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Surface modification of polyurethane films by plasma and ultraviolet light to improve haemocompatibility for artificial heart valves

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ABSTRACT

Prosthetic cardiac valves implantation is a common procedure used to treat heart valve diseases. Although there are different prostheses already available in the market (either mechanical or bioprosthetic), their use presents several problems, specifically concerning thrombogenicity and structural failure. Recently, some progresses have been achieved in developing heart valves based on synthetic materials with special emphasis in polymers. Among them, polyurethanes are one of the most commonly used for the production of these devices.

Herein, Elastollan® 1180A50, a thermoplastic polyurethane (TPU), was used to formulate films whose surfaces were modified by grafting 2-hydroxyethylmethacrylate (HEMA) either by ultra-violet (UV) or by plasma treatment. All films were analyzed before and after grafting. X-ray photoelectron spectroscopy (XPS) measurements were used to evaluate TPU surfaces functionalization. HEMA grafting was confirmed by the increase of the hydroxyl (OH) groups' concentration at the surface of the films. Atomic force microscopy (AFM) analysis was done to evaluate the surface topography of the biomaterials. Results showed that the roughness of the surface decreased when HEMA was grafted, especially for plasma treated samples.

After grafting the films' hydrophilicity was improved, as well as the polar component of the surface energy, by 15–30%. Hydrophobic recovery studies using milli Q water or PBS were also performed to characterize the stability of the modified surface, showing that the films maintained their surface properties along time. Furthermore, blood-contact tests were performed to evaluate haemolytic and thrombogenic potential. The results obtained for HEMA grafted surfaces, using plasma treatment, confirmed biomaterials biocompatibility and low thrombogenicity. Finally, the cytotoxicity and antibacterial activity of the materials was assessed through *in vitro* assays for both modified films. The obtained results showed enhanced bactericidal activity, especially for the films modified with plasma.

1. Introduction

Valvular heart diseases (VHD) include several heart conditions that can be either congenital or acquired. Acquired VHD comprise degenerative valve diseases (which are the most common in developed countries) and rheumatic heart sickness (mostly common in developing nations) [1].

Nowadays, the number of patients diagnosed with degenerative valve disease is progressively growing with population ageing [2]. In fact, it is estimated that at least one in each eight people over 75 years old will suffer from one kind of VHD, becoming a serious public healthcare problem and a significant economic burden [3].

Some patients suffering from less severe valvular lesions are able to go through their lifetime without ever needing surgical intervention. However, for others, surgery is the only viable solution. Surgical treatment may involve the repair or the replacement of the original damaged valve. The ideal choice would be to keep the original valve [4]. However, for nearly 70% of the cases this procedure is no longer viable and valve substitution must be performed [5]. Currently, nearly 280 000 heart valve substitutes are implanted each year all over the world, in an approximated proportion of 50/50 for mechanical and bioprosthetic valves [6]. Despite the improvements in the design and composition of the commercially available valve prosthesis, mechanical valves have a high associated risk of thrombogenicity, while the bioprosthetic valves may suffer from premature structural failure [7]. Moreover, after a prosthetic valve implantation, a life-threatening complication, known as prosthetic valve endocarditis (PVE), may also occur [8]. PVE is

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usually caused by microorganism infection, especially bacteria and fungi, and its treatment requires medical treatment. However, in advanced stages, antibiotic therapy alone may not be enough to eliminate the infection and prosthesis replacement is required [9].

In order to overcome these problems, synthetic leaflet heart valves have been investigated over the last decades, trying to combine in one material, features like durability and enhanced haemodynamics [10]. Among the different synthetic materials used so far, polyurethanes (PU) have been considered the most suitable for this purpose [11]. PU's properties such as abrasion resistance, affordable manufacturing, chemical stability, durability, elasticity and haemocompatibility are fundamental for their extended applications in the biomedical field [12]. Besides heart valves, they are also used to prepare blood oxygenators, catheters, drug delivery systems, internal lining of artificial hearts, scaffolds for tissue engineering and wound dressing membranes [13]. However, several studies have reported that heart valves produced with PU's, may suffer from premature failure, caused by their suboptimal design and low durability [7]. The production of PUs with different compositions and by applying different manufacturing techniques have resulted in materials with improved properties which have allowed to expand their potential applications [14].

Thermoplastic polyurethanes (TPU) are currently being used for several industrial uses, such as adhesives, coatings and films [15]. Furthermore, previous studies have also shown their suitability for heart valves production [16].

Hereby, a commercial pre-processed polyether-based thermoplastic polyurethane (Elastollan®1180A50), was studied in order to be applied in a near future as a base material for synthetic heart valves manufacture. Elastollan®1180A50 choice was done based on its absence of plasticizers, good heat resistance, high mechanical flexibility, and its ability to be processed by moulding. Moreover, it also exhibits excellent abrasion resistance, toughness, transparency, hydrolytic stability and fungal resistance [17].

