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Antioxidant and hydrophilic poly(lactic acid) fibers obtained through their modification with amines and ferulic acid

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ABSTRACT: The ferulic acid (FA) is a natural antioxidant, abundantly present in plants, which acts as the plant's immune system. In order to take advantage of its properties, a method has been developed, which combines antioxidant FA with bio-based biodegradable poly(lactic acid) fibers and biocompatible hydrophilic polyallylamine, enabling the production of versatile base material that could be used for active anti-inflammatory wound dressings. The fibers are first subjected to aminolysis in order to obtain amino moieties on the surface, able to react with the molecules of FA. Next, the FA was attached to the aminolyzed fibers surface with use of 1-ethyl-3–(3-dimethylaminopropyl) carbodiimide and *N*-hydroxysuccinimide. The anti-inflammatory properties of the modified fibers were assessed using 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay. Presence of FA on the fibers' surface was investigated through X-ray photoelectron spectroscopy analysis and Folin–Ciocalteu (total phenolic content) test. © 2017 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2017**, *134*, 45112.

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INTRODUCTION

Due to raising occurrences of chronic diseases and global aging, the demand for special wound dressings is on a significant rise. It is expected that global wound dressings market will rise to USD 14.9 billion in 2020 at a compound annual growth rate of 7.0%. Particularly, the segment of anti-infection dressings is predicted to show the highest growth rate.[1] To meet the requirements of modern society, we need a modern approach to creating materials which can be used in functional wound dressings.

Since many years most of the disposable products utilized in the medical industry have been based on synthetic polymeric materials. The low production cost and their versatility make synthetic polymers the best choice for producing not only disposable products but also more advanced medical devices. The only problem is their slow decomposition rate, which causes the need for such materials to be disposed by either burning (creating dangerous fumes) or storing in landfills. Both methods pose a threat to the environment, so replacing those polymers by their biodegradable counterparts would be the best solution. Poly(lactic acid) (PLA) is one of best known biodegradable synthetic polymers. It is widely used across industries, but its most important application is in the medical sector. PLA fibers are a great material for wound dressings since they are versatile, biodegradable, biocompatible, have good mechanical properties, and are relatively easy to produce.[2,3] However, poly(lactic acid) is a material of low chemical activity and high hydrophobicity, which makes it difficult to functionalize and alter its properties.[4]

Ferulic acid [(E)-3–(4-hydroxy-3-methoxy-phenyl)prop-2-enoic acid] (FA), is a derivative of cinnamic acid and one of the phenolic acids acting as secondary metabolites in the plants' "immunological system." FA is the most ubiquitous phenolic acid found in nature, present in plant cell walls and is also one of the most active ones,[5,6] i.e., it makes up 70% to 90% of all phenolics present in wheat.[7,8] Due to its chemical structure, FA is characterized by high antioxidant activity,[6,9–12] UV-protection, and anticancer properties.[6,9,13,14] Upon absorption of UV radiation, the double bond adjacent to carboxylic group catalyses the forming of a stable phenoxy radical, which is also supported by the molecule's methoxy and carboxylic

groups. As a result FA can not only protect from free radicals, but also prevent radical chain reactions and lipid peroxidation.[11,15,16] The carboxylic group present in the FA provides a good anchor for the coupling reactions, allowing for attaching antioxidant molecules onto the surface of amine-functionalized fibers. The antioxidant properties of certain substances are known to be strongly linked to the anti-inflammatory effect. Hence, most of the therapeutic potential of FA is described as antioxidizing and anti-inflammatory.[11] FA is one of active components present in the rhizome of *Cimicifuga* genus used in Chinese and Japanese medicine as anti-inflammatory agent.[10,17,18]

The unique properties of both poly(lactic acid) fibers and FA prompted the authors of this study to create a method, which allows to incorporate the phenolic acid onto the surface of the fibers, thus obtaining PLA fibers with antioxidant properties and standarized mechanical properties. There have been but a few reported approaches to phenolics acids being used as modifiers for fibers or polymer materials. Curcio *et al.*[19] combined two very potent antioxidants—gallic acid and catechin with chitosan macromolecules through a free radical-induced grafting procedure. An ascorbic acid/hydrogen peroxide redox pair was employed as the radical initiator. Both samples exhibited very high antioxidant properties. This process was also used by Cho *et al.*[20] to obtain gallic acid-grafted chitosan. Its antioxidant properties were very similar to the results found in Curcio *et al.* Gallic acid as a modifier was also employed by Chuysinuan *et al.*,[21] but in different manner: Chuysinuan *et al.* used gallic acid as an constituent of poly(L-lactic acid) spinning solution, to obtain electrospun fibrous mats with antioxidant properties. Arrua *et al.*[22] established a method of including caffeic acid (CA) [3–(3,4-dihydroxyphenyl)-2-propenoic acid] which is also a very potent antioxidant. The authors synthesized a chloride derivative of CA in the form of caffeoyl chloride and immobilized it on the surface of polypropylene films grafted with hydroxyethyl methacrylate as a (PP-*g*-HEMA) monomer. The obtained films were characterized by very good antioxidant properties and effectivity in preventing oxidation of ascorbic acid in samples of real orange juice. Another approach was presented by Chuysinuan *et al.*[23] In this approach 1,6-hexamethylenediamine (HMD) was used as an "anchor" between the surface of the surface of PLA nonwoven nanofibers and CA molecules. It was reported that the obtained nonwovens exhibited relatively high antioxidant activity and good wettability. However, after the treatment, their tensile strength decreased by more than 75%.

As for now there is no method reported, that utilizes FA as modifying agent. The aim of the present study was to provide a solution allowing to introduce FA onto the surface of poly(lactic acid) fibers, while retaining their mechanical properties and therefore to obtain a versatile fiber that can be used in active wound dressing system. The other desired effect was an increase in the hydrophilicity of the fibers.

