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Composite Biomaterials Based on Poly(L-Lactic Acid) and Functionalized Cellulose Nanocrystals

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Abstract: The biocomposite films were prepared from poly(L-lactic acid) and cellulose nanocrystals. To improve interfacial compatibility of hydrophilic cellulose nanocrystals with hydrophobic matrix polymer as well as to provide the osteoconductive properties, cellulose was functionalized with poly(glutamic acid). The modified cellulose nanocrystals were better distributed and less aggregated within the matrix, which was testified by scanning electron, optical and polarized light microscopy. According to mechanical tests, composites filled with nanocrystals modified with PGlu demonstrated higher values of Young's modulus, elongation at break and tensile strength. Incubation of composite materials in model buffer solutions for 30 weeks followed with staining of Ca^{2+} deposits with Alizarin Red S assay testified better mineralization of materials containing PGlu-modified cellulose nanocrystals as filler. As the result of *in vivo* experiment, the developed composite materials showed less level of inflammation in comparison with pure polymer matrix and composites filled with non-functionalized cellulose nanocrystals.

Keywords: Biomaterials; polymer composites; biodegradable and biocompatible polymers; poly(L-lactic acid); cellulose nanocrystals; modification

1 Introduction

Cellulose is one the most available renewable polymer in the world. In the past decade, cellulose nanocrystals have attracted the attention as reinforcement filler for the preparation of composites [1]. Besides renewability and relative low cost, cellulose nanocrystals are characterized with a high strength and a low abrasiveness [2]. Taking into account the natural character of cellulose, it is widely considered for preparation of biomedical materials. There are many reports on the preparation of cellulose-containing biomaterials including composites, electrospun nanofibers, hydrogels, sponges, membranes, etc. [2].

An interest in using cellulose micro- and nanocrystals as fillers in biodegradable matrices has increased over the last few years [21–23]. Recently, the preparation of nanocomposites based on aliphatic polyesters

and cellulose micro- and nanocrystals was reported [3–6]. Being biodegradable, aliphatic polyesters are widely used for preparation of biomedical materials. The main obstacles for wide application of this class of polymer in biomedicine are their relatively high hydrophobicity, potential inflammatory side effects and low cell adhesion [7–9]. Thereby, the introduction of hydrophilic polysaccharide particles is needed to obtain the biocomposite material with optimized properties. However, such filler is incompatible with the hydrophobic polyester matrix and it is a serious limitation for preparation of desired biocomposites.

To improve the interfacial biocompatibility of cellulose nanocrystals the number of modification techniques have been developed [10]. For instance, the adsorption of poly(ethylene glycol) [11] and grafting of different polymers [12] were recently reported by some scientific groups. Moreover, there is a range of works devoted to the chemical modifications of cellulose micro- and nanocrystals with poly (lactic acid) [3,13,14] or poly(ε-caprolactone) (PCL) [4]. The grafting of aliphatic polyester to cellulose nanocrystals could be performed via "grafting from" technique, which is based on the utilization of cellulose surface hydroxyls as initiators of in situ polymerization of cyclic esters, such as lactide or caprolactone. As a consequence, the modified nanocrystals demonstrated better distribution in the aliphatic polyester matrices and, in turn, improved mechanical properties of the obtained composites [3,4,13]. The chemical grafting of cellulose nanocrystals with poly(2-ethyl-2-oxazoline) *via* microwave "grafting from" technique was recently reported by Dadkhah et al. [15]. Also, Zoppe et al. corresponded the grafting of poly(N-isopropylacrylamide) *via* control living radical polymerization to cellulose nanocrystals [16]. Besides "grafting from", a "grafting to" technique is also used for the modification of cellulose nanocrystals with various polymers. For instance, there are studies in which the modification of nanocrystalline cellulose with epoxy-terminated poly(ethylene oxide) [17] and amino-terminated poly(2-ethyl-2-oxazoline) [18] were realized. Additionally, the surface hydrophobization can be achieved *via* attachment of low molecular compounds using transesterification with fatty acid methyl ester [19], the modification with maleic or phthalic anhydrides [20] and γ-methacryloxypropyltrimethoxysilane [21]. The current achievements in the field of nanocrystalline cellulose modification can be found in recent reviews [1,12].

In present work, we describe the preparation of composite materials based on poly(L-lactic acid) (PLLA), as a matrix material, and cellulose nanocrystals with covalently attached poly(glutamic acid) (PGlu), as a dispersed phase. Poly(lactic acid) is approved by the Food and Drug Administration (FDA) [22] agency as biomedical polymer and, as a result, it is widely used in diverse applications in biomedicine [23,24]. Earlier, Karaman et al. reported that poly(lactic acid)/poly(lactic acid-co-glycolic acid) nanofibers modified with glutamic acid rich peptide demonstrated osteoconductive properties [25]. The ability to induce mineralization is one of the key features of materials proposed for bone tissue regeneration. In order to have simpler and more reproducible model of the material on the first stages of the research we have focused ourselves on the fabrication of composite films and their characterization to assess the properties of the proposed biocomposite materials and expediency of their further deeper investigation as 3D scaffolds. Here, the formation of biocomposite films containing PGlu-functionalized cellulose nanocrystals have been performed and their morphology, ability to attract calcium ions, mechanical and biological properties of developed materials have been discussed. The properties of interest were compared with those of pure PLLA and PLLA films containing non-modified cellulose nanocrystals.

