Study of the adhesive properties versus stability/aging of hernia repair meshes after deposition of RF activated plasma polymerized acrylic acid coating

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ABSTRACT

In order to confer adhesive properties to commercial polypropylene (PP) meshes, a surface plasma-induced deposition of poly-(acrylic acid) (PPAA) is performed. Once biomaterials were functionalized, different post-deposition treatments (i.e. water washing and/or thermal treatments) were investigated with the aim of monitoring the coating degradation (and therefore the loss of adhesion) after 3 months of aging in both humid/oxidant (air) and inert (nitrogen) atmospheres. A wide physicochemical characterization was carried out in order to evaluate the functionalization effectiveness and the adhesive coating homogeneity by means of static water drop shape analysis and several spectroscopies (namely, FTIR, UV–Visible and X-ray Photoemission Spectroscopy). The modification of the adhesion properties after post-deposition treatments as well as aging under different storage atmospheres were investigated by means of Atomic Force Microscopy (AFM) used in Force/Distance (F/D) mode. This technique confirms itself as a powerful tool for unveiling the surface adhesion capacity as well as the homogeneity of the functional coatings along the fibers. Results obtained evidenced that post-deposition treatments are mandatory in order to remove all oligomers produced during the plasma-treatment, whereas aging tests evidenced that these devices can be simply stored in presence of air for at least three months without a meaningful degradation of the original properties.

1. Introduction

The use of polymeric meshes during the hernia-repair surgical operations is a routine procedure [1–4], although still nowadays there are different phenomena which can cause hernioplasty complications, for instance surgical infections or the post-operative prosthesis displacement with detachment of the polymeric device, collapse of the anchoring sutures and reformation of the hernia disease [5].

In order to solve these issues, thus reducing the risk related to hernioplasty failure, the surface modification of standard polymeric meshes seems to be a promising route to follow [6]. Among others, plasma treatments have recently gained much attention, thanks to their ability to induce modifications only at the surface level without altering the intrinsic physicochemical and mechanical properties of the prosthetic biomaterial [7–12].

Recently, the plasma deposition technique has been successfully used to functionalize polymeric materials with plasma poly-(acrylic acid) (PPAA) layers for clinical purposes, such as exploiting —COOH groups in covalent binding of c-DNA probes [13] or as substrates for the adhesion of human cells colonies in a non-specific manner [14–15]. Moreover, the adoption of a thin coating, produced by plasma deposition, avoid the lack of the open porosity between the biomaterials fibers necessary for an optimal tissue infiltration and proper mesh integration in the biological tissue [5].

In detail, the plasma polymerization process conditions which guarantee a good compromise between the maintenance of the functional groups (in this case, the carboxylic groups of the polyacrylic acid) and the increase of the coatings mechanical properties (obtained synthesizing highly branched and cross-linked PPAA), can be reached by working following a "monomer-deficient regime", as described by Yasuda et al. [16], using a pulsed-plasma deposition in order to obtain a better control of the surface reactivity and the film chemistry [17]. The monomer-deficient domain corresponds to a high input power/gas

flow ratio (W/F), based on the following equation:

$$R_m/F = Gexp^{(-Ea/(W/F))} \qquad (1)$$

where R_m is the mass deposition rate, F is the total gas flow, G is a constant (which comprises the decomposition rate of the monomer, that strongly depends on the plasma apparatus), E_a is the apparent activation energy, W is the input power and W/F is the average energy per particle [18–20].

However, even though all these precautions have been taken into account, the stability over time and atmospheric conditions of such functional plasma-polymerized thin coatings is still a limiting factor, which significantly reduces the application of these smart devices in the biomedical fields. In this context, the use of atomic force microscopy in force/distance (AFM F/D) mode can be a powerful tool for evaluating both the stability of such adhesive thin coatings and how incorrect post-deposition treatments can dramatically alter the plasma-induced surface properties [21–22].

Therefore, in this paper, we extend our previous work [23], focused on the surface functionalization of polypropylene (PP) meshes by an adhesive thin-coatings made by plasma-polymerized polyacrylic acid (PPAA), considering the effects of post-deposition treatments (i.e. no treatment, water washing and water washing followed by a thermal treatment) and following the coating stability after 3 months of aging in humid/oxidant (air) and inert (nitrogen) atmospheres. Among all the physicochemical techniques here selected, AFM in F/D mode confirms to be a very useful tool to optimize the post-deposition procedure, making these functional devices suitable for a real and feasible industrial scaling-up.

2. Experimental

2.1. Biomedical devices and reagents

Monofilament PP meshes for surgical applications were provided by Herniamesh® S.r.l. (Chivasso, Italy). Lightweight (ca. 30 g m^{-2}) PP flat rectangular meshes probes were 6 × 11 cm, with 0.32 ± 10% mm of thickness, and fibers diameter of 80 ± 10% μm. Other reagents used were: Acrylic acid monomer (AA, CH$_2$CH(COOH), Sigma Aldrich, purity 99%, CAS 79-10-7), Toluidine Blue O (TBO, C$_{15}$H$_{16}$ClN$_3$S, Sigma, Technical Grade purity, CAS 92-31-9), Sodium hydroxide (NaOH, Fluka, purity 97%, CAS 1310-73-2), and Acetic acid (CH$_3$COOH, Aldrich, purity 99.7%, CAS 64-19-7). All chemicals were used without purification.

