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RESEARCH PAPER

Polylactic acid (PLA)/Silver-NP/VitaminE bionanocomposite electrospun nanofibers with antibacterial and antioxidant activity

Bogdanel Silvestru Munteanu · Zeynep Aytac · Gina M. Pricope · Tamer Uyar · Cornelia Vasile

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Abstract The antibacterial property of silver nanoparticles (Ag-NPs) and the antioxidant activity of Vitamin E have been combined by incorporation of these two active components within polylactic acid (PLA) nanofibers via electrospinning (PLA/Ag-NP/VitaminE nanofibers). The morphological and structural characterizations of PLA/Ag-NP/VitaminE nanofibers were performed by Scanning Electron Microscopy (SEM), Transmission Electron Microscopy and X-ray diffraction. The average fiber diameter was 140 ± 60 nm, and the size of the Ag-NP was 2.7 ± 1.5 nm. PLA/Ag-NP/VitaminE nanofibers inhibited growth of *Escherichia coli*, *Listeria monocytogenes* and *Salmonella typhymurium* up to 100 %. The amount of released Ag ions from the nanofibers immersed in aqueous solution was determined by Inductively Coupled Plasma Mass Spectrometry, and it has been observed that the release of Ag ions was kept approximately constant after 10 days of immersion. The antioxidant activity of PLA/Ag-NP/VitaminE nanofibers was evaluated according to DPPH (2,2-diphenyl-1-picrylhydrazyl) method and determined as 94 %. The results of the tests on fresh apple and apple juice indicated that the PLA/Ag/VitaminE nanofiber membrane actively reduced the polyphenol oxidase activity. The multifunctional electrospun PLA nanofibers incorporating Ag-NP and Vitamin E may be quite applicable in food packaging due to the extremely large surface area of nanofibers along with antibacterial and antioxidant activities. These materials could find application in food industry as a potential preservative packaging for fruits and juices.

Keywords Electrospinning · Nanofiber · Polylactic acid (PLA) · Silver nanoparticles · Vitamin E · Antibacterial · Antioxidant

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Introduction

Electrospinning is a cost-effective method, applicable for a wide range of polymers in solution or in melt state (Wendorff et al. 2012; Ramakrishna 2005; Doshi

and Reneker 2007), by which nanofibers/nanowebs with very high surface/volume ratio and nanoporous structures can be obtained, which allow the entrapment of bioactive molecules. The electrospun nanofibers are used in various application areas including filtration, wound dressing, tissue scaffold, drug delivery, energy, catalysis, sensors, environmental, agriculture, and etc. (Wendorff et al. 2012; Ramakrishna 2005) and also proved to have potential applications in food and food packaging as building/reinforcement element of environmentally friendly composites for food packaging material and as building elements of the food matrix for imitation/artificial foods. The electrospinning of nanofibers from biopolymers, biocompatible polymers, or edible polymers is often chosen for food and food packaging applications (Kayaci and Uyar 2012; Kayaci et al. 2013a, 2013b; Fernandez et al. 2009).

In addition, electrospun nanofibers incorporating active agents with antibacterial and/or antioxidant properties which are required for food packaging have been investigated (Kayaci and Uyar 2012; Kayaci et al. 2013a, 2013b; Fernandez et al. 2009). For food safety, such functionality will prevent the invasion of bacteria and micro-organisms and will have a significant impact on shelf-life extension and food safety of products. For instance, high temperature stability and slow release of volatile food additives such as vanillin (Kayaci and Uyar 2012) and eugenol (Kayaci et al. 2013a) by cyclodextrin inclusion complexation encapsulated in electrospun polyvinyl alcohol (PVA) nanofibers were achieved. Glucose oxidase activated the lactoperoxidase (a naturally occurring antimicrobial system in milk) when immobilized in PLA nanofibers (Zhou and Lim 2009).

As regards to biomedical field, materials with antibacterial and antioxidant properties have applications in wound healing (Lee et al. 2007), tissue engineering, as implant coating films, catheters, urethral and ureteral stents, urological implants, and other medical devices. Antibacterial activity against *Staphylococcus aureus* (*S.aureus*) and *Escherichia coli* (*E.coli*) of polyurethane composite nanofibers containing ciprofloxacin, a fluoroquinolone antibiotic was studied (Choi et al. 2013). The incorporation of curcumin into ultra-fine cellulose acetate nanofibers imparted the antioxidant activity to the resulting fiber mats (Suwantong et al. 2010). Curcumin-loaded polycaprolactone nanofiber matrix is bioactive and has potential as a wound dressing with antioxidant and anti-inflammatory properties (Merrell et al. 2009).

PLA is compatible with many foods, such as dairy products, beverage, fresh meat products, and ready meals. Electrospun PLA nanofibrous webs incorporating triclosan/cyclodextrin inclusion complexes have shown efficient antibacterial activity against Gram positive and Gram negative bacteria (Kayaci et al. 2013b). With regards to biomedical applications, nanofibrous mats from poly(L-lactide) (PLLA) and gelatin/PLLA solutions showed controlled evaporative water loss, promoted fluid drainage ability, and excellent biocompatibility with application as wound dressing (Gu et al. 2009).

