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Protocols

Superficial physicochemical properties of polyurethane biomaterials as osteogenic regulators in human mesenchymal stem cells fates

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ABSTRACT

All of the cells' interactions are done through their surfaces. Evaluation of surface physicochemical scaffolds along with other factors is important and determines the fate of stem cells. In this work, biodegradable and biocompatible polyester/polyether based polyurethanes (PUs) were synthesized by polycaprolactone diol (PCL) and poly (tetra methylene ether) glycol (PTMEG) as the soft segment. To assess better the impact of surface parameters such as stiffness and roughness effects on osteogenic differentiation of the human mesenchymal stem cell (hMSC), the dimension effect of substrates was eliminated and two-dimensional membranes were produced by synthesized polyurethane. Surface and bulk properties of prepared 2D membranes such as surface chemistry, roughness, stiffness and tensile behavior were evaluated by Attenuated total reflectance Fourier transform infrared (ATR-FTIR), atomic force microscopy (AFM) and tensile behavior. The prepared 2D PU films had suitable hydrophilicity, biodegradability, water absorption, surface roughness and bulk strength. The hMSCs showed greater osteogenesis expression in PU substrates with more roughness and stiffness than others. The results demonstrated that surface parameters along with other differentiation cues have a synergistic effect on stem cells fates.

1. Introduction

The fate of stem cells as one of the three main components (cells, extracellular matrix (ECM) and signals) for tissue engineering is dependent on the cellular niche. Therefore, understanding those mechanisms underlying the self-renewal and differentiation of stem cells including the study of cell communication pathways and the endogenous stem cell niche is at the center of attention. Cells can sense the surface properties of the substrate that are located by receptors on their surfaces. Recent progress have been made in this field to understand the importance and influence of the surface properties on the cell fates [1,2]. Evaluating the effectiveness of engineered surfaces is critical in order to develop better techniques for controlling cell fates, cell- and matrix-based assays.

Therefore, artificial ECMs as engineered niches must be modified in confronting any type of stem cells in order to minimize remodeling [3–5]. On the other hand, it has been confirmed that the substrate properties alter cell behavior such as cell adhesion, cell proliferation, and cell differentiation [6–9]. Cellular fates are controlled by the following parameters [9]:

- Interaction with matrix proteins (surface of matrix),
- Soluble and secreted factors,
- Other cell types in stem cell niches or the microenvironment.

All of the existing parameters are converted into biological encodes by surface communications and are transferred to the cell nucleus. Therefore, proper interactions between the microenvironment and its components with stem cells are very important.

Selection and preparation of a suitable substrate with controllable properties, that well imitate a cellular niche, is a major challenge in tissue engineering. Some of the synthetic and natural polymers with different properties (biological, physical and mechanical properties) according to their applications are suitable materials for different biomedical applications [10–12]. The polyurethanes (PUs) are very important groups of polymers because of their engineering features such as good mechanical

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properties, biocompatibility, blood compatibility and biodegradability [13–15]. These polymers have alternating hard and soft blocks that can lead to separate microphases under appropriate conditions to form hard and soft domains [16–18]. Thus, a change in any of the polyurethane synthetic components can bring different properties and can be a prepared polymer with the desired behavior. As a result, PUs are an option to examine the impact of the physicochemical behavior of substrates on stemness and cell fates [19,20].

Elastomeric character and extra strength in PUs are referred to soft and hard segments, respectively [21], in the event that degradation of PUs is extremely dependent on the polyols as a soft segment structure [22,23]. The polyols used in the synthesis of biodegradable PUs include poly (propylene glycol) (PPG), poly (ethylene glycol) (PEG), poly (lactic acid) (PLA), poly (caprolactone) (PCL), and glycolic acid, among others [24,25]. A combination of two or more types of polyols can cause variety in the properties and applications for PUs [26]. The PUs are prone to hydrolytic degradation and therefore are employed in scaffolds designed for tissue engineering. The PUs made from aliphatic polyesters are expected to be one of the most economically competitive biodegradable polymers. The difference in the surface phase structure and surface chemistry of these polyurethanes was possibly responsible for the surface and bulk degradation [27].

Moreover, the bulk structure, two-phase microstructure, average macrodiol length, and interdomain spacing also have impacts on the degradation. The PCL, which is a linear polyol synthesized with ring opening polymerization (ROP) [11], is frequently used as the soft segment in degradable polyurethanes because it can be hydrolyzed and its degradation products are non-toxic and can be metabolized [28,29]. Poly (tetramethylene ether) glycol (PTMEG) is an etheric polyol with biocompatible properties for the synthesis of PUs with high mechanical properties [30]. From aromatic and aliphatic isocyanates used in the synthesis of polyurethanes, aliphatic and cyclic-aliphatic isocyanates yield PUs that are less rigid but have better oxidative and ultraviolet stabilities. Some of these materials include 1, 4-butanediisocyanate (BDI), 1, 6-hexamethylene diisocyanate (HDI) and isophoronediisocyanate (IPDI), among others. HDI is often used in the preparation of biomedical PUs with relatively non-toxic degradation by-products such as diamine 1, 6- hexanediamine [29].

