POLYMERIC SCAFFOLDS FOR TISSUE ENGINEERING APPLICATIONS

Ayse Zehra AROGUZ

Chemistry Department, Engineering Faculty, Istanbul University – Cerrahpasa, Avcilar, Istanbul, 34320, Turkey, aroguz@istanbul.edu.tr

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Corresponding Author:
Ayse Zehra Aroguz, Chemistry Department, Engineering Faculty, Istanbul University - Cerrahpasa, Avcilar, Istanbul, 34320, Turkey.
aroguz@istanbul.edu.tr

ABSTRACT

Polymeric materials are commonly used for many purposes in Tissue Engineering Applications. In particular, they are used as scaffolds which are specially prepared in cell growth studies as well as drug loading and release systems. In drug delivery and controlled release systems functional, scaffolds are widely used in order to send the drug to its target region. On the other hand, in cell growth systems patterned polymeric scaffolds are prepared and used to allow the cells to grow at a certain region with a particular form. For this purpose, different techniques are used for the adhesion of cells onto the material surfaces.

In this study, patterned scaffolds from various polymers as Polymethylacrylate (PAM), Polystyrene (PS) and polyvinylchloride (PVC) were prepared using micro contact printing with the soft Lithographic Technique. The prepared materials were morphologically analyzed and cell growth was followed by using electron scanning microscope (SEM). Poly (dimethylsiloxane) (PDMS) molds were prepared in different shapes and used as stamp materials to transfer the designed patterns. The cell growth on these patterned surfaces was followed after seeding L929 mouse fibroblasts cells. Neutral Red Uptake Assay was applied to observe cell growth. The cell growth experiments showed that the cells were attached to the patterned surfaces and a significant increase in cell growth on the surfaces were observed.

Keywords: patterned polymer, scaffold, biotechnology, cell growth, stamp.

INTRODUCTION

In cell growth systems, cells are grown in a certain order on the patterned surfaces. Therefore, various techniques are used for the proper adhesion of cells onto these pattern surfaces. Biomaterials as basic elements are commonly used for the enhancement of cell seeding, cell proliferation and cell deposition in the extra-cellular matrix (ECM). (Liedtke et al, 2019, Wolf et al., 2012). In most tissues, the migration of cells takes place within 3D environments. These environments are complex and have several challenges, because of the adaptation to the changing properties of the environment (Yamada and Sixt, 2019).

Extracellular matrix (ECM) materials derived from natural tissues exhibit superior

for the biocompatibility of cultured cells and for more favorable immune responses (Zhu, et al., 2019). It is known that scaffold-guided cell organization is an important criterion for the regeneration and maturation of the tissues (Higuchi et al., 2013, Li et al., 2017). In vivo tissue-forming cells on patterned scaffolds are closely regulated by their physicochemical properties such as porosity, pore size, stiffness, bio-activities, pore interconnectivity (Lane et al., 2014). These properties provide a homogenous distribution of the seeded cells. The transferring of the nutrients into the cells attached on the surface of the scaffold can thus be facilitated (Ludovica et al., 2018). Park and coworkers studied on the functional composite fabricated by biopolymer and they were used their materials for medical applications. Smith and Grande worked on the functionate scaffolds for the applications of musculoskeletal regenerative process (Smith and Grand, 2015). Biopolymer-based functional composites were prepared in the literature for medical applications (Park, 2017; Liu et al., 2009).

In this work, biopolymeric scaffolds were prepared and used for the cell growth systems. For this purpose, microscope lamellas were used as main holder materials and covered with different polymers as Polymethylacrylate (PAM), Polystyrene (PS) and polyvinylchloride (PVC) which are then followed by patterning process. The patterned surfaces were used for the cell proliferation and their results were compared. For the patterning process, poly(dimethylsiloxane) (PDMS) as molds were originally prepared and used for the transferring of the pattern. L929 mouse fibroblasts were used during the cell seeding process and observed using Neutral Red Uptake Assay.