The presented method consists of a two-step modification process. In the first step, the PLA fibers surface is aminolyzed to increase its attaching ability to FA and the amount of active sites able to accept the modifier. The second step includes the actual modification with FA. For the aminolysis two different amines were used—HMD and poly(allylamine chydrochloride) (PAH). As a diamine, HMD is able to attach both to PLA fibers surface and molecules of the modifier. In the process of aminolysis one of the amine group of HMD reacts with the ester groups of PLA, leaving the other amine moiety free for further reactions and thus enriching the surface of PLA fibers with active amine groups. This way HMD can act as an anchor between PLA fibers surface and FA molecules, that are attached to the free amine active sites in the further modification process.

PAH is a cationic polyelectrolyte containing amine groups in its chain structure. It is biocompatible and does not exhibit any cytotoxic effects.[24] It is also characterized by strong hydrophilicity. Application of polymer as an alternative to diamine increases the amount of active amine groups on the fibers surface. Compared to aminolysis that uses short molecules (like HMD), it also allows to omit a decrease in fibers tensile strength.[25,26] In its native form, PAH does not react with the PLA. To allow reaction of the PAH and ester groups of PLA, the ammonium groups ($-NH^{3+}$) in PAH need to be converted into free primary amine groups (NH_2). An aqueous solution of PAH was thus treated with sodium hydroxide (NaOH) to receive pH level 10 to 11, resulting in conversion of PAH to poly allylamine (PAH*). The PAH* solution was then used for aminolysis. Obtained fibers were characterized by significant increase of hydrophilicity and higher amount of free amine groups as compared with fibers aminolyzed with HMD.

To support the reaction between amino groups on the surface of the amine-endowed PLA fibers and FA, the latter is reacted with 1-ethyl-3–(3-dimethylaminopropyl)carbodiimide and *N*-hydroxysuccinimide (NHS). The obtained PLA–FA fibers are analyzed with several analytical methods. The total phenolic content of the material is determined by the Folin–Ciocalteu test. The antioxidant activities are evaluated by the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH*) scavenging assay. The wetting behavior of the fibers' surfaces are determined by measuring the static water contact angles and assessment of hygroscopicity. The presence of HMD, PAH, and FA on the surface is determined using X-ray photoelectron spectroscopy (XPS). Using XPS for analysing the fibers was a novel approach and thus needed a specific way of preparing the sample.

Both approaches resulted in a successful attachment of FA to the surface of poly(lactic acid) fibers. The obtained fibers were characterized by prominent antioxidant activity and increased hydrophilicity.

EXPERIMENTAL

Materials

PLA fibers were manufactured in the Institute of Biopolymers and Chemical Fibers in Łódź, Poland. The fibers have the following parameters: linear mass 2.84 dtex, length 60 mm, tenacity 24 cN/tex, and elongation 64%. To allow further

modification, all fibers were carefully cleaned from aperture. PAH, HMD, FA, *N,N*-dimethylformamide (DMF), NHS, and DPPH were purchased from Sigma-Aldrich (Poznań, Poland). EDC was obtained from ROTH (Zielona Góra, Poland). Dichloromethane and the Folin–Ciocalteu reagent were supplied by Merck (Warsaw, Poland). Ethanol was provided by POCh SA (Gliwice, Poland). Methanol and propan-2-ol were purchased from Chempur (Ruda Śląska, Poland). Acid violet 6B (CAS Registry Number 1694–09-3) (test dye) was provided by Boruta-Zachem SA (Zgierz, Poland). All the chemicals except test dyes were of analytical reagent grade and used without further purification.

Aminolysis of PLA Fibers

Aminolysis of the fibers' surface was conducted with two methods.

1. The fibers (500 mg) were immersed in a 0.04 mol L^{-1} PAH water solution (1:10 w/w) with pH of 11.2 (adjusted by addition of NaOH) at room temperature for 60 min. After the treatment, the samples were washed with large quantity of deionized (DI) water and dried at room temperature.
2. The fibers (500 mg) were placed in an aqueous ethanol solution (1:1 v/v) for 2 h and washed with large amount of DI water. After that the fibers were immersed in in 0.04 mol L^{-1} HMD/isopropanol solution for 10 min. The aminolized fibers were then rinsed with DI water for 24h.

Grafting the Amine-Functionalized Fiber Samples with FA

Prior to the coupling process, 250 mg of FA was dissolved in aqueous ethanol solution (1:1 v/v) and placed in an ice-water bath equipped with a magnetic stirrer. The FA was subsequently activated by adding 169 mg of EDC and later on 102 mg of NHS. The aminolyzed fibers were immersed in the activated FA solution, stirred in ice-water bath for 30 min and kept to react at room temperature over 24 h. After that time FA-grafted fibers were rinsed several times with ethanol and dried at room temperature. The method is based on the approach suggested by Chuysinuan *et al.*[23] with major changes (authors use different base material and active agent).

Test Dyeing

Initially the aminolysis effects were assessed using a quick, but efficient method of test dyeing. The used dye, Acid violet 6B, easily bonds to amino groups. Hence, it can be used to analyse the presence of amino groups on the fiber surfaces. Amine-functionalized and unmodified reference fiber samples were immersed in 0.1% Acid violet 6B solution at pH = 3 and stirred at 45 °C for 30 min. Afterwards the samples were washed several times with distilled water and dried at room temperature.