Silver nanoparticles (Ag-NPs) can be used as a broad-spectrum antibacterial agent for both Gram positive and Gram negative bacteria in biomedical and food packaging applications. Because of their high reactivity originating from the large surface to volume ratio, Ag-NP can effectively eliminate bacteria and yeasts even at rather low concentrations (Gangadharan et al. 2010). A very low concentration of silver (as low as 1.69 μg/mL Ag) gave antibacterial performance in the study of Panácek and others (Panácek et al. 2006). Furthermore, antibacterial activity of Ag-NP was found to be dependent on the size of silver particles, since the only nanoparticles that present a direct interaction with the bacteria preferentially have a diameter of approximately 1–10 nm (Morones et al. 2005). The smaller particle size provides improved antibacterial activity (Espinosa-Cristóbal et al. 2009).

Antibacterial property of electrospun nanofibers containing Ag-NP was reported by numerous studies such as PLA/Ag-NP fibers against *S.aureus* and *E.coli* (Xu et al. 2006), poly(-ethylene oxide)/Ag-NP fibers intermixed with polyurethane fibers against *E.coli* (Tijing et al. 2012), polyacrylonitrile/Ag-NP fibers against Gram positive *Bacillus cereus* and Gram negative *Escherichia coli* micro-organisms (Shi et al. 2011), and nylon-6/AG-NP nanofibers against both Gram negative *Escherichia coli* and Gram positive *S.aureus* (Pant et al. 2012).

In this paper, antioxidant activity of Vitamin E—a fat soluble antioxidant (Traber and Atkinson 2007)—was combined with antibacterial property of Ag-NP in electrospun PLA nanofibers in order to obtain multifunctional biomaterials. Antibacterial activity of nanofibers was determined by well-known standard methods whereas the antioxidant activity of nanofibers

was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. In addition to antibacterial and antioxidant tests, nanofibers were characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), and inductively coupled plasma mass spectrometry (ICP-MS) as well.

Experimental

Materials

Polylactic acid (PLA, 2002D, Nature Works) was purchased from Nature Works, USA, with a number average molecular weight of 106 kDa and polydispersity index of 1.64 (determined by GPC). Vitamin E (α-tocopherol), $AgNO_3$ as silver salt, dichloromethane (DCM, extra pure), N,N-dimethylformamide (DMF, 99 %), methanol (extra pure), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich. The water used in experiments was distilled from a Millipore milli-Q ultrapure water system.

Preparation of silver nanoparticles (Ag-NPs)

$AgNO_3$ was mixed for 5 min in DMF and subsequently poured into the previously prepared 10 % (w/v) PLA solution in DCM. DMF and DCM were in the volumetric ratio of 3/7 (v/v). The concentration of $AgNO_3$ was adjusted to have 1 % (w/w, with respect to polymer) Ag-NP in the resulting PLA/Ag-NP/VitaminE nanofibers. After 48 h stirring in dark 5 % (w/w, with respect to polymer), Vitamin E in liquid form was added. After 80 min of the addition of Vitamin E, the solution became dark brown but not turbid. In this process, the reduction of silver salts by DMF can be monitored from the color change of the solution.

Electrospinning of nanofibers

The experimental set-up for electrospinning device consists of a direct current high voltage power supply (Spellman SL series), a metal plate collector, a syringe pump (Model: KDS 101, KD Scientific), and a syringe oriented with the needle perpendicular to the metal plate. The high voltage is applied between the metal plate and the syringe needle. The polymer solution was loaded into the syringe. The solution is extruded from the needle tip by the syringe pump at a constant flow rate. At the point of ejection (the needle tip) a polymer jet is formed as a result of the electric charge repulsion outgoing the solution surface tension. Electrospinning parameters for obtaining bead-free and uniform PLA nanofibers and PLA/Ag-NP/VitaminE nanofibers were adjusted as: flow rate 16.7 μL/min, needle tip-collector distance 10 cm, voltage 15 kV, and needle diameter 0.6 mm. Electrospinning was performed at 23 °C and at 18 % relative humidity in an enclosed Plexiglas box.

Electrospinning was also effectuated using various concentration of Vitamin E (from 0–5.5 %) and Ag-NP from 0.1–1.5 %). According to other experiments of ours, the increased Vitamin E concentration leads to significant decrease of viscosity, and also the capacity to obtain nanofibres is reduced (Dumitriu et al. 2014), while the increase of the Ag-NP concentration above 0.5–1 % affects the biocompatibility and cell proliferation (Vasile et al. 2013). After several trials, optima concentrations of 5 % Vitamin E and 1 % Ag-NP were chosen.

Measurements and characterization

Rheometer (Physica MCR 301, Anton Paar) equipped with a cone/plate accessory (spindle type CP 40-2) was used at a constant shear rate of 100 1/sec, at 22 °C to measure the viscosity of the solutions. The conductivity of the solutions was measured with Multiparameter meter InoLab® Multi 720 (WTW) at RT.

