Short communication

Cellular orientation on micro-patterned biocompatible PHBV film

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ABSTRACT

Cellular orientation control is important for tissue regeneration. Design of oriented structures for cells with suitable features can be used in tissue engineering. One of the methods of cellular orientation with the aim of regenerating which damaged tissues is utilizing oriented biocompatible substrates. Different methods can be used to design these structures such as utilizing magnetic fields, electrospinned oriented fibers or methods such as directional solidification. This paper investigates the influence of grooved substrate-mediated physical guidance on the growth and alignment of cells in vitro. A novel technique was developed to fabricate microgrooves on biodegradable polymer substrates made of poly hydroxyl buthyratevalerate. Solvent-castings were used to transfer micro patterns from quartz and silicon substrates onto biodegradable polymer films. The cells were successfully oriented on micro grooved polymeric substrate which can be used for axon guidance and nerve regeneration.

Keywords: Micro Patterned Polymers; Biodegradable Polymers; PHBV; Fibroblast Cells

INTRODUCTION

Cellular Orientation can be efficient for reconstruction of damaged tissues and organs such as nerve tissues. One of the methods of cellular orientation is utilizing oriented biocompatible substrates and scaffolds. Micro patterning is an example of oriented substrates designing.

In this physical method, the groove structures are designed. The grooved structure designed on the biocompatible materials has provided suitable condition for cellular growth and orientation [3–6]. In these cellular designs, substrate should be proportionate in terms of adhesion and cell growth, size and type of cells in order to place cells properly in these oriented grooves. The cells exhibit sensitivity to the dimensions of the microgrooves [7-10]. Grooved structures which are created via this physical method basically agreed with incompatible and non polymeric materials that are not useful for regeneration of damaged tissues [11]. The dependence upon physical contact potentially limits the patterning area, and though these physical patterning methods orient Schwann cells, portions of the substrate surface are not available for colonization. Thus, chemical modification by micro contact printing is attractive; the printing process is quicker, easier, and allows cells to modify the substrate surface as needed [12-15]. Today biopolymers are used frequently in scaffold design for reconstruction of damaged tissues. Among these biopolymers, biocompatible and biodegradable synthetic polymers are notable to mention. Biodegradable synthetic polymers are commonly used in tissue engineering.

Among these synthetic polymers, PHBV microbial polyester can be noted to that is biocompatible and biodegradable copolymer. PHBV is a material with suitable properties for cellular growth and adhesion with controllable degradation characteristics and suitable for scaffolds design. Designing such structures can be used frequently for reconstruction of

tissues like nerve due to requirements of axons in the regular orientation [16].

MATERIALS AND METHODS

Substrate fabrication

Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) containing 5 mol% of 3-hydroxyvalerate with 680,000 molecular weight was purchased from Sigma Chemical Co. Chloroform and 2,2,2-trifluoroethanol (TFE) to prepare PHBV solution were purchased from Sigma-Aldrich Chemical and used as received without further purification. Quartz substrates were etched using reactive ion etching (RIE) through the mask, leaving behind long rectangular areas capped by chrome. RIE is a dry developed process with an etch rate of about 30 nm/min and a resolution of 2 µm. After the chrome was removed from the quartz substrate, the quartz substrates were used as a micro dyes for transferring the geometric microgrooves to the biodegradable polymer. Films were produced by casting solutions of PHBV in chloroform and spin casting them onto the micro-patterned silicon wafers. The PHBV solution was spun on the substrate at 1800 rpm for 1min. After drying for 24 h, the films were floated off the silicon wafers onto the surface of water and used.

Cell investigations

The fibroblast cell suspension (L929) of mouse tail was prepared on the ISO10993 standard basis. The fibroblast cell suspension was transferred to flask ($25cm^2$) containing 5 ml DMEM (2Mm l-glutamine, penicillin (100 lu/ml), streptavidin (100µl/ml)) and FBS 10%. The suspension was, then, inserted into an incubator (5%Co2, 37°C). The fibroblast cells were proliferated in the flask and were washed using FBS/EDTA. Then, the trypsin enzyme/EDTA was added in to the flask (4°C), and remained in the incubator for 2 min. The culture media (FBS/DMEM) was added to the flask the cells were gently pipetted. The cell suspension was transferred to the falcon tube (15ml) and centrifuged (1410 rpm) for 5 min. The solution was removed, and the precipitation was transferred to a new flask (75 cm^2) for re-culturing. The surface of the samples was well cleaned by using cotton and alcohol. Pieces (0.5 × 0.5 cm) of the main sample was cut and placed in one of the Petri dish wall using a sterilized pincer. *50000 cells*/*well* were seeded into a 12-*well* culture plate, removed by pipette and were poured on the main samples. Then, all of the samples were placed in the memmert incubator at 37 °C for 48 hours. The samples were removed from the incubator after 48 hours, and were studied through Nikon Eclipse Ts-100 photonic microscope.

