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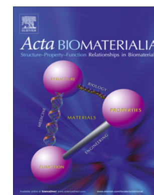

A thermoresponsive, micro-roughened cell culture surface

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ABSTRACT

Surface topography has been shown to play a major role in cell behavior, but has yet to be seriously exploited in the field of cell surface engineering. In the present work, surface roughness has been used in combination with the thermoresponsive polymer polyisopropylacrylamide (PIPAAm) to generate cell sheets with tailored biochemical properties. Micro-roughened polystyrene (PS) with 1.5–5.5 μm features was derivatized with PIPAAm to form a cell culture surface for the growth of human fibroblast cell sheets that exhibit a modified cytoskeleton and extracellular matrix. Fibroblasts cell sheets cultured on the rough surfaces had fewer actin stress fibers and twice the average fibronectin (FN) fibril formation when compared to cell sheets on flat substrates. The cell sheets harvested from the roughened PS were collected after only 2 days of culture and detached from the PIPAAm grafted surface in <1 h after cooling the culture system. The simple and rapid method for generating cell sheets with increased FN fibril formation has applications in tissue grafts or wound repair and has demonstrated that the thermoresponsive surface can be used for reliable cell sheet formation.

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1. Introduction

The thermoresponsive polymer polyisopropylacrylamide (PIPAAm) is a well-established, smart biomaterial capable of sustaining adherent cell cultures at 37 °C, but releasing cells and their extracellular matrix (ECM) when cooled below 32 °C [1–4]. At warmer temperatures, above PIPAAm's lowest critical solution temperature (LCST), the polymer dehydrates and collapses at the surface. When cooled, the polymer rehydrates and expands [5]. As a result, cells cultured on the thermoresponsive polymer are gently released into media when the temperature drops below the LCST [6]. Common methods of releasing cells from a culture substrate include use of the enzyme trypsin or mechanical scraping, but both methods damage the cells and ECM, and in addition compromise cellular function [7,8]. These methods also release the cells individually or in clumps, and many of the cell–cell linkages or interactions are lost. PIPAAm surfaces avoid these problems by releasing the cells with the ECM intact, and if cells are confluent, they are released as a single sheet [9–11]. The single sheets have the possibility to combine with other cell sheets for more complex tissue engineering [12]. Retaining the ECM supports the cell sheet shape and can ensure reattachment of the sheet onto other cells or surfaces. The cell sheet layer contains no other scaffolding or synthetic material once detached, and therefore is readily incorporated into in vivo systems with no loss of biocompatibility [13].

PIPAAm has been adapted for cell culture on the surface of biomaterials by a number of research groups. The polymer has been synthesized with other monomers for copolymerization to induce differences in surface wettability and changes in the LCST [14] or terminated with biomolecules for increased cell adhesion [15,16]. Although research with PIPAAm is extensive, there have been few reports on using thermoresponsive polymers in combination with 3-D or surface-modified scaffolds [17,18]. None of these reports have investigated the impact of surface microstructure or 3-D scaffolding on ECM formation for cell sheets. Surface topography has been well documented to control cell adhesion, morphology, gene expression and ECM deposition [19–21]. Combined with PIPAAm, altered surface architecture can be used to generate cell sheets with different biochemical properties and user-defined ECM profiles. One of the main components of ECM, fibronectin (FN), is arranged into fibrillar matrix networks in a process known as fibrillogenesis. FN fibrillar assembly is mediated by transmembrane integrin binding, which is crucial for cell growth and survival [22] and vital for facilitating wound healing [23]. The cell–material interface can be engineered for control of the FN fibrils in a cell sheet. Microsurface topography alters cytoskeletal rearrangement and integrin binding [24] and therefore directly influences ECM composition and arrangement. Cell sheets with ECM that closely mimics the in vivo microenvironment might be expected to be more relevant for complex tissue formation or for a tissue graft to aid in closing a wound.

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The research presented has exploited PIPAAm's thermoresponsive properties to engineer a defined micro-rough surface on polystyrene (PS) for cell sheet formation and control of cellular ECM. The microtopography is produced using a simple templating technique to generate 1.5–5.5 μm features on the surface. The rough PS surface is modified with an amine-terminated alkoxy silane, followed by secondary modification and UV-initiated grafting of PIPAAm to the surface. The PIPAAm-grafted PS surface (PIPAAm-PS) was characterized with atomic force microscopy (AFM) and infrared (IR) spectroscopy. The chemically and physically defined surface was used for cell culture with human fibroblasts to generate cell sheets with an ECM structure that was compared to cell sheets cultured on physically unmodified PS. Analysis of cellular FN and f-actin in the sheets was performed with fluorescence microscopy and statistical analysis of FN density and anisotropic calculations of actin filaments. By physically modifying the surface we have fabricated two significantly different types of cell sheets from the same cell type. The technique constitutes a new bottom-up approach for cell sheet engineering. To the best of our knowledge, this is the first report using a thermoresponsive surface in combination with surface micro-roughness to generate cell sheets with specific cytoskeleton and ECM profiles.

