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Microcontact printing of polydopamine on thermally expandable hydrogels for controlled cell adhesion and delivery of geometrically defined microtissues

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ABSTRACT

Scaffold-free harvest of microtissue with a defined structure has received a great deal of interest in cell-based assay and regenerative medicine. In this study, we developed thermally expandable hydrogels with spatially controlled cell adhesive patterns for rapid harvest of geometrically controlled microtissue. We patterned polydopamine (PD) on to the hydrogel via microcontact printing (μCP), in linear shapes with widths of 50, 100 and 200 μm. The hydrogels facilitated formation of spatially controlled strip-like microtissue of human dermal fibroblasts (HDFBs). It was possible to harvest and translocate microtissues with controlled widths of 61.4 ± 14.7, 104.3 ± 15.6, and 186.6 ± 22.3 μm from the hydrogel to glass substrates by conformal contact upon expansion of the hydrogel in response to a temperature change from 37 to 4 °C, preserving high viability, extracellular matrix, and junction proteins. Microtissues were readily translocated *in vivo* to the subcutaneous tissue of mouse. The microtissues were further utilized as a simple assay model for monitoring of contraction in response to ROCK1 inhibitor. Collectively, micro-sized patterning of PD on the thermally expandable hydrogels via μCP holds promise for the development of microtissue harvesting systems that can be employed to *ex vivo* tissue assay and cell-based therapy.

Statement of significance

Harvest of artificial tissue with controlled cellular arrangement independently from external materials has been widely studied in cell-based assay and regenerative medicine. In this study, we developed scaffold-free harvest system of microtissues with anisotropic arrangement and controlled width by exploiting thermally expandable hydrogels with cell-adhesive patterns of polydopamine formed by simple microcontact printing. Cultured strips of human dermal fibroblasts on the hydrogels were rapidly delivered to various targets ranging from flat coverglass to mice subcutaneous tissue by thermal expansion of the hydrogel at 4 °C for 10 min. These were further utilized as a drug screening model responding to ROCK1 inhibitor, which imply its versatile applicability.

1. Introduction

Hierarchical arrangement of microstructures in natural tissues plays important functional roles [1]. For examples, anisotropic arrangement of skeletal muscle and neuronal cells facilitates efficient movement and signal propagation along their direction, respectively [2–4]. In addition, morphogenesis during embryonic development is affected by spatially controlled differentiation of stem cells [5]. Therefore, many biomaterials formed by exploiting microfabrication techniques such as micro-molding, soft lithography, and e-beam lithography have been used to recapitulate structure-related function and study functional modulation [6]. For example, the migrating direction of 3T3-fibroblasts was

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controlled by creating ratchet geometric patterns composed of asymmetric or symmetric triangles, while neuronal differentiation of human mesenchymal stem cells depended on the width of linear cell adhesive patterns [7,8]. However, there is still great demand in regenerative medicine for the development of systems in which engineered tissue with a defined structure can be delivered to the target without scaffolds for implantation [9]. This system is extremely useful as a model to study the cytotoxicity or efficacy of pharmacological reagents with minimal interference from external environmental factors [10].

Stimuli-responsive materials have attracted a great deal of attention for scaffold-free harvest and delivery of microtissues. Most pioneering work has involved the use of poly(N-isopropylacrylamide) (NIPAAm) brush-grafted on cell culture dishes, allowing spontaneous detachment of cell sheets in response to temperature decrease from 37 °C to below the lower critical solution temperature (LCST) of NIPAAm [9]. Recently, we also reported that thermally expandable hydrogels can be utilized for rapid harvest and delivery of cell sheets to a target substrate upon decreasing the temperature from 37 to 4 °C [11–13]. In particular, the systems using thermally expandable hydrogels have the advantage that cell sheets or microtissues can be delivered directly from the hydrogel to various targets ranging from flat glass coverslips to curved tissue defects in a simple and reproducible manner. However, spatially controlled cell adhesion on the surface of the thermally expandable hydrogels for the creation of microtissues with distinct structural features, and delivery of these microtissues to targets have yet to be achieved.

Microcontact printing (μCP) is a useful method to prepare a surface with chemically confined micro-patterns of ink molecules [14]. Chemical patterning relies on the balance of interactivity of ink molecules toward a poly(dimethylsiloxane) (PDMS) stamp and a target substrate; a PDMS stamp has a low surface free energy (22 dyn/cm^2), resulting in transfer of ink to a target with higher surface free energy [14,15]. Various types of biomolecules including proteins, peptides, and DNAs have been extensively used to control cell adhesion [16,17]. However, hydrogels are not favorable as target substrates for μCP of biomolecules from PDMS because they are too soft or tacky to withstand strong physical pressure. In addition, low interactivity with ink and diffusion of printed molecules often result in poor efficacy [18,19]. Thus, several processes such as chemical modification of hydrogels for higher interaction with ink molecules, sequential delivery from pre-printed materials such as poly(vinyl alcohol) film or glass slide, and printing on freeze-dried hydrogels to avoid sticking and to increase stiffness have been invented to improve patterning [18–22].

