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CIPROFLOXACIN – MODIFIED DEGRADABLE HYBRID POLYURETHANE -POLYLACTIDE POROUS SCAFFOLDS DEVELOPED FOR POTENTIAL USE AS AN ANTIBACTERIAL SCAFFOLD FOR REGENERATION OF SKIN

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Abstract

The aim of performed studies was to fabricate an antibacterial and degradable scaffold that may be used in the field of skin regeneration. To reach the degradation criterion the biocompatible polyurethane (PUR), obtained by using amorphous macrodiol α,ω-dihydroxy(ethylene-butylene adipate) macrodiol (PEBA), was used and processed with so-called "fast-degradable" polymer polylactide (PLA) (5 wt% or 10 wt%). To meet the antibacterial requirement obtained hybrid PUR-PLA scaffolds (HPPS) were modified with ciprofloxacin (Cipro) (2 wt% or 5 wt%), the fluoroquinolone antibiotic inhibiting growth of bacteria such as *Pseudomonas aeruginosa*, *Escherichia Coli* and *Staphylococcus aureus*, which are main cause of wound infections. Obtained unmodified and Cipro-modified HPPS were studied towards their chemical composition to detect presence or absence of characteristic functional groups of PUR, PLA and Cipro, and as well to indicate the participation of hydrogen bonds in the HPPS structure in dependence on PLA addition and ciprofloxacin modification. Mechanical properties were studied to determine the possible application of HPPS as a skin tissue scaffold. Scanning electron microscopy (SEM) was used to study morphology of unmodified and Cipro-modified HPPS and to performed elementary analysis by using energy-dispersive x-ray spectroscopy (EDX) of obtained materials. Finally, the microbiological tests were performed to indicate the antibacterial effect of Cipro-modified HPPS on *S.aureus* growth. Performed studies showed that Cipro-modified HPPS, obtained by using 5 % of PLA, possess suitable mechanical characteristic, morphology, degradation rate and demanded antimicrobial properties to be further developed as a potential scaffolds for skin tissue engineering.

Keywords: polyurethane, polylactide, tissue engineering, skin scaffold, antibacterial, degradable, medical

Introduction

Skin injuries, wounds, burns and damages of epidermis take place by the variety of reasons like contact with hot water, excessive exposition to the sun, different chemicals and flames or can be the result of certain skin diseases (1,2). Not suitable treated wounds, burns and injuries may end with a bacterial infection and even death in the worst case. If the epidermis damage is large and deep application of the wound dressing may not be sufficient enough for natural skin regeneration. Thus, functional skin tissue scaffolds (STS) are being developed to treat large and deep skin defects (1,2). Fabrication of the STS is one of the tissue engineering (TE) tasks. TE deals with fabrication of biologic substitutes that will restore maintain and improve tissue functions following damage either by disease or traumatic processes. The general principles of TE involve combining living cells with natural or synthetic scaffold to build a three-dimensional (3D) living construct, which is functionally, structurally and mechanically equal to (or better) than the tissue that is to be replaced (3). The development of such implantable construct requires a careful selection of the biomaterial used for scaffold fabrication. The tissue scaffold should meet strict requirements and act as extracellular matrix (ECM), which surrounds cells in the body and support cells proliferation (4).

Biomaterials of polymer origin are employed in the tissue scaffolds manufacturing to replace various tissues and organs (5–7). Polymeric materials play a key role in the studies for skin tissue regeneration. To the most commonly used synthetic polymers in this field belongs PURs (8–10), PLA (11), polycaprolactone (PCL) (12,13) ply(glicolide) (PGA) (14).

The versatile synthesis of PURs provides biocompatibile, antithrombogenic and biodegradable materials(15), which are used in a huge variety of medical devices including endotracheal tubes, vascular grafts, elements of artificial hearts, membranes for dialysis, adhesives for bone regeneration and materials for dental recovering (16–18). PURs are one of the most popular biomaterials applied for controlled and targeted delivery of drugs in medical devices (19).

Due to the unique segmented structure of PURs their properties can be modified according to the selected requirements (15). Biodegradation profile is one of them. According to the literature PURs usually need over 6 months to lose 30% of their initial mass *in vivo*, what is suitable according to the tissue regeneration requirements (20,21). It was reported that degradation of scaffold has to be controlled in such a way that its physicochemical and mechanical properties will be maintained at least from 3 to 6months. Between 1st and 3rd month cells are constantly proliferating and between 3rd and 6th month regeneration takes place *in situ*. Henceforth, the scaffold matrix may start losing its mechanical properties and should be metabolized by the body without foreign body reaction approximately between 12 and 18 months (22–25). What is worth to be mentioned here is that the degradation products of PUR have to be nontoxic and canalizable in natural life cycles (26). The degradation rate may be controlled at the different levels. For example by the application of “fast-degradable” materials like PLA or PEG (27,28), which blended with PURs significantly improve their degradation rate. Montini-Ballarin et al.

(27) observed that electrospun PUR grafts were losing only 63% of mass after 34 weeks in hydrolytic degradation performed in PBS, while PUR blends with PLLA were degrading faster: blend PUR/PLLA=50/50 and PUR/PLLA=10/90 lost 74% and 90% of their initial mass respectively after 8 weeks of the study.

Poly(L-lactic acid) (PLLA) is FDA approved biodegradable polyester commonly used in biomedical applications, such as drug delivery systems, tissue engineering, and biomedical devices that exhibit semi-crystalline structure (29–31). PLLA possesses high elastic modulus required to withstand high pressure and flow without collapse or degradation until tissue develops and matures *in vivo*. PLLA has a mechanical response similar to collagen (31). Degradation process of PLA occurs by hydrolysis and lead to a decrease in the macromolecules of average length when water reacts with the ester unions present in the polymeric chains (32–34). In initial stage the amorphous regions of PLA react with water molecules (their segments are more flexible). This leads to an increase in chains mobility, which forms new more defective crystals. The degradation rate of PLA can be accelerated because of the autocatalytic effect caused by the acidic nature of the generated carboxylic end-groups. At the final stage the hydrolysis takes place at the crystalline regions (35,36). On the other hand, PURs which consist of alternating soft (SS) and hard segments (HS) can be designed as biodegradable materials as well (37). SS are usually engaged to introduce chemical bonds susceptible to degradation, and therefore alternate the material degradation rate. In contrary, HS are often degraded through enzymatic mechanisms (37). Although degradation mechanisms depend on both the PUR soft and hard segments, there are certain mechanisms common to the majority of biodegradable/bioresorbable PURs (27,28). As it was described by Montini-Ballarin et al. ester bonds are hydrolyzed in α-hydroxy acid oligomers as degradation products, as well as in fragments containing urethane and urea with acid terminal groups. The composition of the prepolymer or macrodiol has shown to control the *in vitro* degradation rate of PUR (38). When soft segments are composed of ester units, the degradation process is mainly the same as the one mentioned before for PLA. In addition it was observed that PURs with amorphous SS degrade faster than others with semi-crystalline SS. Additional degradation of urethane and urea units to free polyamines could take place, depending on the diisocyanate used (38).

Infections related to the biomaterials are often observed with artificial implants and in many cases result in the failure of the devices (38). Various substances known as toxins, proteases and pro-inflammatory molecules, may cause an excessive and prolonged inflammatory response of the host tissues by the bacterial colonization and subsequent infection (39,40). This can seriously interfere with the wound healing process (41). Thus the large focus is to design a skin tissue scaffold that is intrinsically infection-resistant (42). Ideal antimicrobial skin tissue scaffold should represent certain features such as provision of a moist environment to enhance healing (43), provide broad-spectrum of antimicrobial activity (bacterial growth inhibition) (44), effective absorption of the wound exudates (45), ensure suitable wound humidity (46), enable formation of new tissue with no scars (47), and be permeable for gases and delivery of nutrients (48). Therefore, the proper care of skin wounds, burns and injuries is important

for prevention of microbial infection and trans-epidermal water loss, which lead to accelerated wound regeneration (49). Thus, restoration of skin barrier is crucial importance in the treatment of injuries. To meet the requirements of antibacterial skin scaffold the templates are modified with antibiotics e.g. coming from the fluoroquinolones group (50).

