Materials Science and Engineering C 72 (2017) 113–117

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Electrospun polyurethane membranes for Tissue Engineering applications

Laís P. Gabriel [a,b,*], Ana Amélia Rodrigues [a,c], Milton Macedo [a,b], André L. Jardini [a,b], Rubens Maciel Filho [a,b]

[a] National Institute of Biofabrication, Campinas, Brazil
[b] Department of Chemical Engineering, University of Campinas, Campinas, Brazil
[c] Department of Medical Sciences, University of Campinas, Campinas, Brazil

ARTICLE INFO

Article history:
Received 26 August 2016
Received in revised form 27 October 2016
Accepted 4 November 2016
Available online 16 November 2016

Keywords:
Electrospinning
Fibroblast proliferation
Implants
Polyurethane

ABSTRACT

Tissue Engineering proposes, among other things, tissue regeneration using scaffolds integrated with biological molecules, growth factors or cells for such regeneration. In this research, polyurethane membranes were prepared using the electrospinning technique in order to obtain membranes to be applied in Tissue Engineering, such as epithelial, drug delivery or cardiac applications. The influence of fibers on the structure and morphology of the membranes was studied using scanning electron microscopy (SEM), the structure was evaluated by Fourier transform infrared spectroscopy (FT-IR), and the thermal stability was analyzed by thermogravimetry analysis (TGA). *In vitro* cells attachment and proliferation was investigated by SEM, and *in vitro* cell viability was studied by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assays and Live/Dead® assays. It was found that the membranes present an homogeneous morphology, high porosity, high surface area/volume ratio, it was also observed a random fiber network. The thermal analysis showed that the membrane degradation started at 254 °C. *In vitro* evaluation of fibroblasts cells showed that fibroblasts spread over the membrane surface after 24, 48 and 72 h of culture. This study supports the investigation of electrospun polyurethane membranes as biocompatible scaffolds for Tissue Engineering applications and provides some guidelines for improved biomaterials with desired properties.

1. Introduction

Tissue Engineering (TE) has emerged as a technology with potential for regeneration of tissues through scaffolds and provides the basis as a possible option for organs development. Tridimensional biomimetic scaffolds have been applied in TE because of their nanoscaled architecture, similar to the extracellular matrix (ECM). However, the synthesis of adequate scaffolds to a cell culture is a challenge to be overcome. Scaffolds should have a porous structure, appropriate pore size, interconnectivity and must be biocompatible [1]. Typical scaffolds designs have included foams, sponges, films and membranes.

The use of polymeric membranes in the medical area has grown considerably. From the membranes, it is possible to obtain artificial skin [2], bandages [3], angioplasty balloons, intra-gastric balloons, and neural connections [4].

The skin wounds market has been considered the most successful market in the TE area, so far [5]. This is a large market composed by products to heal damaged skin, severe burns, diabetic and pressure ulcers. Skin wounds may appear as a result of accidents, but also as a result of surgical incisions. Pressure ulcers may be caused by immobility, and cause infections or more severe complications. Patients with chronic diseases such as diabetes and obesity are also subject to more difficult wound healing. In the United States, it is estimated that, about 6.5 million cases per year of skin ulcer [6], about 285 million people had diabetes mellitus in 2010 and it is estimated >360 million people by 2030 will have diabetes mellitus [7,8]. It is worthwhile mentioning, that with population aging, it is expected to have a significant increase in such illnesses as well as in the skin wounds in general due to more skin sensibility.

Nowadays, several polymers have been applied as membranes for TE, including poly(lactic acid) (PLA) [9], poly(glycolic acid) (PGA), poly(lactic-co-glycolic acid) (PLGA) [10], polycaprolactone (PCL) and polyurethane (PU). Among them, polyurethanes (PUs) are of particular interest due to the mechanical properties, processability, flexibility and biocompatibility, associated with their versatile structure. The soft segment of the segmented structure of PUs can be modified in order to obtain a flexible structure, leading to lower friction with the host tissue improving the artificial tissue integration.

The final property of the membrane is not only function of the polymer, but also of the manufacture process. Among possible techniques, electrospinning was identified as a suitable procedure for TE due the possibility of obtain scaffolds that can mimic the extracellular matrix. It is an attractive technique, but many factors determine the success of

* Corresponding author at: 500 Albert Einstein Avenue, Campinas, São Paulo, Brazil.
E-mail address: lagabriel@gmail.com (L.P. Gabriel).

http://dx.doi.org/10.1016/j.msec.2016.11.057
0928-4931/

the fiber production, from the solution parameters, such as, concentration, conductivity and viscosity, the controlled variables, such as, the flow rate, the electrical field strength, the distance between the tip and the collector, and the laboratory environment parameters, such as temperature, humidity and air velocity [11–13]. However, this technique presents limitations based on scarce cell infiltration and ingrowth, inadequate mechanical strength [14], possible toxicity due to the residual solvent, and slow production rate. The optimum pore sizes for tissues are in the range 100–500 μm [15] and the electrospinning technique enables to obtain pore sizes in this range.

The synthesis of electrospun polyurethanes is relatively new and associating with the fact that PU is a promising polymer to support cell adhesion due to its mechanical resistance, it is interesting to investigate the potential of this material. Moreover, there have been few studies proving that this is a viable and promising technique for the fabrication of membranes. In addition, PU demonstrated to have good barrier properties and oxygen permeability, ideal for membrane applications [16]. For fibroblasts culture, the optimal pore size was found to be 20 μm [17], at the same time, a large pore size of 500 μm was found to be excellent for fibroblast vascular tissue ingrowth [18]. However, few *in vivo* studies of electrospun PU scaffold were done [19]. Electrospun polyurethane have been applied as wound dressings [20], shape memory devices [21], drug delivery device [22], stents [23], aortic valve [24], bandages [16] and grafts [25].

