surfaces

MDPI

Article

Improvement in Biological Performance of Poly(Lactic Acid)-Based Materials via Single-Point Surface Modification with Glycopolymer

Viktor Korzhikov-Vlakh [1,*], Ekaterina Sinitsyna [1,2], Kirill Arkhipov [1], Mariia Levit [2], Evgenia Korzhikova-Vlakh [1,2,*] and Tatiana Tennikova [1]

[1] Institute of Chemistry, Saint-Petersburg State University, Universitetskiy pr. 26, Peterhof, 198504 St. Petersburg, Russia; kat_sinitsyna@mail.ru (E.S.); tennikova@mail.ru (T.T.)
[2] Institute of Macromolecular Compounds, Branch of St. Petersburg Institute of Nuclear Physics Named after B.P. Konstantinov of the National Research Center "Kurchatov Institute", Bolshoi pr. 31, 199004 St. Petersburg, Russia; musia_1@yahoo.com
* Correspondence: v.korzhikov-vlakh@spbu.ru (V.K.-V.); e.korzhikova-vlakh@spbu.ru (E.K.-V.)

Abstract: As a promising polymer for the production of biomaterials and drug delivery systems, poly(lactic acid) (PLA) is characterized by its relative hydrophobicity, as well as its chemical and biological inertness. Here, we aimed to improve the biological properties of PLA-based materials via the covalent attachment of a hydrophilic biocompatible glycopolymer, namely poly(2-deoxy-*N*-methacrylamido-*D*-glucose) (PMAG) on their surface. PMAG is a water-soluble polymer that contains glucose units in its side chains, which are responsible for good biocompatibility and the ability to attach bioactive molecules. In the developed protocol, PMAG was synthesized by controlled radical polymerization in the presence of a reversible addition–fragmentation chain transfer (RAFT) agent, followed by the conversion of glycopolymer terminal dithiobenzoate functionality into a primary amino group (PMAG-NH_2). PLA-based films served as model aliphatic polyester materials for developing the surface biofunctionalization protocol. According to that, PMAG-NH_2 covalent immobilization was carried out after alkali treatment, allowing the generation of the surface-located carboxyl groups and their activation. The developed modification method provided a one-point attachment of hydrophilic PMAG to the hydrophobic PLA surface. PMAG samples, which differed by the degree of polymerization, and the variation of polymer concentration in the reaction medium were applied to investigate the modification efficacy and grafting density. The developed single-point polymer grafting approach provided the efficient functionalization with a grafting density in the range of 5–23 nmol/cm^2. The neat and modified polymer films were characterized by a number of methods, namely atomic force microscopy, thermogravimetric analysis, ellipsometry, and contact angle measurements. In addition, an ArgGlyAsp-containing peptide (RGD peptide) was conjugated to the PMAG macromolecules grafted on the surface of PLA films. It was shown that both surface modification with PMAG and with PMAG-RGD peptide enhanced the adhesion and growth of mesenchymal stem cells as compared to a neat PLA surface.

Keywords: synthetic glycopolymer; covalent modification; polymer grafting to PLA surface; surface hydrophilization; functionalization; cell adhesion

Citation: Korzhikov-Vlakh, V.; Sinitsyna, E.; Arkhipov, K.; Levit, M.; Korzhikova-Vlakh, E.; Tennikova, T. Improvement in Biological Performance of Poly(Lactic Acid)-Based Materials via Single-Point Surface Modification with Glycopolymer. *Surfaces* **2024**, *7*, 1008–1028. https://doi.org/10.3390/surfaces7040067

Academic Editor: Jinde Zhang

Received: 26 October 2024
Revised: 20 November 2024
Accepted: 26 November 2024
Published: 1 December 2024

1. Introduction

As a promising polymer for the fabrication of biomedical materials, aliphatic polyesters reveal a number of disadvantages associated with the chemical structure of the polymer chain [1]. The strong features of aliphatic polyesters such as poly(lactic acid) (PLA), poly(lactide-*co*-glycolide) (PLGA), poly(ε-caprolactone) (PCL), poly(3/4-hydroxybutyrate) (PHB), etc., include biocompatibility, biodegradability, moderate mechanical properties, thermoplasticity, and ease of processing [2–4]. However, relative hydrophobicity (contact

angle $\geq 80°$), low adhesion of the cells, and chemical inertness, which make it difficult to functionalize the surface with bioactive compounds, are the main disadvantages of materials based on aliphatic polyesters [5–7]. To overcome these obstacles, various methods for the surface modification of these materials are being developed, with the main objective of the hydrophilization and biological functionalization of the surface to induce the required biological effect [8,9].