Fluoroquinolones are well-established broad spectrum antibiotics (51,52) having potent bactericidal activity against most common pathogens which are prevalent at wound site such as *S. aureus*, *P. aeruginosa*, *E. Coli* etc. (53). Performed microbiological studies revealed that ciprofloxacin is relevant antimicrobial agent and works against the bacterial species such as *E. coli, S. aureus* (50,54), the main species responsible for wounds infections according to references . Among them, Ciprofloxacin is one of the most widely used fluoroquinolones to treat a variety of bacterial infections. Its low minimal inhibitory concentration for both Gram-positive and Gram-negative bacteria that cause wound infections and the frequency of spontaneous resistance to ciprofloxacin is very low (56).

In this paper we described the fabrication process of hybrid PUR-PLA scaffolds containing 5 wt% or 10 wt% of PLA, to improve the scaffold degradability. PURs used in this study were synthesized by the use of amorphous polyester α,ω-dihydroxy(ethylene-butylene adipate) (PEBA) and aliphatic diisocyanate (1,6-hexamethylene diisocyanate, HDI) (8) according to the references reporting better degradability of PURs containing amorphous macrodiols (27,28) and non-toxic degradation products of PURs obtained by using aliphatic diisocyanates (38,57). These PURs were characterized in our previous work and recognized as biocompatible (8). Obtained hybrid PUR-PLA scaffolds (HPPS) were modified with ciprofloxacin (Cipro), a fluoroquinolone antibiotic, which has an inhibitory effect on the *S. aureus* growth, which is one of the bacterial species responsible for most common wound infections (37% of all species isolated from the wounds was S.aureus) (55). Obtained unmodified and Cipro-modified HPPS were characterized by the FTIR spectroscopy, to determine the chemical functional groups of unmodified HPPS and the presence of bonded ciprofloxacin in Cipro-modified HPPS. Mechanical properties (tensile strength and elongation at break) of HPPS were studied to indicate their potential application as a skin tissue scaffold. Scanning electron microscopy (SEM) was done to observe presence of Cipro-modifier at the surface of Cipro-modified HPPS in comparison to unmodified HPPS. Short-term interactions with selected media and optical microscopy were performed to determine the degradation characteristic of obtained unmodified and Cipro-modified HPPS. Finally, the microbiological tests were performed to indicate the antibacterial effect of Cipro modification of HPPS. Performed tests showed that obtained materials may be suitable candidates for skin tissue engineering.

Experimental

Fabrication of porous Hybrid Polyurethane-Polyester Porous Scaffolds (HPPS)

The fabrication procedure of HPPS was similar to described in our previous paper (10). PUR, reported by Kucińska-Lipka et al. (8) was dissolved in dimethylsulfoxide (DMSO) at 20 wt% concentration. PLA (Mw=2000, Sigma Aldrich) was dissolved in DMSO at the same concentration (20 wt%). PLA solution was then mixed with PUR solution at concentration of 5 wt% or 10 wt% (per mass of PUR). Solution PUR-PLA mixture was mixed with the use of magnetic stirrer at 60°C for 24h. Then, sodium chloride (NaCl, POCH, Poland) of crystals size in the range 0.6–0.4 μm was added to the PUR solution until complete solution saturation occurred (high viscosity of mixture). Formulated PUR-PLA-salt mixture was transferred between the flat stainless steel molds and pressed at hydraulic press (ZUP Nysa) for 3 min at 4,9 MPa pressure (at 20°C) to reach uniform distribution of the mixture at the molds. Molds were placed at the refrigerator set at -20°C overnight to direct the solvent crystallization (58–60). Then HPPS were removed from the mold and immersed in warm (40–50°C) bidistilled water, where for 7 days the solvent and the sodium particles were washed out. Water was changed twice a day. Finally, samples of HPPS were dried at 50◦C for 24 h and used for modification and testing. Symbols of samples were given in Table 1.

Modification of HPPS with antibacterial agent from the group of fluoroquinolones

The antibacterial factor ciprofloxacin (Sigma Aldrich) approved by FDA, was used in this study in hydrochloride form. The HPPS modification was as follows; Gelatin solution (5 wt%) in DMSO containing 2 wt% or 5 wt% of C was prepared. Gelatin was used for two reasons: one was to increase the solution viscosity, and the other was to improve the biocompatibility after implementation of HPPS. HPPS were cut into samples of 40 mm^3 volume. Samples were placed in the 24-well culturing plates (Bionovo, Legnica, Poland), immersed in 3 ml of Cipro-gelatin solutions containing 2 wt% or 5 wt% of Cipro and left for 24h under vacuum at 20°C to penetrate fully the HPPS. After that time Cipro-modified HPPS were dried overnight in laboratory drier set at 60°C and used for examination. Symbols of samples were given in Table 1.

Samples symbols

Table1 shows symbols of obtained samples with their brief explanation.

Table 1.Symbols of unmodified and Cipro-modified HPPS with brief explanation

Symbol	Explanation
PUR/10PLA/0C	Unmodified HPPS obtained with 10 wt% of PLA
PUR/10PLA/2C	HPPS obtained with 10 wt% of PLA, modified with 2 wt% of AF
PUR/10PLA/5C	HPPS obtained with 10 wt% of PLA, modified with 5 wt% of AF
PUR/5PLA/0C	Unmodified HPPS obtained with 5 wt% of PLA
PUR/5PLA/2C	HPPS obtained with 5 wt% of PLA, modified with 2 wt% of AF
PUR/5PLA/5C	HPPS obtained with 5 wt% of PLA, modified with 5 wt% of AF

Characterization

Fourier transform infrared spectroscopy (FTIR)

The FTIR analysis was performed with the use of Nicolet 8700 Spectrometer in the spectral range from 4000 to 500 cm^{-1} averaging 256 scans with a resolution of 4 cm^{-1}.

Mechanical properties

Tensile strength (T_{Sb}) and elongation at break (eb) were studied by using the universal testing machine Zwick & Roell Z020 according to PN-EN ISO 527-2:2012 with a crosshead sped of 100 mm/min and measuring path of 60,35.

Optical microscopy

Unmodified and Cipro-modified HPPS were studied by Digital Microscope U800X at 800x magnification. Optical microscopy (OM) studies were performed before and after short-term degradation studies with selected media. Initial morphological characterization was done by using program ImageJ® software (U.S. National Institutes of Health, Bethesda, MA, USA).

Scanning Electron Microscopy (SEM)

SEM of unmodified and Cipro-modified HPPS was performed by using FEI Quanta 250 FEG at accelerating voltage of 10 kV. Samples were covered with 15 nm layer of gold in sputter-coater Leica EM SCD 500. SEM images were analyzed by ImageJ® software (U.S. National Institutes of Health, Bethesda, MA, USA).

Energy-dispersive X-ray (EDX) spectroscopy was performed to study elemental analysis and chemical composition of unmodified and Cipro-modifiedHPPS

Short-term degradation studies in selected media

The short-term degradation studies of obtained unmodified and Cipro-modified HPPS were performed in selected media: 2N HCl, 5M NaOH and 0,1M $CoCl_2$ in 20% H_2O_2. It is a standard procedure previously reported in the literature (61,62). PURs were cut into round samples of 0,5 cm^2 area. Prepared samples were dried and weighed in thermobalance (RADWAG MAX50/SX) set at 60°C. Then, 6 samples of each studied PUR materials were placed in 24-well cell culture plate filled with selected media: oxidative solution of 0,1M $CoCl_2$/20% H_2O_2; acidic solution of 2N HCl or basic solution of 5M NaOH. Samples were incubated in selected media at 37°C. Mass changes of samples were examined after 15 days for oxidative, acidic and basic media. Samples mass changes measurements were as follows: samples were taken out from the container and put into a paper sheet to reduce the medium excess. Then, samples were placed in the thermobalance (set at 60°C) where were dried to the constant mass and weighed. Mass loss was calculated by the formula 1. The results are arithmetic mean of six measurements.

$$S = \left(\frac{m_i - m_0}{m_0} \right) \cdot 100\% \quad (1)$$

Where*:*

m_i - sample weight after 1, 3, 7, 14 days and 1,2,3 and 6 months of incubation (g)

m_0- sample weight before the test (g)

Microbiological tests

Antibacterial activity of unmodified and Cipro-modifiedHPPS was tested by using three bacterial strains belonging to species: *Escherichia coli* (Gram negative), *Staphylococcus aureus* (Gram positive) and *Pseudomonas aeruginosa* (Gram negative), respectively, which are potentially Cipro-sensitive bacterial species. The bacterial strains were obtained from a collection of the Department of Molecular Biotechnology and Microbiology, Gdańsk University of Technology.