The purpose of this study was to prepare PU membranes *via* the electrospinning apparatus and to investigate the produced membrane through morphological, structural, thermal properties and biological response through *in vitro* cell viability and adhesion assays.

2. Materials and methods

2.1. Materials

All chemicals were of analytical grade, purchased by Sigma Aldrich and were used without further purification. PUs in pellets form (medical grade SG85A) was kindly provided by Lubrizol Advanced Materials. This is an aliphatic poly(ether-urethane) prepared from poly(tetramethylene glycol) (PTMG), hexamethylene diisocyanate (HMDI) and butanediol (BDO) as chain extender.

2.2. Methods

2.2.1. PU electrospinning solution preparation

PU stock solution was prepared by dissolving PU in chloroform at a concentration of 2 g/L during sonication (Ultrasonic clear, Unique, São Paulo, Brazil) for 2 h.

2.2.2. Preparation of the electrospun membranes

The electrospinning apparatus employed in this research was designed and built-up by the National Institute of Biofabrication (INCT-Biofabris), where it is located, and presents more operational flexibility compared to commercial rigs. It consisted of a syringe pump, a high-voltage direct-current power supplier (Testtech) generating a positive dc voltage up to 30 kV, and a grounded collector that was covered with aluminum foil. The solution was loaded into a syringe, and a positive electrode was clipped onto the syringe needle. The feeding rate of the polymer solution was controlled by a syringe pump, and the solutions were electrospun onto the collector. The syringe pump was set at a volume flow rate of 7 mL/h, the applied voltage was 18 kV, the tip-to-collector distance was 10 cm, and all solution preparations and electrospinning were carried out at room temperature.

2.3. Material characterization

2.3.1. Morphological and structural characterization

SEM analysis was performed in a Scanning Electron Microscope (model LEO 440i; Leo Electron Microscopy; Cambridge, England). The voltage used was 20 kV and the current was 100 pA. A metallic SC7620 Sputter Coater was used for coating the sample with gold.

2.3.2. Structural characterization

FT-IR studies were conducted at room temperature using a Thermo Scientific Nicolet 6700 FT-IR Spectrophotometer (Nicolet Instrument Inc., Madison, WI, USA), connected with an ATR accessory. Spectra were collected from 40 scans, within a range of 675–4000 cm^{-1} using a resolution of 4 cm^{-1}. All spectra were ATR corrected using Smart Omni Sampler (Thermo Scientific, Madison, WI).

2.3.3. Thermal characterization

The investigation of the PU thermal stability was carried out by TGA, model TGA-50 (Shimadzu Instruments Kyoto, Japan). A sample mass of about 15 mg was heated under a flowing nitrogen atmosphere (50 mL/min) from 30 to 600 °C, at a heating rate of 10 °C/min.

2.3.4. Fibroblasts cell culture

VERO fibroblasts cells (African green monkey kidney fibroblasts) were provided by Adolfo Lutz Institute (São Paulo, Brazil). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS, Gibco). Growth media contained 100 units/mL penicillin and streptomycin and cells were cultured at 37 °C, humidified, 5% CO_2/95% air environment. Cells were used at population numbers below 5. The DMEM-LG with 10% of phenol was used as positive control of toxicity (PCT) and the polystyrene extract was used as negative control of toxicity (NCT).

2.3.5. Evaluation of fibroblasts cells using SEM

VERO cells were seeded on the PU surface with a final density of 3×10^6 cells/mL. After growth time of 24, 48 and 72 h, the samples were fixed in glutaraldehyde 2.5% in 0.1 M sodium cacodilate buffer for about 2 h. The samples were washed in PBS and then in water for 15 min. Afterwards, they were dehydrated in a graded series of ethanol (50%, 70%, 95% and 100%), critical point dried with CO_2 (Balzers, CTD-030), and sputter coated with gold in a SC7620 Sputter Coater apparatus. The cell-seeded PU scaffolds were characterized by Scanning Electron Microscope (SEM, model 440i Leo, Zeiss) operated at 20 kV and 100 pA. Statistical study was carried out using the Image J Software.

2.3.6. In vitro cell viability by MTT assays

To determine the toxicity of the polyurethane and the nanocomposite, cells were seeded onto 96-well culture plates at 1×10^6 cells/mL and were incubated for 24 h. After that, cells were incubated with 100 μL of the aforementioned cell culture medium for 24, 48 and 72 h. The amount of formazan crystals formed was measured after 4 h of exposure to the MTT solution in dimethyl sulfoxide (DMSO) and absorbance values were measured at 595 nm by a scanning multi well spectrophotometer plate reader (Microplate Reader F5, Molecular Probes).

The data are presented as mean ± S.E.M. Statistical analysis of the data was carried out using ANOVA, followed by Tukey's HSD *post hoc* test (equal variances). Differences were considered statistically significant when the p-value was <0.05.

2.3.7. In vitro cell viability by Live/Dead assays

A viability study on PU membranes was performed with a Live/Dead assay kit (Kit Live/Dead® Viability Cytotoxicity Molecular Probes TM). The cells were seeded (1×10^3 cells/mL) into a 96-well plate and incubated with DMEM-LG containing 10% of FBS at 37 °C for 24 h. After this period, the membranes were incubated with the cells for 24, 48 and 72 h. A Live/Dead fluorescence assay kit was applied to qualify the