Like other polymer materials, aliphatic polyester materials can be modified by adsorption (physical modification) or the covalent attachment (chemical modification) of (macro)molecules of interest [7]. Being hydrophobic, aliphatic polyester-based materials can be modified by simple adsorption of proteins like fibronectin, collagen, vitronectin, thrombospondin, tenascin, laminin, etc., to control surface–cell interaction [7,10,11]. Although physical modification is a simple way to coat a surface, passive adsorption forms only an unstable coverage that can be competitively replaced by other (macro)molecules present in a complex biological system. Thus, the surface composition may be unstable and susceptible to change when such polymer-based biomaterials are used. An interesting approach to surface modification of poly(L-lactic acid) (PLLA) materials was proposed by Zhu et al. [12]. The authors prepared PLLA films and modified them by stereocomplexation with poly(D-lactic acid-co-glucose) (PDLAG). The modification was performed by simply immersing the PLLA film in PDLAG solution to obtain surface-modified PLLA films. The presence of glucose moieties on the surface provided a noticeable hydrophilization of the latter, where the contact angle decreased from 84° for pure PLLA to 69–60° for PLLA–PDLAG depending on the time of immersion.

In addition to adsorption, plasma treatment is widely used to make the surface more hydrophilic, functional, and capable of promoting cell adhesion. Oxygen, nitrogen, or anhydrous ammonia are most commonly used as a plasma for such modifications [13–15]. While treatment with oxygen and nitrogen just provides hydrophilization, which creates a favorable microenvironment for cells, treatment with ammonia generates amino groups on the surface of materials, which can help to anchor biomacromolecules due to ionic and hydrogen bonding interactions, as well as by covalent modification. For example, Yang et al. used this technique to achieve the hydrophilization of PLA and PLGA scaffolds and improve the adhesion of human skin fibroblasts to the surface of modified materials [16]. Ammonia plasma treatment has also been shown to be useful for improving the anchoring of collagen on the surface of PLA-based scaffolds, which resulted in enhanced mouse fibroblast adhesion [17].

A simpler and easily accessible way to generate reactive functionality on the surface of aliphatic polyesters is alkaline hydrolysis of the polyester surface layer. In this case, the surface becomes enriched with carboxyl and hydroxyl groups [18]. The former can be activated and put into reaction with primary amino groups of macromolecules and biomolecules. For example, after the activation of the carboxyl groups, the PLA surface can be modified with chitosan. Chitosan-modified PLA materials have been shown to improve the attachment and proliferation of rat osteoblasts [19]. In addition to alkaline hydrolysis, aminolysis can be used to introduce reactive amine groups onto the PLA surface. For example, 1,6-hexamethylenediamine has been used for aminolysis, followed by a conjugation with biocompatible macromolecules such as gelatin, chitosan, or collagen [20]. Such a modification provided the positive effect of accelerating endothelium regeneration in vitro.

The covalent grafting of functional synthetic polymers is also a convenient tool for further modifications of the surface with the bioactive molecules. For instance, the grafting of poly(methacrylic acid) (PMAA) to the PLA surface using atom transfer radical polymerization (ATRP) produced a negatively charged coating that did not promote cell adhesion. Further modification of PMAA-grafted PLA with gelatin promoted a significant improvement in cell–surface interaction [21]. Photoinduced grafting of poly(acrylic acid) (PAA) to the PLA surface was described by Steffens et al. [22]. For this purpose, plasma-activated PLA films were immersed in an aqueous solution of acrylic acid and exposed to UV light.