2. Materials and methods

2.1. Materials

Polymer casts were prepared using polydimethylsiloxane (PDMS) from a Sylgard-184 kit from Dow Corning (Midland, MI). Wet/dry, 4000 grit, silicon carbide (SiC) paper (Part No. 40400014) was purchased from Struers, Inc. (Cleveland, OH). Sterile Petri dishes for cell culture (Part No. 25384-302) were purchased from VWR (Radnor, PA). *N*-Isopropylacrylamide (NIPAAm), benzyl alcohol, benzophenone and glutaraldehyde (GA) were purchased from Alfa Aesar (Ward Hill, MA). 3-Aminopropyltrimethoxysilane (APTMS) was purchased from Gelest, Inc. (Morrisville, PA). Dulbecco's Modified Eagle's Medium: Nutrient Mixture F-12 (DMEM/F12), phosphate-buffered saline (PBS) and penicillin–streptomycin (P/S) were purchased from Cellgro (Manassas, VA). Hyclone Fetal Clone III (FBS) was purchased from Fisher Scientific (Pittsburg, PA). Normal goat serum was purchased from Rockland Immunochemicals (Gilbertsville, PA). H-300 fibronectin primary antibody and goat anti-rabbit IgG-FITC secondary antibody were purchased from Santa Cruz Biotechnology (Dallas, TX). Rhodamine phalloidin was purchased from Biotium Inc. (Hayward, CA). Deionized water came from a Millipore (Billerica, MA) Synergy UV water purification system with a resistivity of 18 MΩ. Ethanol and methanol were purchased from Pharmco-AAPER (Brookfield, CT).

2.2. Fabrication of micro-roughened PDMS surfaces

The SiC paper was sonicated in ethanol for 10 min, rinsed with fresh ethanol, dried in nitrogen and baked for 15 min at 70 °C to remove any remaining solvent that might be absorbed in the SiC paper. Degassed PDMS pre-polymer (10:1; elastomer:hardener) was poured over the clean SiC paper and placed under reduced pressure (100 mmHg) for 1 h. The paper and PDMS were baked at 70 °C for a minimum of 6 h. After cooling, the paper was gently peeled away from the PDMS cast (rough PDMS).

2.3. Fabrication of micro-roughened PS surfaces

Micro-roughened PS was fabricated with a modified protocol developed by Isenberg et al. [25]. The rough PDMS was clamped, modified side down, into a commercially available tissue culture Petri dish between two aluminum plates. The PDMS, dish and plates were heated to 110 °C for 1 h, removed and allowed to cool to room temperature. The PDMS and metal plates were removed, leaving the roughened PS. The complete fabrication procedure can be seen in Fig. 1. The rough PS was then pressed with a heated metal punch to generate PS discs 1.5 cm in diameter. Unmodified PS underwent no templating procedure, but was punched into discs. Templating the PS by pressing the SiC paper directly into the PS was tested, but few features were transferred using this method. The compression of the rough PDMS layer in between the aluminum plates and the PS dish was more successful at producing micro-roughness on the PS surface.

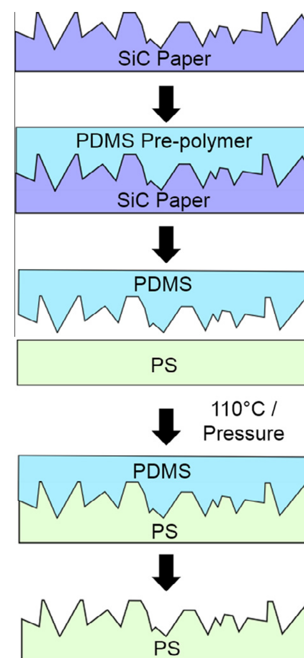

Fig. 1. Fabrication process of micro-roughened PS. PDMS pre-polymer was cured on SiC paper. The physically modified PDMS was then used to template tissue culture PS using heat and pressure. The final PS substrate had microscale surface features.

2.4. PIPAAm-PS

The PS discs were cleaned by sonication in an ethanol bath for 5 min and dried in a stream of nitrogen. The substrate surfaces were incubated in a 50% v/v APTMS solution in ethanol for 20 min. APTMS-PS was rinsed with ethanol, dried in a stream of nitrogen and incubated in a 0.5% v/v GA solution in PBS for 30 min for formation of imine Schiff base [26]. The GA-PS discs were removed, rinsed with water, dried in a stream of nitrogen and immediately incubated in a 12% w/v NIPAAm, 0.5% w/v bis-acrylamide, 0.5% v/v benzyl alcohol, 3% w/v benzophenone and 0.5 mM $NaIO_4$ (taken from aqueous 0.1 M $NaIO_4$) solution in methanol. The system was placed on an ice bath and irradiated with a UV lamp (RPR-3000 Å lamp, SNE Ultraviolet Co., Branford, CT) with a measured power of 5.1 mW cm^{-2} for 1 h. Lamp power was measured at the distance of sample irradiation (~4.0 cm) using a Gentec-EO (Lake Oswego, OR) Solo2 Laser Power and Energy Meter. The PIPAAm-PS discs were removed, rinsed with methanol, ethanol, sonicated in ethanol for 1 min and dried in a stream of nitrogen. The discs were soaked in water for 3 h with water replaced every hour to remove any residual NIPAAm not grafted to the surface, rinsed with ethanol and dried in a stream of nitrogen.

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