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Development of micropatterned surfaces of poly(butylene succinate) by micromolding for guided tissue engineering

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ABSTRACT

Native tissues present complex architectures at the micro- and nanoscale that dictate their biological function. Several microfabrication techniques have been employed for engineering polymeric surfaces that could replicate in vitro these micro- and nanofeatures. In this study, biomimetic surfaces of poly(butylene succinate) (PBS) were engineered by a micromolding technique. After the optimization of the system parameters, 20 surfaces with different combinations of groove and ridge sizes were developed and characterized by scanning electron microscopy (SEM). The influence of the engineered microfeatures over the viability and attachment of human adipose derived adult stem cells (hASCs) was evaluated. hASCs cultured onto the engineered surfaces were demonstrated to remain viable for all tested patterns. SEM and immunostaining showed adequate attachment and spreading of the stem cells for all the patterned groove/ridge combinations. This study indicated that it is possible to engineer micropatterned surfaces of PBS and that the developed structures could have great potential for tissue engineering where cell alignment is an essential requisite.

1. Introduction

One of the major motivations for the increasing effort spent on designing and developing micro- and nanostructured surfaces and materials for tissue engineering strategies is that natural tissues and the associated extracellular matrices (ECMs) are composed of micro- and nanoscaled elements [1,2]. In fact, when an implant first contacts the host environment, a layer of proteins immediately covers the surface of the implant [3]. The adsorptive behavior of these proteins is highly dependent on the surface properties, including its micro- and nanostructure [4,5], as well as on the material chemistry [6,7]. This surface-specific adjustment can result in the presentation of different regions of the proteins to cells, ultimately determining the success of the implant.

The micro- and nanoscale biological elements present in the ECM arrange themselves in specific architectures, essential for normal tissue function. An outstanding example is the organization of fibroblasts and cardiomyocytes in native myocardial tissue. These cells align themselves and assemble in parallel arrays in a way that is critical to obtain the electrical and mechanical properties of the heart [8]. Similarly, collagen fibers in the bone are aligned structures that provide bone with the tensile strength necessary to ensure the functionality of the tissue [9]. Thus, while developing engineered tissues it is of major importance to replicate the native microarchitecture, namely the controlled cellular alignment, and therefore to modulate in vitro the tissue function.

Several microfabrication techniques allow replication of the microarchitecture of tissues, modulating in vitro the cell shape, function or differentiation. Specifically, several different methods have been reported for cellular alignment, namely micropatterning of molecules [10], fabricating fibrous scaffolds by electrospinning [11–13] or engineering microchannels using soft-lithography methodologies [14,15]. Substrates with micropatterned adhesive proteins provide tight control over the cell attachment process. However, these patterned surfaces consist of a two-dimensional (2-D) substrate to culture cells. Three-dimensional (3-D) structures with multiple opportunities for cell attachment have been developed using various technologies, including electrospinning [11–13]. It has been reported that cells are able to align along the fibers within the 3-D network [12]. In order to more precisely control the overall orientation of cells, microchannels have been fabricated using micromolding or photolithography methods. Cardiac organoids have been formed within microengineered channels as a result of the alignment of cardiomyocytes [14].

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In this work we report a simple method to control the alignment of human adipose stem cells (hASCs) by microengineering the surface of a poly(butylene succinate) (PBS) polymeric surface. PBS is an aliphatic polyester that has shown to be biodegradable [16,17]. It has been processed by our group into discs [18] and fibers [19], showing promising results for bone [20] and cartilage [21] tissue engineering, both in vitro [19,21] and in vivo [20]. Herein, a micromolding technique was employed to fabricate microfeatures with different groove/ridge sizes onto the polyester PBS surface. This system can be used as an in vitro model for the study of the biological performance of cells in different patterned surfaces. The described system is applicable to a variety of cell types, and it might prove possible to incorporate the lessons learnt from this system into device design of engineered tissues with specific cell shape and elongation in vitro, as observed in collagen fibers in bone.

2. Experimental

2.1. Materials and solutions

The polymeric material used for the preparation of the substrates in this study was commercially available PBS (Bionolle 1050, Showa High Polymer Co. Ltd., Tokyo, Japan). Substrates for the micropatterns of PBS were processed into circular discs (10 mm diameter, 1.5 mm height) by conventional injection molding technology using optimized processing conditions, as described elsewhere [18]. For the development of the patterns, PBS pellets were dissolved at 0.5% and 2% (w/v), with a pure solvent of either dichloromethane (DIM, Sigma) or trichloromethane (TIM, VWR). A polydimethylsiloxane (PDMS) mold was kindly provided by Professor Hong Hong Lee (School of Chemical Engineering, Seoul National University). The PDMS mold was fabricated with 20 patterns with various combinations of groove and ridge sizes, enabling the effect of a range of scales of the patterns on the biological activity of cells on those substrates to be determined.