Then, the carboxyl groups of PAA were activated and involved in the conjugation with horseradish peroxidase or RGD peptide to enhance cell adhesion. The hydrophilization of the surface of the PLA-based films by their covalent modification with polyacrylamide and alginate hydrogels has been also reported [23]. In the case of polyacrylamide, the authors partially hydrolyzed the surface to form carboxyl groups, then activated and modified them sequentially with ethylenediamine and glycidyl methacrylate (GMA) to introduce unsaturated double bonds. This was followed by the copolymerization of acrylamide with N,N′-methylenebisacrylamide on the surface of methacrylated PLA. Surface modification of PLA-based films with alginate was performed after the functionalization of PLA with ethylenediamine and the activation of the carboxyl groups of alginic acid by crosslinking with lysine as a bifunctional linker. Instead of the reported two-step modification of the surface-hydrolyzed PLA-based materials with ethylenediamine and GMA, methacrylation can be performed in one step by simply using 2-aminoethyl methacrylate [24]. Ninaya et al. proposed an approach for the covalent surface modification of PLA microspheres based on the γ-irradiation of microspheres to generate free radicals in the system, which then interact with monomers, namely acrylic acid, acrylamide, or maleic anhydride, and initiate grafting [25]. The authors showed that the extent of grafting depends on the irradiation dose and dose rate, as well as on the monomer used in the grafting process. Recently, the single-point modification of PLA materials with PLys via a thiol-ene click reaction for spatially controlled cell adhesion has been reported [26].

Similar to PEG, neutral glycopolymers can be used to functionalize the surface of nanoparticle-based drug delivery systems to provide stability in the body's environment (plasma) and protect them from capture by macrophages (stealth effect) [27,28]. In addition to surface hydrophilization and providing the stealth effect, PMAG can be used for the covalent attachment of specific bioactive compounds such as polylysine, bone morphogenetic factor 2 (BMP2), or RGD peptides, which can be used to control cell behavior [29,30]. Such peptides are widely used to modify materials, including polyester-based ones, to be used in bone tissue engineering and regenerative medicine [31–35]. Polylysine and RGD peptides are known to enhance cell adhesion and proliferation [31,36], and BMPs are responsible for osteodifferentiation [32].

In our previous work, we have studied the modification of PLA and PCL films with a bioinspired synthetic glycopolymer, namely poly(2-deoxy-N-methacrylamido-D-glucose) (PMAG) [37]. This polymer is soluble in water and biocompatible. The previously proposed covalent modification method involved several steps, including the following: (i) Alkaline hydrolysis of the surface of aliphatic polyester-based materials; (ii) activation of the generated carboxyl groups; (iii) surface modification with ethylenediamine; (iv) modification of the aminated surface with modified PMAG containing aldehyde groups; and (v) reduction of the formed aldimine bonds (Schiff bases) with sodium borohydride. As a result, this modification led to the multipoint attachment of each PMAG macromolecule to the surface of the material.

In this study, a novel approach to the functionalization of PLA-based materials using PMAG is proposed. In contrast to the previously developed method, which was based on the application of PMAG obtained by free-radical polymerization and thus possessing high dispersity (Đ = 2.2), in the current work, we synthesized PMAG by RAFT polymerization. This allowed us, firstly, to obtain well-defined glycopolymer macromolecules and, secondly, to perform post-polymerization modification of terminal functionality to obtain macromolecules with reactive amino groups, namely PMAG-NH_2. The obtained PMAG-NH_2 macromolecules reacted with the activated surface carboxyl groups that were preliminarily generated on the PLA surface via treatment with alkali solution. Unlike the previously described approach [37], here, we describe the hydrophilic polymer attachment protocol with a reduced number of steps—three instead of five. Moreover, the developed approach results in different structuring of PMAG on the surface—uniform single-point brush-like PMAG attachment instead of the previously described [37] random multipoint polymer chemosorption. In addition, the RGD peptide was conjugated to the grafted PMAG macro-

molecules on the surface of the PLA films. The obtained PLA-PMAG and PLA-PMAG (RGD) films were evaluated for the adhesion of mesenchymal stem cells (MSCs). The mechanistic scheme summarizing the general idea of the study is shown in Figure 1.

