAvidin-labelled polystyrene nanoparticles.⁸ A limitation of this technique is that they cannot control the size and shape of the patches, potentially interfering with robust interactions between the cell and the biological environment.

Here, we establish photolithographic patterning of a polyethylene glycol diacrylate (PEGDA) hydrogels as a simple technique to produce patches on cell surfaces. The cell patch approach is based on the eosin mediated photopolymerization of PEGDA, first demonstrated on cell surfaces by the Hubbell lab.¹³ The irradiation of visible light (530 nm) on surface immobilized eosin Y in the presence of triethanolamine (TEA) initiates PEGDA gel formation adjacent to the cell surface. While the Hubbell lab loaded high levels of eosin through nonspecific eosin absorption, our lab has previously synthesized 100 nm thick, non-patterned PEGDA films on cells with lower eosin loadings. Low levels of eosin conjugation (1,600 – 54,000 eosin/ μm^2) are achieved by contacting a streptavidin-eosin conjugate with a cell surface specifically labeled with biotinylated antibodies or nonspecifically labeled with a biotinylated NHS species.¹⁴

In this work, we pattern these coatings on a cell for the first time using standard photolithographic techniques. We first characterize PEGDA hydrogel formation on a microarray of biotinylated proteins on glass. The thickness, specificity, and spatial accuracy of PEGDA pattern formation was evaluated at different irradiation times, intensities, and mask positions. After pattern gelation trends are established on a glass substrate, we then apply nanoparticle loaded patches to A549 cells (adenocarcinomic human alveolar basal epithelial cells). As a potential application, the homing of tumor cells to pre-existing tumor sites^{15, 16} offers an opportunity to direct the transport of drug-loaded cells to tumors. A549 cells have been frequently used as an invasive immortalized cell line for non-small cell lung cancer.^{17, 18} The thickness and feature resolution of PEGDA patches deposited on the surface of A549 cells was characterized, and the effects of PEGDA pattern formation on cell viability were evaluated. In all, this study investigates the key parameters governing the formation of cellular patches through surface-mediated PEGDA photolithography. When compared to existing patch strategies, the simplicity of this approach decreases the aggregate time and complexity for cellular patch production.

EXPERIMENTAL SECTION

Materials

Epoxy functionalized slides were obtained from CEL Associates. Bovine serum albumin (BSA), eosin-5-isothiocyanate, phosphate-buffered saline tablets, triethanolamine, PEG diacrylate 575, 1-vinyl-2-pyrrolidinone and Whatman chip-clip were obtained from Sigma Aldrich (St Louis, MO). Poly (ethylene glycol) diacrylate 8000 was purchased from Alfa Aesar. Ethyl alcohol (200 proof) was purchased from Pharmco-aaper. Biotinylated bovine serum albumin, streptavidin, trypsin/EDTA, ethidium homodimer-1, yellow-green or Nile red fluorescent spheres, collagen I rat protein sulfo-NHS-LC-biotin, 16% paraformaldehyde aqueous solution and DAPI were purchased from Thermo Fisher Scientific. LED lamp with 530 nm wavelength (M530L2-C1) and neutral density filter (NE260B) were purchased from Thorlabs. Biotin anti-human EpCAM was purchased from BioLegend. The photomask used for surface patterning was a chrome-coated glass mask purchased from Louisville Photomask. RPMI-1640 cell culture media was purchased from Cellgro and supplemented with 10 v/v% fetal bovine serum (FBS, Gibco, Carlsbad, CA), 100 U/mL Penicillin, 10 mg/mL Streptomycin (Gibco) prior to use. Adenocarcinomic human alveolar basal epithelial cells (A549) were purchased from Lonza (Basel, Switzerland). 1X PBS was prepared by dissolving phosphate buffered saline tablets in ultrapure water according to the manufacturer protocol. Streptavidin-eosin was prepared as described previously¹⁹. PBSA was prepared by adding 1 mg/mL bovine serum albumin to 1X PBS.