Surface Chemical Composition

Surface chemical compositions were determined by XPS analysis using an Axis Ultra photoelectron spectrometer (Kratos Analytical, Manchester, UK). The spectrometer was equipped with a monochromatic Al-Kα ($h\nu = 1486.6$ eV) X-ray source of 300 W at 15 kV. The kinetic energy of photoelectrons was determined with a hemispheric analyzer set to pass energy of 160 eV for wide-scan spectra and 20 eV for high-resolution spectra. During all measurements, a low-energy electron source combined with a magnetic immersion lens was used to prevent the electrostatic charging of the sample. Later, all recorded peaks were shifted by the same required value to set the C 1 s peak to 285.00 eV. The quantitative elemental compositions were determined from peak areas using experimentally determined sensitivity factors and the spectrometer transmission function. Spectrum background was subtracted according to Shirley.[27] The high-resolution spectra were deconvoluted with the Kratos spectra deconvolution software. The free parameters of the component peaks were their binding energy, height, full width at half maximum, and the Gaussian–Lorentzian ratio.

For the analysis, the fiber samples were measured as bundles. To prevent artifacts arising from the sample holder, the bundles were prepared over a hole in the sample holder.

Total Phenolic Content

The total phenolic contents of unmodified and modified fibers was assessed using the slightly modified Folin–Ciocalteu method.[22] Hundred milligrams of fibers, 1 mL of Folin–Ciocalteu reagent, and 10 mL of distilled water were placed in a volumetric flask and stirred for 3 min. Afterward 4 mL of 2% Na_2CO_3 and 10 mL of water were added to obtain the final volume of 25 mL. Then, the solution was kept at room temperature for 48 h. Employing a Perkin-Elmer Lambda 2 UV-Vis spectrometer (PerkinElmer Inc., Waltham, MA) the absorbance of the prepared samples was determined at 760 nm and compared to a reference sample of unmodified fibers prepared under the same reaction conditions. To determine the amount of phenolic groups on the surface of the fibers, a calibration curve was prepared using standard solutions containing 5 to 25 μmol L^{-1} FA. The results were expressed as micromoles of FA per gram of fibers (μmol g^{-1}).

Antioxidant Activity

The antioxidant activities of neat and modified fibers were evaluated using the slightly modified DPPH assay.[21] Each sample (100 mg of fibers) was dissolved in 10 mL of a 7:3 v/v DCM/DMF solution. Then, the solution was diluted with 10 mL of methanol. 1 mL of each sample was transferred to separate vials. To each of the vials 3 mL of ethanol solution of DPPH (100 μmol L^{-1}) was added and the mixture was incubated in darkness at room temperature for 30 min. After incubation the absorbance (A_{sample}) of the solution was spectrophotometrically measured at 517 nm with a Perkin-Elmer Lambda 2 UV-vis spectrometer (PerkinElmer Inc., Waltham, MA). The absorbance of a solution made from nonmodified sample ($A_{control}$) was also measured as a reference. The antioxidant activity (% AA) was expressed as the percentage of the absorbance value for DPPH radical species which was decreased in comparison with that of the reference sample, according to the following equation:

$$\%AA = \frac{A_{control} - A_{sample}}{A_{control}} \cdot 100\% \quad (1)$$

The correlation between the total phenolic content on the surface and the fibers' antioxidant activity was determined using Pearson's correlation coefficient.

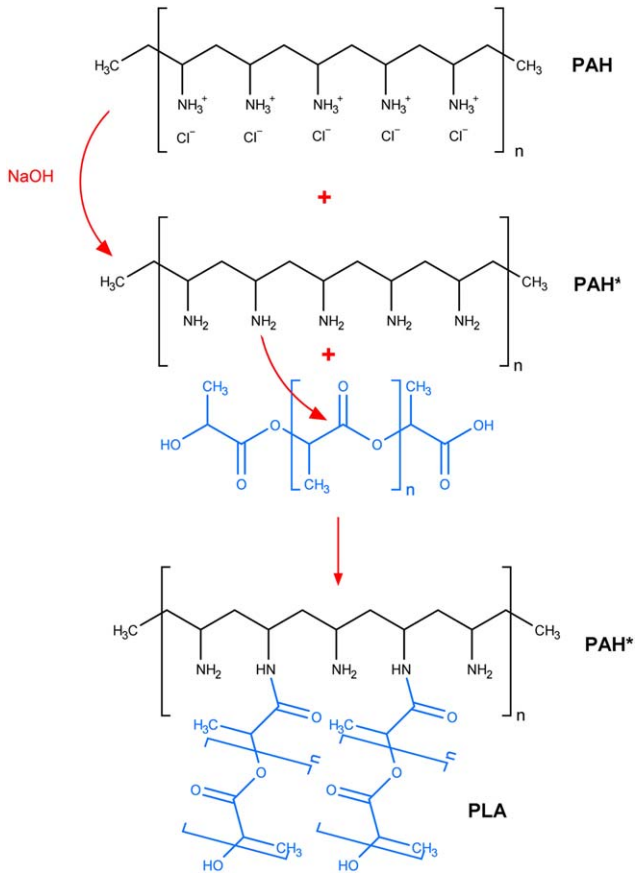

Figure 1. Schematic overview of the aminolysis of the PLA fibers' surface with PAH.[30] [Color figure can be viewed at wileyonlinelibrary.com]

SEM Microscope Imagining

The microscope imagining of fiber's surface was performed by using FEI NOVA NanoSEM 230 Scanning Electron Microscope (FEI Company, Hillsboro, OR), equipped with an EDAX Apollo SDD EDS detector (FEI Company, Hillsboro, OR).

Static Water Contact Angle

Static water contact angles of neat and modified PLA fibers were measured at room temperature using a Krüss DSA 100 drop shape analyzer (Krüss GmbH, Hamburg, Germany). From each fiber sample, three square plates with fibers arranged in parallel were produced. Twenty droplets (10 μL) of distilled water (surface tension 72.8 mN m^{-1} at 23 °C) were placed randomly at different positions on the flat surface of the plates. After setting the droplets on the surface and after no noticeable change in their shapes, the projected images of the droplets' shape were analyzed to calculate the static contact angle values (θ).