2 Experimental Part

2.1 Materials

L-lactide, tin octoate, and agents for modification were purchased from Sigma-Aldrich (Munich, Germany). Nanocrystalline cellulose was a product of Blue Goose Biorefineries Inc. (Saskatoon, SK, Canada). Toluene, chloroform, methanol and other organic solvents used for polymer synthesis, modification and preparation of films were from Vecton (St. Petersburg, Russia). All solvents were

distilled before application. THF used as eluent for size exclusion chromatography (SEC) analysis was of HPLC grade and purchased from Merck (Darmstadt, Germany). Polystyrene standards (from 2000 to 400000) were purchased from Waters (Milford, MA, USA) and used for column calibration in SEC. Before SEC analysis, sample solutions were filtered through a Millipore Merck syringe PTFE membrane filter (0.45 μm) (Darmstadt, Germany). PGlu was synthesized in IMC RAS *via* the ring-opening polymerization of γ-O-benzyl-α-glutamic acid N-carboxyanhydride, and then deprotected with TFA/TFMSA, as described elsewhere [26,27]. The obtained PGlu possessed the following characteristics: M_w = 2100 (weight average molecular weight), M_n = 2000 (number average molecular weight), $Đ$ = 1.05 (dispersity), amount of residual benzyl groups was equal to 22% (according to ^{1}H NMR) [28].

2.2 Methods

2.2.1 Polymer Synthesis and Characterization

PLLA was synthesized in bulk at 130°C *via* the ring-opening polymerization of L-lactide. [Monomer]/[Sn(Oct)$_2$] ratio was equal to 2500. Polymerization was conducted in vacuum-processed Schlenk tubes during 12 h. After synthesis, polymer was dissolved in chloroform, and precipitated in cold methanol. Then it was filtered and washed with a fresh portion of methanol, and dried in vacuum. The molecular weights (M_w and M_n) and dispersity ($Đ$) were determined by SEC with the use of a Shimadzu HPLC system (Shimadzu Corporation, Tokyo, Japan) consisting of a pump LC-10AD VP, system controller SCL-10A VP, and refractometric detector RID-10A (Canby, OR, USA) supplied with a Rheodyne 725i injection valve (Rheodyne, Rohnert Park, CA, USA) and two columns of Agilent PLgel MIXED-D (7.5 × 300 mm, 5 μм) (Santa-Clara, CA, USA). Analyses were performed in THF at 40°C and at a flow rate of 1.0 mL/min. Data processing was fulfilled using LC solution software (version 1.25, Shimadzu Corporation, Kyoto, Japan). The intrinsic viscosities of synthesized polymers solutions (in $CHCl_3$) with different concentrations were measured using Ostwald's capillary viscosimeter (Neva-Reaktiv, St. Petersburg, Russia).

2.2.2 Manufacturing of Films and Mechanical Properties' Study

Polymer solution in chloroform (5% , 6.5 mL) was poured onto cellophane film fixed within the glass cylinder (i.d. = 75 mm) and left for 12 h at room temperature and normal atmospheric pressure for chloroform evaporation. Then, the cellophane was removed, and the obtained polymer films were dried at 50°C until constant weight (7 days). To prepare composite films, neat or modified cellulose nanocrystals were dispersed in polymer solution in chloroform under ultrasonication (Sonopuls HD2070, Bandelin, Germany). 5, 10, and 15 wt% of filler were added into PLLA matrix. Other manipulations were the same as for pure polymer films.

AG-100kNX Plus Shimadzu universal mechanical system (Tokyo, Japan) was used to study the mechanical characteristics of the films under uniaxial extension mode. The band-like specimens of 2 mm × 20 mm were tested at extension speed of 10 mm/min.

2.2.3 Microscopy

Surface morphology was analyzed by scanning electron microscopy (SEM) using Zeiss AURIGA Laser (Oberkochen, Germany). Before the registration of SEM images, the surfaces of film specimens were additionally deposited with conductive carbon layers. Polarized light microscopy was carried out by means of a Leica DM4500 P instrument (Leica Microsystems, Wetzlar, Germany) in transmittance mode. Optical microscopy was performed using Nikon Eclipse E200 (Tokyo, Japan).

2.2.4 Mineralization Study

The investigation was carried out using simulated media containing Ca^{2+} and PO_4^{3-} ions at concentrations known for body fluids [29]. The specimens were placed into separate wells of a microtiter plate and washed with double distilled water. One cycle included the incubation of specimens for 5 days

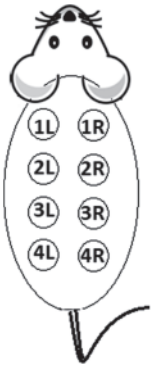
in 3 mM aqueous $CaCl_2$ solution, 2 days in water, 5 days with 3 mM aqueous NaH_2PO_4 solution and again 2 days with water. One Ca^{2+}/PO_4^{3-} cycle took 2 weeks and was repeated 15 times. The temperature at all steps kept at 37°C. After final careful washing, the tested materials were dyed with 2% aqueous alizarin red S solution for 40 min. The specimens washed up to colorless washing solutions were analyzed by an optical microscopy.