From the literature it is known that graft copolymerization of polyurethanes with hydrophilic vinyl monomers, such as acrylic acid [18], acrylamide [19] and 2-hydroxyethylacrylate [20], is an appropriate method to enhance surface hydrophilicity, and improve its haemocompatibility [21].

Herein, 2-hydroxyethylmethacrylate (HEMA) was grafted onto the surface of Elastollan®1180A50 to increase its hydrophilicity and improve its biological properties. Plasma and UV irradiation were used to perform the modification of films' surface. Furthermore, several parameters were assessed, such as their chemical surface functionalities, roughness, antibacterial activity, blood compatibility, cytotoxicity, hydrophilicity, hydrophobic recovery, surface energy and thrombogenicity to evaluate their potential for being used in heart valve fabrication.

2. Experimental

2.1. Materials

Bacterial strain *Escherichia coli* (*E. coli*) DH5α was purchased from ATCC. Elastollan®1180A50 was obtained from BASF. Foetal bovine serum (FBS) was acquired from Biochrom AG (Berlin, Germany). Human Fibroblast Cells (Normal Human Dermal Fibroblasts adult, criopreserved cells) were bought from PromoCell (Labclinics, S.A.; Barcelona, Spain). LB agar was obtained from Pronadise. Irgacure®2959 was kindly given by CIBA (Ciba Specialty Chemicals, Basel, Switzerland). 3-(4,5-Dimethylthiazol-2-yl)-5-(3carboxymethoxyphenyl)-2-(4-sulphofenyl)-2H-tetrazolium, inner salt (MTS), amphotericin B, dimethylformamide (DMF), Dulbecco's modified Eagle's medium (DMEM-F12), ethylenediaminetetraacetic acid (EDTA), 2-hydroxyethylmethacrylate (HEMA), isopropyl alcohol, L-glutamine, penicillin G, phosphate-buffered saline solution (PBS), streptomycin, and trypsin were acquired from Sigma–Aldrich (Sintra, Portugal).

2.2. Methods

2.2.1. Films preparation

Elastollan®1180A50 films were prepared by solvent evaporation. Elastollan®1180A50 was dissolved in DMF to a 10% (w/v) TPU solution. This solution was poured into glass Petri plates. Then, the Petri dishes were stored in an oven at 60 °C, for 24 h. Subsequently, films were removed from the dishes and ultrasonically cleaned with isopropyl alcohol for 15 min, prior to surface grafting experiments.

2.2.2. Argon plasma grafting

A laboratory and small-scale production plasma system FEMTO (low pressure plasma), manufactured by Diener Electronics, with a stainless steel plasma chamber of 100 mm diameter and 270 mm length, was used for the plasma surface modification experiments. TPU films were placed at 80 mm from the electrode and were plasma treated with Argon, in a pressure chamber of 0.6 mbar, for 3 min and applying 100 W of power to the electrodes to generate the plasma [22]. Then, the plasma-treated TPU films were dipped into a 10% (v/v) aqueous solution of HEMA and introduced in an oven at 60 °C, for 1 h. Finally, the modified films (TPU-Ar-HEMA) were washed abundantly with deionized water and dried until constant weight was obtained.

2.2.3. UV grafting with Irgacure®2959

For the UV grafting, films were previously activated with UV light in a 0.5% photoinitiator (Irgacure®2959) aqueous solution for 30 min. Afterwards, they were removed and dipped into a 10% (v/v) HEMA aqueous solution. Then, samples were irradiated with UV light during 30 min and the modified films were obtained (TPU-UV-HEMA).

In both steps of the modification, films were irradiated using a Mineralight® Lamp, Model UVGL-48, in the 254 nm wavelength setting. This generated a power of 6 Watt and the samples were placed at a distance of 4 cm from the light source.

2.3. Characterization techniques

2.3.1. X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) measurements were made with a VGS ESCALAB 200A spectrometer with an Al Kα X-ray source. The operation conditions were set to 15 kV. The binding energy scale was fixed by assigning a binding energy of 285.0 eV to the $-CH_2-$ carbon (1s) peak. The samples were analyzed at a take-off angle of 0° relative to the normal of the surface. The C1s and O2s envelopes were analyzed and peak-fitted using a combination of Gaussian and Lorentzian peak shapes obtained from the XPS peak 4.1 software.

2.3.2. Atomic force microscopy

Atomic force microscopy (AFM) analysis of the samples was performed in a Nanoscope IVa Veeco Metrology using the tapping mode (scan size 4.0 μm, scan rate 1.0 Hz). The average roughness (Ra) was calculated directly from the AFM images.

2.3.3. Analysis of contact angle and surface free energy

The contact angle and surface energy measurements were performed at room temperature in an OCA 20 contact angle measurement unit from Dataphysics. Surface free energy (γ_S) values as well as the dispersive (γ_S^D) and polar (γ_S^P) components were obtained according to the Owens–Wendt–Rabel and Kaelbe