RESULTS

An effective technique was developed for fabricating microgrooves with dimension and spacing on biodegradable PHBV films using solvent casting technique. To our knowledge, this is the first time that this simple but effective technique has been used for fabricating patterned films of biodegradable polymers with fibroblast cell. Figure 1 shows microgrooved quartziferous die with dimensions of 10/20/2 µm .Figure 2 shows SEM images of the microgrooved molded PHBV film with die. This figures shows good grooves molded on the polymer surface.

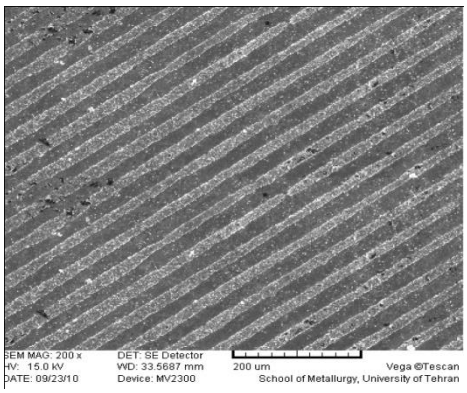

Figure 1. microgrooved quartziferous die with dimensions of 10/20/2 µm

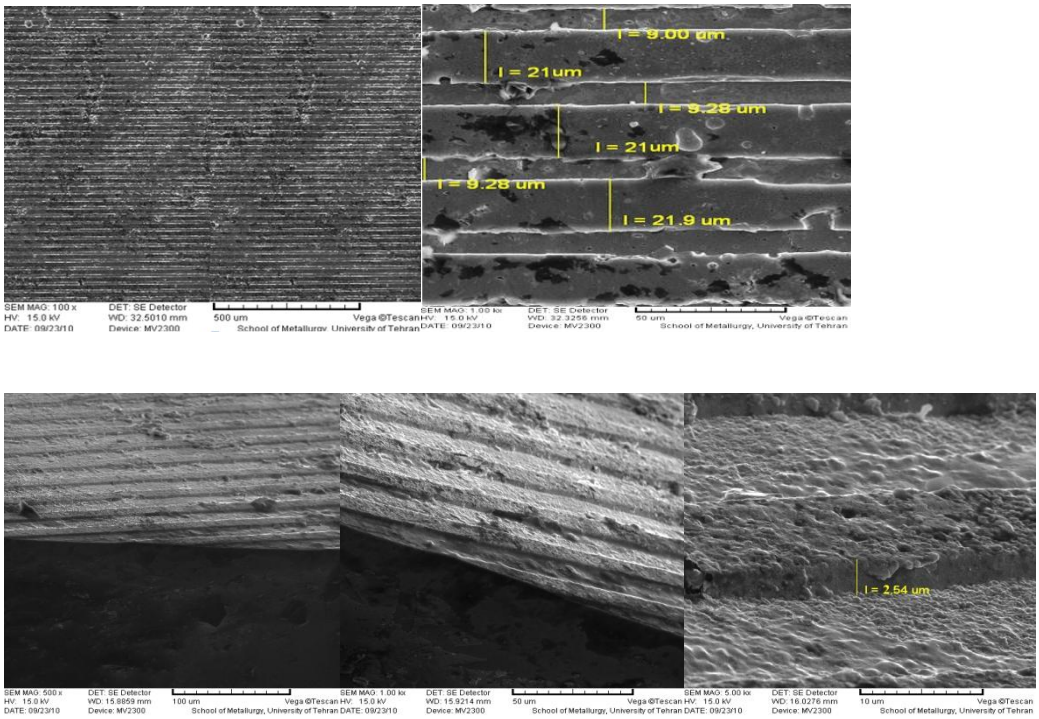

Figure 2. SEM images of the micro-grooved molded PHBV film with die.

Cells cultured on the micro-grooved film and incubated for 48 h. Figure 3 shows good attachment and cellular growth. Orientation of cells along micro-grooved PHBV film well showed.

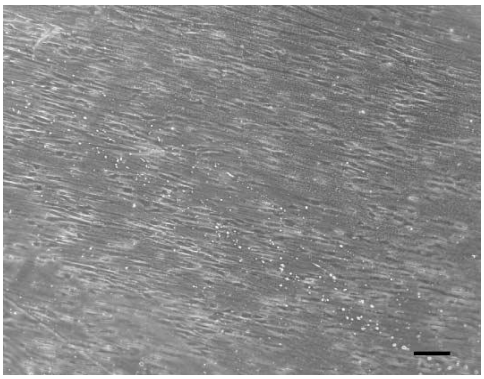

Figure3. Alignment of fibroblast cells along microgrooves on a PHBV film (initial groove dimensions of 10/20/2 µm) after culture. Bar 50 µm.

Novel patterning technique based on reactive ion etching coupled with compression solvent casting was developed to produce micro-grooved biodegradable polymer substrates. Micro-grooved biocompatible polymer substrates were found to provide physical and chemical guidance to cells and enhance their adhesion and alignment on the substrates in vitro. These substrates are of potential significance in promoting tissue regeneration especially peripheral nerve regeneration.

ACKNOWLEDGEMENT

This project was supported by *Young Researchers Club - Islamic Azad University*- Tonekabon Branch, Tonekabon, Iran.

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