2.2.5 Biological Experiments

All experiments with animals were organized and fulfilled according to the principles of humane care of animals with the consent and approval of the Bioethical Review Committee of the Research Institute of Phtisiopulmonology (Statement #46 from 18.04.2018). Male rats (Wistar line, 11 weeks old, average weight 310 ± 16 g) were purchased from the Federal State Unitary Company "Nursery Rappolovo" (Rappolovo, Leningrad Region, Russia). Prior to the study the animals were quarantined for 14 days and monitored daily by visual clinical inspection. Six clinically healthy rats were involved in the experiments. The rats were given standard diet fortified in protein and vitamins (Furage Ltd., St. Petersburg, Russia), as recommended by Russian Health Ministry edict #1179 for animal experimentation, plus water ad libitum. The rats were kept at the vivarium of Research Institute of Phthisiopulmonology under standard conditions in full accordance with the "Rules of an establishment, equipment and maintenance of experimental biological clinics", approved by Russian Federation National standard R53434-2009 and by the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (CETS No. 123, 1991). In particular, they were kept at 12 h light/12 h darkness conditions, air temperature between 23-25°C and 50–70% relative air humidity. Air exchange (12–15 volumes/hour) was carried out by inflow–outflow ventilation, with daily air sterilization by UV light. The rats were divided in two groups of three animals each and housed in polycarbonate cages with a floor area of 600.0 cm^2 per animal.

The subcutaneous implantation was carried out in a rat back following the rules of aseptic operation and in accordance to the scheme presented in Fig. 1. The round-shaped film specimens (5 mm in diameter and 100 ± 10 μm in thickness) were prepared from larger films with the application of mandrel and stored at 4°C. Before implantation, films were sterilized in a laminar flow box "Laminar-C"-1,2-ME (EuroLab, St. Petersburg, Russia) by incubation in 70% ethanol solution for 1 hour. After that, the specimens were repeatedly washed 10 times with PBS. All manipulations were performed under general anesthesia using a 0.2 wt.% solution of rometar (Xylazinum, Biovet, Czech Republic) at a dose of 0.1 mL per 100 g of animal weight. The anesthetic was injected into the thigh muscle of the rats. 10-mm incisions were cut on the dorsal section of rats and specimens were implanted under the muscular layer of the dermis. The incisions were sutured using retention suture (Lintex, Russia). To prevent postoperative complications, Cefamesin® (20 mg/kg, intramuscularly, dissolved in solution of 0.25 wt.% Procaine, 5 days; Pharm-Sintez, Russia) was applied. The rats were monitored daily during 4 weeks. The weight of the animals was a parameter to control their health. At the end of the experiment, the animals were euthanized with Zoletil® (*Virbac*, Carros, France) (total dose—60 mg/kg; active components: tyletamine hydrochloride and zolazepam hydrochloride; Virbach CA, France). A macroscopic study of model animals was carried out *via* an inspection of the implantation sites.

The skin sections at the implantation places were resected together with neighboring tissues. The specimens were fixed with 10% formalin buffer solution (pH 7.4) for 24 h and embedded in paraffin using an automatic Excelsior AS Tissue Processor, ThermoFischer Scientific (Waltham, MA, USA) and commercial medium IsoPREP Biovitrum (St. Petersburg, Russia). Finally, they were impregnated with HISTOMIX medium, Biovitrum (St. Petersburg, Russia). The prepared blocks were sliced into 3-μm thick sections by rotary microtome HM 325 ThermoFisher Scientific (Waltham, MA, USA). All slices were stained with hematoxylin-eosin according to the protocol of the manufacturer Biovitrum



Code	Group 1	Group 2
1L, 1R	Pure PLLA	Pure PLLA
2L, 2R	PLLA + 5% cellulose	PLLA + 5% cellulose/PGlu
3L, 3R	PLLA + 10% cellulose	PLLA + 10% cellulose/PGlu
4L, 4R	PLLA + 15% cellulose	PLLA + 15% cellulose/PGlu

Figure 1: Scheme of material's implantation. The total number of repeats for composite materials was 6; for pure aliphatic polyester specimens it was 12. *Abbreviations:* PLLA – poly(L-lactic acid), PGlu – poly(glutamic acid)

(St. Petersburg, Russia). The analysis was carried out with the use of transmitted-light bright field AxioLab Zeiss microscope (Carl Zeiss, Jena, Germany) in 10 fields of view in areas oriented in the same plane.

Morphometric study was fulfilled using a Carl Zeiss Axio Imager AZ microscope (Jena, Germany) supplied with the Axio Vision software for the analysis of images (Carl Zeiss, Jena, Germany). The average parameter value for each sample as well as the average parameter value for the group were calculated. The data were analyzed using Student's test (t-test), and $p < 0.05$ was set as the level of statistical significance.

2.3 Statistical Analysis

To analyze the statistical significance among the groups, one-way analysis of variants (ANOVA) in Excel with the XLSTAT was used. Data were expressed as mean ±SD and n = 6 for mechanical study and for in vivo experiments of composites and 12 for in vivo experiments of pure PLLA specimens. $p \leq 0.05$ was counted as a statistically significant.

3 Results and Discussion

3.1 Manufacturing of Composite Films

Poly(L-lactic acid) was used as a matrix polymer for manufacturing of films. It was synthesized by ring-opening polymerization of L-lactide catalyzed with tin octoate. The polymerization was performed in bulk for 12 h at 130°C. The obtained polymer was analyzed by SEC and viscosimetry. The PLLA yield and characteristics are presented in Tab. 1.