Hygroscopicity

The hygroscopicity of unmodified and modified samples and also HMD and PAH was determined using the desiccator method, according to Polish standard PN-P-04635:1980—"Textiles—Testing methods—Determination of Hygroscopicity."[28] The modified samples were placed in two desiccators, one with relative humidity (RH) of 45%, second with RH of 100% for 48 h. After adjusting the moisture balance, which was characterized by constant masses, the samples were weighted with an accuracy of 0.0001 g ± 1 g. The hygroscopicity (H) was calculated according to eq. (2).

$$H = \frac{m_w - m_d}{m_d} \cdot 100\% \quad (2)$$

where m_d was the mass of the sample placed in 45% RH, and m_w was the mass of the sample placed in 100% RH.

Tensile Strength

The tensile strength of the fibers after aminolysis was measured by an Instron 5544 single-column electromechanical testing system (Instron, Norwood, MA) with a maximum load of 2 kN. The instrument was equipped with a computer control unit and grips specially designed for fibers. The measurements were performed according to ISO 5079:1995 standard "Textile fibers—Determination of breaking force and elongation at break of individual fibers"[29] with crosshead speed of 1 mm/min. For each fiber 20 measurements were performed.

RESULTS AND DISCUSSION

To facilitate the reaction between PLA fibers surface and FA, an introduction of amine groups was necessary. It was obtained by aminolysis of fibers surface with HMD and PAH.

Aminolysis

In the first step of modification, PAH and HMD were both attached to the PLA fibers surface by aminolysis.

As it has been mentioned earlier, due to the protonation of the amino groups the native PAH does not react with the native PLA. Thus, NaOH at pH = 11.2 was employed to convert the amino hydrochloride groups (—$N^+H_3 \cdot Cl^-$) into primary amino groups (—NH_2). Since the formation of carboxylic amides is thermodynamically preferred, the moieties of the amino groups along the polyallylamine molecules are able to react with carboxylic ester groups of the PLA fibers. During that aminolysis reaction, stable amide linkages (—CONH—) between the PAH* and the PLA fibers are formed. A large part of the amino group is not involved in the linking reaction and remains available for further functionalization (Figure 1). Similar aminolysis mechanisms are present in the reaction between HMD and surface of poly(lactic acid) fibers. The end groups of poly(lactic acid) macromolecules react with the diamines, forming stable amide bonds.

The presence of amino groups was assessed by the simple and quick Acid violet 6B dyeing test. The different coloration shown in Figure 2 clearly indicates that different amounts of

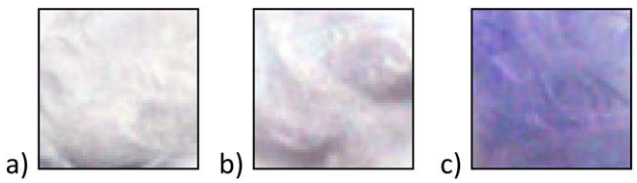

Figure 2. Photographs of PLA fiber samples after carrying out test dyeing with Acid violet 6B: (a) neat fibers without amino groups on the sample surface, (b) fibers aminolyzed with HMD, and (c) fibers aminolyzed with PAH*. [Color figure can be viewed at wileyonlinelibrary.com]

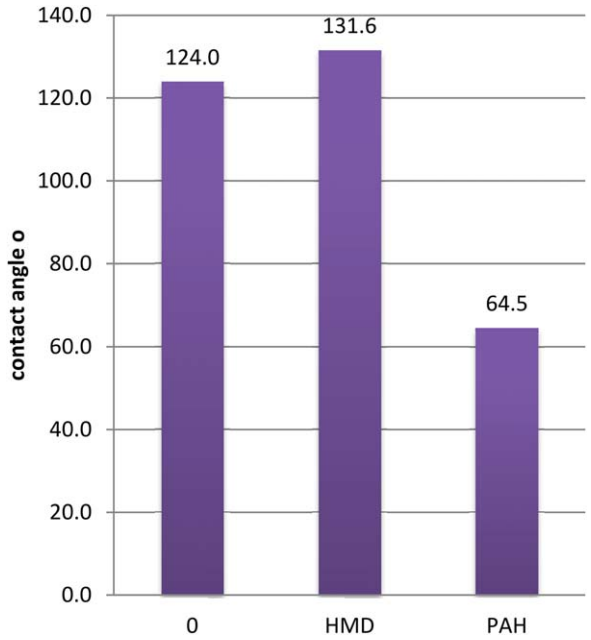

Figure 3. Results of the static contact angle measurements carried out on neat (0), HMD-modified (HMD), and PAH-modified (PAH) fiber samples. [Color figure can be viewed at wileyonlinelibrary.com]

amino groups are present on the PLA fiber's surfaces that have reacted with PAH*. Also sample treated with HMD exhibit slight coloration allowing to conclude, that the reaction was also successful.

XPS results confirmed the findings of the simple test dyeing. Particularly, in contrast to the unmodified PLA fibers the wide-scan spectra recorded from the HMD- and PAH-modified fiber samples showed the presence of nitrogen (N 1 s peaks in Figure 8). Additionally, the PAH-functionalized sample also contained considerable amounts of chlorine (Cl 2p and Cl 2 s peaks), which results from adsorbed hydrochloride ions.

