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BIOTECHNOLOGICAL APPLICATIONS OF SYNTHESIZED PATTERNED POLYMERIC SCAFFOLDS

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ABSTRACT

Recently, there has been a significant progress regarding the biotechnological applications within the field of tissue engineering. One of such applications concerns the preparation of patterned polymeric scaffolds for patterned cell growth. Cell growth systems that make use of surface micro structuring and patterning have been gaining important traction as a result of their capacity to finely control cell growth and promote substrate adhesion. Being one of the numerous micro structuring techniques, soft lithography is a simple technique for the preparation of nano-patterned polymeric scaffolds and was used in several studies to create patterned biopolymers on lamellas. In these studies, various lamellas were covered with different biopolymers and patterned usingpoly(dimethylsiloxane) (PDMS) stamps which were prepared by passing UV light through a printed photomask that contained the desired pattern. The stamp replicates the opposite of the master. The same polymer master can be used many times without degradation. In cell viability was observed on the prepared materials in this work. Viability of cell growth on these biopolymers was then studied with L929 mouse fibroblasts using Neutral Red Uptake Assay. The results showed a significant increase in cell growth on the patterned surfaces of the biopolymers.

Keywords: biopolymer, scaffold, tissue engineering, cell growth.

INTRODUCTION

Biomaterials are essential elements for the enhancement of cell seeding, cell proliferation and cell deposition in the extra-cellular matrix (ECM) (Celebi et al., 2011). Pore size, pore interconnectivity and mechanical rigidity are important properties while preparing scaffolds. These parameters provide a homogenous distribution of the seeded cells and make it easier to transfer nutrients into the cell with scaffold material being used as delivery systems (Chocholata et al., 2019, Ludovica et al., 2018). Scaffolds can be affected by the surrounding liquid environment. In tissue engineering, there is a need for scaffold materials, on which cells can develop and grow (Wintermantel, 1996). These materials are made from biocompatible polymers because cells should grow in a natural environment.

The extracellular matrix (ECM) plays an important role in the controlling of cell behaviors such as cell adhesion, cell orientation and migration onto the scaffold surface. For cell growth experiments, functional groups on polymer surface exhibit some advantages over many other materials (O'Brien, 2011). Using microstructured and patterned polymeric surfaces is important in cell growth systems because this pattern helps control where the cell would grow. There are different techniques used to prepare patterned polymeric scaffolds. Cell proliferation studies on patterned polymeric materials have accelerated in recent years with the development of nanotechnology (Guillaume, et al., 2017, Zhang and Kohn, 2012, Shrirao, 2017). Soft lithography technique is used for patterning on polymer surfaces. In this technique, patterned soft elastomeric PDMS molds are used (Guillaume, et al., 2017). This method has useful properties including, biocompatibility, low toxicity, low cost, effective chemical inertness, and mechanical flexibility. In this method, a pattern

is formed on a polymer surface by the means of an elastomeric mold. PDMS molds used for soft lithography are produced by the classical photolithography method.

The aim of this study was to produce scaffolds using biocompatible polymers. These scaffolds were patterned and cell growth experiments were performed on these materials to investigate the adhesion and proliferation of the cells. For this purpose, PDMS molds were prepared and used as stamps to transfer patterns onto the polymeric surfaces. The cell growth studies were performed on polymer coated glass materials.

MATERIAL AND METHODS

Poly(dimethyl siloxane) (PDMS) was purchased from Dow Corning Corporation. Poly (ethylene glycol dimetacrylate) (PEG-DMA) (Mw= 550 $gmol^{-1}$), 2,2 dimethoxy-2 phenyl acetophenone (DMPA) (UV initiator) were obtained from Sigma Aldrich. 1-Octadecanethiol ($C_{18}H_{38}S$) (Mw=286 $gmol^{-1}$) was supplied by Merck company. Neutral red was purchased from Sigma. All the solvents used in this work were purchased from Merck company and used without further purification.

