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Antifungal bionanocomposites based on poly(lactic acid) and silver nanoparticles for potential medical devices

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Abstract

Novel biocompatible and antimicrobial composites based on poly(lactic acid) (PLA), hydrolyzed collagen (HC) and silver nanoparticles (AgNPs) were prepared by melt processing. Tributyl o-acetyl citrate (ATBC) was used as plasticizer, for improving the processability of PLA. The influence of HC and AgNPs on the PLA bionanocomposites was investigated in terms of biocompatibility, antifungal activity and water contact angle measurements. Surface morphology by SEM and the identification of AgNPs by UV-Vis were also presented. The investigated bionanocomposites exhibited the characteristic plasmonic effect of silver nanoparticles. All composites showed a high degree of biocompatibility. Sample containing HC 5 wt.% and AgNPs showed a significant antifungal property, inhibiting fungal adhesion and mature biofilm development. The increased hydrophilicity determined by contact angle analysis for the PLA/HC10/AgNPs composite did no contributed significantly to improving of anti-adherence effect. 10 wt.% HC promoted fungal colonization and mature biofilm development.

Keywords: *biopolymers, hydrophilicity, antimicrobial, biocompatibility*

Introduction

In the last two decades has been found that the number of nosocomial infections increased significantly and most of these infections are associated with implantable medical devices (IMDs) (SOUSA et al., 2011 [1]). The use of IMDs has become indispensable in all fields of

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medicine being used for diagnostic or therapeutic (VON EIFF et al., 2005 [2]). The risk associated with the use of polymeric IMDs derived from the possibility of their contamination with bacteria and fungi followed by microbial biofilm associated infections, especially common in the immunocompromised host organisms. These infections are responsible for the increased rates of mortality and hospitalization cost of treatment (KAALI et al., 2011 [3]) due to increased antimicrobial resistance, for example, when microbial cells exist in the biofilm they are 1000 times more resistant to antibiotics than are planktonic cells (CERI et al., 1999 [4], DONLAN and COSTERTON, 2002 [5]) and host defense mechanisms resistance.

The most common microorganism that are involved in infection associated to IMDs are Coagulase Negative Staphylococci (CoNS), particularly *S. epidermidis* (slime positive), *S. aureus, Ps. aeruginosa, E. coli* and *Candida* species (KATSIKOGIANNI and MISSIRLIS, 2004 [6]). A relatively small number of *Candida* species are pathogenic for humans. All are opportunistic pathogens and the immunocompromised hosts being sensitive to their attack. The principal pathogen of the genus is *Candida albicans*. IMDs as stents, shunts, prostheses, endotracheal tubes, pacemakers, and various types of catheters are frequently colonized of *Candida* spp. (DOUGLAS, 2003 [7]).

Silver and its compounds have been studied for many years for their antibacterial activity (SHAMELI et al., 2010 [8]). Silver antimicrobial activities depend of the form in which it is used. It has been shown that antimicrobial efficacy is enhanced by using of nanoparticles and incorporating them into polymer matrices (MONTEIRO et al., 2009 [9]). Recently studies have showed that silver nanoparticles (AgNPs) have efficient and broad-spectrum antibacterial ability with minimal cytotoxicity to human cells and these represent a new strategy for antimicrobial agents (MA et al., 2011 [10], RÂPĂ et al., 2013 [11]).

Poly (lactic acid) (PLA) is an aliphatic polyester approved by the US Food and Drug Administration (FDA) for direct contact with biological fluids (ARMENTANO et al., 2013 [12]). PLA can be processed by film casting, extrusion, blow molding, and fiber spinning due to its greater thermal processability. PLA is a semi-crystalline polymer which has a glass transition temperature (T_g) of 55-59 ^oC and the melting temperature (T_m) of 175-180 ^oC, has good mechanical strength and good thermoplastic processability (XIAO et al., 2012 [13]). Furthermore, PLA is a biodegradable and biocompatible polymer and is highly studied for applications in the medical field for controlled release systems, biodegradable sutures and surgical implants (SMITH, 2005 [14]). For these reasons, PLA was chosen in this study for designing of novel antifungal bionanocomposites for medical application together with AgNPs incorporating into these.

The main uses of PLA have been limited by its brittleness, slow degradation profile, and poor hydrophilicity (XIAO et al., 2012 [13]). To improve the ductility of PLA-based materials, a large number of investigations have been made to modify PLA properties via plasticization (HASSOUNA et al., 2011 [15]). Biocompatible molecules such as oligomeric lactic acid, oligomeric citrate ester, oligomeric PEG, and glycerol represent the plasticizers for modification of PLA properties (XIAO et al., 2012 [13]).

In order to improve biocompatibility of PLA, natural polymers allowing good attachment to cells are introduced. Therefore, collagen, a natural polymer, is increasingly being used for tissue repair engineering (WAHL and CZERNUSZKA, 2006 [16]; VROMAN and TIGHZERT, 2009 [17]) and drug delivery (RUSZCZAK and FRIESS, 2003 [18]). The collagen is enzymatically degradable and is reabsorbed by the body (CUI et al., 2015 [19]).