All bacterial strains were cultivated in 20 ml of fresh and sterile LB medium (Luria Broth). LB medium containing, g/L: casein peptone 10.0; yeast extract 5.0; NaCl 10.0 dissolved in deionized water. Cultivations were carried out in 200 ml sterile Erlenmeyer flasks, on a rotary shaker at 170 rpm at 37°C for 18-24 h. After the incubation time, 100 µl of each bacterial strain culture was transferred into 10 ml of sterile LB medium in 100 ml sterile Erlenmeyer flasks. Next, bacterial strains cultivations were carried out on a rotary shaker at 170 rpm at 37°C to get the log phase of bacterial growth (OD_{600} values 0.4-0.7). For determination of antibacterial activities, 100 µl of each bacterial strain suspensions in log phase of growth were placed on sterile LA medium with a sterile glass rod. LA medium containing, g/L: casein peptone 10.0; yeast extract 5.0; NaCl 10.0; agar 15.0. Prior the examination, unmodified and Cipro-modified HPPS were sterilized by the exposition to UV radiation for 30 minutes and placed on plates with sterile tweezers. Sterile samples of unmodified and Cipro-modified HPPS scaffolds were placed on the bacterial cultures on LA plates and incubated at 37°C for 24 h. After the incubation, the presence or absence of growth inhibition zones around samples of unmodified and Cipro-modified HPPS their diameter was measured. All analysis were done in triplicate.

Results

Fourier Transform Infrared Spectroscopy

Fig. 1 shows the FTIR spectra of ciprofloaxin used for HPPS modification and FTIR spectra of unmodified and Cipro-modified(2 wt% or 5 wt%) HPPS, which were obtained by using 5 wt% or 10 wt% of PLA.

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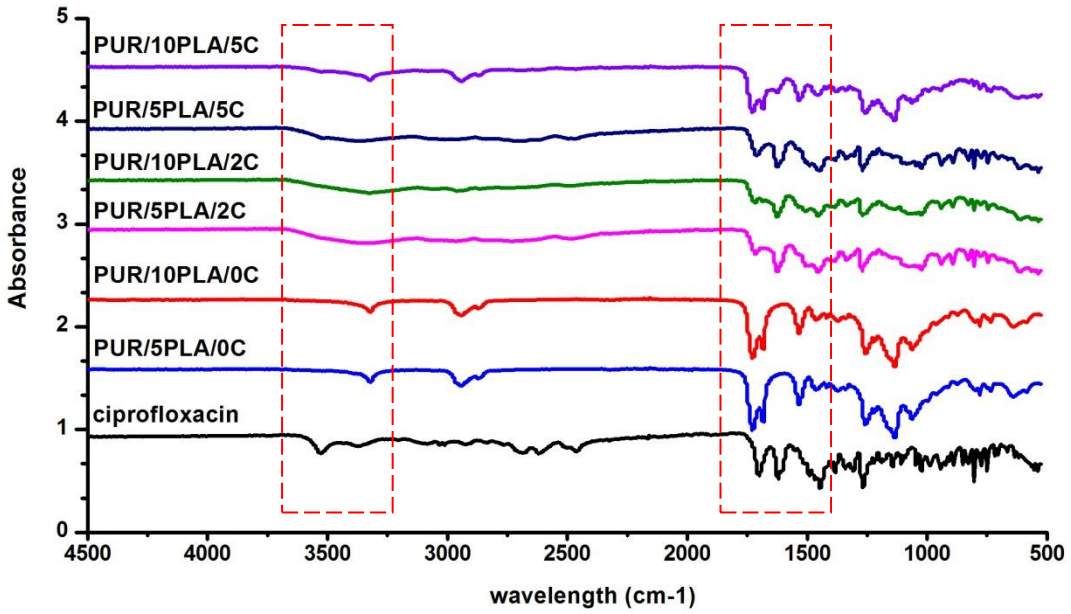

Fig. 1 The FTIR spectra of ciprofloxacin used for HPPS modification and FTIR spectra of unmodified or Cipro-modified (2 wt% or 5 wt%) HPPS, obtained by using 5 wt% or 10 wt% of PLA

To analyze the spectra of ciprofloaxin and unmodified and Cipro-modified HPPS (Fig. 1) the book of Silverstein et al.(63) and the scientific paper of Tan et al. (63) and Yilgor et al. (64) were used.

The narrow peak detected in case of unmodified HPPS (PUR/10PLA/0C and PUR/5PLA/0C) (Fig. 1) at 3328 cm^{-1} corresponded to the stretching of NH group present in urethane linkages. Bands observed at 2941 cm^{-1} and 2864 cm^{-1} indicated stretching of aliphatic asymmetric and symmetric CH_3 and CH_2 groups present in HPPS, coming from PUR components (macrodiol and diisocyanate) and PLA chemical structure. At 1725 cm^{-1} was observed stretching of carbonyl groups present in PLA structure. Further band was indicated at 1681 cm^{-1} related to the presence of urethane linkages in obtained HPPS. The confirmation of urethane linkage presence in the HPPS structure was the band observed at 1522 cm^{-1} concerning stretching of C-N. At 1466 cm^{-1} and 1373 cm^{-1} were observed bands indicating deformation of CH_3 and CH_2 groups of HPPS. Between 1262 cm^{-1} and 1053 cm^{-1} was recognized stretching of –C(O)-O- and C-O- of HPPS coming mainly from PUR macrodiol and PLA structure. Between 778 cm^{-1} and 586 cm^{-1} were indicated out of plane deformation of CH_3, CH_2 and NH and OH as well.

In terms of Cipro-modified HPPS with 2 wt% or 5 wt% of ciprofloxacin (Fig. 1) (PUR/10PLA/5C, PUR/5PLA/5C and PUR/10PLA/2C, PUR/5PLA/2C) the arrangement of the bands was similar to those observed for unmodified HPPS and ciprofloaxin: Between 3667-3123 cm^{-1} was identified stretching of COOH group present in the ciprofloxacin and stretching of NH groups of both ciprofloxacin and in the HPPS structure. In the range of 3116-2886 cm^{-1} were noted stretching of aromatic and cycloaliphatic rings present in the structure of ciprofloxacin, and the asymmetric and symmetric stretching of aliphatic CH_3 and CH_2 groups present in HPPS (macrodiol and diisocyanate of PUR and in PLA structure).

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Between 2721-2479 cm^{-1} was noted stretching of double bonds present in aromatic ring. At 1714 cm^{-1} was indicated stretching of carbonyl groups of PLA. At 1620 cm^{-1} was observed band described as stretching of urethane linkages and stretching of C-N confirming presence of urethane linkage in HPPS structure. At 1449 cm^{-1} was observed stretching of the rings present in ciprofloxacin. Between 1383 cm^{-1} and 1268 cm^{-1} were observed aromatic ring overtones related to the aromatic ring substitution. Between 1169 cm^{-1} and 1015 cm^{-1} were observed stretching of -C(O)-O- and -C-O-. Between 938-520 cm^{-1} were indicated out of plane deformation of CH_3, CH_2 and NH and OH as well.

Mechanical properties

Fig. 2a and Fig. 2b showed tensile strength (T_{Sb}) and elongation at break (e_b) of obtained unmodified and Cipro-modified(2 wt% or 5 wt%) HPPS, containing 5 wt% or 10 wt% of PLA.