Scanning electron microscopy (SEM) images were recorded using a FEI—Quanta 200 FEG microscope. Prior to SEM imaging, nanofibers mounted on metal stubs with double-sided adhesive tape were coated with 5 nm Au/Pd (PECS-682) to minimize charging. The fiber diameter of nanofibers was measured directly from the SEM images ($n \geq 100$) and average fiber diameter (AFD) was calculated.

Transmission electron microscopy (TEM) images were taken using a FEI-Tecnai G2F30 microscope. HC200 grids were attached on the aluminum foil, and the nanofibers were directly electrospun onto the grids. TEM images were acquired in bright field, while scanning transmission electron microscopy (STEM) images were recorded in dark field.

X-ray diffraction (XRD) analysis of the nanofibers was performed by XRD (PANalytical X'Pert powder diffractometer) using Cu Kα (λ $K\alpha = 1.54$ Å) radiation in a 2θ range 10–70°.

Inductively coupled plasma mass spectrometry (ICP-MS) measurements were performed using a quadrupole Thermo Scientific XSERIES 2 ICP-MS ICP-MS instrument. 20 mg PLA/Ag-NP/VitaminE nanofibers immersed in 30 mL deionised water. At certain time intervals (for 20 days), 100 μL samples were extracted from PLA/Ag-NP/VitaminE nanofibers immersed aqueous solution, and equal amount of the water was refilled. 50 μL of this sample was diluted with 10 mL solution of 2 % nitric acid/water. Calibration curve was drawn in a concentrations range: 0.3, 0.6, 1.25, 2.5, and 5.0 ppb.

Antibacterial activity

Antimicrobial tests were effectuated according to standard methods SR ISO 16649-2/2007—Microbiology of alimentary and animal products. The experimental protocol for testing antimicrobial efficiency against *Escherichia coli*, *Salmonella typhymurium*, and *Listeria monocytogenes* consists in the following stages: sterilization of samples; ATCC culture bacteria contamination; inoculation and incubation performed for 24 and 48 h at 44 °C; and identifying target germs.

Sterilization of the samples was made in autoclave at 110 °C, 0.5 bars for 20 min.

Preparation of ATCC cultures was done by: seeding the average pre-enrichment and incubation at 37 °C for 24 h; counting the colonies in 0.1 mL culture by selective culture medium separation; and seeding of 0.1 mL bacterial culture ATCC using sterile swab samples surface.

Identifying target germs: The following standardized methods of bacteriology procedures were used, according to standards in force: SR ISO 16649—*Escherichia coli;* horizontal method for β -glucuronidase-pozitive *Escherichia coli* cuantification—Part 2: Colonies counting at 44 °C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide according to "Minerals Modified Glutamate Broth" (Cat. 1365) producing blue or green–blue collonies on agar glucuronide; SR EN ISO 11290—*Listeria monocytogenes;* SR EN ISO 6579/2003/AC/2004/AC/2006, Amd.1:2007—*Salmonella sp.*

Antioxidant activity

(a) DPPH method

The antioxidant activity test was carried out for PLA nanofibers and PLA/Ag-NP/VitaminE nanofibers. 3 mL of 10^{-4} M DPPH solution prepared in methanol and in the presence of the nanofibers the decrease in absorbance of DPPH solution at 517 nm after 30 min of reaction was measured via UV–VIS NIR Spectroscopy (Varian Cary 5000). The photographs of the solutions were taken after the measurement.

(b) The antioxidant activity of membranes was tested on fresh apple and apple juice by following both sensorial analysis and properties of juice as UV absorbance, pH and conductivity. A drop of apple juice was dropped on the PLA, PLA/Ag, and PLA/Ag/Vit E nanofiber membranes. At certain intervals (after 1, 2, 3, 4, 24, 48, and 72 h), 50 μL were extracted from the juice drop, and the pH, conductivity, and absorbance at 450 nm were recorded. As reference (blank) sample was used a juice drop placed on PET (polyethylene terephthalate) substrate. The pH and conductivity were measured using WTW InoLab pH/ION/Cond 750 multiparameter meter, and the absorbances were measured with a SPECORD 205-222A652 spectrophotometer.

Results and discussion

Preparation of silver nanoparticles (Ag-NPs) was done by reduction with N,N-dimethylformamide (DMF) which can act as a powerful reductant for silver salts (Pastoriza-Santos and Liz-Marzan 1999) and was explained in detail under materials and methods section. The reduction process proceeds at a meaningful rate even when performed at room temperature, in the dark and can be observed as a time changing yellow color of the solution due to the plasmon resonance absorption. Visual observation shows that, as the reaction takes place, the color shifts from light yellow to dark brown due to the plasmon resonance absorption (Pastoriza-Santos and Liz-Marzán 2000).