2.2. Plasma polymerization apparatus and treatment conditions

Plasma-polymerized Poly-(Acrylic-Acid) (PPAA) thin films deposition was performed as previously reported [23]. The samples were introduced in a Plasma Enhanced Chemical Vapor Deposition (PECVD) reactor (Chamber Base Pressure = 3.7 ± 0.2 Pa; Radio frequency RF = 13.56 MHz) equipped with a delivery frame suitable to inject vapors coming from liquid reactants (monomer precursors). The reactor is a cylindrical closed chamber (320 mm wide and 200 mm height) made by stainless steel. Argon was selected as gas-carrier in order to sustain plasma discharge, for all experiments. The complete treatment consists of a two-steps process: i) the surface etching by continuous Ar plasma discharge (for cleaning the fibers surface and promoting the adhesion of the PPAA coating); ii) AA polymerization by pulsed plasma discharge. Surface etching step was performed by flowing Ar gas (flow = 20 sccm), applying a discharge RF power of 50 W, for 5 min at a total pressure of 30.7 Pa. Polymerization was performed by admitting acrylic acid vapors (flow = 3 sccm) diluted in Ar (flow = 20 sccm), applying a discharge Radio Frequency (RF) power of 200 W, a duty cycle of 10% (on time = 10 ms, off time = 90 ms) for 10 min at a total pressure of 19.3 Pa. The expected thickness of PPAA layer for the used experimental conditions was of ca. 100 ± 20 nm, measured on a flat surface [13].

PPAA-functionalized samples were treated in two different post-deposition conditions in order to evidence the best preparation procedure: 1) incubation: samples were soaked in deionized water Milli-Q grade (dH$_2$O) for 30 min under oscillating shaking in order to remove unstable surface oligomers formed at the end of the plasma process, 2) incubation and thermal treatment: incubated samples were dried under N$_2$ flux and heated at 80 °C for 1 h in oven. These samples were compared with the untreated ones. Moreover, two different storage atmospheres were tested: inert (nitrogen) and humid/oxidant (air).

Functionalized samples were coded with the acronym PPAA_Xy_Z, where X indicates the post-deposition treatments (namely: W for water incubated, WT for the water incubated/thermally treated and 0 for untreated samples), y indicates the analyzed mesh-side (i.e. front = a and back = b, if applicable) and Z indicates the aging storage atmosphere (namely: N for 3 months aged samples stored in nitrogen, A for 3 months aged samples stored in air and 0 for non-stored as-functionalized samples). Original PP non-functionalized mesh was taken as reference material and coded as Ref.

2.3. TBO titration and quantification of carboxylic functionality density

The amount of —COOH functionalities of PPAA was tested with Toluidine Blue O (TBO) colorimetric determination. The amino group contained in TBO molecule reacts specifically with a surface carboxylic group according to a 1:1 ratio [24]. PPAA-functionalized samples were contacted with 3 mL of 0.5 mM TBO aqueous solution (pH = 10) at 37 °C, for 5 h, in the dark. To remove the unreacted dye, meshes were rinsed with copious amount of 0.1 mM NaOH solution. Afterwards, samples were transferred in 1.5 mL of 50% v/v acetic acid solution and shaken for 10 min, in order to completely release the TBO linked to the carboxylic functional groups. Colorimetric dosing of TBO (and consequently of carboxylic groups) was performed by using a double beam UV–Vis-NIR Varian Cary 5000 spectrophotometer, in transmission mode. All the spectra were obtained by using a quartz cuvette and monitoring the $\lambda_{max} = 633$ nm. A solution of 50% v/v acetic acid was used as reference background. The experiments were repeated twice. The evaluation of carboxylic functionality density (in terms of groups cm^{-2} of mesh) was performed through the Lambert-Beer law on the basis of an external calibration with TBO solutions (at known concentrations and volumes) and a multiplex replication of experimental values. Finally, the –COOH amount was normalized for the geometric surface of mesh samples used for the analysis (i.e. 4 cm^2). External calibration curve is reported in Figure S1.

2.4. Physicochemical characterization techniques

Static (sessile drop) water contact angle (WCA) measurements were performed by using OCAH200 (Dataphysics, Instruments GmbH). 1.5 μL of dH$_2$O was spotted onto the meshes surfaces at Room Temperature (RT) and images of the droplets were captured. SCA20 software was used to fit drop profiles through the Young-Laplace method and, indeed, to calculate contact angles between fitted function and baseline. The instrument automatically calculates the contact angle, allowing to roughly estimate the hydrophilic/hydrophobic behavior of the material surface. At least three drops were dispensed for each sample.

Fourier Transform Infrared Spectra (FTIR) spectra were recorded in Attenuated Total Reflection mode (ATR, using a diamond cell for single reflection) in a Bruker Vector 22 spectrophotometer equipped with Globar source, DTGS detector and working with 128 scans at 4 cm^{-1} of resolution in the range 4000–400 cm^{-1}. ATR-FTIR spectra were obtained on single fibers repeating the acquisition for three times.

X-ray Photoemission Spectroscopy (XPS) studies were carried out by a Versa Probe 5000 from PHI electronics, using Summit as software. Spectra were analyzed using Fityk software. Al Kα radiation (1486.6 eV), having a beam diameter of 100 μm, was used as X-ray source. C1s and O1s (not discussed) signals were analyzed. All core-