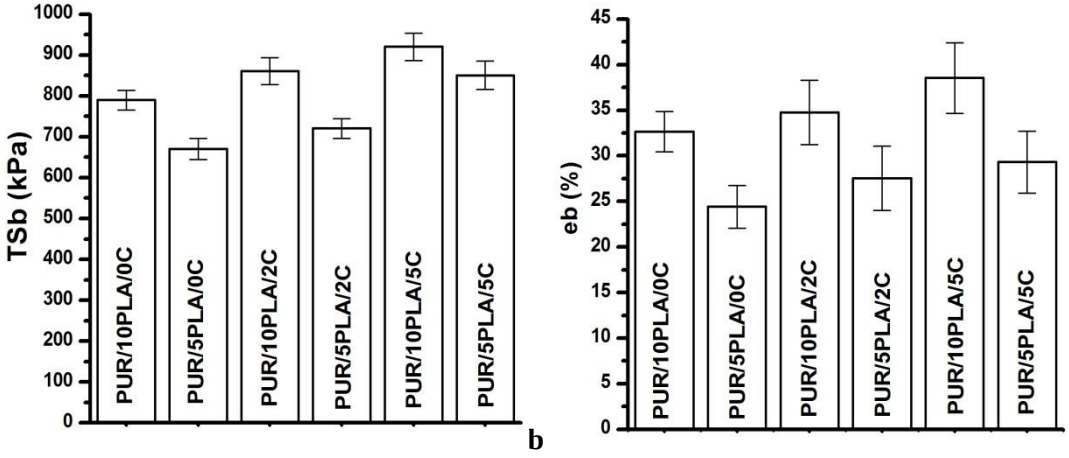

Fig. 2 Tensile strength (TSb) and elongation at break (eb) of unmodified and Cipro-modifiedHPPS

TSb of PUR/5PLA/0C (Fig. 2a) was 670±26 kPa, and eb (Fig. 2b) was 24±2%. The HPPS modification with ciprofloxacin significantly increased T_{Sb} of obtained Cipro-modified HPPS (PUR/5PLA/2C=720±24 kPa, PUR/5PLA/5C=850±34 kPa) and slightly increased their eb (PUR/5PLA/2C=28±4%, PUR/5PLA/5C=30±3%). The T_{Sb} of PUR/10PLA/0C was 790±24 kPa and eb was 32±2%. Application of ciprofloxacin modification in HPPS caused large improvement of TSb (PUR/10PLA/2C=860±33 kPa and for PUR/10PLA/5C=920±33 kPa) as it was observed in case of HPPS containing 5 wt% of PLA. The eb increased slightly (PUR/10PLA/2C=34±5%, PUR/10PLA/5C=39±4%). The HPPS, which contained 5 wt% of PLA had lower T_{Sb} than HPPS containing 10 wt% of PLA, but in case of eb no significant improvement was noted.

Scanning Electron Microscopy

Fig. 3 showed SEM images of unmodified and Cipro-modified(2 wt% or 5 wt%) HPPS obtained by using 5 wt% or 10 wt% of PLA. At Fig. 3 was presented as well the image of ciprofloaxicin used for HPPS modification. From Fig. 4a to Fig.4d were showed the results of EDX analysis performed during SEM studies of unmodified and Cipro-modified HPPS. Fig. 5 shows the EDX spectra of ciprofloxacin.

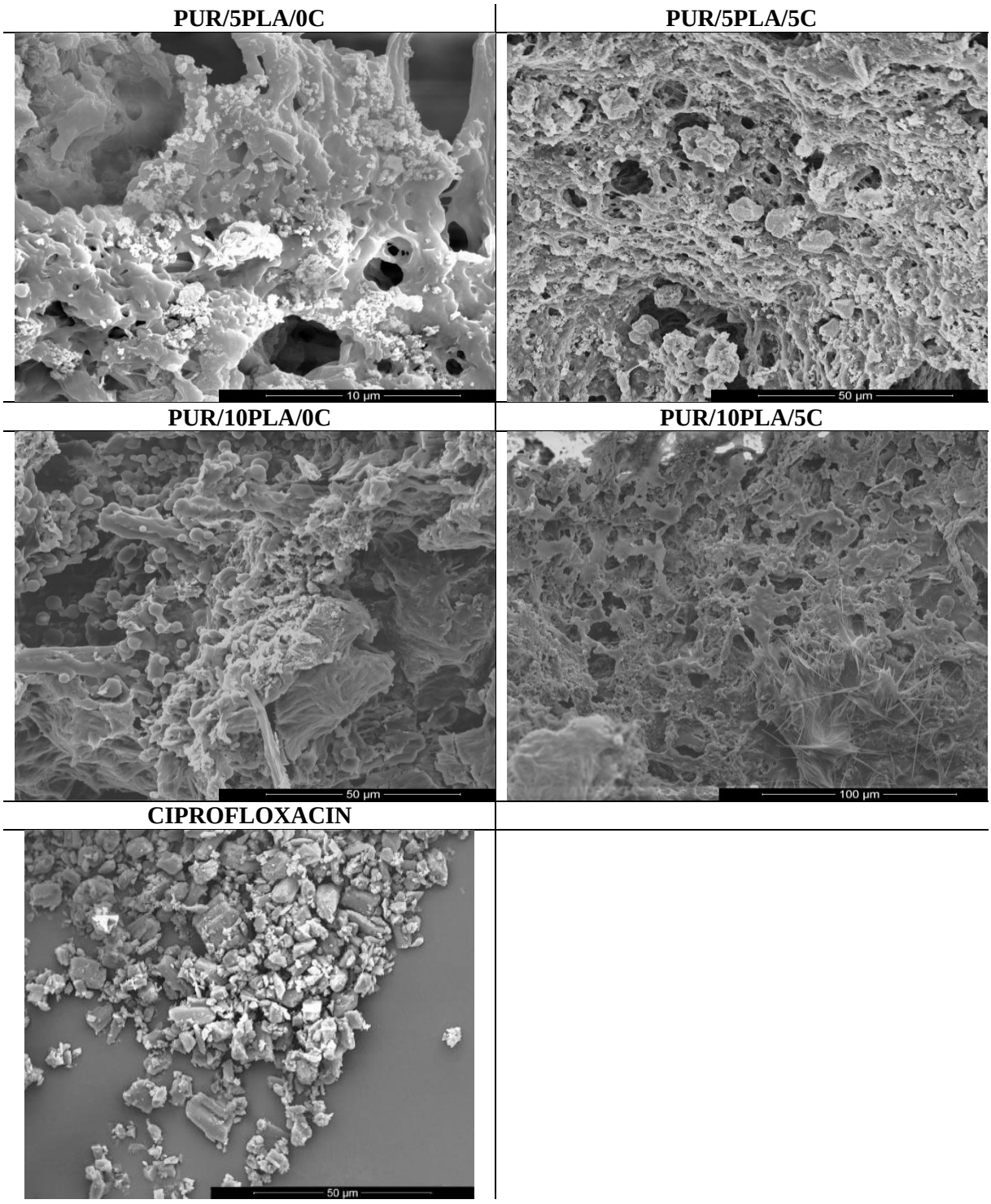

Fig. 3 SEM images of unmodified and C-mosidief (2 wt% or 5 wt%) HPPS obtained by using 5 wt% or 10 wt% of PLA and SEM image of ciprofloaxin used for HPPS modification.

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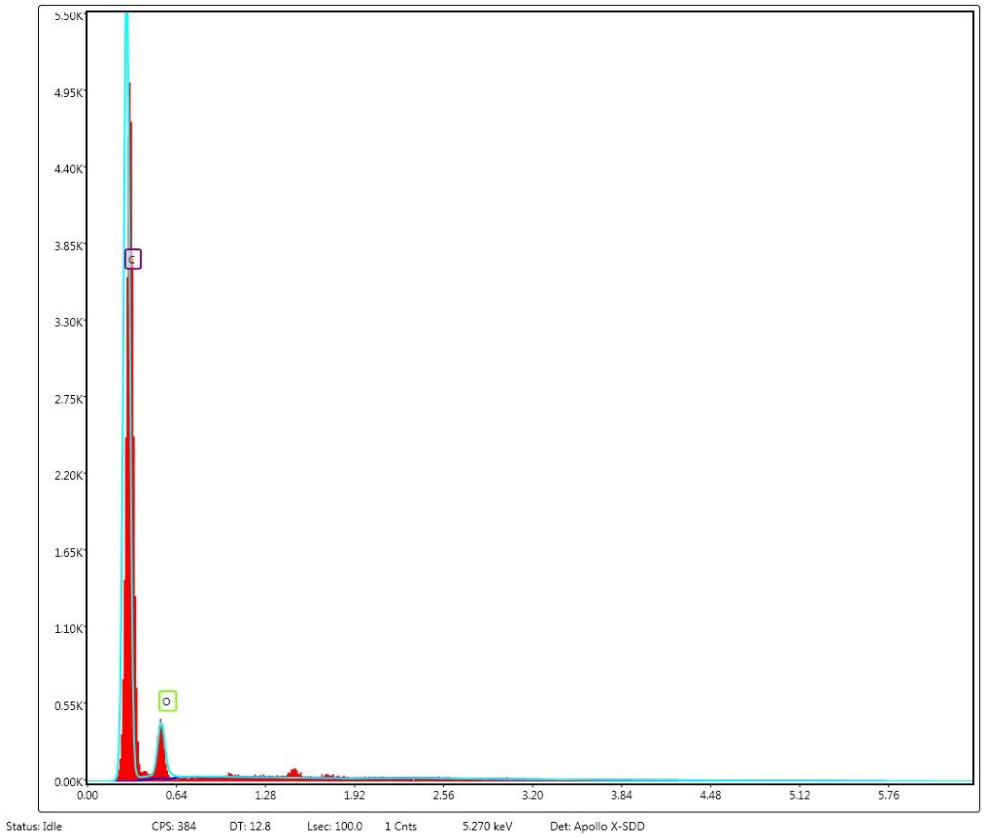