2.2. Preparation of micropatterned PBS surfaces

The PDMS mold and the PBS polymeric solution were heated to the same temperature. 20 μl of PBS solution were pipetted on top of the pattern present on the PDMS mold. An injection-molded disc of PBS was placed over the PBS solution as a substrate for the PBS pattern. A weight was used to facilitate the migration of the PBS solution by capillarity through the PDMS micropatterns. As the solvent evaporates (~2 min), the micropatterns of the PDMS are transferred to the PBS injection-molded discs (substrate) and the PBS disc is gently removed from the surface of the PDMS mold (Fig. 1). Three parameters of the system were varied in order to evaluate their influence over the fabricated microfeatures: (i) the temperature of both the PDMS mold and the PBS solution (~20 and ~100 °C); (ii) the concentration of the PBS solution (0.5% and 2%, w/v); and (3) the solvent used to dissolve PBS pellets (DIM or TIM). Twenty patterns with different groove/ridge size combinations were fabricated. Micropatterned PBS surfaces were analyzed by scanning electron microscopy (SEM) using a Leica Cambridge S-360 (Leica Cambridge, UK). All specimens were precoated with a conductive layer of sputtered gold. SEM micrographs were taken at an accelerating voltage of 15 kV at a number of magnifications. The width of the grooves and ridges of the patterns was calculated using an image analysis software (NIH Image J, $n = 4$). The depth of the grooves was constant for all micropatterns (~1.2 μm). Six PBS micropatterns from the 20 developed were selected for further biological studies (Nos. 3, 4, 6, 8, 15, 20) to obtain a good coverage of the range of dimensions that may have a stronger effect over the cells.

2.3. Attachment and proliferation of human adipose stem cells

2.3.1. Cell culture

In order to observe the cell response on the micropatterned PBS surfaces, primary hASCs were seeded on the engineered surfaces. hASCs were isolated as described elsewhere [22]. Briefly, stem cells were isolated from human adipose tissue samples by the enzymatic digestion of the tissue with 0.2% collagenase type IA in phosphate-buffer saline (PBS, Sigma) for 60 min at 37 °C under gentle stirring. Digested tissue was filtered and adherent cells selected after centrifugation steps. Cells were expanded in Dulbecco's Modified Eagle's Medium (DMEM, Sigma) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Biochrom AG) and 1% antibiotic (Gibco) at 37 °C in a humidified atmosphere with 5% CO_2 until a sufficient number for the study was obtained. Three samples per pattern were placed in 24-well plates and 1.5 ml of the cell suspension was seeded onto the PBS micropatterned surfaces using a density of 3.3×10^4 cells ml^{-1}. Seeded cells were incubated in a humidified atmosphere at 37 °C and 5% of CO_2 for 1 and 3 days in order to evaluate cell morphology upon spreading. TCPS was used as a control surface.

2.3.2. Cell viability assay

Cell viability was evaluated during culture time (1 and 3 days) by quantifying the metabolic activity of hASCs, using Alamar Blue® (Invitrogen). Alamar Blue can be reduced in active cell mitochondria, changing the solution color from blue to a bright red. Alamar Blue stock solution was diluted with culture medium without phenol red. Analysis was performed according to the manufacturer's

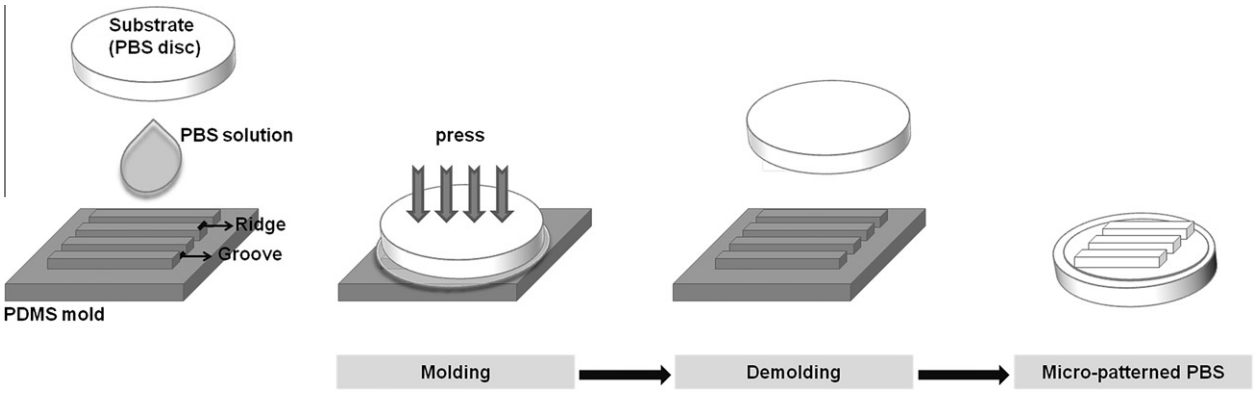

Fig. 1. Schematic representation of the preparation of micropatterned PBS surface.

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