Table 1: Characteristics of PLLA used for manufacturing of films

SEC				
M_n	M_w	$Đ$	η, dl/g	Yield, %
50800	82300	1.62	0.97	90

To obtain composite materials, 5, 10 and 15 mass% of the neat and modified cellulose nanocrystals were used as fillers. Hydrodynamic diameter of cellulose nanocrystals used in this work was 128 ± 7 nm (commercial suspension). The surface of cellulose nanocrystals was charged negatively and had ζ-potential equal to –23 mV. Recently, we developed the procedure to modify cellulose nanocrystals with

poly(glutamic acid) (PGlu) containing 22% of residual benzyl groups. For this, the cellulose units were initially oxidized with sodium periodate to generate aldehyde groups, which then were brought into interaction with terminal amino group of poly(glutamic acid) [28]. Finally, formed Schiff's base was reduced with sodium borohydride. The immobilization capacity was evaluated [28] and appeared to be 225 ± 13 mg/g of cellulose nanocrystals which corresponded to 90 ± 6% of initial mass of poly(glutamic acid) used for modification. It should be also noted that the residual benzyl groups in the PGlu structure were left in order to increase the hydrophobic interactions with the polyester matrix.

Composite films were manufactured *via* casting of the obtained PLLA solution in chloroform onto the cellophane support and further solvent evaporation. The dispersion of neat cellulose nanocrystals in PLLA solution prepared in chloroform was accompanied with noticeable aggregation of hydrophilic filler. Moreover, the aggregation enhanced with the amount of nanocrystalline cellulose particles introduced into the system. To diminish aggregation before film casting, we used extensive ultrasonication. However, even in that case the aggregation took place and aggregates were observed in final composite materials (see Section 3.2). In turn, application of cellulose nanocrystals modified with poly(glutamic acid) showed much less aggregation and filler distribution in the matrix polymer was much more even.

3.2 Scanning Electron, Optical and Polarized Light Microscopy of Composite Materials

The obtained composite films were investigated with the use of different modes of microscopic analysis to evaluate the evenness of composite materials. Fig. 2 illustrates the SEM images, which were registered for the surfaces of pure and composite films. One can observe the increasing of surface irregular roughness, which followed the addition of neat cellulose nanocrystals to the PLLA matrix. Such roughness was more pronounced when the amount of filler was increased. In turn, the application of cellulose nanocrystals modified with PGlu did not led to such effects. In the latter case, the surface possessed much more regular morphology, which could result from less aggregation of filler.

The results obtained by SEM were in good agreement with optical microscopy images obtained in transmitted and reflected light (Fig. 3). The PLLA films filled with neat cellulose nanocrystals could be characterized by higher content of visible large aggregates in comparison with composites containing filler modified with PGlu.

The images of pure PLLA and its composites obtained by polarized light microscopy are shown in Fig. 4. Being crystalline polymer, PLLA forms fine large spherulites. Compared to pure PLLA, the morphology of its composites with neat cellulose nanocrystals is of considerable faultiness. The spherulites of composite containing 5 wt% of neat cellulose is much smaller and faultier in comparison with pure PLLA. An increase of the neat cellulose nanocrystals content in PLLA matrix led to the evident disorder of PLLA crystallinity structure. The result should contribute to the inferior dispersibility of neat cellulose within the PLLA phase. At the same time, an introduction of modified filler at concentration of 5 wt% favored to the better distribution of cellulose nanocrystals in PLLA matrices and spherulite morphology was better preserved. Further increase of modified filler amount was also followed by PLLA morphology disordering. However, it was not as dramatic as for composites with non-modified cellulose nanocrystals.

Thus, the modification of cellulose nanocrystals with poly(glutamic acid) containing 22% of residual benzyl groups improved the compatibility of hydrophilic filler with hydrophobic PLLA matrix.

3.3 Mechanical Properties

Mechanical properties of the films were studied under conditions of uniaxial extension of band-like samples. Young's moduli *(E)*, tensile strengths (σ_b) and elongation at break (ε_b) were determined for pure PLLA and its composites (Fig. 5). The results obtained for the non-filled PLLA films are in agreement with published data (E ~2.5–4 GPa, σ_b ~59–66 MPa, ε_b ~4–19%) [30,31]. For all composite films

