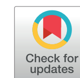
Bioactive Materials 3 (2018) 167–173

Contents lists available at ScienceDirect

Bioactive Materials

journal homepage: http://www.keaipublishing.com/en/journals/bioactive-materials/

Bioactive polydimethylsiloxane surface for optimal human mesenchymal stem cell sheet culture



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ARTICLE INFO

Article history:
Received 20 November 2017
Received in revised form
22 January 2018
Accepted 26 January 2018
Available online 14 February 2018

Keywords:
Cell sheet engineering
Human mesenchymal stem cell
Polydimethylsiloxane
Layer-by-layer grafting
Cell adhesion

ABSTRACT

Human mesenchymal stem cell (hMSC) sheets hold great potential in engineering three-dimensional (3D) completely biological tissues for diverse applications. Conventional cell sheet culturing methods employing thermoresponsive surfaces are cost ineffective, and rely heavily on available facilities. In this study, a cost-effective method of layer-by-layer grafting was utilized for covalently binding a homogenous collagen I layer on a commonly used polydimethylsiloxane (PDMS) substrate surface in order to improve its cell adhesion as well as the uniformity of the resulting hMSC cell sheet. Results showed that a homogenous collagen I layer was obtained via this grafting method, which improved hMSC adhesion and attachment through reliable collagen I binding sites. By utilizing this low-cost method, a uniform hMSC sheet was generated. This technology potentially allows for mass production of hMSC sheets to fulfill the demand of thick hMSC constructs for tissue engineering and biomanufacturing applications.
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1. Introduction

Creating suitable materials for tissue engineering is challenging due to the lack of bioactivity and biocompatibility for most synthetic materials [1]. Scaffold-free tissue engineering constructs have gained popularity because they eliminate the potential release of regeneration discouraging molecules from the scaffold [2]. Pioneered by Okano group, cell sheet engineering is an effective technology to create cell-dense constructs with preserved extracellular matrix (ECM) contents [3]. Those ECM biomacromolecules are bioactive, and can be tailored to have unique properties by employing specific cell types [4,5].

Human mesenchymal stem cells (hMSCs) can be easily obtained from bone marrow, adipose tissues, and peripheral blood. They can also be extensively expanded *in vitro* to fulfill substantial demand [6]. Moreover, hMSCs are multi-potent, immune-modulatory, and regenerative [7]. These unique properties make hMSC sheets especially attractive in engineering three-dimensional (3D) completely biological tissues for diverse applications. Our previous studies have demonstrated that hMSC sheets can be further vascularized to fabricate 3D prevascularized constructs for full thickness skin wound repair [8,9]. Importantly, to meet the stringent standard for engineering or biomanufacturing functional 3D tissues, the hMSC sheet needs to be complete and highly uniform.

To obtain a complete cell sheet, including the hMSC sheet, cells have been seeded on a transferable surface other than the regular cell culture treated plastic, in order to minimize the damage to cells, ECM structure, as well as the cell-ECM connection caused by cell detachment [10]. The thermoresponsive surface approach is well accepted because it allows for cell detachment without external force via shifting of the surface wettability at certain temperatures [11]. However, the fabrication process was cost-intensive and facility-dependent. In addition, the thermoresponsive surface needs to be grafted with cell adhesive molecules to ensure cell attachment [12]. Considering the high cost of materials and efforts for the long-term culture of cell sheet, it is crucial to develop an economical and stable substrate surface for easily harvesting intact cell sheets.

Polydimethylsiloxane (PDMS) has been employed in biological applications as an inert, stable and biocompatible material. Nevertheless, PDMS is hydrophobic and has poor cell adhesion. Plasma etching followed by collagen adsorption is the conventional method for converting PDMS from hydrophobic to hydrophilic to enhance cell adhesion. The collagen was accumulated on the surface via weak forces such as electrostatic, hydrophobic, and van der

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Peer review under responsibility of KeAi Communications Co., Ltd.

https://doi.org/10.1016/j.bioactmat.2018.01.005
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Waals. Therefore, it is fairly easy for the collagen molecules to leach into the solution, resulting in a non-uniform collagen coating [13]. We have found in our previous research that this could eventually lead to a patchy cell sheet, which severely impedes its application for biomanufacturing functional 3D completely biological tissues.

Layer-by-layer grafting of (3-aminopropyl)triethoxy silane (APTES), glutaraldehyde, and collagen I has been developed to covalently bind collagen on the PDMS surface for enhancing cell adhesion and proliferation [14]. In this study, we further demonstrated that the covalent grafting method significantly improved the collagen distribution on PDMS surface, which led to higher hMSC sheet formation efficiency and lower defect rate for long-term hMSC sheet culture. By utilizing this low-cost method, uniform hMSC sheets could be fabricated, allowing massive cell sheet production to fulfill the demand of for completely biological tissue engineering and biomanufacturing applications.