The aim of the present study is to obtain the antimicrobial and biocompatible polymeric composites with potential applications in fabrication of IMDs. Biocompatibility, antibiofilm

effect and hydrophilicity surface properties of novel antifungal bionanocomposites were investigated. Surface morphology and the identification of AgNPs were also presented.

Materials and methods

Materials

The PLA used in this study was 2002D from NatureWorks LLC, UK; tributyl *o*-acetyl citrate (ATBC) was supplied by PROVIRON, (Belgium); hydrolyzed collagen (HC) was acquired from Sigma-Aldrich; silver nanoparticles (AgNPs) were acquired from US Nanomaterials Research, Inc.; it consists of 99.99 % Ag covered with 0.2 % PVP and is characterized by a particle size of 30-50 nm.

Sabouraud broth medium was obtained from Merck (Germany); Sabouraud agar medium was acquired from Acumedia (Bucharest, Romania); Phosphate Buffered Saline (PBS) was obtained from Biochrom (Germany); Fetal Bovine Serum (FBS) was obtained from Sigma-Aldrich (USA).

Candida albicans ATCC 10231 fungal strain was used from the collection of the Department of Microbiology of the Faculty of Biology (University of Bucharest, Romania), it was grown overnight on Sabouraud agar medium. Fungal suspensions of 0.5 McFarland were obtained in sterile physiological water.

Bionanocomposites preparation

In this study we aimed to achieve the antimicrobial and biocompatible polymeric mixtures. In order to improve the flexibility of PLA, a content of 20% ATBC was used. Also, our purpose was to increase the biocompatibility of the mixtures by using of HC at loading of 5 wt. % and 10 wt. % respectively. AgNPs in content of 1 wt.% was used as antimicrobial agent. It has therefore been achieved antimicrobial bionanocomposites based on PLA, hydrolyzed collagen and silver nanoparticles by melt blending procedure at a temperature of 170^{o}C using a Brabender Plastograph. The compositions and the appearance of bionanocomposites are showed in Table 1.

Table 1. The composition and the appearance of investigated bionanocomposites

Code sample	Composition, wt.%
PLA/ATBC	PLA:ATBC = 80:20
PLA/HC5	(PLA:ATBC):HC = (80:20):5
PLA/HC5/AgNPs	[(PLA:ATBC):HC]:AgNPs [(80:20):5]:1
PLA/HC10/AgNPs	[(PLA:ATBC):HC]:AgNPs = [(80:20):10]:1

From the melted mixtures, films and sheets were obtained for testing by using a 200P COLIN 164 Press at temperature of 170 ºC and pressure of 125 atm.

Surface plasmon resonance study

The presence of silver nanoparticles was identified by UV-Vis absorption spectroscopy using a HELIOS ALPHA UV-Vis spectrophotometer. The spectra were recorded in the wavelength range of 235-800 nm at a resolution of 1 nm, using air as references.

Surface morphology study

SEM analysis was performed on a FEI electron microscope, using secondary electron beams with energies of 30 keV, on samples covered with a thin silver layer.

Contact angle study

The bionanocomposites's surface hydrophilicity was determined by goniometric method using the KSV Instrument's CAM 101, equipped with high speed digital video camera (C 200-HS; KSV – Finland). The measurements were made by using as test liquid distilled water, which was dropped in small volume (4µl) at room temperature on the material surface. Values of the water contact angle (WCA) for 30 seconds continuously were registered. Three drops measurements were performed for each film and average values with standard deviation were presented.

In vitro cytotoxicity test

The cytotoxicity of the bionanocomposites was evaluated according to the EN ISO 10993-5 standard [20] using the direct contact method on a fibroblast cell line (NCTC clone L929) cultivated in Minimum Essential Medium (MEM) containing 10 % Fetal Bovine Serum (FBS) and 2mM L-glutamine, 100 U/mL penicillin, 100µg/mL streptomycin and 500 µg/mL neomycin.

Cell suspension was seeded in 24-well culture plates at a density of 4 x 10^4 NCTC cells/mL and incubated in a humidified 5% CO_2 atmosphere, at 37ºC, for 24h. Test samples were added in triplicate manner on confluent monolayer of L929 mouse fibroblast cells and plates were incubated in standard conditions for other 24h and 48h; the cell viability was quantitatively determined by MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay (MOSSMAN, 1983 [21]).

Briefly, the culture medium in each well was replaced with MTT solution and the plates were incubated for 3h at 37ºC. The MTT solution from the wells was replaced with isopropanol followed by gentle shaking to solubilize the formazan crystals. Absorbance of colored solution was read at 570 nm using a microplate reader Berthold Mithras LB 940 (Germany).

The measured optical density (OD) is directly proportional to the number of viable cells in the tested cell culture and the results were calculated according to the formula (1):

$$\%\ cell\ viability = \frac{OD\ sample}{OD\ control} x\ 100 \qquad (1)$$

Untreated cells served as control group considered as 100% viable cells. The experiment was performed in triplicate for each sample.

The cell morphology examination was preformed after incubation 24 h for the attachment of cells in wells, the medium was renewed, the samples were added and the cells were incubated for 48 h under appropriate conditions. Subsequently the cells were fixed in Bouin and Hematoxilin–Eozin stained. The cultures were visualized by light microscopy using an inverted microscope Carl Zeiss Axio Observer D1 (×20) and images were taken with the digital camera Axio Cam MRc (Germany).