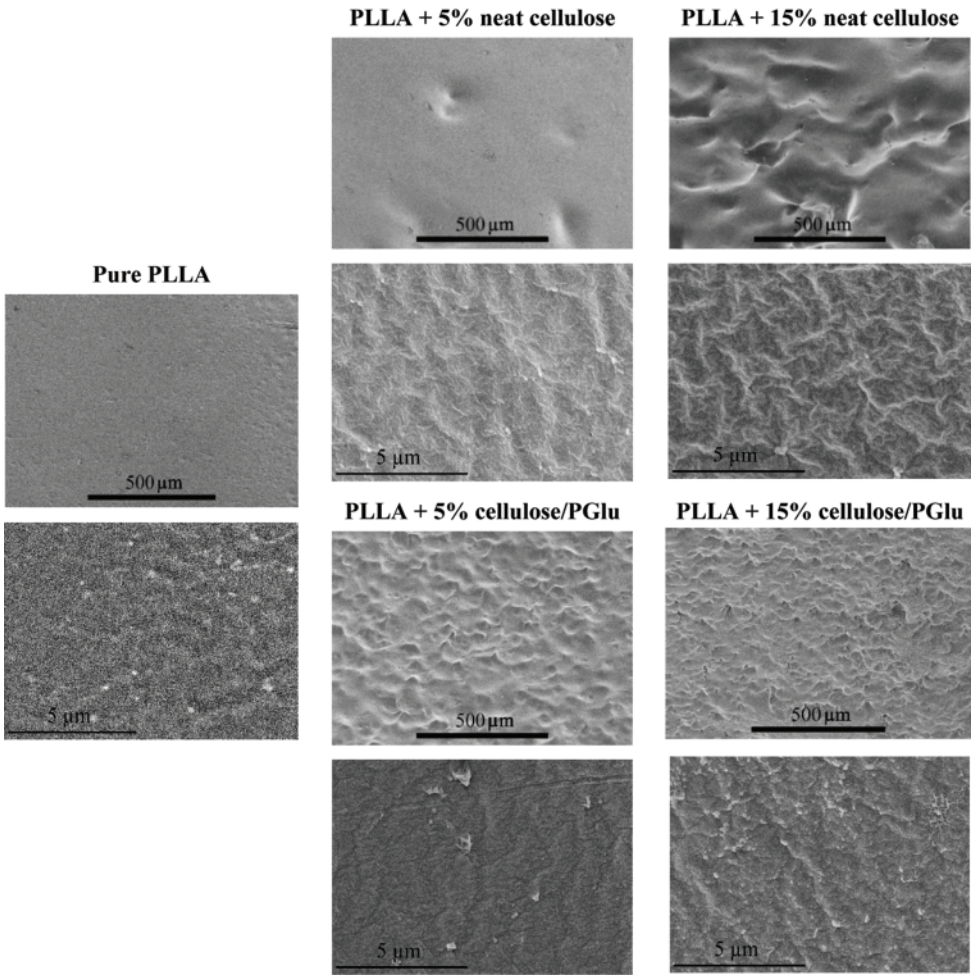


Figure 2: SEM images of pure PLLA and its composites with neat and modified cellulose nanocrystals at different magnifications

containing neat cellulose nanocrystals as filler, Young's moduli were twice lower than for pure PLLA material. As opposite to those, all composites based on PLLA filled with cellulose nanocrystals modified by PGlu kept the values of Young's moduli at the same level as pure polymer films.

For both kinds of composites, the reduction in tensile strength was established. However, the decrease in σ_b values for composites prepared with the use of neat cellulose was twice lower than for those filled with modified cellulose nanocrystals. In turn, both kinds of composites were characterized with the reduced and comparable elongation at break values. It can be a result of the formation of zones with accentuated fragility caused by the material inhomogeneity due to the filler introduction. In spite of the observed decrease of tensile strength and elongation at break values for PLLA filled both with neat and modified cellulose nanocrystals as compared with pure PLLA, the PLLA composites with modified cellulose nanocrystals exceed other materials in their ability to mineralization and demonstrated the enhanced biological properties. These are discussed below.

3.4 In Vitro Mineralization

To study the mineralization ability of composite materials, they were exposed sequentially in Ca^{2+}- or in PO_4^{3-}-containing solutions, respectively. The experiment lasted during 30 weeks. The mineralization was

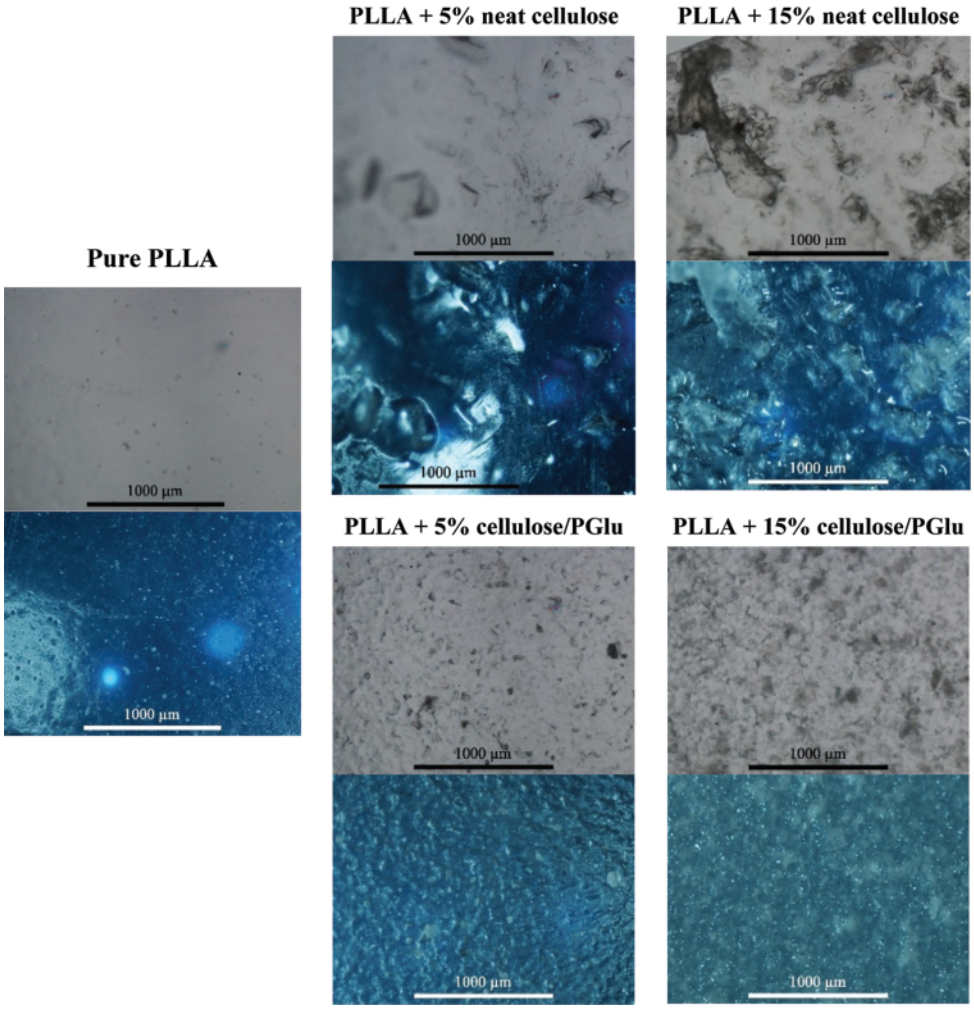


Figure 3: Images of optical transmitted light (gray) and reflected (blue) microscopy of pure PLLA and its composites with neat and modified cellulose nanocrystals