Morphology analysis of nanofibers

Scanning electron microscope (SEM) images and diameter distributions of polylactic acid (PLA) nanofibers and PLA/Ag-NP/VitaminE nanofibers are shown

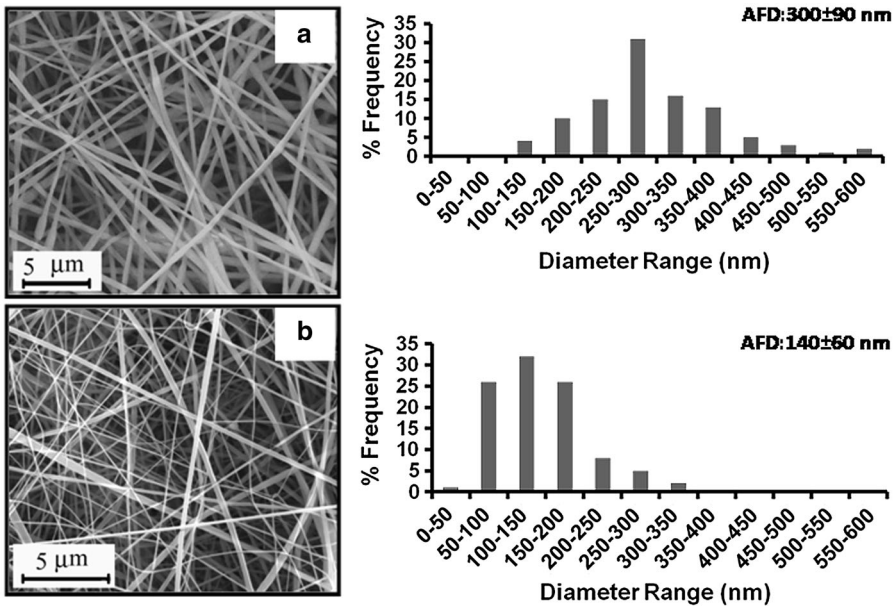

Fig. 1 Representative SEM images and fiber diameter distributions with average fiber diameter (AFD) of the electrospun **a** PLA and **b** PLA/Ag-NP/VitaminE nanofibers

in Fig. 1. The average fiber diameter (AFD) was smaller, and diameter distribution was narrower for PLA/Ag-NP/VitaminE nanofibers (140 ± 60 nm) (Fig. 1b) in comparison with AFD of PLA nanofibers (300 ± 90 nm) (Fig. 1a). The thinner diameter of PLA/Ag-NP/VitaminE nanofibers can be explained by the lower solution viscosity and higher solution conductivity of the PLA/Ag-NP/VitaminE solutions than PLA solution (Table 1) (Wendorff et al. 2012; Ramakrishna 2005; Uyar and Besenbacher 2008). The addition of $AgNO_3$ possibly enhances the charge density of the PLA solution. In addition, the presence of Ag-NP and Vitamin E in PLA solution probably disturbed the chain entanglements of the polymer chains and therefore lower the viscosity of the solution. Hence, PLA/Ag-NP/VitaminE solution having lower viscosity and higher conductivity compared to PLA solution (Table 1) yielded much thinner fibers because of the increased stretching of the jet during the electrospinning. This behavior is very common for the electrospinning of polymer solutions where the low viscosity and high conductivity resulted in much thinner fibers (Uyar and Besenbacher 2008). In addition, SEM images showed that the addition of Ag and Vitamin E did not deteriorate the fiber morphology of PLA nanofibers.

Table 1 The representative characteristics of solutions used for electrospinning (composition, viscosity, and conductivity) and of the obtained nanofibers (average diameter, morphology, and antioxidant activity)

	PLA	PLA/Ag-NP/VitaminE
PLA content[a] (w/v)	10	10
Ag content[b] (w/w)	–	1
Vitamin E content[b] (w/w)	–	5
Viscosity (Pa·s)	0.408	0.182
Conductivity (μS/cm)	8.82	65.00
Average fiber diameter (nm)	300 ± 90	140 ± 60
Fiber morphology	Bead-free nanofibers	Bead-free nanofibers
Antioxidant activity (%)	4	94

[a] With respect to solvent

[b] With respect to polymer (PLA)

Transmission electron microscopy (TEM) and scanning transmission electron microscopy (STEM) images of PLA/Ag-NP/VitaminE nanofibers and diameter distribution for the Ag-NP in PLA/Ag-NP/VitaminE nanofibers are presented in Fig. 2. The TEM