Eosin conjugation of biotin-immobilized microarrays

Biotinylated microarrays consisted of an epoxide-coated glass substrate, and serial dilutions of biotinylated bovine serum albumin (bBSA, from 1000 to 0.105 μg per mL in PBS) were printed on array surface by GMS Affymetrix 417TM Arrayer. Each concentration of bBSA was duplicated into two spots. After array printing, the slides were dried overnight at room temperature and then were stored in a microscope slide storage box until use. For each microarray experiment, the bBSA-printed slide was loaded in Whatman Chip-Clip slide

holder, and each Chip-Clip array well was blocked with PBSA for 45 min to reduce non-specific interaction on unprinted sites. Array wells were then incubated in streptavidin-eosin (25 $\mu\text{g/mL}$ in PBS) for 30 min.

Culture and eosin photo-initiator conjugation of A549 cells

The A549 cell line was cultured in RPMI-1640 medium containing 10v/v% fetal bovine serum (FBS) and 1% streptomycin/penicillin at 37°C incubator with 5% CO_2 . Cells were cultured between 80 and 90 % confluency, and trypsinized prior to use with 0.25% trypsin-EDTA (Thermo Fisher) in sterile PBS for 5 min. Glass microscope slides (25mm $\times$ 75mm) were sterilized in a conical tube by immersion in 200 proof ethanol overnight before cell culture. After drying and sterilization, slides placed in a 100 mm petri dish were rinsed with sterilized PBS twice and incubated with 415 μL collagen I rat protein solution in 20 mL PBS at room temperature for 1 h. Approximately 5 million cells were cultured on the collagen-covered slides within a cell culture dish. After cell attachment, cells were cultured until >90% confluency for polymerization studies.

A549-cultured slides were gradually lowered in temperature prior to incubations on ice. Specifically, slides in culture dishes were incubated in 25 mL fresh cold medium and placed at room temperature for 10 min and then on ice for 20 min, washed with ice cold PBS and then loaded into a Whatman slide holder. Each cell well on Whatman Chip-Clip was rinsed with 400 μL PBS three times. After rinsing, a freshly prepared biotinylation solution of 1 mM sulfo-NHS-LC-biotin was added to the cells for 30 min. Biotinylated cells with NHS-biotin were rinsed with cold PBS three times and incubated with 35 $\mu\text{g/mL}$ streptavidin-eosin in PBS for 30 min. After photoinitiator conjugation, cell slides were rinsed with PBS three times.

Non-patterned polymerization of PEGDA on biotin-immobilized microarrays

PEGDA monomer was composed of 25 w/v% PEG diacrylate 575, 21 or 210 mM triethanolamine, and 35 mM vinyl pyrrolidinone. Irradiation conditions were 20, 30, or 40mW/cm². The thickness of PEGDA hydrogel on microarray system was observed by Dektak 6M stylus surface profilometer. The estimation of PEGDA hydrogel thickness by fluorophore was performed by the addition of yellow green fluospheres (2.5 v/v%, Life Technology) into the PEGDA monomer solution. The fluorescence of yellow green fluospheres was obtained by Nikon Eclipse Ti-U microscope with filter set of 470/535 excitation /emission. Fluorescence intensity was quantified by image analysis using Image J software.

Photo-lithographic polymerization of PEGDA hydrogel patches on biotin-immobilized microarray or adherent live cells

For PEGDA hydrogel patches formation, bBSA-immobilized epoxy microarray or biotinylated A549 cell surface provided binding sites for streptavidin-eosin conjugation. For photolithographic polymerization, the slide (microarray or cell slide) was separated from the photomask with two 40 μm thick spacers, and this assembly was held together with binder clips. The photomask features consisted of 10 μm thick chrome stripes separated by 28 μm spaces. For microarray polymerization, a neutral density filter was attached under the glass

slide to minimize reflection of the light, and the monomer solution was a solution of 25 w/v % PEG diacrylate 575, 21 or 210mM triethanolamine, 35 mM vinyl pyrrolidinone and 2.5v/v% yellow green Fluospheres in deionized water. For patch polymerization on live A549 cells, the monomer solution contained 10 w/v% PEG diacrylate 8000, 21 mM triethanolamine, 35 mM vinyl pyrrolidinone and 2.5 v/v% yellow green fluospheres in PBS, and the pH was adjusted to 7.4 with the addition of 1.2 molar hydrochloride, as needed. Monomer solutions were bubbled with ultrapure nitrogen in the dark for 10 min before photopolymerization. For polymerization, the PEGDA monomer solution was pipetted into the space between the slide and photomask. The whole photomask assembly system was purged with nitrogen for 3 min. The photomask-assembled slide was then irradiated under visible-light exposure with peak wavelength of 530 nm with continuous ultra-pure nitrogen purge. The desired light intensity and irradiation time period was selected as a parameter for polymerization. After irradiation, microarray slides were gently rinsed with deionized water, blown dry with house air, and stored in a microscope slide box. Polymerized cell slides were gently removed from the photomask assembly, re-loaded into Whatman chip-clip, and rinsed with PBS three times. Images of hydrogel patches were obtained by epi-fluorescence microscopy (Nikon Eclipse Ti-U).

