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Developing a biomaterial interface based on poly(lactic acid) via plasma-assisted covalent anchorage of D-glucosamine and its potential for tissue regeneration

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ABSTRACT

The aim of this study was to develop the potential tissue engineering applications of D-glucosamine (GlcN) immobilized onto the surface of a biodegradable matrix in order to induce a desired biological effect at biointerfaces. Thus, for sample preparation we used a novel multistep physicochemical approach. In the first step the poly(lactic acid) (PLA) films were exposed to a low pressure plasma in air atmosphere, followed by radical graft copolymerization with acrylic acid to yield a carboxyl-functionalized spacer layer on the PLA surface. The carboxyl groups were then coupled to GlcN molecules via the carbodiimide chemistry. The developed surfaces were characterized by X-ray Photoelectron Spectroscopy (XPS), Contact angle measurements and Atomic Force Microscopy (AFM). A preliminary study on the proliferation of fibroblasts on the developed surfaces was performed using the NIH/3T3 cell line.

1. Introduction

Optimization of polymer surface functionalities can control the biological processes such as cell adhesion, proliferation, viability and enhanced extracellular matrix (ECM) secretion functions at biointerfaces [1,2]. Two main strategies in surface modification of polymeric materials are often applied. Firstly, the material surface properties such as chemical composition, wettability, surface charge and roughness, etc. are tailored in such a way that the adsorbed proteins can maintain their normal bioactivities. However, this method cannot induce specific cell behaviors due to the nonspecific protein absorption. The second strategy involves direct immobilization of certain biomolecules onto the biomaterial surfaces in order to induce specific cellular response. Several classes of bioactive molecules, such as proteins, lipids and carbohydrates have been recently utilized for this purpose [3].

Bioabsorbable polymers are considered a suitable alternative to the improvement and development of numerous applications in medicine [4,5]. Poly(lactic acid) (PLA) is one of the most promising biopolymers due to the fact that the monomer "lactic acid" is produced from nontoxic renewable feedstock and it is naturally occurring organic acid as well. The excellent biocompatibility, thermoplastic processability and mechanical properties of PLA widened its use in a variety of biomedical applications. However, as any other synthetic polymer, its surface lacks to bioactive motifs that can be used for specific biorecognition in tissue engineering applications.

Several surface modification techniques have been developed to create new functionalities on polymer surfaces as a precursor for the covalent attachment of bioactive compounds [6]. Among them, plasma treatment is an effective and economical surface modification technique for many materials and of growing interests in biomedical engineering [7,8]. Plasma is a gaseous mixture of charged particles, neutral and excited atoms and molecules, radicals and photons. Practically, non-thermal or "cold" plasma which is composed of low temperature particles (charged and neutral molecular and atomic species) and relatively high temperature electrons is convenient for the treatment of heat-sensitive materials such as biodegradable polymers [9,10]. Moreover, a unique advantage of plasma modification is that the surface functionalities and characteristics can conveniently be tailored on a nanome-

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ter scale without losing desirable physical characteristics of bulk polymer. Plasma can impart the surface with different functionalities and active centers in the form of radicals, peroxides, and hydroperoxides. These active centers can induce a radical graft polymerization in a next step. Finally, the introduced functionalities can be utilized to immobilize relevant biomolecules in order to impart bioactivity to the base polymer.

In particular, carbohydrates are ideal biomolecules for material surface functionalization due to their polyhydroxylated nature. In addition, they are able to convey biological information [11,12]. Carbohydrates are directly implicated in recognition processes including adhesion between cells, adhesion of cells to the extracellular matrix, and specific recognition of cells by one another. Thus, the immobilization of carbohydrates onto polymeric surfaces can be an optimum process for improving simultaneously hydrophilicity, cell adhesion and bioactivity [13–15].

D-Glucosamine (GlcN) is a naturally occurring amino-sugar and it is considered a prominent precursor in the biochemical synthesis of glycosylated proteins and lipids. Therefore, the immobilization of GlcN should be a safe approach for biomaterial surface developments. According to the literature, chitosan based materials are relevant candidates in the field of biomaterials, especially for tissue engineering [16,17]. However, the studies on GlcN which represents a contributing monomeric unit in the backbone of chitosan are limited. Recently, GlcN has been used to develop glycosylated polymeric surfaces for different biomedical applications. For example, Russo et al. [18] grafted GlcN onto poly(ε-caprolactone) in a single step process of polymer aminolysis for enhancing density and spreading of hMSCs. Wang and Lan [19] developed a glycosylated surface on poly(3-hydroxybutyrate-co-4-hydroxybutyrate) membrane by the chemical attachment of GlcN through a multi-step technique for the selective adsorption of low density lipoprotein.