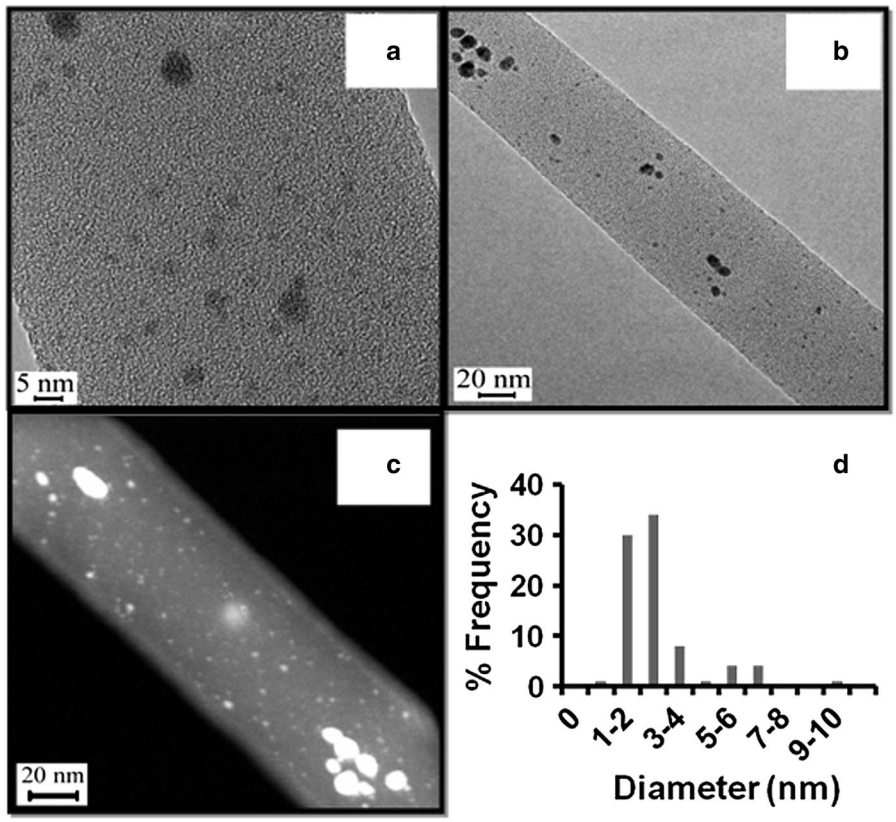

Fig. 2 Representative TEM images **a** higher magnification **b** lower magnification and **c** STEM image of PLA/Ag-NP/VitaminE nanofibers and **d** size distribution for the Ag-NP in PLA/Ag-NP/VitaminE nanofibers

and STEM images of PLA/Ag-NP/VitaminE nanofibers confirmed the presence of Ag-NP in the nanofiber matrix. In addition, Ag-NPs were mostly distributed uniformly within the nanofiber matrix along with few aggregations. The average size of the Ag-NP was determined as 2.7 ± 1.5 nm (Fig. 2c). Although the majority of the Ag-NP was around 2 nm, very few bigger Ag-NPs up to 9 nm were observed in STEM and TEM images (Fig. 2b, c).

Crystalline structure of nanofibers

X-ray diffraction (XRD) of PLA/Ag-NP/VitaminE nanofibers is given in Fig. 3. The XRD patterns of the PLA/Ag-NP/VitaminE nanofibers show the peak of metallic silver with face-centered cubic (fcc) structure. The peak at $2\theta = 38.1°$ corresponds to the three d-spacing (111) (He et al. 2002). The peak at 35.4° can be attributed to the strain-induced crystallization of PLA (Chen et al. 2011). Also, the crystalline phase of PLA nanofibers is responsible for appearance of the peaks at 16.7° (Tábi et al. 2010) and 27.8° (Srithep

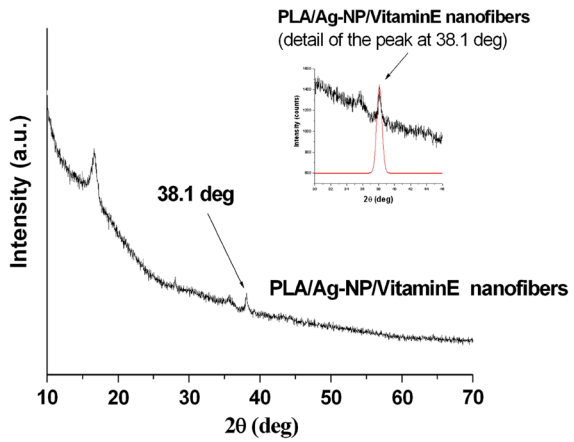

Fig. 3 XRD pattern of PLA/Ag-NP/VitaminE nanofibers with the Lorentz fitted profile

et al. 2013), respectively. The detail of the Lorentz fitted peak at 38.1° is shown as inset Fig. 3. The broad nature of this XRD peak can be attributed to the small dimensions (nano-size) of the Ag particles.

Debye-Sherrer formula was used to estimate the average size of the Ag-NP

$$D = \frac{K \cdot \lambda}{B \cdot \cos\theta}, \quad (1)$$

where λ is the wavelength of the X rays (Cu λ $K\alpha = 1.54$ Å); K is a dimensionless constant that may range from 0.89 to 1.39 depending on the specific geometry of the scattering objects; B is the width of the XRD peak at half maximum; θ is the diffraction angle; and D is the size of the crystalline domains. For $K = 0.9, B = 0.043$ rad, $2\theta = 38.09°$, $\lambda = 0.154$ nm the formula yields for D approximately 4.0 nm. We can assume that the size of the silver nanoparticles is equal or higher than 4.0 nm, which is well correlated with the value obtained from TEM images.