Soft Lithography and Microcontact Printing

Soft lithography technique was developed by Whitesides and his co-worker (Kumar and Whitesides, 1993, Xia and Whitesides, 1997). Micro-contact printing method is the most commonly used soft lithography technique for the preparation of biomaterials in tissue engineering applications. In this method, the PDMS layer is first dipped in a special solution which is called molecular “ink” and then this ink is transferred onto the polymer surface by the micro-contact printing technique.

Photoresist Patterning

First, the pattern is produced and printed out (called photomask) using Auto Cad computer program. The photomask is placed over the photoresist polymer coated on the microscope glass and the photomask pattern is homogeneously transferred onto the photoresist surface. PEG-DMA in methanol (50% w) and 2,2 dimethoxy-2 phenyl acetophenone (1% w, as UV initiator) were used as the photoresist polymers. The UV light (λ=365 nm) first passed through the photomask pattern in the bright regions and reached onto the photoresist polymer surface. UV-light hardened these parts by forming cross-linking bonds. The regions where the light did not pass remained in liquid phase. These liquid parts were removed from the surface by washing and the resulting patterned surface is dried. The pattern on the photomask is then passed to the photoresist polymer surface.

Preparation of the mold

PDMS solution was first prepared by the mixing of liquid PDMS with curing agent which one it should be added (10/1). The homogenous solution was poured onto the patterned PEG-DMA. For the curing process this material was kept at 60°C overnight. After the curing process, the mold was gently removed from the top of the surface of the substrate.

Preparation of patterned surface

The mold material in this technique acts as a helping tool. In cell growth studies, the mold material is first treated with alkanethiol solution and then contacted with a polymer coated glass for a certain period of time. The main task of the mold is to transfer the alkanethiol solution which has cell attractive properties to the polymer covered surface. Thus, the surface is both patterned and provides a suitable environment for the growth of cells in these patterns. Figure 1 shows schematically pattern transfer onto the substrate by using PDMS stamp.

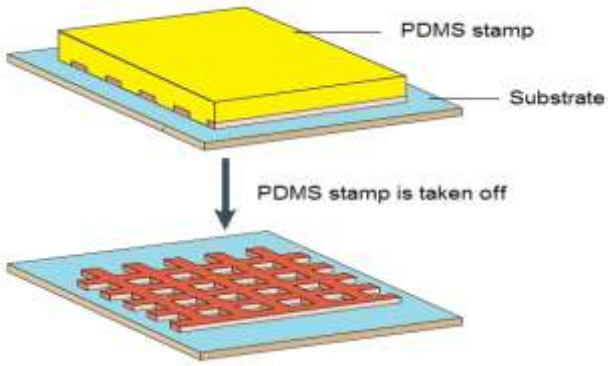

Figure 1. Transfering of pattern.

Cell Seeding on the Patterned Surface

In the cell cultivation studies, Neutral Red Assay method was carried out on the patterned polymer coated glass material by planting 100000 cells on average (Repetto et al., 2008). The scaffolds were first sterilized with an ethanol solution (70%). In this study, L929 mouse fibroblast cells were used for seeding. First the cells were seeded on the prepared materials. After seeding cells onto the cell culture plates containing scaffolds with patterned surfaces, the plates were incubated for 72 h in 5% CO_2 incubator at 37 °C. The seeded cells were attached on the patterned surface and grown on the patterned regions. Neutral red solution was applied to the grown cells. The samples were then incubated in Phosphate Buffer Solution (PBS) solution for another 2 hours. During this time the living cells absorbed the neutral red. The neutral red is internalized only by living cells. The internalized dye was solubilized in 1 mL of ethanol/H_2O (1/1) (including of 1% acetic acid) solution. The amount of solution absorbed by the cells is directly proportional to the number of cells and gives a qualitative result. The dye inside the cells was measured spectrophotometrically at 550 nm. By using the cell number vs absorbance graph, the number of grown cells on the patterned materials was estimated.