Statistical analysis for microarray hydrogel polymerization

The fluorescence intensity of nanoparticle-loaded hydrogel patterns was quantitated through image analysis using the Image J software. For determination of the hydrogel pattern accuracy, the normalized sum of squared error and mean peak intensity were calculated by a custom Matlab program. The experimental data of fluorescence intensity distribution was normalized and were input into a custom Matlab script to fit a Gaussian function to the fluorescence data. The midpoint of the Gaussian curve was considered the midpoint of the pattern. The mean peak intensity was determined by the mean of the four data points closest to the pattern midpoint. The normalized ideal pattern is defined as a 10 μm wide step function with a magnitude equal to 1. The normalized pattern data was determined by scaling the fluorescent intensity data to a maximum of 1. The normalized sum of the squared error (Normalized SSE) is defined as the squared difference between normalized pattern data and normalized ideal pattern.

Membrane integrity assay after hydrogel patch polymerization on A549 cells

After polymerization and rinsing, photopatterned cells were incubated in 2 μM ethidium homodimer (Life Technologies) diluted in 3 v/v% fetal bovine serum in PBS for 30 min. Single channel images were obtained by epi-fluorescence microscopy (Nikon Eclipse Ti-U) and merged by Image J software.

PEGDA hydrogel formation on fixed A549 cells

A549 cells were grown to ~90% confluency on sterilized glass microscopy slides. Cells were washed 3 times with cold PBS, then fixed with 1% paraformaldehyde for 10 min, then washed 3 times again with cold PBS. Slides were blocked with 1% BSA in PBS for 40 min. Cells were stained with 10nM DAPI in PBS for 10 minutes and rinsed 3 times with PBS. Slides were then immersed in biotin anti-human EpCAM at 1:100 dilution in PBS+ 1%BSA for 40 minutes. Slides were rinsed 1 time with PBS+ 1%BSA then immersed in PBS

+ 1%BSA with 25 μ g/mL SA-EITC for 30 minutes. The slides were then rinsed 3 times with PBS and clamped to a chromium/glass photomask with a 40 μ m spacer. A monomer precursor solution was injected into the photomask setup which consisted of 420 mM PEG-diacrylate (Mn ~575), 210mM triethanolamine, 35mM vinyl pyrrolidinone, and 0.05wt% Nile red Fluosphere nanoparticles. The entire setup was then exposed to 10mW/cm² green LED light (Thorlabs) for 10 minutes and rinsed 3 times with DI water. After drying, the slides were imaged with an inverted epifluorescent microscope at 20X objective.

RESULTS AND DISCUSSION

Cellular patch fabrication typically consists of patterned layer-by-layer coating fabrication, adhesion onto the cell surface, and release of the assembly from the substrate.^{8, 10} In this study, we provide a unique approach for the formation of a drug-loaded polymer patches through direct photopolymerization of a nanoparticle-loaded polymer depot on the surface of a live cell. We expect the photo-polymerization of hydrogel cellular patch to have the following attributes: (a) patch fabrication in a physiologically-compatible environment; (b) direct hydrogel production onto a portion of cell membrane without multistep coating prefabrication; (c) highly controllable patch geometry fine-tuned by irradiation conditions and photomask features. To create geometrically-controlled polymeric film on the cell's surface, we take advantage of the high affinity between biotin and streptavidin to localize eosin on the cell surface. Cell surface biotinylation is attained through labeling surface proteins with NHS-biotin, and the biotinylated cell surface is contacted with streptavidin-eosin to immobilize low concentrations of eosin on the surface. A photomask with striped chrome features is placed above adherent A549 cells, and the gap between the cells and the photomask is filled with a PEGDA monomer solution. The surface-mediated photopolymerization of PEGDA hydrogel patches proceeds with green light irradiation at a wavelength of 530 nm (Fig. 1).