MATERIALS AND METHODES

In this work, dimethoxy 2,2- phenyl-2 acetophenone (DMPA) as UV initiator and Poly (ethylene glycol dimetacrylate) (PEG-DMA) with M_w 550 $gmol^{-1}$ were purchased from Sigma Aldrich. Poly(dimethyl siloxane) (PDMS) used as stamp was obtained from Dow Corning Corporation. Hexadecanethiol ($C_{16}H_{34}S$) and 1-Octadecanethiol ($C_{18}H_{38}S$) (M_w = 286 $gmol^{-1}$) were supplied from Merck company. Neutral red and all the solvents used in this work were purchased from Merck.

Soft Lithography Process

Soft lithography is one of the common processes for the preparing pattern surfaces. In this technique elastomeric materials are used as stamps, molds or photomasks. Because elastomeric materials are used, this technique is named as soft lithography which was developed by Whitesides and his colleagues (Xia and Whitesides, 1998, Jiang et al., 2003; Jiang and Whitesides, 2003). PDMS is commonly used in patterning processes for the cell growth experiments. Micro-contact printing technique in soft lithography process is preferred for the fabrication of biomaterials as scaffold in tissue engineering studies. Molecular ink was prepared first and PDMS mold is immersed into the molecular ink and contact with the polymer surface the pattern is then transferred onto the polymer surface as seen in Figure 1.

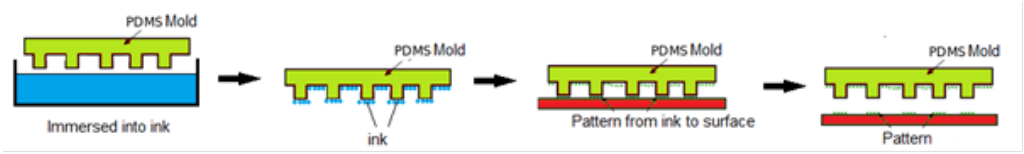

Figure 1. Pattern transfer from molecular ink onto the polymer surface.

In this work patterns were produced by using Auto-Cad computer program and printed out with a high-resolution printer. Then, the pattern is placed over the polymer

surface which was covered on the microscope glass and the photomask pattern is homogeneously transferred onto the photoresist surface. In this step, the polymer should be light-sensitive polymer which is called photoresist polymer. For this purpose, PEG-DMA was used as the photoresist polymer. The UV light (λ=365 nm) passed through the pattern in the bright area and reached onto the photoresist polymer surface. The bright area hardened after being exposed to the UV-light while the rest of the region remained in liquid phase. The polymer surface was washed, and the liquid parts were removed. After drying, the polymer patterned surface was obtained. PDMS solution was covered on the patterned surface and kept at 60°C for 24h. After drying PDMS was removed from the surface and the patterned stamp was obtained. These stamps were used for the patterning of the other polymers covered on the microscope glass.

Preparation of the patterned surface

The stamp PDMS was first immersed into the alkanethiole (undecanethiol) solution and contact with polymer surface. Alkanethiol which is absorbed by the cells is used as molecular ink. Therefore, by using PDMS stamp the polymer surface is covered with alkanethiole solution with a certain pattern after immersing ink. Figure 1 shows schematically the pattern transfer onto the polymer surface to make by cells visible.

Cell seeding on the Patterned polymer Surface

Neutral Red is used for the cell growth studies. Certain amount cells (approximately 100,000 cells) are seeded on the patterned polymer surface after sterilization. L929 mouse fibroblast cells were used for the cell growth studies. After seeding the cells, the patterned surfaces were incubated for 3 days in a CO_2 incubator at 37 °C. Then, neutral red dye was applied to the cells and adsorbed by the living cells. The concentration of adsorbed amount of dye is proportional to the number of living cells. The neutral red concentration was obtained by measuring the absorbance value of dye solution using a UV-spectrophotometer (at 550 nm).

RESULTS AND DISCUSSIONS

The results are comparatively given in the bar diagram in Figure 2 for the covered samples with different polymers as Polymethylacrylate (PAM), Polystyrene (PS) and polyvinylchloride (PVC), respectively. PEG-DMA covered surface was used to compare the cell proliferation results.