RESULTS AND DISCUSSIONS

Cell growth studies

The grown cells on the rectangular pattern surfaces are shown in Fig. 2

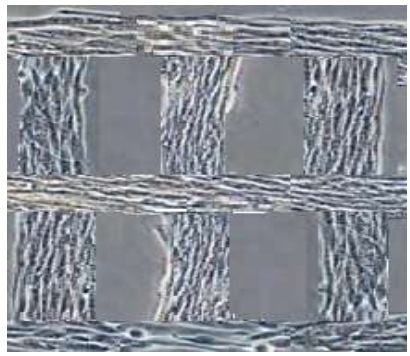

Figure 2. Cell grown up on rectangular patterning surface.

The comparative results of the cell growth experiments obtained from the different polymer coated samples are seen in Fig. 3. Column 1 represents the cell growth on the glass coated with polyvinylchloride (PVC). In this experiment undecanethiole was used. PEG-DMA covered surface (column 2) represents effective result for the cell growth experiments. Column 3 relates to PVC samples using hexadecanethiole. It was observed that the effect of the kind of alkanethiol (either hexadecane or undecanethiole) on PVC coated samples was not significant.

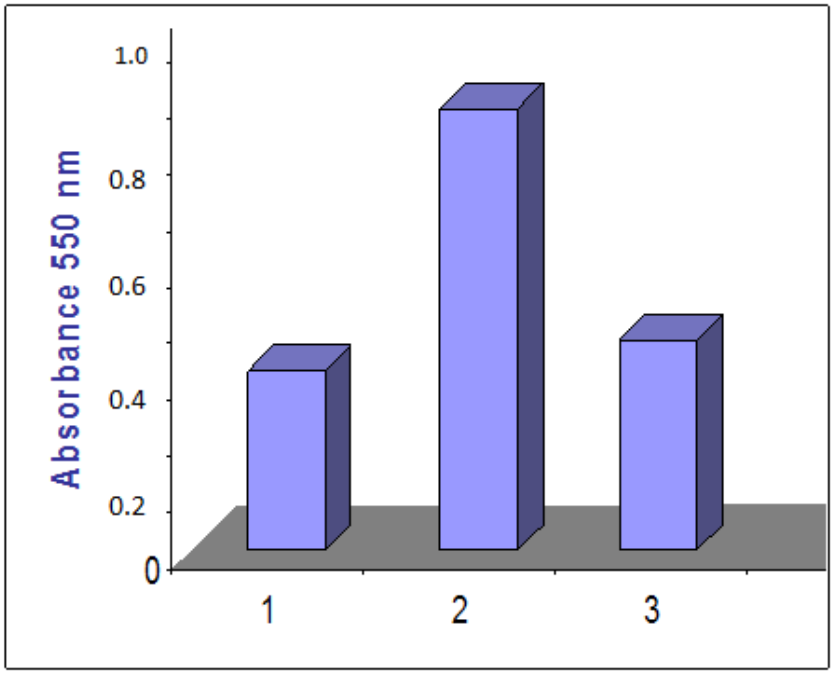

Figure 3. Comparative studies for cell growth. (1) polyvinylchloride (PVC) coated surface, undecanethiol. (2) PEG-DMA coated surface, hexadecanethiole. (3) Hexadecanethiole with patterned polyvinylchloride (PVC) coated surface.

The cell growth exists for all samples prepared in this study as shown in Fig.3. The maximum result was obtained on PEG-DMA coated surface using hexadecanethiole.

CONCLUSIONS

In this work, micro patterned surfaces were fabricated by using the soft lithography technique. Microcontact printing technology was preffered to transfer the produced pattern. The patterned materials were used as scaffolds for cell growth experiments. PDMS molds were originally produced and used to transfer the pattern onto the polymer coated surfaces. PVC and PEG-DMA polymers are used as coated polymers. The results obtained from the morphological analyse show that the patterns were succesfully transferred from the PDMS stamps to the polymer coated materials. After cell cultivation, cell growth experiments were performed to observe the growth of cells on the patterns. Cell attachment and cell growth were observed on all prepared samples. Cell viability results showed that these materials are suitable for cell growth studies.

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