Despite the polar carboxylic ester groups in the backbone the PLA polymer the unmodified fiber samples had a clearly hydrophobic surface (Figure 3). The application of HMD did not increase the hydrophilicity. Rather, the water contact angle has been slightly increased. As confirmed by the XPS and the dyeing test results, the amount of HMD bonded to the PLA surface is rather small. Furthermore, we have to remember, that only some part of amine groups is accessible on the surface, the rest was used for amide bonds creation. It seems to be also necessary to evaluate the contribution of the surface topography to the contact angle value measured. According to the model by Wenzel[31] for a hydrophobic surface the static contact angle value is increased by increasing the surface roughness, but the surface roughness reduces the contact angle value measured on a hydrophilic surface.

The attachment of the PAH* polymer significantly decreased the contact angle of the fiber sample surface—to 64.5° measured on the PAH*-modified sample. From the XPS results, it can be concluded that a considerable amount of primary amino groups were present on the fiber surface. These polar groups are accessible to water, and due to their intrinsic hydrophilic character they can be solvated. The steric accessibility of the amino groups is also a key requirement for their successful reaction with the FA molecules.

The determination of hygroscopicity using the desiccator method confirmed the results of the contact angle measurements. While the unmodified fiber sample consumed only traces of water the amine-modified samples showed higher capacities to absorb water vapor (Figure 4). The high hygroscopicity of the PAH*-modified fiber sample is coherent with the low contact angle and the hydrophilic character found for the fiber surface. The results of wetting and hygroscopicity measurements were visibly different for the HMD-modified sample. The applied polar groups enabled the absorption of water from the atmosphere, but they did not increase the wettability of the fiber sample. Obviously, a small amount of applied amino groups cannot change the intrinsically hydrophobic properties of the unmodified fiber surface into a surface with hydrophilic properties. According to the model proposed by Wenzel, the increase of surface roughness would contribute to the hydrophobic character of the sample surface. Actually, scanning electron micrographs of the aminolyzed fibers showed small changes in the topography of the fiber surface. Compared to the unmodified fiber, the aminolyzed fiber's surface is characterized by a slightly increased roughness (Figure 5). Due to the modification, in the case of intrinsically hydrophobic surfaces, the roughness increased the contact angle value (HMD-modified sample) while the capillary of the intrinsically hydrophilic surface increased the wettability of the fiber sample (PAH-modified sample).

In order to study the influence of the roughness on the mechanical properties of aminolyzed PLA fibers the tensile strength was

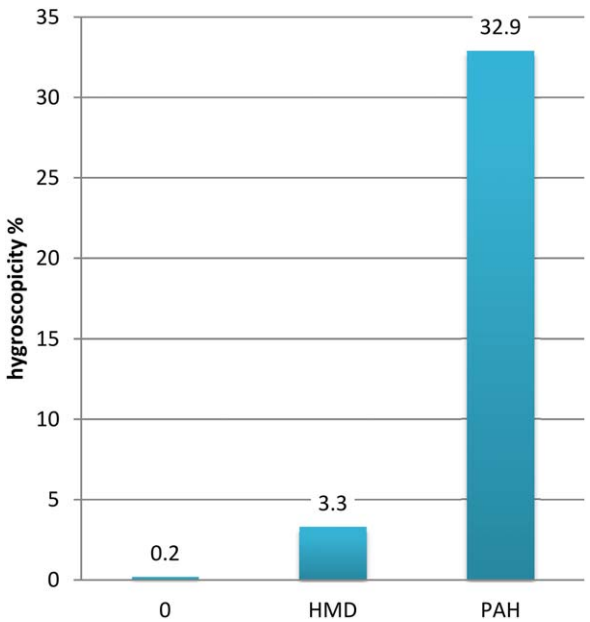

Figure 4. Results of the hygroscopicity measurements carried out on unmodified (0), HMD-modified (HMD), and PAH-modified (PAH) fiber samples. [Color figure can be viewed at wileyonlinelibrary.com]

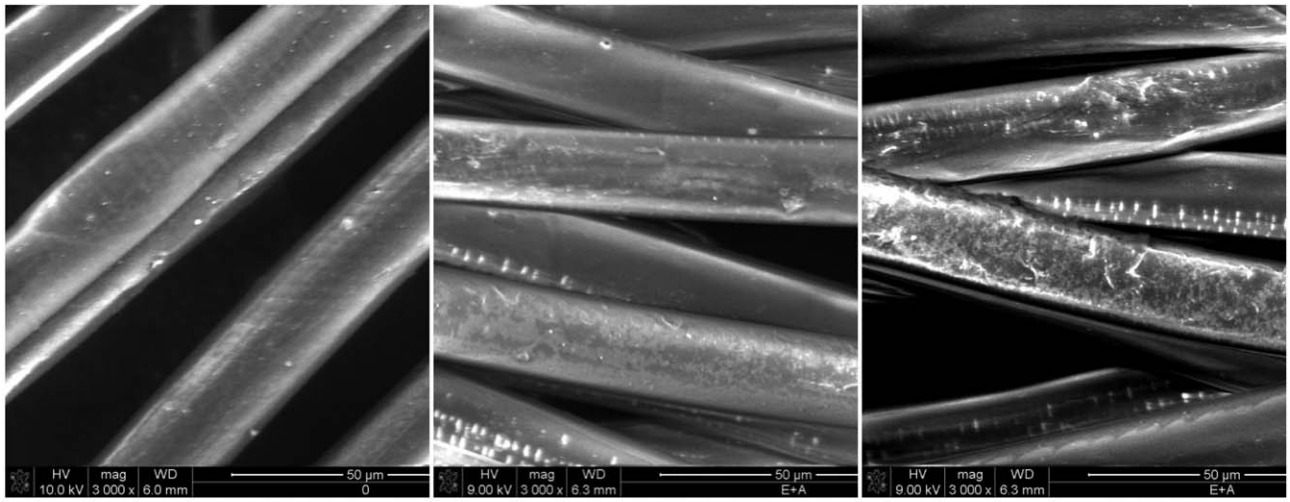

Figure 5. Scanning of electron micrographs (SEM) of fibers before and after the aminolysis (magnification ×3000).

measured. As shown in Figure 6 the modification with amines did not significantly affect the tenacity and elongation.