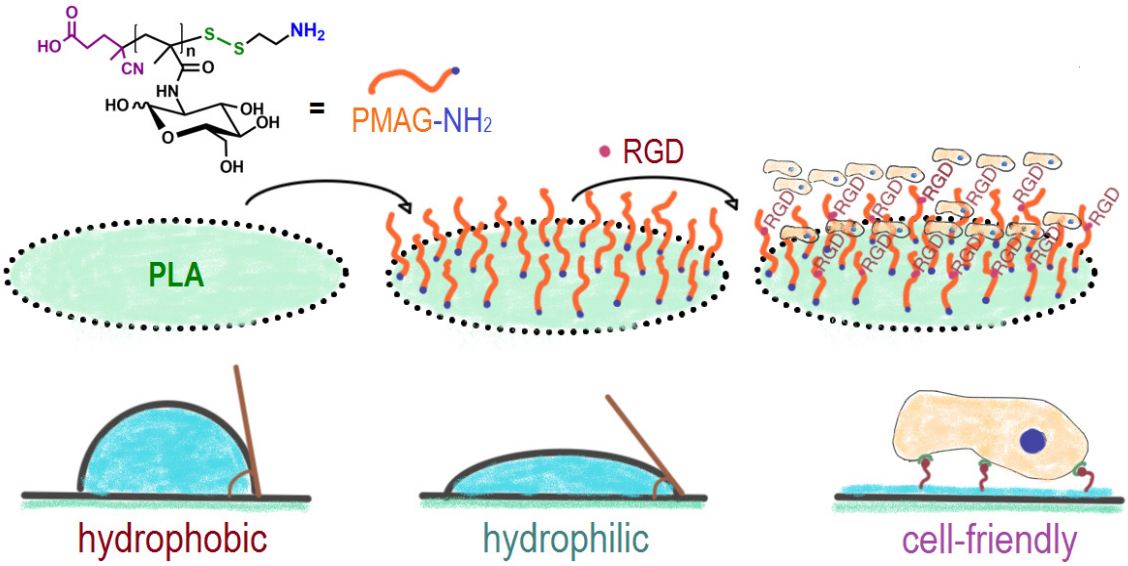

Figure 1. Scheme summarizing the general idea of the study.

2. Materials and Methods

2.1. Materials

D,L-lactide (99%), methacryloyl chloride (97%), D-glucosamine hydrochloride (≥99%), tin (II) octoate (92.5–100%), 2,2′-azobis (isobutyronitrile) (AIBN, 98%), 4-cyano-4-((phenylcarbonothiol)thio)pentanoic acid (100%), and 2,4,6-trinitrobenzenesulfonic acid (TNBS, 5% aqueous solution), sodium periodate (99.8), sodium borohydride (≥98%) were purchased from Sigma-Aldrich (Darmstadt, Germany).

Organic solvents such as chloroform, methanol, dimethylformamide (DMF), diethyl ether, as well as chemically pure acetic acid, triethylamine, ninhydrin, and dry sodium hydroxide were purchased from Vecton (St. Petersburg, Russia). L-Glycine was a product of Reanal (Budapest, Hungary). Deuterated solvents $CDCl_3$ and D_2O were produced by Astrakhim (St. Petersburg, Russia). The salts used for the preparation of buffer solutions were of analytical grade and were purchased from Vekton (St. Petersburg, Russia). MAG was synthesized using a previously published protocol [38]. The GRGDSP peptide was purchased from the Institute of Experimental Medicine of RAS (St. Petersburg, Russia).

The human adipose-derived mesenchymal stem cells were purchased from the Cell Collection of the Institute of Cytology of Russian Academy of Sciences (St. Petersburg, Russia). Cells were cultivated in alpha-MEM medium (ThermoFisher Scientific, Waltham, MA, USA) containing of 10% human serum (C.C.pro GmbH, Oberdorla, Germany) as well as 0.5% gentamicin (Merck Millipore, Darmstadt, Germany). Calcein-AM was a product of Abcam Ltd. (Cambridge, GB). 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (MTT reagent) was purchased from (ThermoFisher Scientific, Waltham, MA, USA).

2.2. Synthesis and Characterization of PLA

Poly(D,L-lactic acid) was synthesized by ring-opening polymerization in the melt of the monomer, namely racemic D,L-lactide, in the presence of tin (II) octoate. For the synthesis, 5.0 g of pre-crystallized monomer and 5.6 mg of catalyst (([D,L-lactide])/([$Sn(Oct)_2$]) = 5020 [mol/mol]) were placed into a 10 mL Schlenk tube and dissolved in 2 mL of hexane. The content of the flask was vacuumed for 10 min with simultaneous intensive stirring while increasing the temperature to 135 °C using a glycerol bath. *n*-Hexane was removed from the reaction mixture and condensed in a freezing trap.

