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# From biodegradable to long-term polyurethanes: In vitro fibroblasts adhesion and degradation study of electrospun polyurethane membranes

Laís Pellizzer Gabriel*, Ana Amélia Rodrigues**, André Luiz Jardini*, Rubens Maciel Filho*

**(Department of Chemical Engineering, University of Campinas, Brazil)*
*** (Department of Medical Sciences, University of Campinas, Brazil)*

**ABSTRACT**
Among the synthetic polymers, polyurethanes are one of the most important polymers applied in Tissue Engineering (TE). Their segmented block structure enables the control of different properties, such as, biocompatibility, blood compatibility, mechanical properties and also biodegradability. In this work, polyurethane membranes were obtained using the electrospinning apparatus. Fibroblasts cells were seeded on the membrane and the morphology, structure and cell adhesion and proliferation were studied using Scanning Electron Microscopy (SEM). Finally, the degradation behavior of the membranes was investigated by in vitro degradation studies. SEM results showed that the membrane presents high porosity, high surface area:volume ratio, it was observed a random fiber network. In vitro evaluation of fibroblasts cells showed that fibroblasts adhered and spread over the membrane surface and in vitro degradation study showed that the developed membrane can be considered a non-degradable polyurethane. This study supports further investigations of electrospun membranes as long-term devices for TE applications.

***Keywords*:** Biodegradation, implants, in vitro degradation, medical devices, Tissue Engineering.

## I. INTRODUCTION

The implantation of synthetic polymers in the body and their duration as medical devices can be divided in two groups: (1) biodegradable devices (2) biostable devices. Biodegradable devices should provide an initial substrate to cell adhesion, proliferation and differentiation and maintain the mechanical properties while it degrades until the newly bone should be regenerating, releasing non-toxic products to the human body [1]. Biostable devices should maintain the architecture and mechanical properties over time in vivo. In addition, they will not release degradation products in the human body [2].

Recently, different synthetic polymers have been applied as implants in TE, such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), polyhydroxybutyrate (PHB), poly(lactic-co glycolic acid) (PLGA) and polyurethane (PU). PLA, PGA and PHB are biodegradable thermoplastic aliphatic polyesteres.

Among the synthetic polymers, PUs are versatile polymers due to their segmented structure, composed of two thermodynamic incompatible phases [3-4]. The hard segment domain of PUs is based on the diisocyanate and the chain extender applied during the PU synthesis. Diisocyanates can be divided in two groups: (1) aromatic (2) aliphatic diisocyanates. The most common diisocyanates applied are diphenilmethane diisocyanate (MDI) and toluene diisocyanate (TDI), but in the medical field aromatic diisocyanates are considered less biocompatible than PUs based on aliphatic diisocyanates, this happens because the degradation products of PUs based on aromatic diisocyanates are toxic to the human body [5], such as carcinogenic aromatic amines. From that, aliphatic diisocyanates are replacing the aromatic diisocyanates, generating PUs that also presents excellent mechanical properties, better oxidative and ultraviolet stabilities [6]. The most common aliphatic diisocyanates applied in TE are hexamethylene diisocyanate (HDI) and dicyclohexylmethane diisocyanate (HMDI), as they have been reported to degrade into nontoxic decomposition products [7], such as nontoxic amines [8-9], and the degradation products can be metabolized by the Krebs cycle.

The degradation rate of PUs can be easier achieved by introducing hydrolysable chain extenders in the hard segment, such as butanediol (BDO), 1,2-ethanediol and 1,2-ethanediamine and glycerol [7]. Diamines are more reactive than diols or triols. The hydrophilic character of the PU can be easily controlled in this way.

The soft segment domain of PUs is based on the long chain linear diol applied (polyol or macrodiol). They can be classified in polyether, polyesthers, polycaprolactone (PCL) and

polycarbonate. The most common polyols applied are poly (propylene oxide) glycol and copolymers of (propylene/ethylene oxides) glycols but in the medical field PCL, poly(ethylene glycol) (PEG) and glycolid acid have been applied [10].