The release of Ag-NP from nanofibers

The released amount of Ag ions into aqueous solution was determined by inductively coupled plasma mass spectrometry (ICP-MS). The concentration of released silver ions in deionized water was in 0.13–0.44 ppb (μg/L) range after 10 days of fiber immersion; and the concentration of released silver ions at the end of the experiment (20 days) is 0.47 ± 0.009 ppb (Fig. 4). A prolonged and constant rate release of silver cations can inhibit the growth of bacteria when their concentration is above 0.1 μg/L (ppb) (Joyce-Wöhrmann et al. 2000; Joyce-Wöhrmann and Münstedt 1999; Wuhrmann and Zobrist 1958). It can be assumed that at least a partial antibacterial protection is also assured in surrounding biological fluid. Due to the fact that the most silver remains in fibers after immersion, the antibacterial effect of such fibers will be mainly confined to the bacteria adhering intimately to the PLA fibers (Parolo et al. 2011). Moreover, the European Food Safety Agency (EFSA) stated that in order to attain antibacterial effects, migrated ions ought to be in the range of the legal limit of 50 μg Ag^+/kg food (Fernandez et al. 2010). Thus, our material could be safe for food packaging because its release is lower than legal limit. It can be supposed that the antibacterial activity of such nanofibers will be limited to the media in direct contact with the fibers.

Antibacterial activity of nanofibers

The results of the antimicrobial testing of the studied nanocomposites against *Escherichia coli*, *Salmonella typhymurium*, and *Listeria monocytogenes* are presented in Table 2 and Fig. 5.

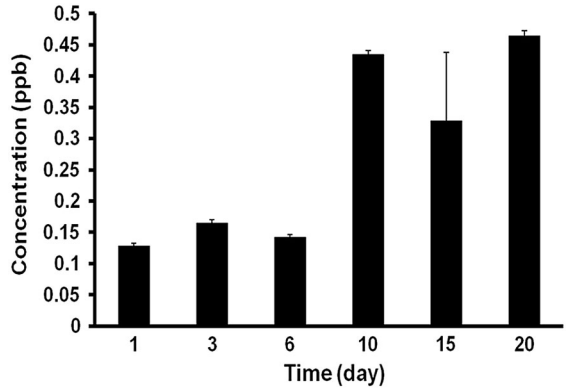

Fig. 4 The amount of silver released in aqueous solution measured by ICP-MS

Table 2 The bacteria growth inactivation in the presence of PLA nanocomposite films, in percent of the control

Sample	% Inhibition 24 h	% Inhibition 48 h
Escherichia coli		
PLA	34.9	52.7
PLA/Ag	93	99
PLA/Ag/VitaminE	87	100
Listeria monocytogenes		
PLA	30.8	40.4
PLA/Ag	100	100
PLA/Ag/VitaminE	100	100
Salmonella typhymurium		
PLA	43.87	55.48
PLA/Ag	100	100
PLA/Ag/VitaminE	100	100

The results indicated that Ag/PLA-NC possessed a strong antibacterial activity *against E.coli*, *Listeria monocytogenes*, and *Salmonela typhymurium* in accordance with literature data (Kamyar et al. 2010). Scientific data report also about antibacterial activity of PLA/Ag system against other bacteria as *Staphylococcus aureus* (Espinosa-Cristóbal et al. 2009; Mohiti-Asli et al. 2014) *P. aeruginosa*, *C. albicans*, *A. niger*, and *S. cerevisiae* (Melaiye et al. 2005). Inhibitory effect of PLA could be explained by acidic pH imparted by it to the medium (pH-2) which is not favorable to bacteria growth and due to the residual content of lactic acid. Some exceptions found at 48 h of incubation are not only due to the antimicrobial activity but also due to the

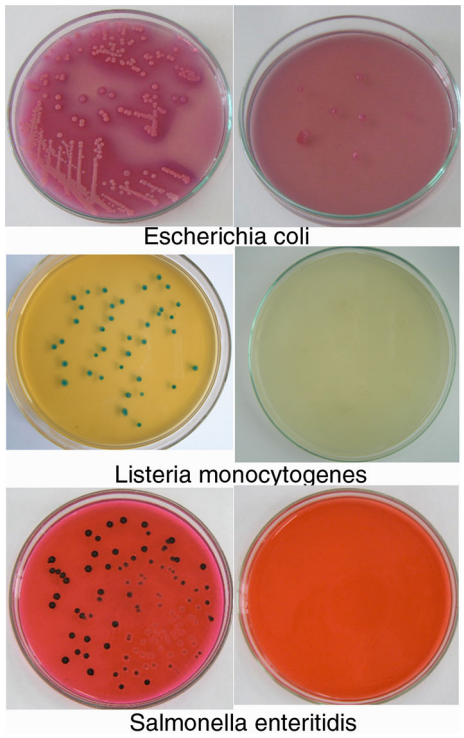

Fig. 5 Microscopical aspects of the colonies of *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella typhymurium* grown over PLA/Ag/VitaminE

reduction of the viability of the tested bacteria in the absence of the nutrition medium. Thus, PLA/Ag/VitaminE nanofibers can be used as an antibacterial scaffold for tissue engineering and medical application as wound dressings for infected wound sites.