Modification of Aminolyzed Fibers with FA

To add antioxidant and anti-inflammatory properties to the fibers, the natural antioxidant FA, was attached to the fibers' surface. The reactivity of the carboxylic group of FA was activated sequentially with EDC and NHS and reacted with amino moieties of the PAH* and HMD pre-modified fiber surfaces (Figure 7).

The fiber samples obtained were comprehensively characterized by various analytical and physicochemical methods as well as application tests.

Elemental Composition of the Fiber Surface. The XPS method allows the qualitative and quantitative analysis of the elemental composition of layers that form the surface or are close to the surface. Its maximum information depth is not more than 8 nm. Hence, XPS was used to evaluate the success of the modification reactions carried out on the PLA fiber's surface. Figure 8 shows the wide-scan and high-resolution C 1 s and N 1 s element spectra, which were recorded from the differently modified fiber samples. As expected, the unmodified PLA fiber did not contain any traces of nitrogen. Trace amounts of nitrogen found in the modified fiber samples resulted from the aminolysis carried out with HMD ($[N]:[C]|_{spec} = 0.011$) and PAH ($[N]:[C]|_{spec} = 0.014$).

According to the chemical structure of PLA, the C 1 s spectrum should show four component peaks. However, a fifth component peak was necessary to deconvolute the C 1 s spectrum and fit the sum curve over all component peaks to the curve recorded. Component peak *A* arose from saturated hydrocarbons, such as the methyl groups $-{}^{A}CH_3$ of the PLA molecules. Component peak *E* showed the carbonyl carbon atoms of the carboxylate ester groups ($O={}^{E}\underline{C}-O-C$). The corresponding alcohol-sided carbon atoms ($O={}^{E}\underline{C}-O-{}^{C}\underline{C}$) contributed to component peak *C*. For esters, the intensity of that component peak should equal the intensity of component peak *E*. The excess of component peak *C* resulted from the presence of nonesterified OH groups. Also, component peak *F*, having an intensity equal to the excess of component peak *C*, showed the presence of nonesterified groups, namely carboxylic acid groups ($O={}^{F}\underline{C}-OH$) and their corresponding carboxylates ($O={}^{F}\underline{C}-O^{-}\leftrightarrow{}^{-}O-{}^{F}\underline{C}=O$). Carbon atoms in α-position to the carbonyl carbon of the ester groups ($-{}^{B}\underline{C}-{}^{E}COO$) were separated as component peak *B*.

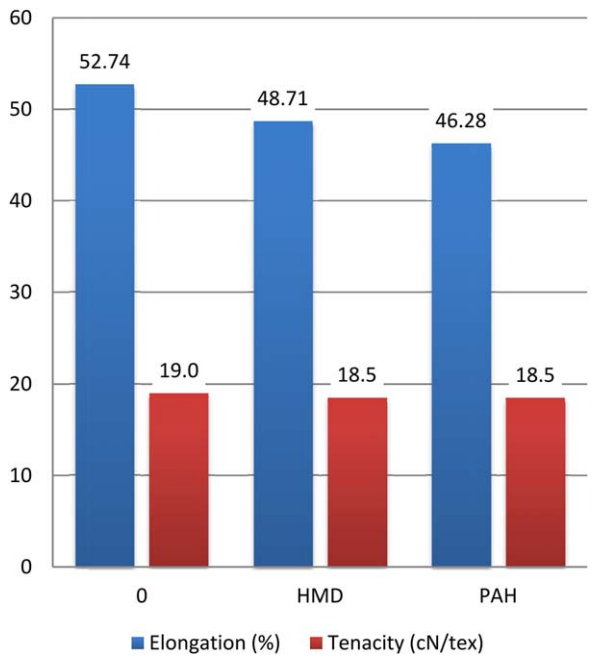

Figure 6. The mechanical properties of the unmodified (0), HMD-modified (HMD), and PAH-modified (PAH) fiber samples. Standard deviations for tenacity stand at: 0: 1.04; HMD: 1.34; PAH: 1.58, for elongation at break: 0: 3.95; HMD: 4.87; PAH: 4.49. [Color figure can be viewed at wileyonlinelibrary.com]