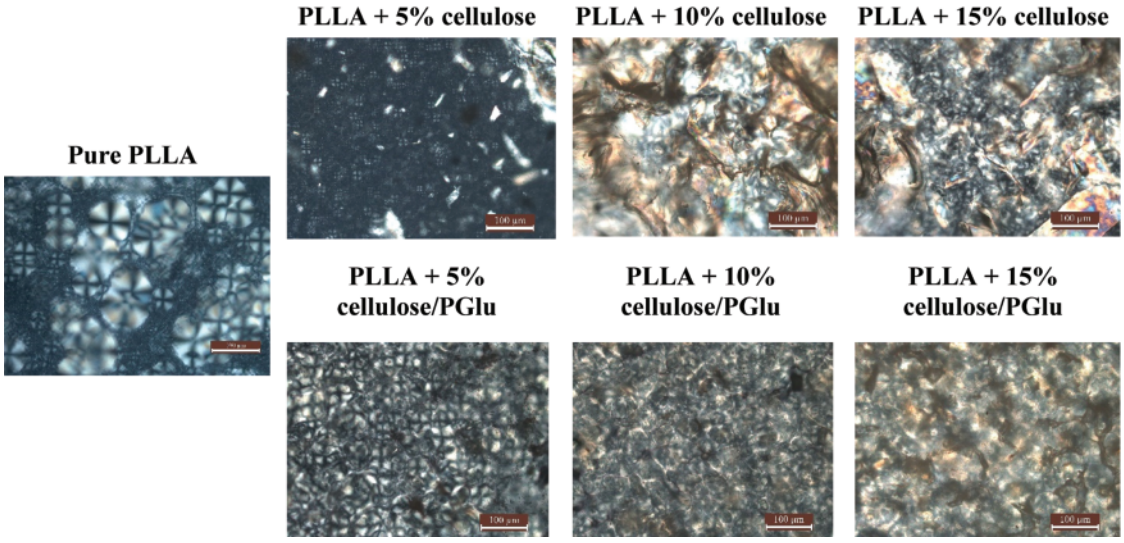


Figure 4: Images of polarized light microscopy of pure PLLA and its composites with neat and modified cellulose nanocrystals