In vitro fungal biofilm development

Biofilm formation was evaluated using a static model for monospecific biofilms development (ANGHEL et al., 2013 [22]). Bionanocomposites with (1x1) cm dimensions were sterilized by UV (for 15 minutes on each side), then were placed in a 24-well plate containing 1.5 mL inoculums with standardized density of 1:100 from 0.5 McFarland of *Candida albicans* ATCC 10231, prepared in Sabouraud broth medium. The samples were incubated for 24 h at 37^{o}C and at the end of the incubation period, the specimens were placed on fresh Sabouraud broth medium and incubated for another 24 h, 48 h and 72 h. Quantification of biofilms was performed by viable cell count assay for the CFU (colony

forming units) determination. After each incubation period, the samples were gently washed with sterile PBS, in order not to destroy the biofilm and then they were placed into a 2 ml Eppendorf tube containing 1 ml PBS. The samples were vortexed a period time of 60 s for the dispersion of biofilm cells into the suspension. Serial ten-fold microdilutions were achieved and spotted on Sabouraud agar medium for viable cells counts assay. The experiment was performed in triplicate for each sample.

Results and discussions

Surface plasmon resonance study

UV-vis spectra of the PLA/HC5/AgNPs and PLA/HC10/AgNPs films were recorded and compared with the spectra of the PLA/ATBC and PLA/HC5 (Figure 1).

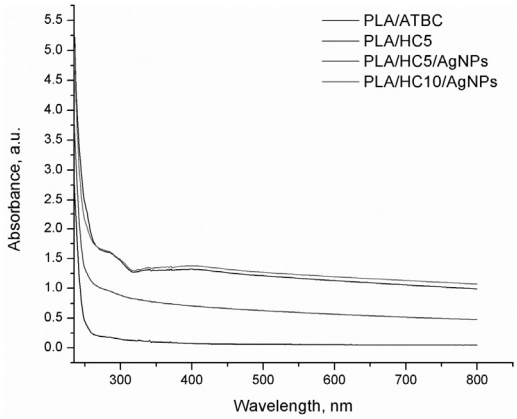

Figure 1. UV-vis spectra of the bionanocomposites films

The observed absorbance peak at 415 nm for PLA/HC5/AgNPs and PLA/HC10/AgNPs films is attributed to the plasmon resonance of the AgNPs, indicating their dispersion in the polymer mixture. Also, other studies have been reported peaks at 400-430 nm which correspond to plasmon resonance of silver nanoparticles (LIU et al. 2009 [23]; CHITTE et al., 2012 [24]; VIVEKANANDHAN et al., 2012 [25]; KRSTIĆ et al., 2014 [26]; LI et al., 2010 [27]; SULAIMAN et al., 2014 [28]). Broadening of bands of the AgNPs incorporated into the polymer matrix could be associated to AgNPs agglomeration, phenomenon observed by (CHITTE et al., 2012 [24]) and also confirmed by SEM analysis.

Surface morphology study

SEM images for the prepared bionanocomposites are shown in Figure 2.

The samples showed a homogeneous morphology, the plasticizer and the hydrolyzed collagen being well dispersed into the polymeric matrix (Figures 2A and 2B). In the case of PLA/HC5/AgNPs and PLA/HC10/AgNPs films, the aggregated silver nanoparticles were observed as bright parts in polymeric matrix (Figures 4C and 4D). This is in good agreement with the results of the plasmon resonance study, where the broadening bands by incorporating of AgNPs into the polymer matrix was observed. Also, it should be noted that silver nanoparticles were well dispersed in composite matrix and its average size was recorded by SEM as being 227.6 nm and 234.9 nm, a higher size compared with the values from the data sheet. Maybe it is possible that under processing time, the AgNPs were agglomerated. Some aggregation or bigger size particles of silver are also observed by (KANMANI and RHIM, 2014 [29]).