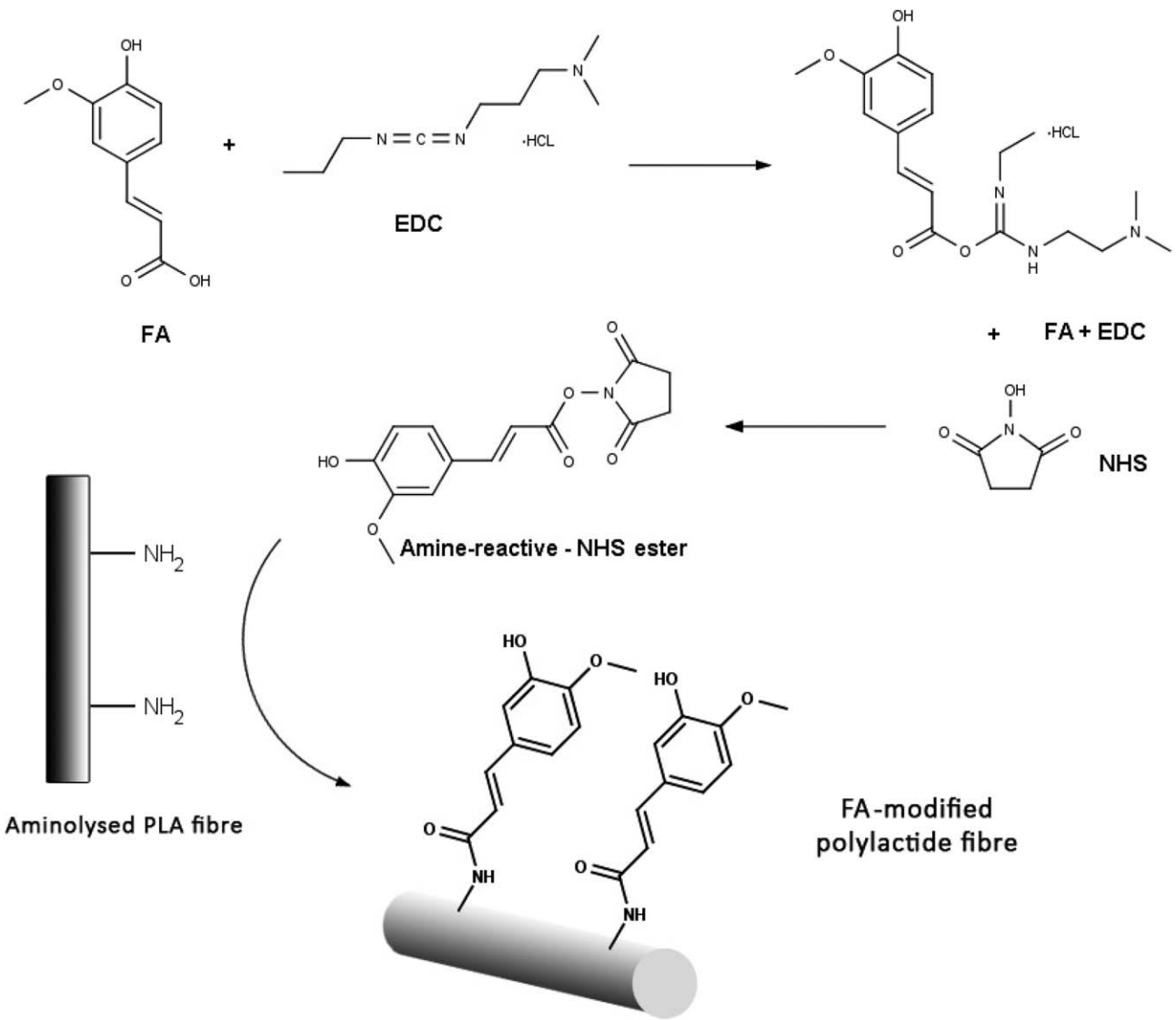

Figure 7. Scheme of the reaction between FA and PLA fibers endowed with amino moieties (—NH_2). The reactivity of the carboxylic groups of the FA molecules was improved by their reaction with EDC or NHS.[30]

As mentioned above, the aminolysis with HMD and PAH introduced moietes of nitrogen-containing groups onto the fiber surface. Photoelectrons that escaped from the carbon atoms of the amine-like ^{B}C–N bonds contribute to component peak *B*. Both of the two C 1 s spectra recorded from the modified fiber samples showed an additional component peak *D* representing amide groups (O=^{D}C–NH–^{B}C), which were formed by the aminolysis of the PLA fibers and the subsequent reactions of the non-reacted amino groups with the carboxylic acid groups of FA. In the case of the HMD-modified PLA fiber sample, the area share of the component peak *D* excellently agreed with the [N]:[C]$|_{spec}$ ratio, which was separately determined from the wide-scan spectrum. All amino groups of the HMD molecules attached to the fiber surface were involved in the aminolysis and the subsequent FA coupling. Corresponding to these findings, the N 1 s spectrum [Figure 8(b), right column] is characterized by a single component peak *K* representing the nitrogen atoms of the amide groups (O=C–^{K}NH–C). The N 1 s spectrum recorded from the PAH-modified fiber sampled showed two nitrogen-containing species. As mentioned, component peak *K* represented the amide groups, which resulted from the aminolysis and the FA coupling. The second component peak *L* appeared due to photoelectrons escaping from nitrogen atoms of protonated amino groups (C–^{L}N^{+}H$_2$), which were not involved neither in the aminolysis nor the FA coupling reaction, thus it proves the incomplete conversion of PAH. Hence, in the corresponding C 1 s spectrum, the intensity of component peak *D* was clearly lower than the [N]:[C]$|_{spec}$ ratio.

Total Phenolic Content. The Folin–Ciocalteu test is one of the most popular methods used to determine the total phenolic content. It utilizes the Folin–Ciocalteu reagent, which is a mixture of sodium molybdate (Na_2MoO_4), sodium tungstate