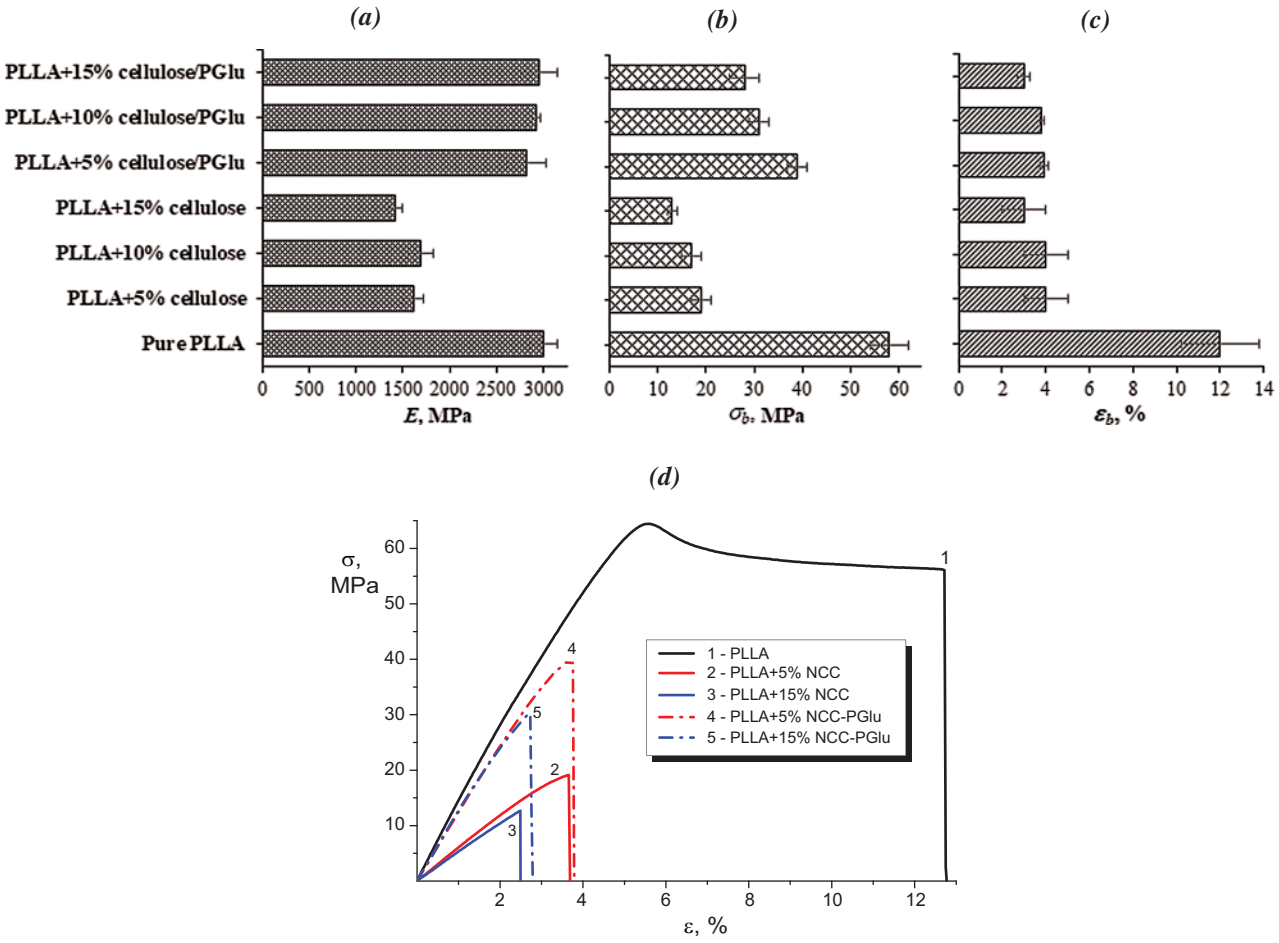


Figure 5: Mechanical properties of pure PLLA and its composites with neat and modified cellulose nanocrystals: *(a-b)* changes in Young's modulus, tensile strength and elongation at break; *(d)* stress-strain curves

evaluated by staining of the films with alizarin red S. Pure PLLA film had not been dyed with alizarin red S that indicated the absence of calcium deposits (Fig. 6).

Light staining was detected for PLLA composites filled with neat cellulose. It can be related to the negative charge of used cellulose nanocrystals which favors to the capture of calcium ions. In turn, PLLA composites containing cellulose nanocrystals modified with poly(glutamic acid) demonstrated considerable mineralization. Moreover, the higher amount of filler was utilized, the more extensive staining was observed. Thus, the modification of cellulose nanocrystals with poly(glutamic acid) favored the elevated capture of calcium ions by the composite material that can be useful for the development of materials for bone tissue regeneration.

3.5 Biocompatibility

The in vivo biocompatibility tests were performed with application of round-shaped specimens (5 mm in diameter and 100 μm in thickness) that were subcutaneously implanted into the back of rats. Each rat was subjected to implantation with 4 specimens of films in each side of their back, 1 film was pure (control) and 3 films were composite materials (Fig. 1). The scaffolds were resected from the animals after four

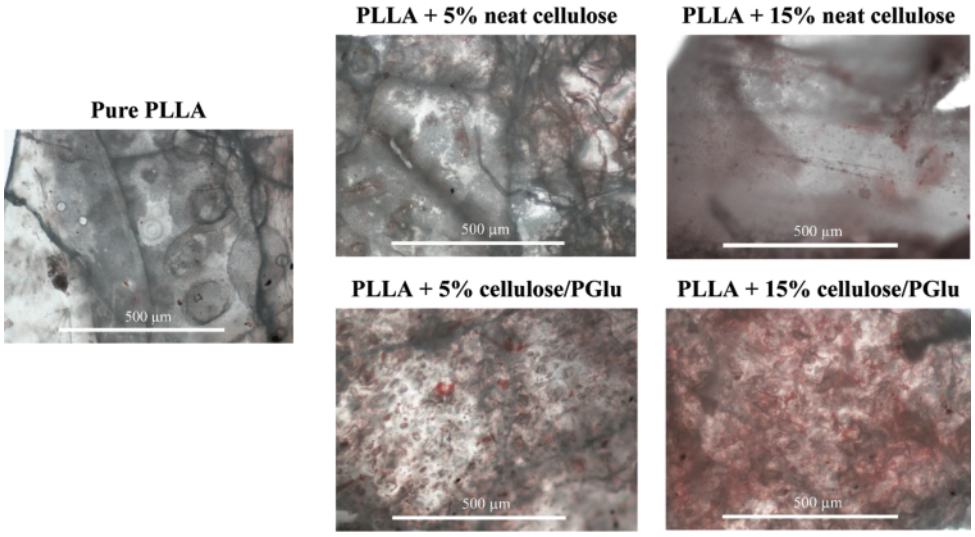


Figure 6: In vitro mineralization study of the pure PLLA and its composites with neat and modified cellulose nanocrystals. The calcium deposits were stained by alizarin red S assay. The intensity of the red color is proportional to the amount of calcium ions on the surface of the films

weeks followed by histological and morphometric analysis of implantation site. Fig. 7 demonstrates the quantitative results of histological examination. It is known that materials with better biocompatibility produce a thinner fibrous capsule [32,33]. All tested materials provided the formation of thin fibrous capsule (less than 100 µm). However, the fibrous capsule thickness of composite materials was by 35-45% lower than for pure PLLA material ($p < 0.05$).

Collagen fibrillogenesis was more intensive for pure PLLA whereas for PLLA containing 10 and 15 wt% of modified cellulose nanocrystals this process was less pronounced ($p = 0.04$). The average number of collagen fibrils was in the range from 12 to 20 for composites and 28 for pure PLLA material (Fig. 7a). As collagen fibrils' number, the number of blood vessels was higher for the pure PLLA (Fig. 7b). In turn, all composite materials demonstrated two times lower number of vessels in comparison with non-filled material ($p = 0.04$).

A histomorphometric analysis of resected implants allowed the detection of macrophages, lymphocytes and fibroblasts (Fig. 7c). Macrophages and lymphocytes indicate the inflammation level. The amounts of macrophages detected in the implantation area were low and similar for all resected specimens. It means that all materials did not provoke an acute inflammation. As to level of lymphocytes, it was twice higher for pure PLLA material in comparison with composite films ($p < 0.04$). However, no evident difference was observed for composites containing non-modified cellulose nanocrystals and these modified with PGlu.