Fig. 4a Selected EDX spectra of PUR/5PLA/0C

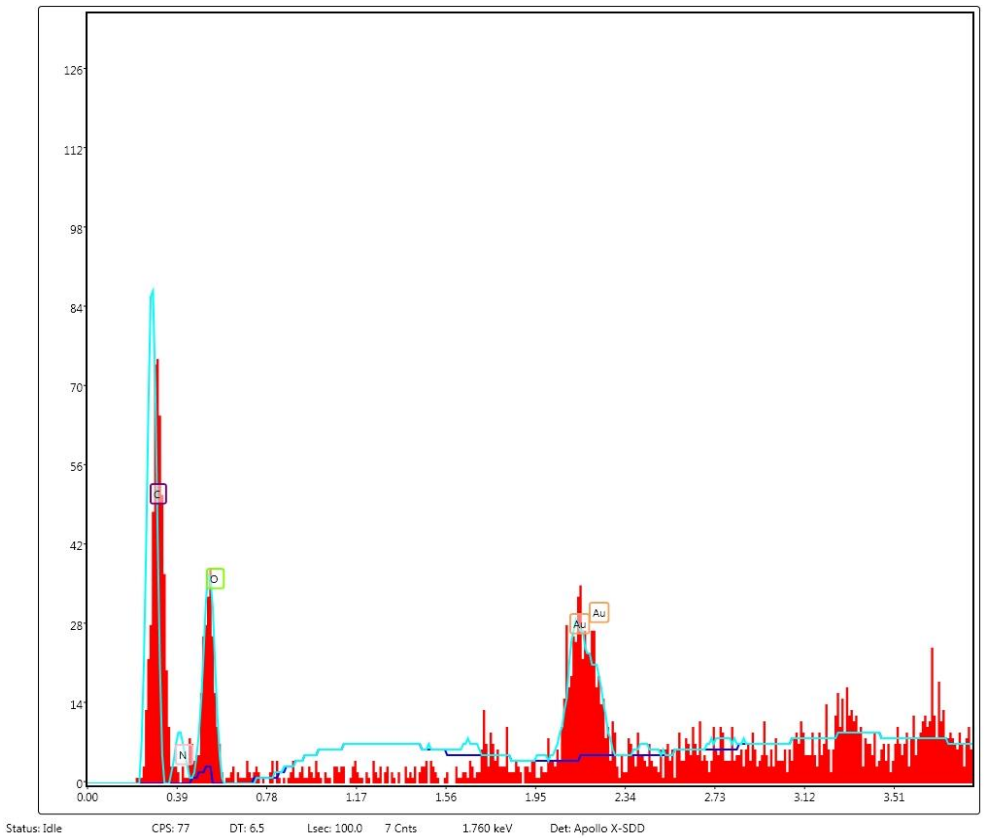

Fig. 4b Selected EDX spectra of PUR/5PLA/5C

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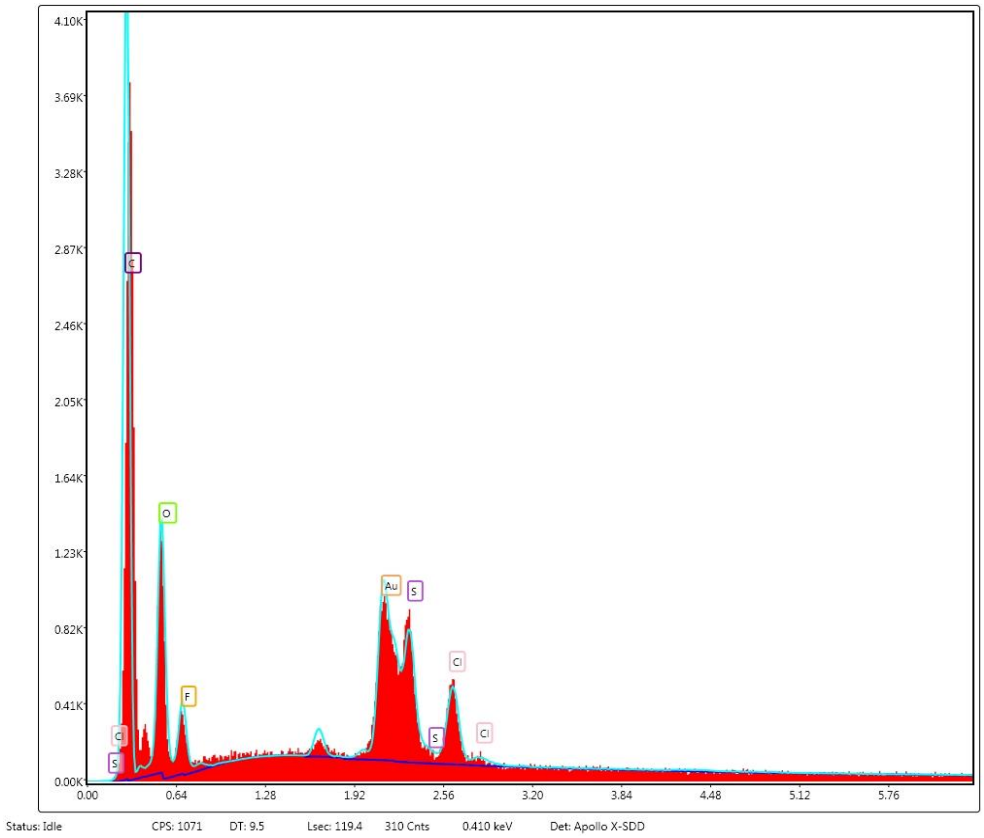