The soft segment has a significant influence on the degradation rate of PUs. In general, the use of polyols with high functionality produces a crosslinked structure and reduces the hydrolytic degradation capacity due to the difficult to the water reach the hydrolytic segments (ester and ether groups) of the PUs [11].

Polyether PUs are recognized as hydrolytically stable at neutral and basic pH and have been applied for long-term applications. However, polyether PU in a combination with metal parts, have been subjected to metal ion oxidation [12]. Polyester PUs can suffer hydrolytic degradation and are no longer used in devices designed for long-term implantation.

PUs based on PCL are used as long-term implantation and can be hydrolyzed and presents non-toxic degradation products and have also been applied for long-term implants (2-4 years) due to degradation rate slower than PLA, PGA and PLGA. Polycarbonate PUs are used in long term implantation and not undergo hydrolytic degradation.

| **Polyol** | **Isocyanate** | **Chain extender** | **Application** | **Degradation character** | **Author** |
|---|---|---|---|---|---|
| Polycaprolactone | HMDI | Putrescine (diamines) | Cardiac TE | Biodegradable | [13] |
| Polycaprolactone | HDI | Piperazine (diamines) | Shape memory polymers | Biodegradable | [14] |
| Polycaprolactone | HDI | Butanediamine | Cardiac TE | Biodegradable | [15] |
| Poly(ethylene glycol) | $H_{12}$MDI | Poly(propylene glycol) (PPG) | TE applications | Biodegradable | [16] |
| Polycaprolactone | HDI | BDO | TE applications | Biodegradable | [17] |
| 1-Butanol | HMDI | Ethylenediamine/ diethylamine | TE applications | Biodegradable | [6] |
| Castor oil | HDI | PEG | Cardiac TE | Biodegradable | [18] |
| Polycaprolactone | HDI | POSS diol | TE applications | Biodegradable | [19] |
| Polycaprolactone | MDI | BDO | Drug delivery | Long-term | [20] |
| Poly(tetramethylene ether) glycol | HMDI | BDO | Drug delivery | Long-term | [20] |
| Polycaprolactone-triol | HDI | Glycerol | TE applications | Biodegradable | [7] |

**Table 1:** Currently monomers and their application in TE.

Table 1 illustrates different types of polyols, diisocyanates, chain extenders, the PU application and the degradation character that are currently being applied in TE.

From the Table 1 it is possible to see that the main polyol applied in TE is the PCL. PCL is a biocompatible polymer with aliphatic ester linkage that is susceptible to be hydrolyzed. Poly(ethylene glycol) (PEG) is another polyol commonly applied in TE. PEG is biocompatible and presents non-toxic degradation products. The main diisocyanate applied is the HDI, due to the aliphatic segment and degrade in non-toxic products; furthermore the addition of BDO to the hard segment is frequently used. Polyhedral oligomeric silsesquioxane (POSS) diol it was also founded as chain extender this is a silicon-oxigen caged structure, it is biocompatible, and presents thermal and oxidative stability [19].

The primary mechanism of PUs degradation is the hydrolysis of ester and urethane groups. The cleavage of the ester bonds occurs via simple hydrolysis generating free carboxylic acids and hydroxyl groups, and may cause the pH decrease [21]. The degradation rate depends on the crystallinity, molecular weight, copolymer composition and morphological structure. The cleavage of urethane groups generates amine and hydroxyl groups, and may cause the pH increase. PUs can also be degradated by oxidation of the ether segments in a presence of enzymes or calcification.

This versatile polymer has enormous potential applications as degradable and non-degradable implants. Along these lines, PUs have gained attention in different applications. In the cardiovascular [13,18] area mostly of the implants are stable, as intraortic balloons, cardiac valves, vascular prostheses and grafts [22-23]. In drug delivery applications, PUs have been applied as long-term intravaginal rings presenting potential to prevent HIV disease, or containing a microbicide [4, 24]. PUs can also been applied as a capsule for oral dosage forms [20]. Bioactive PUs have also been developed by adding nanoparticles or antibiotics in order to promote antibacterial and antimicrobial

properties [25]. Another approach in the PU as wound dressings devices [26], artificial skin and bandages. PU can also be applied on the prevention of biofilm formation in devices.