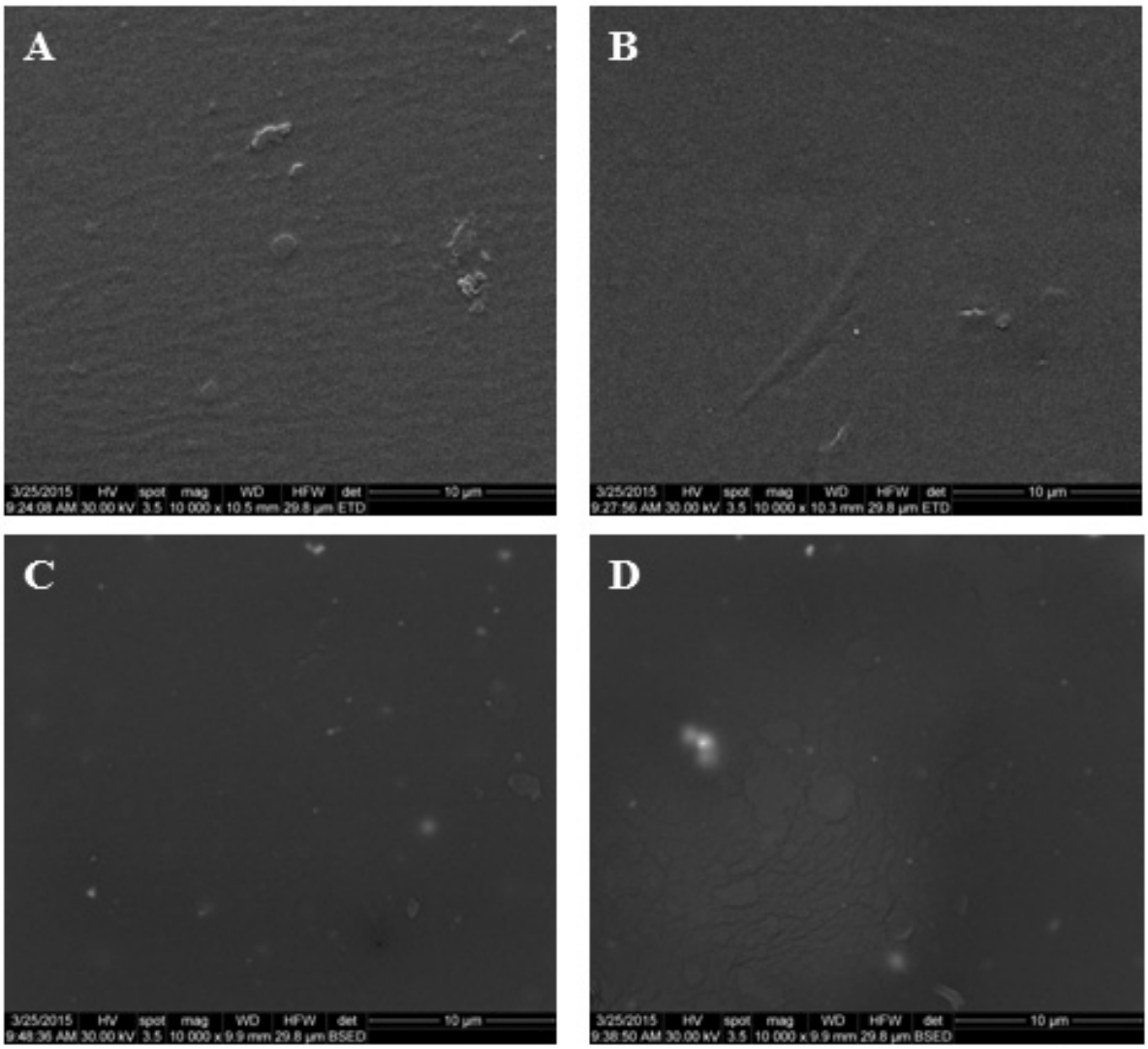

Figure 2. Scanning electron micrograph (SEM) of bionanocomposites
A) PLA/ATBC; B) PLA/HC 5; C) PLA/HC5/AgNPs; D) PLA/HC10/AgNPs
B)

Contact Angle Study

Water contact angle (WCA) is an indicator of the hydrophilic/hydrophobic properties of the polymeric materials. In general, the lower the water contact angle value shows the higher hydrophilicity (RHIM et al, 2006 [30]). More specifically, a WCA less than 90° indicates that wetting of the surface is favorable, while WCA greater than 90° generally means that wetting of the surface is unfavorable. For super hydrophobic surfaces, WCA are usually greater than 150°, showing almost no contact between the liquid drop and the surface (YUAN and LEE, 2013 [31]).

Water contact angles were investigated for films based on PLA, AgNPs and different contents of HC, as are shown in Figure 3. The results were expressed in terms of water contact angle (˚, degree) on the film surface.

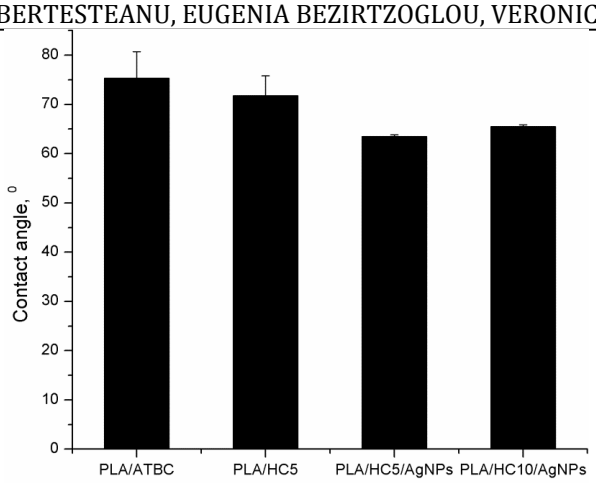

Fig. 3. Water **c**ontact angle values for the bionanocomposites
(Error bars represent standarddeviation)

Water contact angle for plasticized PLA is found to be 75˚. For WCA for neat PLA, a value of 73.36˚ was reported (GIRDTHEP et al. 2014 [32]).Water contact angle value decreased to 73˚ by adding of HC 5 wt.%, due to the hydrophilic character of biopolymer. Incorporation of AgNPs leads to lower contact angles values both for bionanocomposites containing HC 5 wt.% and 10 wt.% with respect to PLA/HC5 sample. This character could be attributed to the hydrophilicity of silver nanoparticles shell from polyvinylpyrrolidone (PVP). At sample containing 10 wt.% HC is expected that AgNPs will decrease more the WCA as against the bionanocomposites containing HC 5 wt%. From Figure 3, is observed a slowly increase of WCA for this sample. This can be explained by an increase of interaction between AgNPs and polymer matrix. Aslo, other authors reported that metallic silver led to increase the hydrophobicity of materials (KANMANI and RHIM, 2014 [29], RHIM et al., 2013 [33]).