Fig. 4c Selected EDX spectra of PUR/10PLA/0C

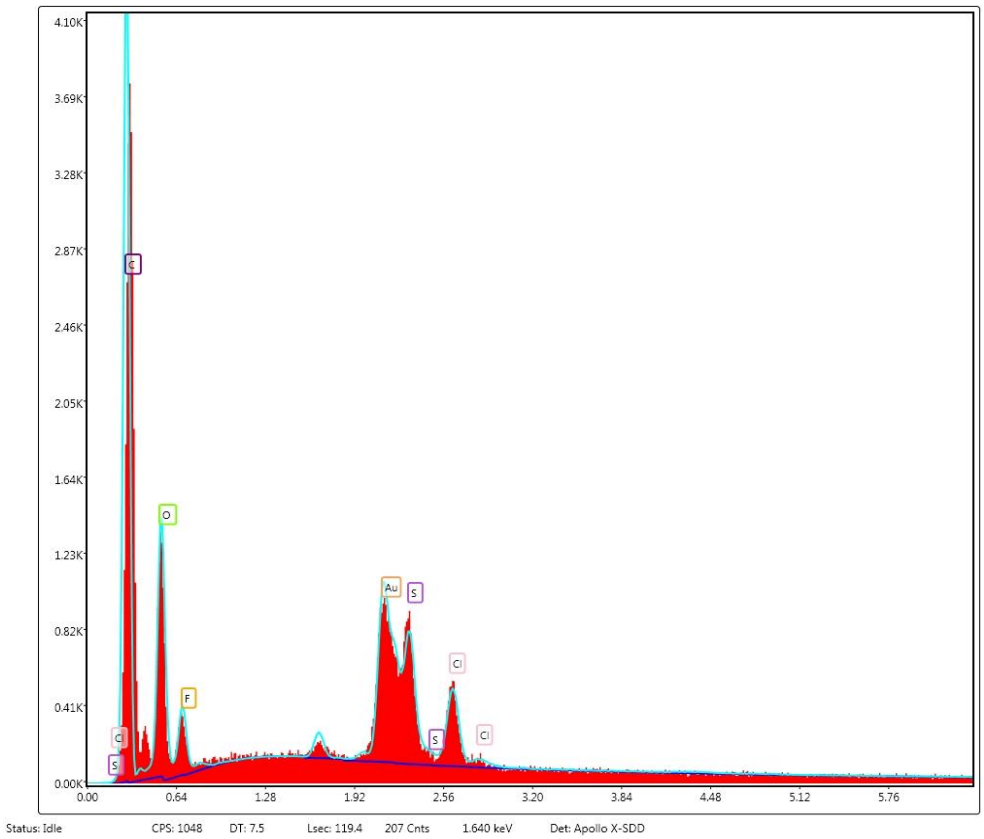

Fig. 4d Selected EDX spectra of PUR/10PLA/5C

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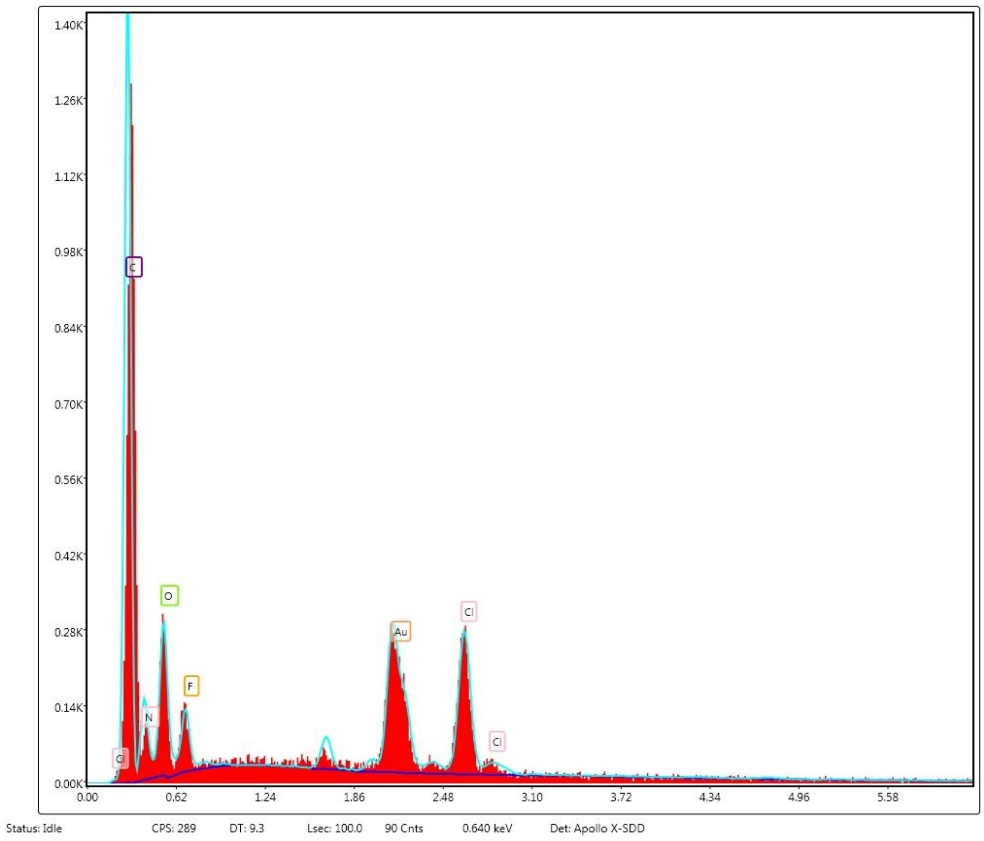

Fig. 5 Selected EDX spectra of ciprofloxacin

SEM images (Fig. 3) confirmed porous structure of unmodified and Cipro-modified HPPS. Porosity of HPPS was given in Table 1.

Table 1. Porosity of unmodified and Cipro-modifiedHPPS

Symbol	Porosity (%)
PUR/5PLA/0C	86%
PUR/5PLA/2C	87%
PUR/5PLA/5C	85%
PUR/10PLA/0C	84%
PUR/10PLA/2C	72%
PUR/10PLA/5C	64%

In case of HPPS obtained by using 5 wt% of PLA the homogenous porous structure (86%) was observed (Fig. 3 and Table 1) of pore sizes in the range of 50-375 µm. Pores were interconnected, what is favorable in case of porous materials dedicated to the tissue engineering. Modification with ciprofloxacin (Fig. 3 and Table 1) didn't cause significant changes in porosity of HPPS containing 5 wt% of PLA (PUR/5PLA/2C=87% and PUR/5PLA/5C=85%) or on the pore sizes (47-320 µm for PUR/5PLA/2C and 32-297 µm for PUR/5PLA/5C).

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For HPPS obtained by using 10 wt% of PLA high % of porosity (84%) was observed as well (Fig. 3 and Table 1). The pores were interconnected and of the sizes in between 67-332 μm. Ciprofloxacin modification (Fig. 3) caused significant change of HPPS morphology. The large decrease of porosity was noted (Fig. 3 and Table 1) (to 72% for PUR/10PLA/2C and to 64% for PUR/10PLA/5C) and as well decrease of pore sizes and even completes closure of pores, which was increasing with the ciprofloxacin amount.

The EDX analysis (Fig. 4a – Fig. 4d) of unmodified and Cipro-modifiedHPPS confirmed presence of chemical elements of PUR and PLA structure: carbon, oxygen, nitrogen. In case of Cipro-modifiedHPPS the EDX analysis revealed presence of ciprofloxacin (Fig. 5). The EDX spectra identify presence of elements like chloride and fluorine characteristic for ciprofloxacin hydrochloride salt used in the study. Presence of gold at the EDX spectra is related to the sputter coating of the HPPS samples prior SEM study.

Short-term interaction with selected media

Fig. 6 showed the % of dry mass remaining after the test of short-term interactions performed with unmodified and Cipro-modified HPPS containing different amount of PLA. Samples were studied after 15 days of incubation in selected media: 2N HCl, 5M KOH and 0,1M $CoCl_2$ in 20% H_2O_2.

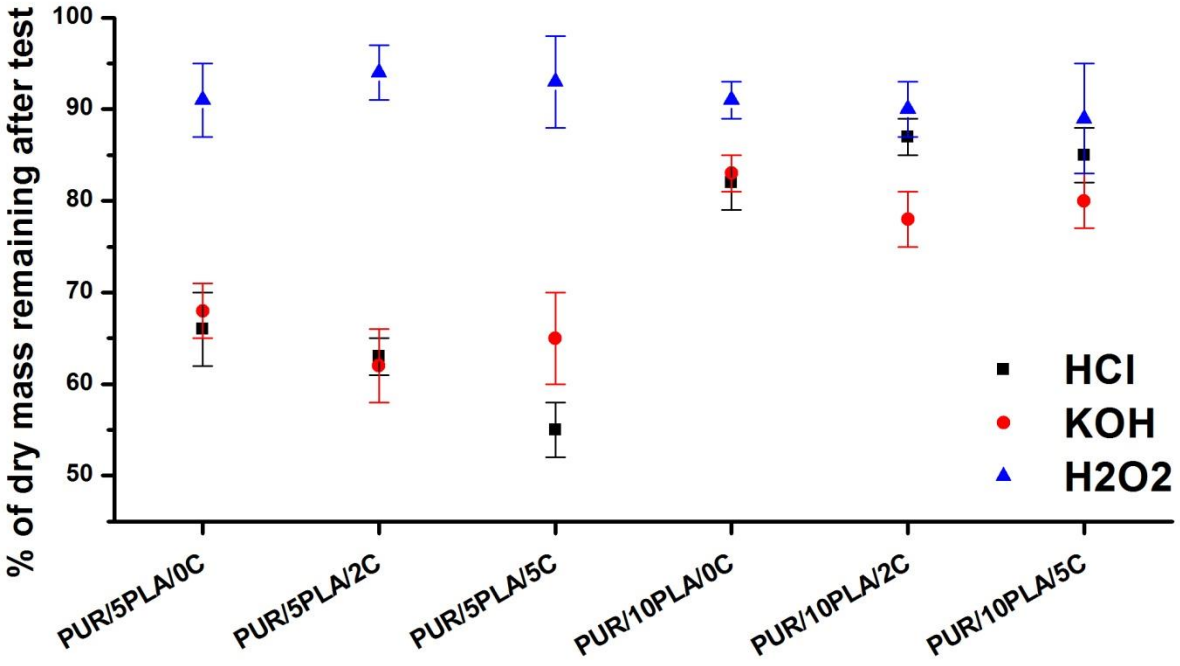

Fig. 6 Dry residue (%) of unmodified and Cipro-modifiedHPPS, obtaind by using 5 wt% or 10 wt% of PLA, after 15 days of incubation in selected media: 2n HCl, 5M KOH, 0,1M $CoCl_2$ in H_2O_2