From the previous summary, it seems that the studies on the cell interactions with GlcN functionalized PLA surfaces prepared by the plasma irradiation route are limited. In the present study, low pressure air plasma was employed to introduce active centers to the PLA film surface. In a subsequent step, such active sites were exploited to initiate the grafting reaction of AAc onto the PLA surface. Lastly, GlcN as a bioactive molecule was chemically immobilized onto the functionalized PLA surface with the aid of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) as a condensing agent. Characterization of the developed PLA surfaces was carried out including chemical structure, wettability, surface energy, and morphology. Finally, adhesion and proliferation of NIH/3T3 mouse embryonic fibroblast cells on the untreated and modified PLA films were investigated in order to verify the applicability of these biomaterials in regenerative medicine.

2. Materials and methods

2.1. Materials

The commercial PLA 4032D, a semi-crystalline grade with the density of 1.24 g/cm^3 and containing around 2% D-LA, was purchased in pellets from Nature Works (Blair, NE). Pellets were dried at 60 °C overnight and then hot-pressed at 180 °C into films with a thickness of ~150 μm, followed by cooling in a press.

Acrylic acid (AAc, 99%) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, 98%) were obtained from Fluka. D-Glucosamine hydrochloride (GlcN, 99%, suitable for cell culture), ethylene glycol (99.8%), diiodomethane (99%) and sodium metabisulfite (99%) were supplied by Sigma-Aldrich. All reagents were used as received without further purification.

2.2. Plasma treatment

Prior to the surface modification by gaseous plasma, the PLA films were cut into rectangles of 4 cm × 5 cm and rinsed with a detergent solution, then with deionized water several times, and finally dried at room temperature for 2 h. PLA films were treated from the both sides under a pressure of 60 Pa in air atmosphere for 120 s with Pico plasma system (Diener, Germany) operating at 40 kHz. The duration of the plasma treatment was optimized by previous experiments by means of water contact angle measurement when equilibrium value was reached for 120 s and prolonged treatment did not show any contact angle value difference. The power and the air feed rate were set to 50 W and 20 sccm in all the experiments.

2.3. Grafting

Immediately after the plasma treatment, the PLA films were immersed into 10 vol% aqueous solution of AAc for 24 h at 30 °C in order to achieve a radical graft polymerization of AAc. The solution contained 0.1 wt% of sodium metabisulfite to inhibit AAc homopolymerization. The AAc graft copolymerization led to the creation of poly(acrylic acid) brushes that are suitable for the immobilization of bioactive agents. After that, the AAc-*g*-PLA films were immersed in deionized water for several hours at room temperature and then washed with a copious amount of water several times to remove any unreacted acrylic acid monomer and poly(acrylic acid) homopolymer. The graft density (μg/cm^2) was determined from Eq. (1):

$$\text{Graft density} = (W_g - W_o)/S \quad (1)$$

where W_o and W_g are the weights of PLA film sample before and after grafting and S is the surface area of the PLA sample.

It is important when carrying out the grafting reaction to keep the ratio between the surface area of the PLA film and the volume of the monomer solution fixed. The PLA surface contains the initiating sites for polymerization and, subsequently, its area is considered a determining factor in the grafting reaction.

2.4. Chemical immobilization of GlcN

AAc grafted PLA sheets were immersed into 0.1 wt% aqueous solution of EDC at 4 °C for 6 h for the activation of carboxyl groups. EDC reacts with carboxylic acid groups to form an active O-acylisourea intermediate that is easily displaced by nucleophilic attack from primary amino groups in the reaction mixture [20]. The activated carboxyl-functionalized PLA samples were then immersed into 1 wt% GlcN aqueous solution for 24 h at 25 °C in order to allow the amide bond formation. Finally, the samples were removed, thoroughly washed with deionized water, and dried for 24 h at room temperature. The scheme of the multistep approach is given in Fig. 1.

2.5. X-ray photoelectron spectroscopy (XPS)

X-ray Photoelectron Spectroscopy (XPS) was conducted using TFA XPS Physical Electronics. The base pressure in the XPS analysis chamber was ≈6×10^{-8} Pa. The samples were excited by X-rays over a 400-μm diameter spot area with a monochromatic Al K$\alpha_{1,2}$ radiation at 1486.6 eV. The emitted photoelectrons were detected by a hemispherical analyzer positioned at a take-off angle of 45°. Survey-scan spectra were obtained at a pass energy of 187.85 and 0.4 eV step resolution. An electron gun was employed for surface neutralization. The elemental concentration analysis was performed over three different positions by MultiPak v7.3.1 software.