Fibroblasts are known to be responsible for tissue renovation process. In our case, pure PLLA and its composites containing neat cellulose nanocrystals were characterized with much more pronounced number of fibroblasts in the area of implantation than in the case of modified nanofiller application ($p < 0.03$).

In summary, it is possible to deduce that all materials did not induce an acute inflammation. However, the composite materials demonstrated lower inflammatory response than pure PLLA matrix. This can be a result of material hydrophilization. Moreover, the composites made of PLLA and cellulose nanocrystals modified with poly(glutamic acid) were characterized with the lowest inflammatory response and the highest biocompatibility among the tested specimens.

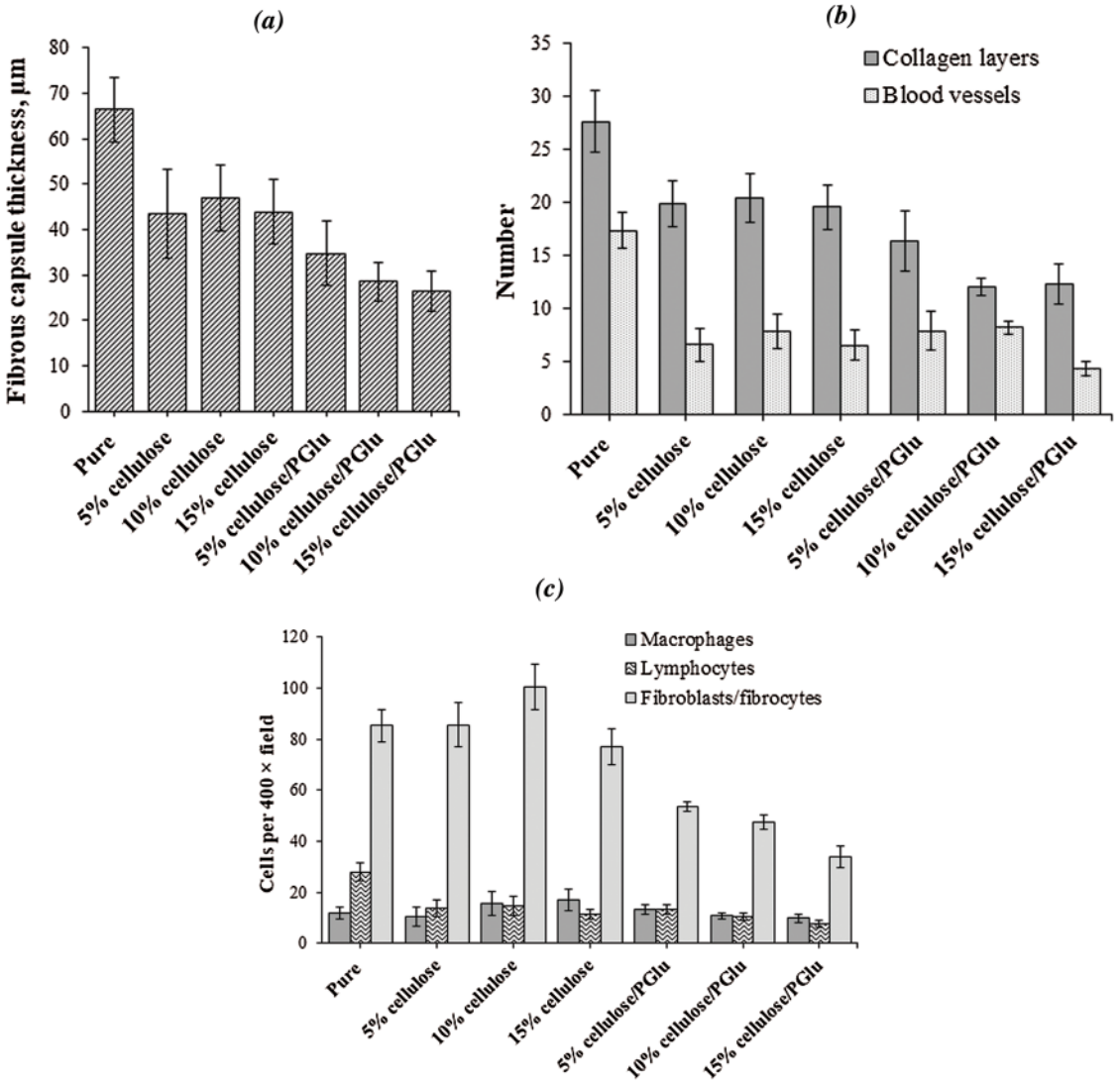


Figure 7: Results of histological analysis of resected specimens after subcutaneous implantation in rats of pure PLLA and its composite films: *(a)* the thickness of fibrous capsule, *(b)* the average number of collagen layers and blood vessels, *(c)* the average number of cells per 400× field, e.g., macrophages, lymphocytes and fibroblasts/fibrocytes

4 Conclusions

Functionalization of cellulose nanocrystals with poly(glutamic acid) allows the improvement of their interfacial compatibility with poly(L-lactic acid). The obtained biocomposite films containing from 5 to 15 wt% of cellulose nanocrystals modified with poly(glutamic acid) were characterized with finer distribution of the filler in the PLLA matrix due to less pronounced aggregation. As a result, the mechanical properties of biocomposites filled with the modified cellulose nanocrystals were improved as compared to those obtained with the application of neat cellulose nanocrystals. Moreover, the cellulose functionalization with poly(glutamic acid) favored the mineralization of developed biocomposites as well as improvement of their biocompatibility.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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