Characterization of PEGDA film polymerization parameters

The fabrication of PEGDA cellular patches requires a careful balance of gelation in irradiated regions with soluble macromolecular species in dark regions. The surface-mediated photopolymerization of PEGDA is controlled by multiple key parameters: the wavelength and intensity of light for photoinitiator excitation, irradiation period, composition of monomer and co-initiator, and the density of photoinitiators on the cell surface. These parameters alter the rate of polymer gelation on the cell membrane. In addition to the complex parameters which govern PEGDA film formation, cells have irregular geometries which limit our capacity to directly measure PEGDA pattern thickness on cells. To better characterize PEGDA film formation for eventual use on live cells, we first utilized an epoxy microarray system without a photomask to characterize how these same parameters dictate PEGDA film formation. Based on these results, we identify target conditions for cellular patch construction.

Biotinylated bovine serum albumin (bBSA) microarrays were prepared with serial biotin concentrations from 0 to 1000 μ g/mL bBSA, where these surfaces were made to represent the possible biotin concentrations on our target cell surfaces.¹⁴ These microarray surfaces

were treated with precursor solution (25 w/v% PEGDA575, 21 or 210mM TEA, 35mM VP and 2.5 w/v% yellow-green nanoparticles in deionized water), and exposed to green light (530 nm) under different irradiation conditions (20, 30, and 40 mW/cm² for 2.5, 5, and 10 min). The profilometric thickness of PEGDA film generated based on bBSA concentration gradient was described in Fig. 2A and 2B. With different irradiation conditions, PEGDA hydrogels were thickest (250 ± 8 nm) under 10 min, 40mW/cm² irradiation using 210 mM TEA (Fig. 2B). With the same TEA concentration and irradiation time, lower irradiation intensity (30mW/cm² and 20mW/cm²) produced lower peak thickness (182 ± 4 and 177 ± 4 nm, respectively). Despite the similar peak thickness at high bBSA, 20mW/cm² irradiation had consistently lower hydrogel thickness with lower bBSA concentration. Furthermore, under 21 mM TEA, same irradiation conditions produced lower hydrogel compared to 210 mM TEA (Fig. 2B). Taken together, these results supported that the thickness of the polymer are predictably controlled by all controllable parameters of the initiation system: the density of eosin conjugation, the irradiation energy, and the amine coininitiator concentration.^{20–22}

Fluorophore-labeled PEGDA for thickness estimation

For the measurement of PEGDA photopatterned coatings, our profilometer lacks adequate resolution for PEGDA film features that are less than 1 μm thick because of stylus tip radius is ~ 12 μm . To overcome this limitation, we correlated the fluorescence of embedded nanoparticles to the thickness of a PEGDA films on large features,¹⁴ and we used this correlation to determine submicron feature geometries by epifluorescent microscopy. We included 2.5 v/v% of yellow-green nanoparticles to become nonspecifically embedded into the hydrogel during polymerization. Fluorescence was highly proportional to the actual PEGDA film thickness from 20 to 150 nm (Fig. 2C). This is consistent with our previous report which used fluorescent intensity to estimate of hydrogel thickness.¹⁴ Furthermore, the fluorescence of yellow green was sufficient to detect polymer thickness below 50 nm, supporting the feasibility of quantifying PEGDA pattern height over the relevant thickness scales for surface mediate polymerization of PEGDA coatings.

Parameters governing the specificity of gelation in irradiated and non-irradiated regions

To better describe the relationship between the irradiation parameters and localized gelation via surface mediated polymerization, a chrome photomask was used to restrict the irradiation to 10 μm stripes on microarrays of biotinylated surfaces (1000 $\mu\text{g/mL}$). Fig. 3A schematically describes the photomask system, and a photograph of the system is provided as Figure S2. The spacers separating the photomask from the microarray were 40 μm thick for all data shown, while preliminary studies with thicker spacers had lower feature resolution (60, 80 and 100 μm , Fig. S2). Fluorescence microscopy was used to observe the spatial distribution of the fluorescence of yellow-green NPs labeled hydrogel patches in a 1 mg/mL bBSA printed microarray spot. Both bright field and fluorescence (ex/em 470/535 nm) microscopy confirm that 10 μm green striped hydrogel patterns were confined to regions within the photoinitiator-bound array spots (Fig 3B). The localization of polymer stripes to circular regions confirmed the polymerization was driven by specific binding of eosin to the microarray spots.