In vitro cytotoxicity test

The cytotoxicity of bionanocomposites was performed by direct contact method used as reference SR EN ISO 10993-5:2009. MTT cell viability assay has served to establish the percentages of viable cells at 24 h and 48 h respectively in the presence of tested samples, the results is represented in Figure 4.

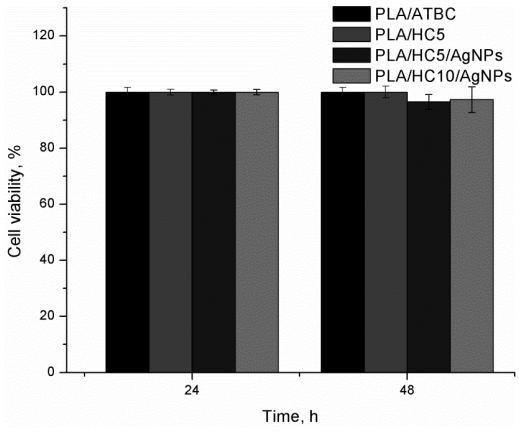

Figure 4. Cell viability of fibroblast cell line (NCTC clone L929) in the presence of prepared bionanocomposites, after 24 h and 48 h (Error bars represent standard deviation)

All composites tested led to a very good cellular viability percentage, this signifies a high degree of biocompatibility in according to the cytotoxicity scale of SR EN ISO 10993-5:2009. 100% cell viability was observed at 24 h for L929 cells treated with all polymeric composites. Also, it was observed an insignificant decrease to cell viability of fibroblast cell line at 48 h, when AgNPs was incorporated in the polymeric composites, this does not change the biocompatible character of the materials and unaltered cell morphology was confirmed. It has been observed that higher collagen content in case of PLA/HC10/AgNPs sample does not make to decline much cell viability at 48 h, therefore we can say that the presences of collagen improves the biocompatibility of composites.

Evaluation of cells morphology (Figure 5) reveals cellular proliferation in the presence of bionanocomposites compared with the control culture, indicating a high degree of biocompatibility.

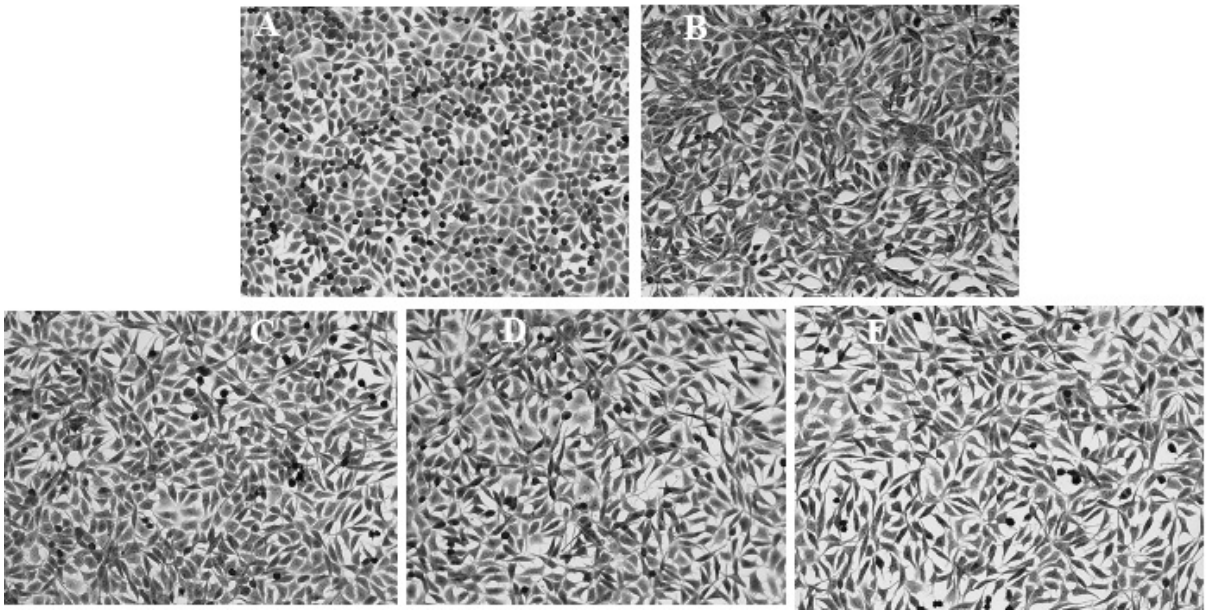

Figure 5. Morphology of fibroblast cell line (NCTC clone L929) after 48 h
A) control culture; B) cells treated with PLA/ATBC sample; C) cells treated with PLA/HC5 sample; D) cells treated with PLA/HC5/AgNPs sample; E) cells treated with PLA/HC10/AgNPs sample. The morphology of the cellular layer obtained in the presence of the tested samples at 48 h was analyzed by optical microscopy (X20).