2. Materials and Methods

2.1. Layer-by layer grafting on PDMS

PDMS substrate was prepared from a SYLGARD 184 Silicone Elastomer Kit (Dow-corning, Midland MI) under manufacturer's instruction. The mixture was poured into a mold, degassed, and cured at 65 °C for 4 h to achieve a homogenous sheet. The PDMS sheets were then punched into 20 mm diameter disks. These PDMS disks were cleaned and dried overnight before being plasma etched for 60 s (radio frequency (RF) power 100 W, chamber pressure 200 mTor, O_2). Treated disks were grafted with the first layer of 10% (3-Aminopropyl)triethoxysilane (APTES, Sigma-Aldrich, St Louis, MO) in ethanol for 2 h followed by two ethanol rinses. The second layer of 2.5% Glutaraldehyde (GA, Sigma-Aldrich) in deionized water was grafted for 1 h followed by two washes in deionized water. Both grafted and non-grafted disks were sterilized under ethanol and UV light for one hour. Sterilized disks were immersed in a collagen I solution (20 μg/mL, Sigma-Aldrich) for 2 h at room temperature. These samples were split up into four groups including plain PDMS control (C), plasma etching only (P), non-grafted (adsorption), and layer-by-layer grafted (grafting) groups.

2.2. Chemical composition characterization

Fourier Transform Infrared Spectroscopy (FTIR) was applied to record the chemical composition of the samples with attenuated total reflectance (ATR) mode. Spectra of pristine PDMS, plasma treated PDMS, plasma treated PDMS with 2 h collagen adsorption were tested to investigate their surface changes in adsorption groups. The pristine PDMS, plasma treated PDMS, APTES modified PDMS, GA coated PDMS after APTES modification and collagen grafted PDMS by layer and layer coating were also tested respectively to compare their surface changes after each grafting step. The samples were fresh made and air dried before test. The spectra were recorded at room temperature in the 700–3700 cm^{-1} range by using a FTIR-ATR spectrometer (Perkin-Elmer, Waltham, MA).

2.3. Surface wettability characterization

Each surface treatment group was evaluated using the static sessile drop method in order to determine the contact angle that water droplets make with the surface. Each treatment group was tested on multiple samples, and in multiple locations on each sample using G10 contact angle measurement system (Krüss, Germany). Deionized water was used to form pendant droplets.

2.4. Surface morphology analysis by atomic force microscopy (AFM)

The surface morphology of all PDMS substrates were characterized by AFM, which was carried out with a Dimension ICON AFM system (Bruker, Billerica, MA). Tapping mode was applied to map the substrate surfaces. Average deviation was evaluated by the cantilever, and considered as surface roughness.

2.5. Cell culture

Bone marrow derived hMSCs were obtained from Texas A&M University Health Sciences Center. Passage 4 hMSCs were seeded on PDMS substrate surfaces at an initial density of 10,000 cells/cm^2. The samples were cultured in complete α-minimum essential medium (α-MEM) containing 20% FBS, 1% glutamine, and 1%penicillin/streptomycin (Thermo Fisher Scientific, Waltham, MA) for up to 14 days with medium changes every 72 h.

2.6. Fluorescent imaging

Samples were obtained after the cell culture and fixed in 3.7% formaldehyde solution (J.T.Baker, Center Valley, PA) for 30 min. Rhodamine phalloidin (Invitrogen, Carlsbad, CA) was used to label actin filaments within hMSCs. Briefly, the cells were blocked with 1% bovine serum albumin (Thermo Fisher Scientific) and incubated with rhodamine phalloidin (1:200 v/v) for 1 h. The cell sheet thickness after 14 days culture was measured by Z-stack scanning using Olympus FV-1000 confocal microscopy. Cell area was derived from these images using ImageJ particle analysis. To do this, the particle size was adjusted to exclude non-cell components, and the contained areas of each image's binary thresholds were measured in order to obtain the final cell area values.

2.7. DNA assay

The DNA content in the samples was determined fluorometrically using PicoGreen assay kit (Life Technologies). Briefly, cells were lysed using proteinase K solution (Sigma-Aldrich) at 37 °C. 100 μL lysed sample from each group was placed in triplicate in a 96-well plate and mixed with 100 μL of Picogreen working solution (Thermo Fisher Scientific), followed by 10 min dark incubation at room temperature. The incubated plate was read by a Fluoroskan Ascent FL fluorescent plate reader (Thermo Fisher Scientific).

2.8. Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

RNA isolation was performed on samples using the RNeasy Mini Kit (Qiagen, Valencia, CA), followed by cDNA synthesis via a reverse transcription kit (Life Technologies, Rockville, MD). The gene expression of cell binding integrins α_2 and β_1 as well as the stemness genes OCT-4 and SOX-2 were analyzed. KiCqStart® SYBR® Green Primers (Sigma-Alderich) were used and the genes analyzed were integrinα_2 gene ITGA-2 (Forward: 5′-GGTGGGGTTAATTCAGTATG; Reverse: 5′-ATATTGGGATGTCTGGGATG), integrin β_1 gene ITGB1(Forward: 5′-ATTCCCTTTCCTCAGAAGTC; Reverse: 5′-TTTTCTTCCATTTTCCCCTG), OCT-4 (Forward: 5′-GATCACCCTGGGATATACAC; Reverse: 5′-GCTTTGCATATCTCCTGAAG), and SOX-2 (Forward: 5′-ATAATAACAATCATCGGCGG; Reverse: 5′-AAAAAGAGAGGCAAACTG). A one step plus system (Applied Bioscience, Waltham, MA) was used for qPCR cycles. Acquired gene expression data of grafting samples was normalized to adsorption samples, and folding difference was obtained by the ΔΔCt method.

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