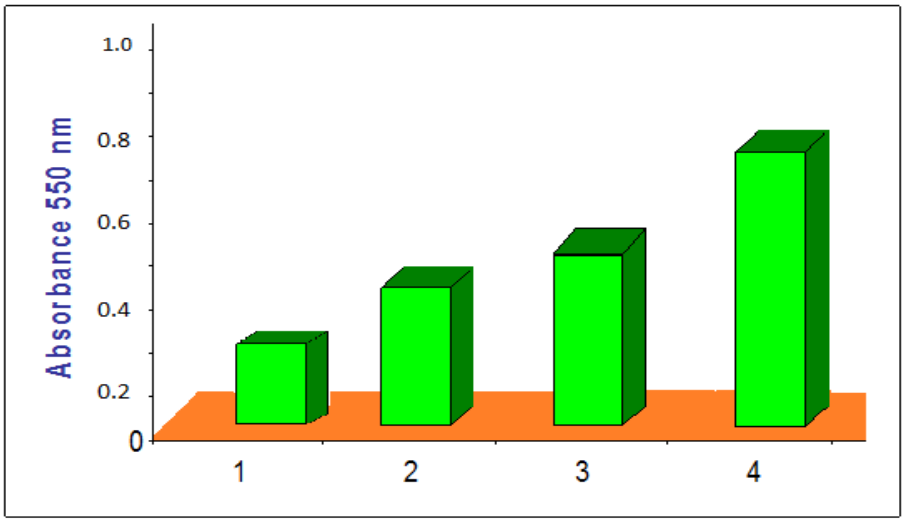

Figure 2. cell growth on different coated surfaces. Undecanethiole were used (1) Polystyrene, (2) PVC, (3) Polymethylacrylate, (4) PEG-DMA

Figure 3 and Figure 4 show the scanning electron microscope results of the grown cells on the patterned polymer surfaces.

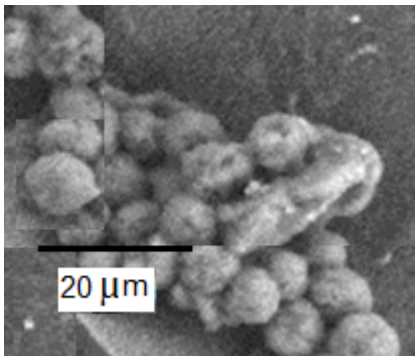

Figure 3. SEM micrographe of the grown cells.

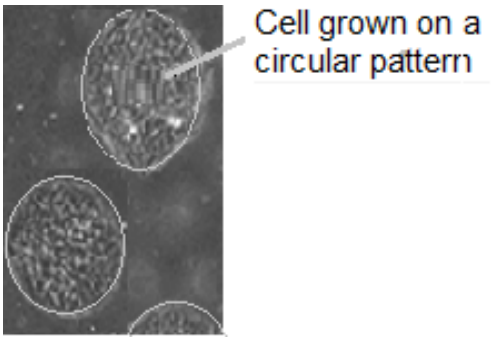

Figure 4. Growing Cells on the circular pattern regions

Cell Growth Studies

The results obtained from cell growth studies on polymer patterns were successful as shown in Fig.3 and Fig.4. As can be seen from the bar diagrams in Fig.2, the best cell growth results were obtained on the patterned PEG-DMA samples. Column 1 represents the cell growth on the glass coated with Polystyrene. Cell proliferation was also observed in the samples of PVC covered surfaces. However, these results were not as successful as PEG-DMA covered and patterned samples.

Compared to the PVC coated sample (column 2) with the Polymethylacrylate covered sample (column 3) less cell growth on PVC surface was observed (Figure 2). It can be seen that cell growth exists for all polymer covered surfaces prepared in this work. The maximum amount of cells was obtained on PEG-DMA coated surface which shows that this polymer is suitable for the cell growth systems. In all these experiments undecanethiole was used as alkanethiole.

CONCLUSIONS

The cell growth studies were performed on the prepared materials to investigate the adhesion of the cells on the patterned regions and grown on these regions. The polydimethylsiloxane (PDMS) as stamp material was originally fabricated and used to pattern transfer onto the polymeric surfaces. It was found that the prepared and patterned materials can effectively be used in engineering and biotechnological application in cell growth studies. The best result was obtained PEG-DMA covered scaffold.

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