This research is an introduction to the effects of physicochemical properties of the polyurethane matrices that can be used in tissue engineering and the impact of these properties on human mesenchymal cells fates. To study the physicochemical effect independent of the scaffold dimension, 2D substrates were prepared. On the other hand, to evaluate the influence of physical and chemical parameters of substrates on osteogenic differentiation of human mesenchymal stem cells (hMSCs), two types of polyols were used in the synthesis of polyurethanes. By changing the compositions of each of these polyols, superficial and bulk properties of the polyurethane such as hydrophilicity, biodegradability, roughness, as well as mechanical and thermal properties have been changed. The effect of these regulating changes is evaluated in the osteogenic differentiation of hMSCs.

2. Experimental

2.1. Materials

The PCL (Mn = 2000 Da, PDI = 1.04) according to the previous work was synthesized and dried in a glass vial under vacuum and magnetic stirring at 100 °C for 120 min [11]. PTMEG (Mn = 1000 Da) from Aldrich was partially dried under vacuum at 80 °C for 24 h. The HDI and 1, 4-Butanediol (BDO) (Merck, Germany) were used as received. All of the materials were dehydrated and stored under vacuum before using. N, N-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were supplied from Merck (Germany) and used as received. Phosphate buffered saline (approximate pH of 7.3) was obtained from Gibco company. Fibroblast cells (SNL76/7) and hMSCs were obtained from the Stem Cell Technology Research Center and maintained in a T-75 culture flask. The cells were maintained in Dulbecco's Modified Eagle's Medium supplemented with 10% (v/v) fetal bovine serum (FBS), penicillin and streptomycin. (3-(4, 5-dimethyl-thiazol- 2-yl)-2, 5-diphenyl tetrazolium bromide) (MTT) was purchased from Sigma. Double-distilled deionized water was used at various stages of the work.

2.2. Synthesis and preparation of polyurethane membranes

The PUs were synthesized using the method reported in the literature [31,32] without the use of catalysts and solvent. Briefly, a mixture of PCL and PTMEG, at different weight ratios, was used as macro diols for the preparation of PUs that is shown in Table 1A. Polyol compositions were added to a glass vial reactor (50 ml) and dehydrated under reduced pressure at nearly 100 °C for 2 h and the temperature was reduced to 85 °C. The diols were reacted with HDI (the overall ratio of NCO to OH was kept at 3:1) and equipped with a magnetic heater-stirrer, without reflux condenser under partial vacuum. The reaction continued until the NCO content reached the theoretical value as determined by dibutyl amine titration according to ASTM D 2572. The time required to complete the reaction of isocyanate groups with two ends of macro diols was 3 h experimentally. Then BDO was added to the synthesized pre-polymers until reactor contents became viscose and stirring cannot continue further. Then the reactor contents were drained into a Teflon petri dish and placed in an oven at 85 °C for 24 h. The PUs were marked as PU-x, where x is the wt% of PCL-diols in the PCL/PTMEG ratio. Then 3 gr of the prepared PU solution (20% w/w PU-x in DMF) was poured into a Teflon mold with dimensions of $10 \times 10\,cm^2$ which was polished by 800-grit sandpaper. The mold was placed in a vacuum oven at 70 °C for 24 h. The PU films were washed three times with distilled water after separation from the mold. The resulted PU films were optically clear and very thin (about of 100–200 μm) when viewed under light microscopy.

2.3. Characterization of the prepared polyurethane membranes

Attenuated total reflectance infrared spectroscopy (ATR-FTIR) was used on the Equinox 55 FTIR spectrometer with 100 scans for a wavelength range of 400–4000 cm^{-1}. Mechanical properties of the PU membranes were evaluated by an Instron tensile testing apparatus (5566-Applied Science Co., Ithaca, NY) at a crosshead speed of 5 mm/min. The scanning electron microscope (SEM), optical microscopy (OM) and atomic force microscopy (AFM) were used to study of morphology, surface structure PU substrates and cell attachment. The membranes were coated with gold without any other modification and morphology of the cells on the membranes were studied by SEM (AIS 2100, Seron Technology, Korea) in the magnification of 26 kV. For the cell cultured sample, the SEM images were obtained by the same instrument with different magnifications. During the evaluation of cell viability in different days, cells were photographed by a Leica Leitz optical microscope (OM) (Leica Inc., Foster City, CA). The surface morphology of PU substrates was investigated using an AFM (Ambios Tech, USA).

To determine the water sorption of the various substrates, the samples were placed in 0.1-mm-thick strips 20 mm × 30 mm in 30 ml of distilled water at 37 °C. The static contact angle (CA) of PU films was measured by the sessile drop method using 2 μl of water droplets and a CCD camera connected to a computer (Dataphysics, OCA15plus). Accelerated (1 mol L^{-1} NaOH and 60 °C) and