All the images presented in Figure 5 representing the cells after 48 h of direct contact with polymeric composites have the specific appearance of the control culture without any degradation aspects of the cell morphology; in conclusion all the samples are biocompatible. The morphology aspects of treated cells are in concordance with the results obtained in the MTT viability assay.

In vitro fungal biofilm development

Viable cell counts on the surface of bionanocomposites developed at 24 h, 48 h and 72 h are plotted in Figure 6.

The obtained results from the assessments of developed biofilms on the surface of bionanocomposites revealed that PLA/HC5/AgNPs sample shows a significant antifungal property, inhibiting fungal adhesion and mature biofilm development. Also, it has been observed in this case that during 48 hours, the biofilm development is much less, as shown by the low number of viable cells included in biofilm (Figure 6).

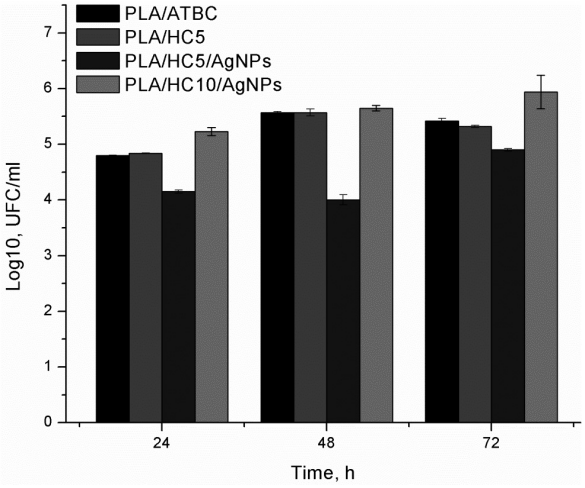

Figure 6. Graphic representation of viable cell counts after removing of *Candida albicans* ATCC 10231 biofilm incorporated cells developed on biocomposites at 24 h, 48 h and 72 h

It is known that hydrophobic polymeric medical devices facilitates both microbial adherence (LAZĂR and CHIFIRIUC, 2010 [34]) as well as the film formation condition (ARCIOLA et al., 2005 [35]), phenomena associated with biofilm formation and resistant infections implicitly. A hydrophilic barrier could be an alternative inhibitory for hydrophobic interactions between the polymer and the microbial surface, well as between polymer and molecules from blood or other body fluids (FRANCOLINI and DONELLI, 2010 [36]). From Figure 6 can be observed that the PLA/HC10/AgNPs sample does not exhibit antimicrobial effect on *Candida albicans*, although it is hydrophile. Therefore, we can say that the increased hydrophilicity determined by contact angle analysis for the PLA/HC10/AgNPs composite has not contributed significantly to improving anti-adherence effect. The presence of AgNPs in the polymer matrix does not confer antibiofilm effect for the PLA/HC10/AgNPs sample, this was evidenced by the increased number of viable cells included in the mature biofilm of 24 h, 48 h and 72 h, when it is compared with PLA/HC5/AgNPs sample. This is in contradiction with other authors that reported antifungal effects of silver nanoparticles on *Candida albicans* (KIM et al., 2009 [37]). Also PLA ultrafine fibers containing nanosilver particles prepared via electrospinning exhibited antibacterial effect (XU et al., 2006 [38]). Maybe it is possible that AgNPs do not migrate at surface material and interact with cell membrane. It was also observed that the higher content of collagen in the composite promotes fungal colonization and mature biofilm development. This behavior could be associated with the use of collagen as a nutrient by fungal cells, probably the collagen in excess (10 wt.%) favors the multiplication rate of the cells adhered. AgNPs from the composite were unable to exhibit inhibitory effect on mature biofilm development. It was reported that secreted aspartyl proteinases (SAPs) from *Candida* hydrolyze many proteins such as: albumin, hemoglobin, keratin, collagen, laminin, fibronectin, mucin, salivary lactoferin, interleukin 1b, cystatin A, and Immunoglobulin A (HUBE et al., 1998 [39]; KHAN et al., 2010 [40]). So, is possible that the collagen hydrolysis products obtained could be used as nutrients by fungal cells tested, the higher content of collagen is proportional to a greater amount of nutrients, associated with a high multiplication rate.

The information provided in this study are important to address in future, they are useful in improving the polymeric materials with antimicrobial properties under development for implantable medical devices.

Conclusions

The results of this study evidenced the preparation of novel antimicrobial and biocompatible polymeric composites based on PLA, collagen and AgNPs by melting procedure. As plasticizer, ATBC was used for improving the melt processability of PLA. HC was 5 wt.% and 10 wt.% and AgNPs was 1 wt.%.

The presence of AgNPs into bionanocomposites was evidenced by UV/Vis spectrometry and SEM analysis. Adding of HC led to biocompatible composites; the biocompatibility increased with content of collagen.