Fig. 6 show that HPPS obtained by using 10 wt% of PLA are less sensitive on the selected environments than those obtained by using 5 wt% of PLA. For unmodified HPPS containing 10 wt% of PLA the dry residue was 82±3 % in the acidic environment, 83±2% in the basic environment and 91±2% in oxidative environment. It shows that 18% and 17% of HPPS containing 10 wt% of PLA degraded in acidic and basic environment respectively, and 9% in the oxidative environment. The ciprofloxacin modification

(both 2 wt% and 5 wt%) of HPPS, containing 10 wt% of PLA, didn't cause significant mass changes. In the acidic environment the mass decrease was in average 13% and 15% respectively when 2 wt% and 5 wt% of ciprofloxacin was added. In the basic environment the mass decrease was 22% and 20% with the increase of the ciprofloxacin amount from 2 wt% to 5 wt% respectively. In the oxidative environment the mass decrease was 10% and 11% for 2 wt% and 5 wt% of ciprofloxacin added respectively.

In case of unmodified HPPS obtained with 5 wt% of PLA the mass decrease was about 15% higher in comparison to the unmodified HPPS obtained with the use of 10 wt% of PLA. Respectively it was as follows: 34 % in acidic environment, 32% in basic environment. In the oxidative environment the mass decrease was comparable to the HPPS samples obtained by using 10 wt% of PLA and equal to the 9%. Introduction of ciprofloxacin in case of HPPS obtained with 5 wt% of PLA has larger influence on the degradation of these materials than in case of HPPS obtained with 10 wt% of PLA. In the acidic environment the mass decrease was 43% and 45% for PUR/5PLA/2C and PUR/5PLA/5C respectively. In basic environment noted mass decrease was 38% and 40% for PUR/5PLA/2C and PUR/5PLA/5C respectively. In the oxidative environment mass decrease was about 10% and comparable the same with mass decrease of Cipro-modifiedHPPS samples obtained with 10 wt% of PLA.

Unmodified and Cipro-modifiedHPPS, which were interacting with acidic and basic environment after 15 days of incubation and drying to the constant mass (at 60°C) were characterized by the high fragility, what caused that they were not possible to use in optical microscopy studies. Such changes were not observed in case of materials after oxidative degradation, which were stable and didn't lost large % of mass. Optical microscopy images before and after short-term interactions with oxidative enviromnet study was presented in Fig. 7. The blue-green color of samples is coming from anhydrous cobalt chloride.

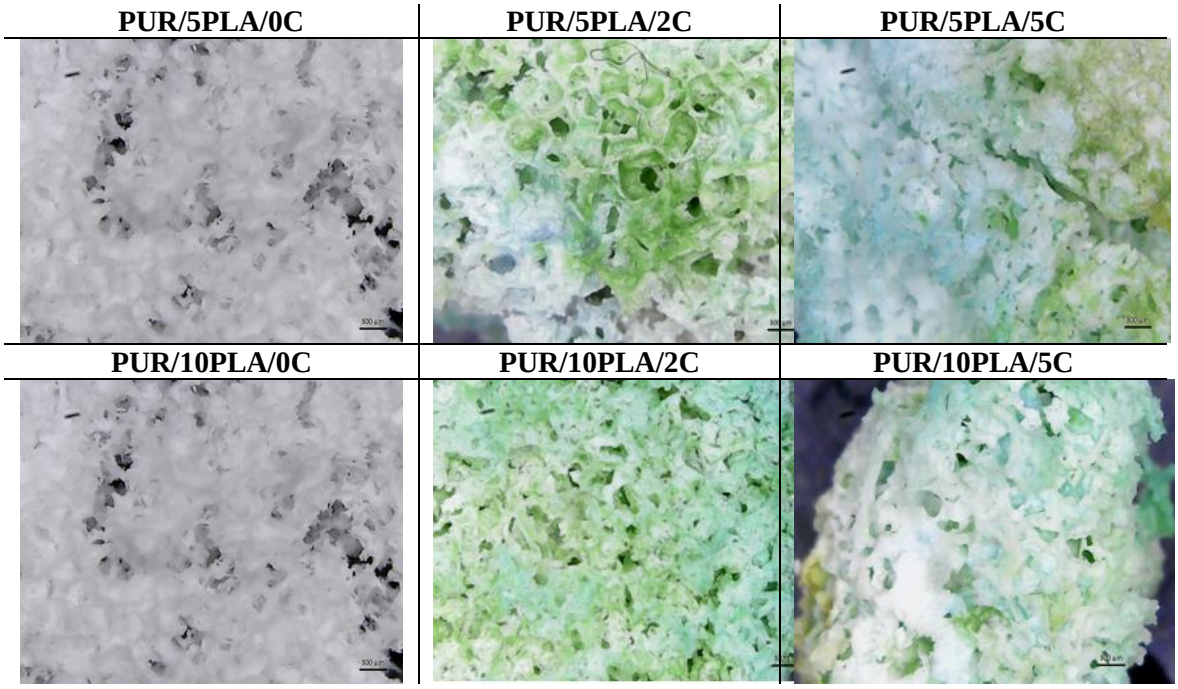

Fig. 7 Optical microscopy images of unmodified and Cipro-modifiedHPPS obtained by using 10 wt% after 15 days of incubation

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Microbiological tests

Performed microbiological tests (Fig. 8) revealed presence of inhibition zones (Table 2.) of *S.aureus* growth when HPPS were modified with ciprofloxacin (2 wt% and 5 wt%). The diameters of inhibition zones were increasing with the amount of ciprofloxacin added to the HPPS.

Tabela 2. *S.aureus inhibition zones detected for unmodified and Cipro-modifiedHPPS*

Symbol	Inhibition zone (mm)
PUR/5PLA/0C	0
PUR/5PLA/2C	15
PUR/5PLA/5C	20
PUR/10PLA/0C	0
PUR/10PLA/2C	16
PUR/10PLA/5C	22

Fig. 8 shows the effect of antimicrobial activity of Cipro-modified HPPS (2 wt% or 5 wt% of ciprofloxacin) against *S.aureus* in comparison to the unmodified HPPS serving as a controls.