The intensity of fluorescence in both irradiated and non-irradiated regions of the array spot was analyzed by imageJ. The intensity was then converted to an estimated thickness based on the fluorescence-thickness relationship curve described in Fig 2C. The thickness at non-irradiated regions was consistently $< 5\text{ nm}$, and was not dependent on light intensity and irradiation period (Fig. S3). To reduce the light reflection at the back side of the glass substrate, a neutral density filter was applied to decrease polymer formation in non-irradiated regions of array spots (Fig S4).

For the irradiated region of the array spot and a 2.5 min irradiation period, there was extremely low thickness ($< 5\text{ nm}$) showing that no observable polymerization occurred for intensities up to and including 40 mW/cm^2 (Fig 4B). For the irradiated regions using light intensities of 30 and 40 mW/cm^2 with times of 5 or 10 min, the thickness was considerably higher ($> 30\text{ nm}$). In contrast, when light intensity was lowered to 20 mW/cm^2 , almost no polymer film was generated. The polymer thickness increased with both light intensity and irradiation time, and these trends are consistent with unpatterned coatings (Fig. 2B). Taken together with, there is a strong relationship between coating thickness and irradiation intensity and time within the irradiated area (Fig 4B), while the non-irradiated area is largely independent (Fig. S3). However, there was lower polymer formation under neutral density filter compared to polymerization without the neutral density filter (Fig. 2 and Fig. 4). The lower thickness is consistent with a lower contribution of reflected light to the irradiation regions, creating a compromise between improved feature sharpness and overall polymer thickness. In all, these trends support a parameter space for successful pattern formation: 5–10 min irradiation at $30\text{--}40\text{ mW/cm}^2$.

Analysis of coating feature edge sharpness

To quantify the accuracy of the pattern transferred from the photomask to the polymer patch, a normalized sum of square error (SSE) was calculated between the experimental topography and an ideal step pattern (Fig 4A and 4B). The SSE of the patterned PEGDA film increased with both irradiation intensity and duration (Fig 4C). In general, the polymer features were larger than the ideal dimensions, and this excess polymerization is attributed to the diffusion of oligomers and actively polymerizing chains into adjacent non-irradiated areas. Our data shows 40 mW/cm^2 , 5min of irradiation generated the PEGDA film with over 50 nm thickness, which is sufficient to load nanoparticles,²³ but also with modest SSE compared to 40 mW/cm^2 , 10 min or 30 mW/cm^2 , 10min. In all, high irradiation intensities coupled with low exposure times are supportive of thick polymer coatings and high feature accuracy.

Parameters governing PEGDA pattern formation and viability on A549 cells

To generate PEGDA film on cell surface, A549 cell surfaces were first biotinylated with NHS- biotin. Then, slides with biotinylated A549 cells were inserted into the same apparatus described in Fig 3A. To minimize monomer toxicity, we used PEGDA 8000 as the monomer unit for cell surface PEGDA hydrogel photopatterning. PEGDA monomers with higher molecular weights have shown to generate less stress and more biocompatible.²⁴ A lower concentration of TEA (21 mM) was used for cell surface PEGDA hydrogel formation because higher concentrations (210 mM) are toxic to the cells.¹¹ PEGDA hydrogel are

successfully patterned onto A549 cells with monomer solution 25 and 15 w/v% PEGDA 8000 polymerized under 40 mW/cm² for 5 min, but these conditions also correlated with significant amount of cell death in the irradiated region where PEGDA polymer was generated (Fig. 5S). 10w/v% of PEGDA 8000 was sufficient to polymerize PEGDA patterns as evidenced by fluorescence signal of yellow green nanoparticle loading, but with much lower cell death rate. Most importantly, A549 cell survival rate was not affected after 48 hours of incubation followed by PEGDA patch generation. As such, photopolymerization is capable of generating partial polymer coatings onto live cells in a rapid manner. Furthermore, the shape of the patch is modified through the selection of an appropriate photomask. Stripes of 10, 20, and 40 μ m pitch and circles of 5 and 20 μ m are easily achieved on cellular substrates (Figure S6).