A significant antifungal property, inhibiting fungal adhesion and mature biofilm development was revealed by PLA/HC5/AgNPs sample. Sample containing collagen 10 wt. % and AgNPs in composition promotes fungal colonization and mature biofilm development.

As result, an optimum content of hydrolyzed collagen and antimicrobial agent is necessary to assure both biocompatibility and antifungal properties of bionanocomposites, as well they will have potential applications in fabrication of implantable medical devices.

Acknowledgements

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References

1. SOUSA C., BOTELHO C., OLIVEIRA R., Nanotechnology applied to medical biofilms control. *Science against microbial pathogens: communicating current research and technological advances,* 878-888 (2011).
2. VON EIFF C., JANSEN B., KOHNEN W., BECKER K., Infections associated with medical devices: pathogenesis, management and prophylaxis. *Drugs*65: 179-214 (2005).
3. KAALI P., STRÖMBERG E., KARLSSON S., Prevention of Biofilm associated Infections and Degradation of Polymeric Materials used in Biomedical Applications. *Pub. In Tech*., 513-540 (2011).
4. CERI H., OLSON M., STREMICK C., READ R.R., MORCK D., BURET A., The calgary biofilm device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. *J. Clin. Microbiol.* 37, 1771-1776 (1999).
5. DONLAN R.M., COSTERTON J.W., Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin Microbiol Rev*., 15:167-193 (2002).
6. KATSIKOGIANNI M., MISSIRLIS Y.F., Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteriamaterial interactions. *European Cells and Materials* 8, 37-57 (2004).
7. DOUGLAS L.J., *Candida* biofilms and their role in infection. *Microbiology*, 11: 30-36 (2003).
8. SHAMELI K., AHMAD M.B., WAN YUNUS W.M.Z., IBRAHIM N.A., JOKAR M., DARROUDI M., Synthesis and Characterization of Silver/Polylactide Nanocomposites. *World Academy of Science, Engineering and Technology* 4: 22-26 (2010).
9. MONTEIRO D.R., GORUP L.F., TAKAMIYA A.S., RUVOLLO-FILHO A.C., RODRIGUES DE CAMARGO E., BARROS BARBOSA D., The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver. *International Journal of Antimicrobial Agents* 34, 103–110 (2009).
10. MA Z., JI H., TAN D., TENG Y., DONG G., ZHOU J., QIU J., ZHANG M., Silver nanoparticles decorated, flexible SiO_2 nanofibers with long-term antibacterial effect as reusable wound cover. *Colloids and Surfaces A: Physicochem. Eng. Aspects* 387: 57– 64 (2011).
11. RÂPĂ M., STOICA P., TĂNASE E.E., GROSU E., VLAD G., Preparation of medical devices with antimicrobial properties, *Journal Of Optoelectronics And Advanced Materials* 15 (7- 8), 807 – 816 (2013).
12. ARMENTANO I., BITINIS N., FORTUNATI E., MATTIOLI S., RESCIGNANO N., VERDEJO R., LOPEZ-MANCHADO M.A., KENNY J.M., Multifunctional nanostructured PLA materials for packagingand tissue engineering, *Progress in Polymer Science* (2013), http://dx.doi.org/10.1016/j. progpolymsci.2013.05.010.