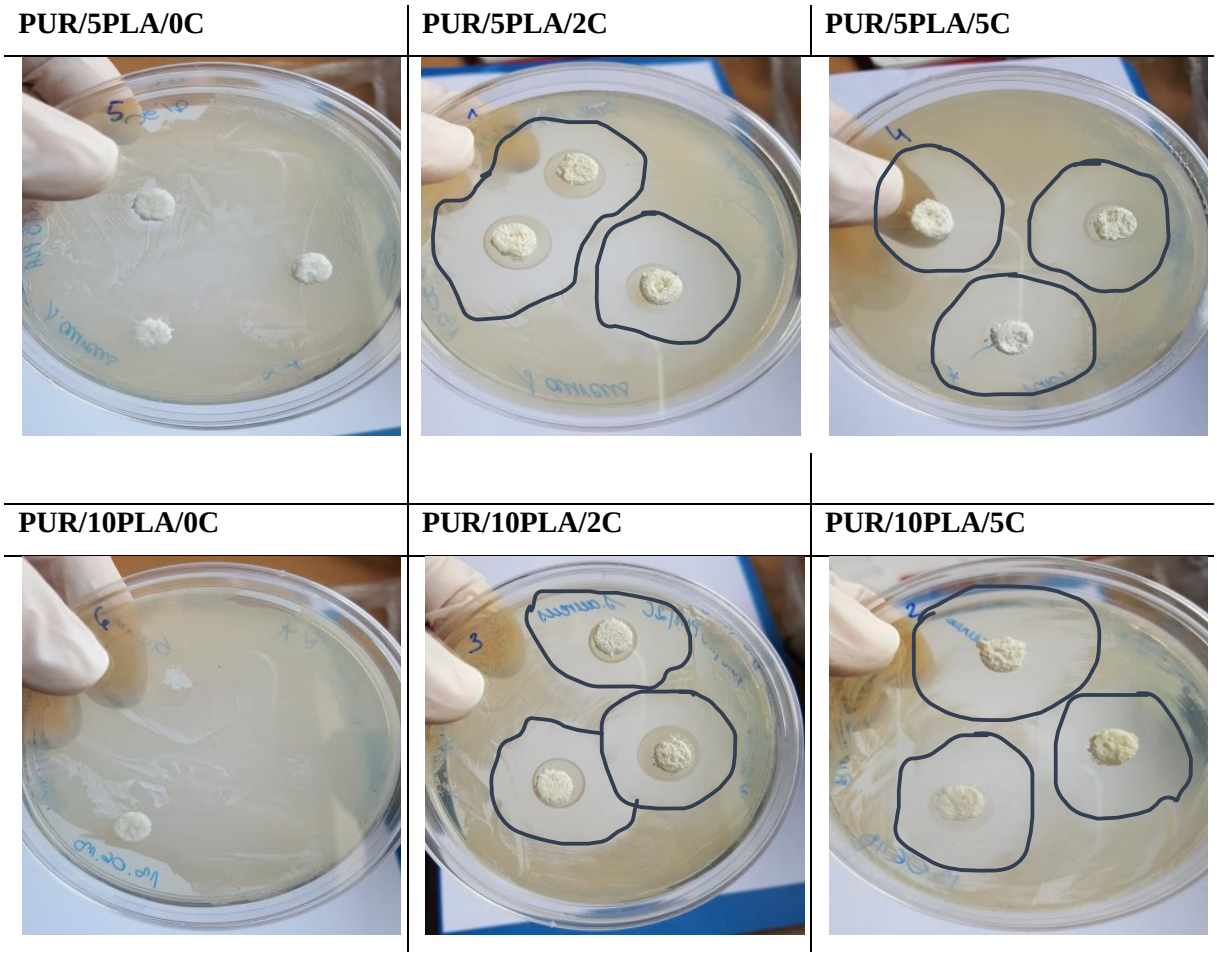

Fig. 8 the effect of antimicrobial activity of Cipro-modifiedHPPS (2 wt% or 5 wt% of ciprofloxacin) against S.aureus.

There was no growth inhibition zones for *E.coli* and *P.aeruginosa*. *P.aeruginosa* frequently was developed as resistant against drugs. Although ciprofloxacin is commonly used antibiotic for

P. aeruginosa there are available reports, which indicate even 30-37% of P.aeruginosa isolates are ciprofloxacin resistant, where among E.coli strains resistance represented approximately 11% (55,65).

Discussion

In this study was described the fabrication process of hybrid PUR-PLA scaffolds (HPPS). These HPPS were containing 5 wt% or 10 wt% of PLA, selected as one of the "fast-degradable" polymers, which when admixed with the PUR were proven to improve its degradation rate (27,28). Moreover, biocompatible PUR used in this study (8,10) was synthesized with amorphous macrodiol PEBA (8), which according to references may improve degradation profile (39) of such HPPS. Obtained HPPS were modified with ciprofloxacin to improve antibacterial effect of HPPS dedicated for skin regeneration. Ciprofloxacin is a fluoroquinolone antibiotic inhibiting *S. aureus* growth, which is one of the bacterial species responsible for most common wound infections (42,48,50,52,53,63). The FTIR analysis of obtained unmodified and Cipro-modified HPPS revealed presence of chemical functional groups characterizing PURs (urethane linkages), PLA (ester linkages) (64) and ciprofloxacin (complex structure) bonded to the HPPS. The EDX analysis confirmed presence of ciprofloxacin in the HPPS systems, what was in good agreement with FTIR studies.

Performed FTIR spectroscopy showed that in case of unmodified HPPS the FTIR bands intensity was growing with the amount of PLA added. The same tendency was noted for Cipro-modified HPPS samples where bands intensity was improving with the increase of ciprofloxacin amount in the HPPS sample. That may suggest formation of additional hydrogen bonds, over those present in the native PUR structure, reinforcing the structure of obtained HPPS (64). Presence of hydrogen bonds, which are increasing with the amount of PLA and ciprofloxacin added, could be an explanation for mechanical properties of obtained HPPS. Scaffolds containing 10 wt% of PLA, revealed largely higher T_{Sb} (670±26 kPa) than those obtained with 5 wt% of PLA (790±24 kPa). Application of ciprofloxacin additionally increased the T_{Sb} value of both HPPS containing 5 wt% (PUR/5PLA/2C=720±24 kPa, PUR/5PLA/5C=850±34 kPa) and 10 wt% of PLA (PUR/10PLA/2C=860±33 kPa, PUR/10PLA/5C=920±33 kPa). Higher amount of hydrogen bonds cause physical crosslinking of the HPPS structure(66). What need to be underlined that from the mechanical point of view both materials (except PUR/10PLA/5C) meet the criteria for skin regeneration. Tensile strength of the skin covering the area of forearm and face is reported to be between 200 - 850 kPa (67), depending on the skin composition, and mean failure strain is 25.45±5.07%(68). From the morphological point of view only HPPS obtained by using 5 wt% of PLA were representing suitable homogenous and interconnected morphology even after ciprofloxacin modification, what was in contrary to the HPPS obtained with the use of 10 wt% of PLA, where porosity was decreasing (even complete closure of pores was observed (69) with the amount of ciprofloxacin added. That is the factor, which disqualify HPPS containing 10 wt% of PLA samples for further tissue engineering applications (69). In terms of degradation rate better performance was noted for HPPS containing 5 wt% of PLA in comparison to the HPPS samples containing 10 wt% of PLA. This could be explained by the presence of reinforcing

hydrogen bonds (64,66) in the HPPS structure; during HPPS fabrication PLA could precipitated to the solution, and later on could be enclosed in the PUR matrix. Due to that PLA particles may act as inactive filler (66), which cause physical hydrogen bonds and strengthen the HPPS structure. In the point of degradation rate better degradation profile was noted for HPPS containing 5 wt% of PLA. The HPPS containing 10 wt% of PLA were more resistant to the selected media. This data are in good agreement with studies performed by Montini Ballarin et al. (27,28). The antibacterial effect against Cipro-sensitive S.aureus strain was depending on the amount of ciprofloxacin added to the HPPS, but not dependent on the % of PLA introduced into the HPPS (See Table 2).

Performed studies revealed that the aim of fabricating degradable and antibacterial Cipro-modified HPPS was achieved. HPPS obtained by using 5% of PLA and modified with Cipro were selected for further development for skin regeneration. These materials were having suitable chemical composition, mechanical properties, degradation profile and morphology to be proposed as a skin tissue scaffold.

Conclusions

In this paper we described the fabrication process of degradable HPPS containing "fast-degradable" polymer – PLA – in the amount of 5 wt% and 10 wt%. To reach antibacterial character of HPPS the samples were modified with ciprofloxacin. Performed studies confirmed that PLA and ciprofloxacin were present in the chemical structure of obtained HPPS. Mechanical tests and morphology studies shows that more suitable characteristic for skin tissue regeneration possess Cipro-modified HPPS containing 5 wt% of PLA. These samples were representing as well better degradation rate in performed short term interactions study with selected media: 2N HCl, 5M KOH and 0,1 M CoCl2 in H_2O_2. On the other hand, the microbiological tests seem to not reveal large differences between Cipro-modified HPPS containing 5 wt% or 10 wt% of PLA. They were representing comparable inhibition zone dimensions, which was increasing with the amount of ciprofloxacin amount added to the HPPS. Thus, performed studies showed that Cipro-modified HPPS samples containing 5 wt% of PLA seems to be suitable to be developed further as a skin tissue scaffold.

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Data Aviability

The raw/processed data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study

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