Recently, the electrospinning technique have gained attention for TE applications due to the possibility of creating medical devices with properties that can mimic the extracellular matrix (ECM) of native tissues due to aligned fiber matrix. The synthesis of electrospun PU is relatively new. Moreover, there have been few studies proving that this is a viable and promising technique for the fabrication of medical devices. This technique presents advantages, such as, high surface area:volume ratio [27], high porosity, small pore size [28], extraordinary length, high porous and interconnected structure for cell attachment and transport of nutrients and oxygen [29]. Electrospun applications is related to the epithelial applications, PU membranes have been applied as artificial skin [30], bandages [31-32], stents [33], grafts [34], and scaffolds [35].

The purpose of this study was to prepare PU membranes using the electrospinning apparatus and to investigate the produced membrane through in vitro cell adhesion after 48 hours and mass loss during 30, 60 and 90 days using the in vitro degradation test. The PU applied in this work is a medical grade commercial elastomer (Tecoflex SG-85A), an aliphatic poly(ether-urethane) prepared from poly(tetramethylene glycol) (PTMG), HMDI and BDO. PTMG is a polyol polyether that exhibits good flexibility properties and it is biocompatible. Their structure and reagents are presented in Figure 1.

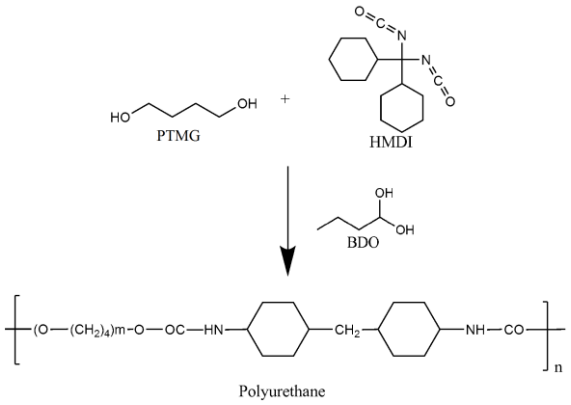


**Figure 1:** Structure of the polyurethane applied in this work.

## II. MATERIALS AND METHODS

### 2.1 Materials

All chemicals were of analytical grade and were used without further purification. PUs in pellets form (medical grade, SG-85A) was kindly provided by Lubrizol Advanced Materials. Chloroform was purchased by Sigma Aldrich.

### 2.2 Methods

#### 2.2.1 Polyurethane solution preparation

PU stock solution was prepared by dissolving 9% of PU in pellets form in chloroform (wt/v) during sonication (Ultrasonic clear, Unique, São Paulo, Brazil) for 2 hours.

#### 2.2.2 Preparation of PU electrospun membranes

The electrospinning apparatus employed in this research was designed and it is located in the National Institute of Biofabrication (INCT-Biofabris), 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.

#### 2.2.3 Morphology evaluation

SEM analysis was performed in a Scanning Electon Microscope (model LEO 440i; Leo Electron Microscopy, Cambridge, England). The applied voltage was 20 kV and the current was 100 pA.

metallic SC7620 Sputter Coater was used for coating the sample with gold.

**2.2.4 In vitro evaluation of cell adhesion**

Vero cells were seeded on the PU surface with a final density of 3 x$10^6$ cells/mL. After growth time of 48 hours, 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 minutes. Afterwards, they were dehydrated in ethanol for 15 min intervals in aqueous 50%, 70%, 95% and 100% ethanol solution and dried at the critical point 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.

**2.2.5 In vitro degradation test**

The degradation experiments were performed following ASTM F1635-11. The dried electrospun polyurethane samples were cut into 2 x 2 $cm^2$ pieces. All the cut specimens had weight of 13.2 ± 0.01 mg and then were placed in a test tube containing 20 mL of pH 7.4 PBS (phosphate-buffered saline) at physiologic temperature (37 $^oC$) to simulate the hydrolytic environment. The tubes were placed in an electronically controlled thermostat and kept in these conditions for 90 days. At regular time intervals, (30, 60 and 90 days), the polyurethanes were taken out from the degradation media and weighted. The samples were washed with distilled water and then vacuum-dried at 37 $^oC$ to constant weight. The mass loss was calculated according to the following equation:

$$Mass\ loss\ (\%) = \frac{w_i - w_d}{w_i} x\ 100$$

Where: $w_i$ and $w_d$ represent the initial weight and dry weight of the samples, respectively.