Antioxidant activity of nanofibers

DPPH method

2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a stable radical in solution and appears purple color absorbing at 517 nm in methanol. The test is based on the principle that DPPH radical on accepting a hydrogen (H) atom from the scavenger molecule i.e., antioxidant, resulting into reduction of DPPH to DPPH2 (Mishra et al. 2012), the purple color changes to yellow with concomitant decrease in absorbance at 517 nm (Chandrasekar et al. 2006). The free radical scavenging activity of the PLA/Ag-NP/VitaminE nanofibers was measured by UV–VIS spectroscopy recording the decrease in absorbance of DPPH solution at 517 nm obtained in the absence and presence of the nanofibers. The antioxidant test results and photograph of the solutions after 30 min of reaction was shown in Fig. 6. Antioxidant activity of PLA nanofibers and PLA/Ag-NP/VitaminE nanofibers are shown in Table 1.

Evaluation of the percentage antioxidant activity was done with formula given below:

$$\text{Antioxidant activity} = (I_{\text{DPPH}} - I_{\text{nanofiber}})/I_{\text{DPPH}} * 100, \quad (2)$$

where I_{DPPH} is absorbance at 517 nm of pure DPPH and $I_{\text{nanofiber}}$ is absorbance at 517 nm of DPPH in the presence of nanofibers. The purple color of solution turned into yellow after 30 min of reaction, which means that there was no more DPPH molecule in PLA/Ag-NP/VitaminE solution. PLA nanofibers exhibited only 4 % of antioxidant activity, whereas PLA/Ag-NP/VitaminE nanofibers have 94 % antioxidant activity. Although PLA nanofibers do not contain any antioxidant agent, they also showed 4 % antioxidant activity. The small antioxidant activity of PLA nanofibers could be due to the residual lactic acid of nanofibers (Theinsathid et al. 2011).

Enzymatic browning of apples

Enzymatic browning of apples (fruits) is a process caused by the oxidation of phenolic compounds into quinones (Queiroz et al. 2008). This reaction is mainly catalyzed by polyphenol oxidase in the presence of oxygen and causes the brown pigmentation together with organoleptic and nutritional modifications in the apple tissues. The polyphenol oxidase activity increases after the fruit peeling or cutting. There are studies showing the antibrowning effect of antioxidants such as cysteine (Arpita et al. 2010), ascorbic acid (Rapeanu et al. 2006), and tropolone (Nunez-Delicado et al. 2007).

The purpose of the following procedure is to evaluate the antibrowning (inhibition of polyphenol oxidase activity) effect of Vitamin E containing PLA fibers on fresh apple and apple juice.

Generally in the presence of the combination of Ag and Vitamin E the apple, and its juice kept their general aspects and properties a longer time period, the change in color or browning do not appear after 48 h, while for other samples the changes appear after

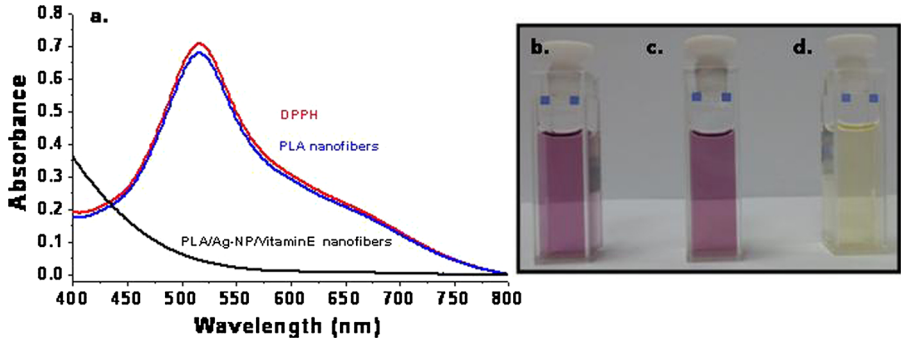

Fig. 6 **a** UV spectra of DPPH solution; in the presence of PLA nanofibers, and PLA/Ag-NP/VitaminE nanofibers; the aspect of **b** the DPPH solution in the absence and **c** the presence of PLA nanofibers, and **d** PLA/Ag-NP/VitaminE nanofibers