13. XIAO L., WANG B., YANG G., GAUTHIER M., *Poly(Lactic Acid)-Based Biomaterials: Synthesis, Modification and Applications, Biomedical Science, Engineering and Technology*, Prof. DHANJOO N. GHISTA (Ed.), ISBN: 978-953-307-471-9, InTech, (2012). Available from: http://www.intechopen.com/books/biomedicalscience-engineering-and-technology/poly-lactic-acid-based-biomaterials-synthesis-modification-andapplications.
14. SMITH R. *Biodegradable polymers for industrial applications*, publised by Woodhead Publishing Limited and CRC Press LLC (2005).
15. HASSOUNA F., RAQUEZ J.M., ADDIEGO F., DUBOIS P., TONIAZZO V., RUCH D., New approach on the development of plasticized polylactide (PLA): Grafting of poly(ethylene glycol) (PEG) via reactive extrusion, *European Polymer Journal* 47, 2134–2144 (2011).
16. WAHL D.A. and CZERNUSZKA J.T., Collagen-hydroxyapatite composites for hard tissue repair. *European cells and Materials* 11: 43-56 (2006).
17. VROMAN I. and TIGHZERT L., Biodegradable Polymers, *Materials 2*, 307-344 (2009).
18. RUSZCZAKA Z., FRIESSC W., Collagen as a carrier for on-site delivery of antibacterial drugs. *Advanced Drug Delivery Reviews* (2003), doi:10.1016/j.addr.2003.08.007.
19. CUI M., LIU L., GUO N., SU R., MA F., Preparation, Cell Compatibility and Degradability of Collagen-Modified Poly (lactic acid). *Molecules 20*, 595-607 (2015). doi:10.3390/molecules20010595.
20. EN ISO ISO 10993 - Biological evaluation of medical devices – Part 5: Tests for cytotoxicity: *in vitro* methods.
21. MOSSMAN, T., Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* 65, 55–63 (1983).
22. ANGHEL I., GRUMEZESCU A.M., HOLBAN A.M., FICAI A., ANGHEL A.G. and CHIFIRIUC M.C., Biohybrid Nanostructured Iron Oxide Nanoparticles and *Satureja hortensis* to Prevent Fungal Biofilm Development, *International Journal* of *Molecular Sciences* 14, 18110-18123 (2013).
23. LIU Y., CHEN S., ZHONG L., WU G., Preparation of high-stable silver nanoparticle dispersion by using sodium alginate as a stabilizer under gamma radiation. *Radiation Physics and Chemistry* 78, 251–255 (2009).
24. CHITTE H.K., BHAT N.V., KARMAKAR N.S., KOTHARI D.C., SHINDE G.N., Synthesis and Characterization of Polymeric Composites Embeded with Silver Nanoparticles. *World Journal of Nano Science and Engineering* 2, 19-24 (2012).
25. VIVEKANANDHAN S., CHRISTENSEN L., MISRA M., MOHANTY A.K., Green Process for Impregnation of Silver Nanoparticles into Microcrystalline Cellulose and Their Antimicrobial Bionanocomposite Films. *Journal of Biomaterials and Nanobiotechnology*, 2012, 3, 371-376.
26. KRSTIĆ J., SPASOJEVIĆ J., RADOSAVLJEVIĆ A., ŠILJEGOVĆ M., KAČAREVIĆ-POPOVIĆ Z., Optical and structural properties of radiolytically in situ synthesized silver nanoparticles stabilized by chitosan/poly(vinylalcohol) blends, *Radiation Physics and Chemistry* 96, 158–166 (2014).
27. Li L.H., DENG J.C., DENG H.R., LIU Z.L., LI X.L., Preparation, characterization and antimicrobial activities of chitosan/Ag/ZnO blend films, *Chemical Engineering Journal* 160, 378–382 (2010).
28. SULAIMAN G.M., ALI E.H., JABBAR I.I., SALEEM A.H., Synthesis, characterization, antibacterial and cytotoxic effects of silver nanoparticles, Digest *Journal of Nanomaterials and Biostructures* 9(2), 787 – 796 (2014).
29. KANMANI P., RHIM J.W., Physical, mechanical and antimicrobial properties of gelatin based active nanocomposite films containing AgNPs and nanoclay, *Food Hydrocolloids* 35, 644-652(2014).
30. RHIM J.W., HONG S.I., PARK H.M., NG P.K.W., Preparation and Characterization of Chitosan-Based Nanocomposite Films with Antimicrobial Activity. *J. Agric. Food Chem*., 54, 5814-5822 (2006).
31. YUAN Y., LEE T.R., *Contact Angle and Wetting Properties.* Eds. G. BRACCO and B. HOLST. *Surface Science Techniques*, Chapter. 1, (2013). DOI 10.1007/978-3-642-34243-1_1.
32. GIRDTHEP S., WORAJITTIPHON P., MOLLOY R., LUMYONG S., LEEJARKPAI T., PUNYODOM W., Biodegradable nanocomposite blown films based on poly(lactic acid) containing silver-loaded kaolinite: A route to controlling moisture barrier property and silver ion release with a prediction of extended shelf life of dried longan, *Polymer* 55, 6776-6788 (2014).
33. RHIM J.W., WANG L.F., HONG S.I., Preparation and characterization of agar/silver nanoparticles composite films with antimicrobial activity, *Food Hydrocolloids* 33, 327-335 (2013).
34. LAZĂR V., CHIFIRIUC M,C.,M edical significances and new therapeutical strategies for biofilm associated infections. *Romanian Archives of Microbiology and Immunology* 69: 125-138 (2010).

35. ARCIOLA C.R., CAMPOCCIA D., GAMBERINI S., BALDASSARRI L., MONTANARO L., Prevalence of cna, fnbA and fnbB adhesin genes among *Staphylococcus aureus* isolates from orthopedic infections associated to different types of implant. *FEMS Microbiol Lett*. 246, 81-86 (2005).
36. FRANCOLINI I., DONELLI G. Preventionand control of biofilm-based medical-device-related infection. *FEMS Immunol. Med. Microbiol*. 59, 227–238 (2010).
37. KIM K.J., SUNG W.S., SUH B.K., MOON S.K., CHOI J.S., KIM J.G., Antifungal activity and mode of action of silver nano-particles on *Candida albicans, Biometals*, 22, 235–242 (2009).
38. XU X., YANG Q., WANG Y., YU H., CHEN X., JING X., Biodegradable electrospun poly(L-lactide) fibers containing antibacterial silver nanoparticles, *European Polymer Journal* 42, 2081–2087 (2006).
39. HUBE B., RUCHEL R., MONOD M., SANGLARD D., ODDS F.C., Functional aspects of secreted *Candida proteinases*. *Adv Exp Med Biol* 436, 339–344 (1998).
40. KHAN M.S.A., AHMAD I., AQIL F., OWAIS M., SHAHID M., MUSARRAT J., Virulence and Pathogenicity of Fungal Pathogens with Special Reference to *Candida albicans*. Chapter 2, *Combating Fungal Infections Problems and Remedy*, 21-45, (2010), DOI 10.1007/978-3-642-12173-9_2.