CONCLUSIONS

This study provides a feasible approach to control the shape of PEGDA hydrogel films on live cells. Also, in contrast to existing methods, our approach avoids multiple, time-consuming fabrication processes required in advance of the patch deposition. Most importantly, PEGDA patches formed through photomask-governed photopolymerization does not impact the survival of cells, suggesting the potential use of these coatings as biologically-guided, drug delivery vehicles. Further investigation is justified to determine the effects of these patches on cell biological functions and to conjugate therapeutic nanoparticles into these cell patch matrices for therapeutic applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

PEGDA	polyethylene (glycol) diacrylate
SSE	sum of squared error
NHS-biotin	sulfosuccinimidyl-6- (biotinamido) hexanoate
TEA	triethanolamine
VP	1-vinyl 2-pyrrolidinone
BSA	bovine serum albumin

FBS	fetal bovine serum
bBSA	biotinylated bovine serum albumin

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Figure 1. Schematic description of A549 cell surface patch photopolymerization

(A) Cells are cultured on the collagen-coated slide, and cell surface proteins are targeted and biotinylated by NHS-biotin. (B) Cell surface biotin is conjugated with streptavidin-eosin. (C) The PEGDA monomer solution is injected into the gap between photomask and cell slides. Formation of PEGDA hydrogel patches is photopolymerized with the presence of photomask which allow light only passing through restricted irradiation region to trigger photopolymerization. TEA: triethanolamine; VP: 1-vinyl 2-pyrrolidinone.

Figure 2. Epoxy microarray system for characterizing PEGDA hydrogel formation via photopolymerization

(A) The arrangement of bBSA microarray on epoxy microscope slide. Twelve concentrations of bBSA were printed from 1000 to 0 $\mu\text{g/mL}$, and each concentration has two replicates. (B) Relationship between log bBSA concentration and the thickness of PEGDA 575 hydrogel thickness under various light intensity, TEA concentration, and irradiation time. Data are given as the mean $\pm$ SEM. (C) Relationship between fluorescence and polymer film thickness. Data are given as the mean $\pm$ SEM. The fluorescence was contributed by yellow-green nanoparticle loading in PEGDA 575 hydrogel.

Figure 3. Photo-patterned PEGDA hydrogels in a microarray format

(A) Schematic diagram of the experimental set-up of photomask covering biotinylated-BSA printed epoxy microarray. (B) Left: bright field image of patterned PEGDA hydrogels; Right: epifluorescent image of patterned PEGDA hydrogels stained by green fluorescein. Photomask had a 10 μ m wide irradiated regions. The spacer thickness is 40 μ m. Scale bar is 100 μ m.

Figure 4. Analysis of PEGDA hydrogel formation rate and accuracy

(A) Schematic graph of Matlab analysis for sum of square error for PEGDA of photo-patterning. The dark blue dots are input data of normalized pattern fluorescence. Gaussian fit (the red curve) was used to fit the data to obtain the midpoint of experimental results. Four data points near center were averaged to determine the normalized fluorescence value of ideal pattern profile. SSE was calculated by the square difference between experimental data and ideal pattern value. Light blue line presented the value of SSE for each data point. (B) Relationship of PEGDA575 gel pattern thickness and irradiation period under different light intensity. Nanoparticle-loaded PEGDA hydrogel was polymerized under various light intensity (diamond: 40 mW/cm², square: 30 mW/cm², and circle: 20 mW/cm²) and irradiation period (2.5, 5 and 10 min). Fluorescence of hydrogel pattern was analyzed by imageJ and then converted to polymer thickness according to the relationship between fluorescence and polymer film height. (C) Relationship between normalized SSE and irradiation period under different light intensity. Normalized SSE of hydrogel pattern was analyzed by Matlab. Solid line: 40 mW/cm², dot line: 30 mW/cm², and dash line: 20 mW/cm².

Figure 5. Cell membrane permeability following hydrogel patch formation

The PEGDA 8000 hydrogel patches were polymerized under 40 mW/cm^2 for 5 min. TEA concentration was 21 mM. After polymerization, cell were stained with ethidium homodimer immediately (A), or incubated in medium at 37 Celsius with 5% CO_2 for 12 (B), 24 (C), or 48 hours (D). The green fluorescence resulted from the yellow-green loaded hydrogel patches on the surface of adherent A549 cells. The red fluorescence indicates the complexation of ethidium homodimer with cellular DNA in the nucleus of a compromised cell. Scale bar is 100 μm .