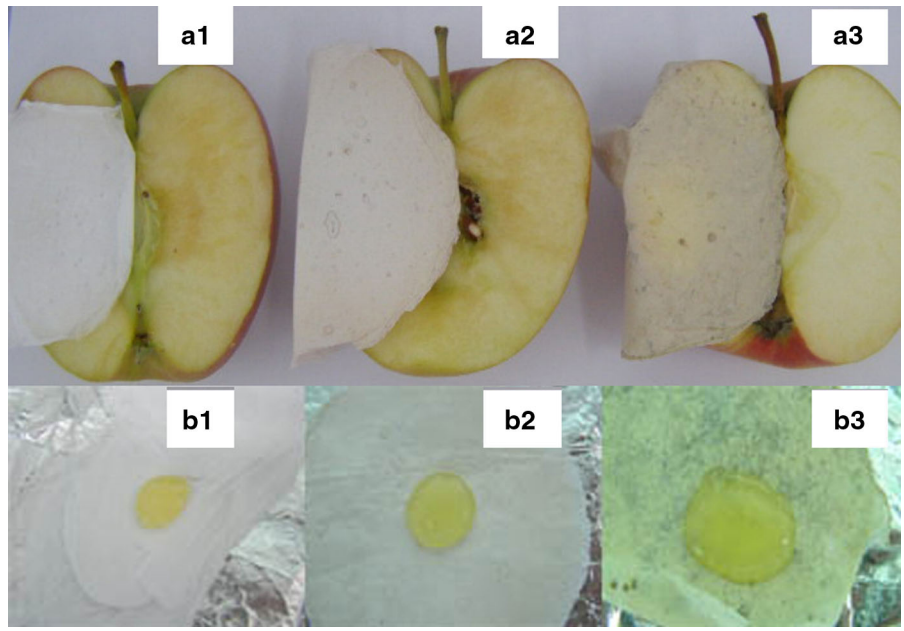

Fig. 7 Aspects of the apples and apple juice in contact with the PLA (a1 and b1), PLA/Ag (a2 and b2), and PLA/Ag/Vitamin E (a3 and b3) membranes after 24 h

24 h or less (2 h for juice placed on PET as commercial packaging material—see Fig. 7, 8).

The inhibition of polyphenol oxidase activity was assayed by measuring the rate of increase in absorbance at 450 nm (Jeong et al. 2008; Arpita et al. 2010). Higher values for absorbance at 450 nm correspond to higher browning/darkening of the juice. The absorbance (Fig. 8a), conductivity (Fig. 8b), and pH (Fig. 8c) insignificantly vary for apple juice placed on PLA/Ag/Vitamin E nanofiber membrane in comparison with PLA and PLA/Ag demonstrating the antioxidant/antibrowning effect of Vitamin E incorporation in PLA. The results indicated that the PLA/Ag/Vitamin E nanofiber membranes actively reduced the polyphenol oxidase activity.

Recently, in a situation similar with the studied system (Ramos et al. 2014a; Ramos et al. 2014b) has been demonstrated that the presence of Ag-NP and thymol antioxidant in PLA matrix also improved its degradability. Degradation of these PLA-based nanocomposites with thymol and silver nanoparticles in composting conditions indicated that the inherent biodegradable character of this biopolymer was improved after such modification. The obtained

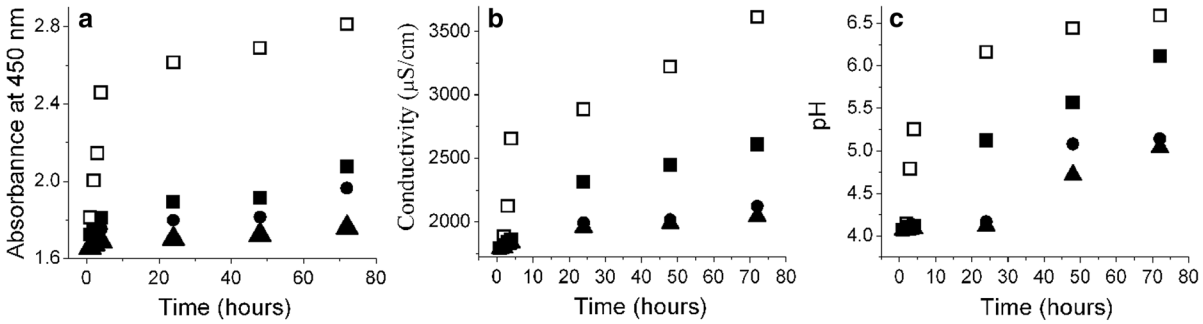

Fig. 8 Variation in time of the absorbance at 450 nm (**a**), pH (**b**) and conductivity of the apple juice in presence of PLA nanofiber membranes in comparison with juice on PET (apple juice on: *unfilled rectangle* PET, *filled rectangle* PLA, *filled circle* PLA/Ag, and *filled triangle* PLA/Ag/Vitamin E)

nanocomposites showed suitable properties to be used as biodegradable active-food packaging systems with antioxidant and antimicrobial effects. Moreover, biodegradable nanocomposites prepared have good potential for food packaging application accomplishing also environmental concerns.

Conclusion

Multifunctional PLA nanofibers containing Ag-NP and VitaminE have been obtained via electrospinning. The characterization of PLA/Ag-NP/VitaminE nanofibers was performed by SEM, TEM and XRD. Ag-NP has average diameter of 2.7 $\pm$ 1.5 nm within the PLA nanofiber matrix with a few aggregates. By incorporating two active agents (Ag-NP and Vitamin E) in the electrospun PLA nanofibers, PLA/Ag-NP/VitaminE nanofibers have shown both antibacterial activity and antioxidant activity. PLA/Ag-NP/VitaminE nanofibers inhibited growth of *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella typhymurium* up to 100 % whereas the antioxidant activity of PLA/Ag-NP/VitaminE nanofibers was determined as 94 %. The results of the tests on fresh apple and apple juice indicated that the PLA/Ag/VitaminE nanofiber membrane actively reduced the polyphenol oxidase activity. The amount of silver released into aqueous solution was measured via ICP-MS and remained constant after 10 days of immersion and this suggested that antibacterial activity of PLA/Ag-NP/VitaminE nanofibers can last for a longer time period. These materials could find application in food industry as a potential preservative packaging for fruits and juices.

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