To achieve our ultimate goal, harvesting microtissues with a controlled structure, we developed chemically patterned and thermally expandable hydrogels using polydopamine (PD) as an ink for μCP onto the hydrogel. Dopamine undergo self-polymerization at basic pH on the surfaces of various materials regardless of surface properties [23]. Under normal *in vitro* cell culture environment, it has been reported that serum proteins (available in cell culture media such as fibronectin, laminin, and vitronectin) can be easily adsorbed onto PD-coated surface, which is the main contributor to enhance cell adhesion [24,25]. Although the mechanism of PD coating on the surface is complex, the physical stacking of polymerized dopamine layer is important for stable coating. We hypothesized that this physically stacked PD layer on micropatterned PDMS could be partially printed onto a hydrogel surface by μCP, and thereby, controlling geometrically controlled cell adhesion. The objectives of the present study were (1) to investigate the effect of μCP of PD on the regulation of cell adhesion and formation of aligned microtissue, (2) to study the delivery of geometrically confined microtissue in response to a temperature change, and (3) to examine potential applications of microtissue under *in vitro* and *in vivo* conditions.

2. Materials and methods

2.1. Materials

Tetronic® 1307 (MW 18000) was purchased from BASF (Ludwigshafen, Germany). *p*-Nitro-phenylchloroformate (PNC), tyramine and Y-27632 were purchased from Sigma Aldrich (St. Louis, MO, USA). Tris-HCl was purchased from IBI Scientific (Peosta, IA, USA). Hydrogen peroxide (H_2O_2), horseradish peroxidase (HRP), dopamine hydrochloride, anti-mouse IgG-biotin conjugate, anti-rabbit IgG-biotin conjugate, and albumin–fluorescein isothiocyanate conjugate (FITC-BSA) were purchased from Sigma Aldrich. Sylgard® 184 silicone elastomer kit was purchased from Dow Corning (Midland, MI, USA) for poly(dimethysiloxane) (PDMS) stamp fabrication. Fetal bovine serum (FBS), trypsin/EDTA, penicillin-streptomycin (PS), and phosphate buffered saline (PBS) were purchased from Wisent (St. Bruno, QC, Canada). A BCA microassay kit (Micro BCA protein assay kit) was obtained from Pierce (Rockford, IL, USA). High glucose Dulbecco's modified Eagle's medium (HG-DMEM) and human dermal fibroblasts (HDFBs) were purchased from Gibco BRL (Carlsbad, CA, USA). Human plasma fibronectin (FN) and mouse anti-fibronectin were obtained from BD Biosciences (Franklin Parks, NJ, USA). Rabbit anti-laminin primary Ab and Rabbit anti-Collagen type IV (Col IV) Ab were purchased from Abcam® (Cambridge, MA, USA). Mouse anti-β-actin primary Ab, anti-rabbit and anti-mouse secondary Ab were purchased from Applied Biological Materials Inc. (Richmond, BC, Canada). Rhodamine-phalloidin, Vybrant™ DiD cell-labeling solution, and LIVE/DEAD® viability/cytotoxicity kits were purchased from Life Technologies Corp. (Grand Island, NY, USA). Mounting medium containing 4′,6-diamidino-2-phenylindole (DAPI) was obtained from Vectasheild® (Burlingame, CA, USA).

2.2. Methods

2.2.1. μCP of PD on hydrogels

To synthesize thermally expandable hydrogels, Tetronic®-tyramine (Tet-TA) was synthesized as described previously [26]. The polymer was dissolved in PBS containing either 0.1% (v/v) H_2O_2 or 0.0025 mg/ml HRP at 15% (w/v). Prepared solutions were mixed at a 1:1 ratio and injected into a glass mold with a 1 mm gap. A micro patterned silicon wafer was prepared by deep reactive ion etching using positive photoresist (AZ7220, AZ Electronic Materials Ltd., Brachbrug, NJ, USA). Sylgard silicone elastomer solution was poured on the silicon wafer with microgrooves (ridges and grooves = 50, 100, 200 μm, depth = 30 μm) and cured at 60 °C for 2 h. To prepare a flat PDMS stamp, the elastomer was cured on a petri dish under the same condition. The cured PDMS stamp was then separated from the wafer and sterilized in 70% EtOH under UV for 30 min. The stamp was washed with distilled water, and then treated with 2 mg/ml of dopamine solution dissolved in 10 mM Tris-HCl buffer (pH 8.5) for 2 h at 37 °C for PD coating. For fluorescence visualization, the PD-coated stamp was treated with 20 μg/ml FITC-BSA solution to prepare a FITC-BSA adsorbed PD layer. The PD-coated PDMS stamp was then placed on the hydrogel prepared in a circular shape (diameter = 8 mm) for 3 min.

Contact printing (CP) of the PD coated flat PDMS stamp on to the hydrogel was assessed by measuring the water contact angle of the stamp. Hydrogel surface with the printed FITC-BSA adsorbed PD layer was observed by fluorescence microscope (TE 2000,