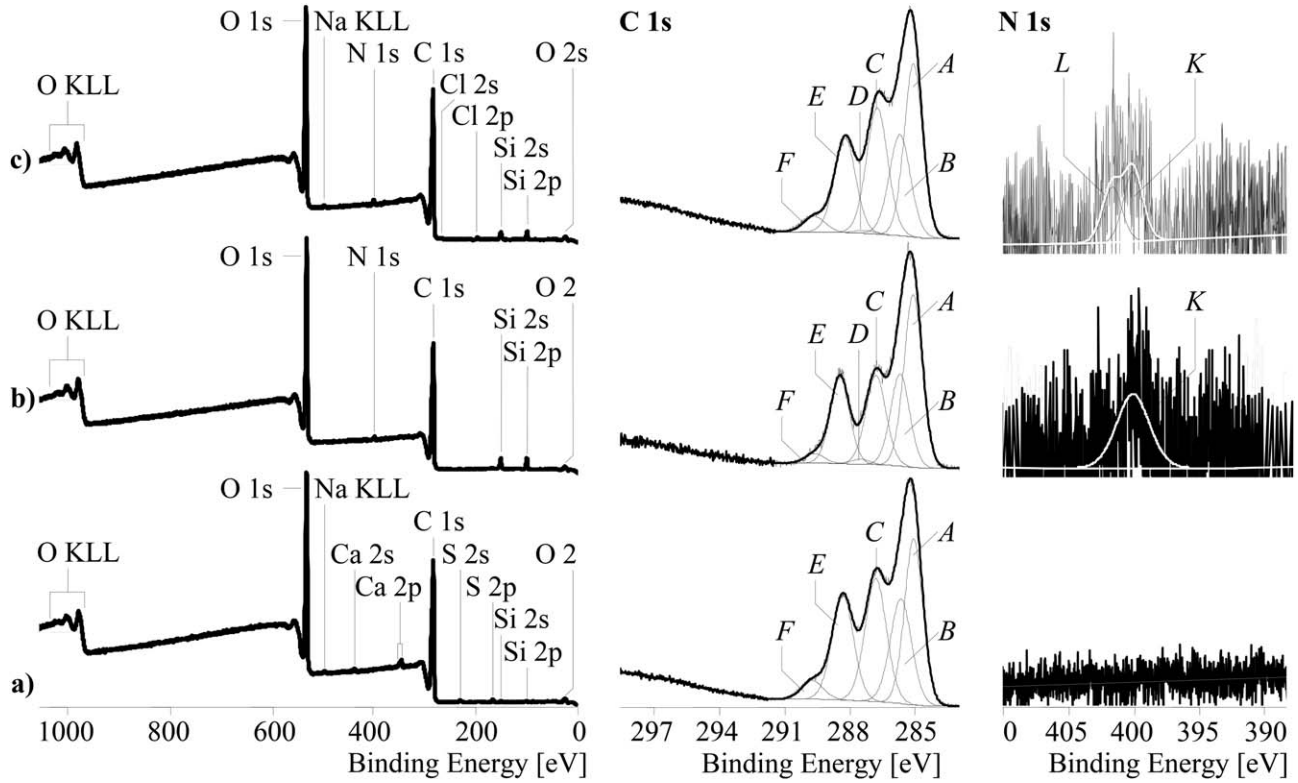

Figure 8. XPS survey (left column), C 1 s (middle column), and N 1 s (right column) high-resolution element spectra of unmodified (a), HMD + FA-modified (b), and PAH + FA-modified (c) PLA fiber samples.

(Na_2WO_4), lithium sulfate (Li_2SO_4), bromine water, and concentrated hydrochloric and phosphoric acids. After the reaction with phenolic moieties, the yellow Folin–Ciocalteu reagent changes to blue, with an absorption maximum between 475 and 450 nm. According to the Beer–Lambert law the absorbance is proportional to amount of phenolic moieties in the sample. Hence, the Folin–Ciocalteu test was employed to quantify the amount of FA, which was attached to the amine-modified PLA fiber samples. Table I showed that the pre-treatment of the PLA fiber with PAH was more effective than the HMD-modification. Comparing the Folin–Ciocalteu test results with the results of the dyeing tests of the differently aminolyzed fiber samples (Figure 2) shows a clear correlation between the amounts of available amino groups and reaction efficacy.

Antioxidant Properties of the Modified Samples. From the point of practical application of fiber samples as wound dressings their antioxidant activities seem to be the most crucial property. The antioxidant activities of the differently modified PLA fiber samples were evaluated by DPPH* scavenging assay. This method allows to determine the percentage of free radicals scavenged by the sample. The antioxidant activity of the fiber sample pre-treated with PAH is about 30% higher than for the HMD-pre-treated sample (Table II).

Hydrophilicity of Obtained Fibers. The wetting behavior of the fiber samples modified with FA has been investigated by measuring contact angle and hygroscopicity. The contact angles as well as hygroscopicity data were not significantly affected by the additional modification step (HMD + FA: $\theta = 135.6°$; hygroscopicity = 4.97%, and PAH + FA: $\theta = 76.5°$; hygroscopicity = 44.92%). The values indicate that the hydrophilic properties of the fibers samples can be mainly controlled by amine modification.

Table I. Results of Folin-Ciocalteu Tests for PLA Fiber Samples Grafted with FA

	Absorbance at λ = 760 nm	Total phenolic content (μmol/g of fibers)
Unmodified	0.0000	**0.0**
HMD + FA	0.6941	**15.2**
PAH + FA	2.6910	**35.2**

Table II. Results of DPPH* Assay for Samples Grafted with FA

	Absorbance at 517 nm	Antioxidant activity (%)
Unmodified	0.4352	**0.0**
HMD + FA	0.1592	**63.4**
PAH + FA	0.0306	**93.0**

CONCLUSIONS

The application of FA as modifier on the surface of PLA fibers equipped them with antioxidant properties. In both methods the samples exhibited significant increase in their antioxidant activity, however, only the PAH approach allowed to obtain a highly hygroscopic material. Also, in the case of PAH, the described synthetic route is based on the use of biocompatible and noncytotoxic materials, such as PLA as substrate material, PAH and naturally occurring FA. By using XPS in combination with the Folin–Ciocalteu and DPPH* test, it was possible to analyze the presence of FA on the surface of the fiber samples. The antioxidant activity of the FA molecules grafted on PAH-modified PLA fibers was increased up to 93%, 63,4% in the case of HMD. Furthermore, the application of the biocompatible PAH also significantly improved the hydrophilicity of the fibers surface, while retaining their mechanical properties. The surface modification of PLA fibers with PAH and the subsequent FA grafting has shown a potential for an active wound dressing material of excellent performance when it comes to reducing inflammation.

For drawing, displaying and characterizing chemical structures, substructures and reactions, Marvin software was used, Marvin 16.9.26.0, 2016, ChemAxon (http://www.chemaxon.com)

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