## III. RESULTS

**3.1 Structure and morphology**

At the end of the process, thermoplastic PU electrospun membrane was obtained. The morphology of the membrane was investigated using SEM. The micrograph image of the membrane is shown in Figure 2.

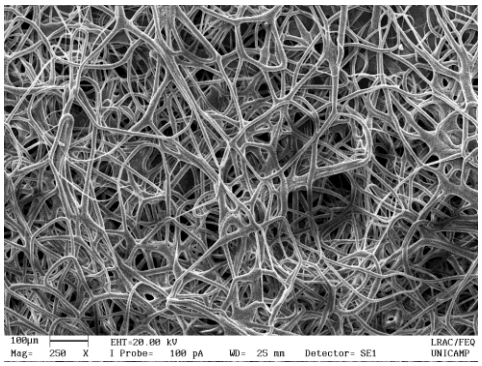


**Figure 2:** SEM image of the electrospun PU.

Electrospun PU showed uniform fiber diameter distribution, presenting high porosity, interconnected and well-distributed pores throughout the membrane. It was observed fiber diameter of approximately 20 micrometers, showing a random fiber networks. It is an unique nanofiber morphology with extremely high surface area to volume ratio characteristic of the production technique.

**3.2 In vitro fibroblasts cell adhesion**

In vitro cell adhesion test was performed to study the fibroblasts adhesion over the membrane surface. Figure 3 shows the cell adhesion after 48 hours of culture.

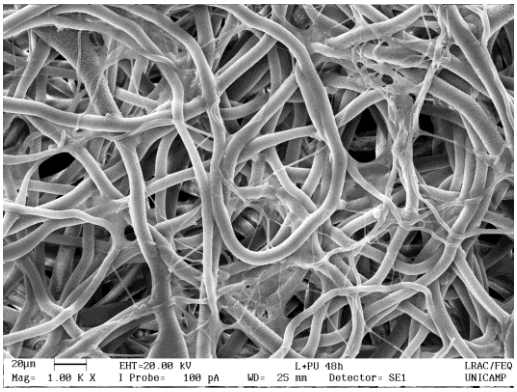


**Figure 3:** In vitro cell adhesion after 48 hours of culture.

The in vitro experiments showed that fibroblasts adhered and spread over the polyurethane surface, following the fiber morphology, generating an extensive network of fibroblasts cells.

**3.3 In vitro degradation study**

The in vitro degradation measurements of the electrospun membranes were performed during 30, 60 and 90 days. Figure 4 depicts the mass loss of the membranes as a function of the degradation time.

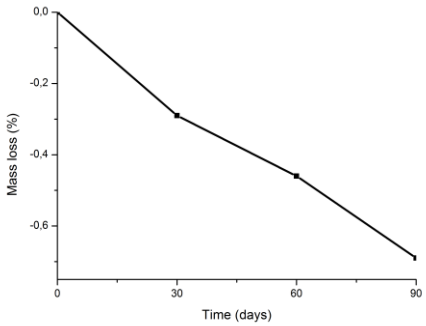


**Figure 4:** Mass loss during in vitro degradation study.

No changes were observed in the surface morphologies of the membranes during the experiment. The surfaces of the samples and the flexibility remained very smooth, with no evidence of any degradation.

After the incubation period, the PU membranes exhibited a very slow weight loss rate. Specifically after 90 days on in vitro incubation, about 0,7% mass loss was observed. Thus, this polyurethane is suitable for long-term applications due to their stability in vitro.

## IV. CONCLUSION

This work successfully prepared electrospun polyurethane membranes by a simple and efficient method. The morphological studies presented an unique nanofiber morphology, exhibiting microfibers with interconnected pores. The in vitro cell adhesion study showed fibroblasts adhesion over the membrane surface. The in vitro degradation investigations showed that this is a biostable polymer. In summary, the membrane developed in this work is very promising for future non-degradable applications in TE, such as ephitelial, drug delivery or cardiac applications.

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