Polymerization was carried out for 4 h at 135 °C. After completion of the synthesis, the reaction product was cooled and dissolved in chloroform. Excess chloroform was removed using a rotary evaporator. The polymer was precipitated into cooled methanol. The sample was dried in the desiccator for 1 h and then dried in a vacuum for 24 h. The yield was 70%.

The characteristic viscosity [η] of PLA was measured using an Ubbelode capillary viscometer with a chamber volume of 5 mL at 25 °C. For this purpose, 150 mg of polymer was dissolved in 9.14 mL of chloroform. To investigate the characteristic viscosity, 5 mL of polymer solution or solvent was loaded to the viscometer. The flow times of the solvent and polymer solutions were then measured sequentially. Dilutions were performed by taking 0.5 mL of polymer solution and adding a 0.5 mL portion of solvent (chloroform) to the viscometer; at each concentration, the flow time was measured 3–4 times until convergence of 0.2 s.

The molecular weight of synthesized PLA was calculated by the Mark–Kuhn–Houwink Equation (1).

$$[\eta] = K \times \left(M_\eta\right)^\alpha \quad (1)$$

where K and α for a solution of poly (D,L-lactic acid) in chloroform at 25 °C are $K = 6.06 \times 10^{-4}$ dL/g and $\alpha = 0.64$ [39]. The characteristic viscosity $[\eta]^{298}$ determined by viscometry was 1.5 dL/g.

2.3. Synthesis and Characterization of PMAG

MAG (0.3805 g), CTA (0.0215 g), and AIBN (0.00316 g) were placed into a 10 mL Schlenk tube equipped with a magnetic stirrer anchor. Next, 3.6 mL of DMF was added to the reaction tube. Before polymerization the mixture was degassed via five freeze–evacuated–thaw cycles and refilled with argon. Next, the Schlenk tube was immersed in a glycerol bath preheated to 70 °C. Polymerization was carried out for 13 h at 70 °C with constant stirring. After the completion of polymerization, the polymer was precipitated into diethyl ether. The filtered polymer was dried in a vacuum at 40 °C for 12 h.

The dried portion of the obtained monomer–polymer mixture was analyzed by ^{1}H NMR spectroscopy in D_2O. A Bruker Avance 400 MHz (Bremen, Germany) instrument was used for ^{1}H NMR spectroscopy. The conversion of the MAG monomer into PMAG was determined by correlation of the integral intensities of the signals belonging to the diastereotopic protons of $=CH_2$ group of the monomer (5.48 ppm) with the protons of the glucose ring (3.4–4.0 ppm) of the polymer in the ^{1}H NMR spectrum of the crude monomer–polymer mixture (Figure S1, Supplementary Materials). The chemical shifts of the protons in the monomer (ppm), 5.48 and 5.66 ppm, correspond to the protons with double bonds of MAG. The chemical shifts of the protons of the PMAG main chain (ppm), 0.93–1.39 and 1.73–1.90 ppm, correspond to the $-CH_3$ and $-CH_2-C$ groups, respectively. The chemical shifts of the protons of the glucose ring (ppm), 3.4–4.0, 5.05–5.30, and 4.78, correspond to C_2H_2-C_6H_6, and C_1H_1 α and β, respectively. The chemical shifts of the protons of CTA (ppm), 1.88, 2.55, 7.52–7.88, correspond to $-CH_2$ of the R group $-CH_2-CH_2-COOH$ and aromatic protons of the Z group, respectively.

The polymer conversion was calculated according to Equation (1):

$$Conversion = (1 - (I(MAG)_{5.48}/I(MAG + PMAG)_{3.4–4.0}) \times 100\% \quad (2)$$

where I is the integral intensity of the proton of the MAG double bond (5.48 ppm) or the sum of the glucose ring protons of the monomer and polymer (3.4–4.0 ppm), respectively.

The number-average (M_n) and weight-average (M_w) molecular weights and dispersity ($Đ$) of PMAG were determined by size exclusion chromatography (SEC). The measurements were performed using a Shimadzu LC-20 Prominence system with a RID 10-A refractometer detector (Kyoto, Japan). A 7.8 × 300 mm Styragel HMW 6E column, bead size 15–20 μm (Waters, Milford, MA, USA), was used. The analysis was performed at 60 °C, using DMF with 0.1 M LiBr as the mobile phase. The eluent flow rate was 0.3 mL/min. The molecular weight characteristics were calculated using a calibration plot constructed using

poly(methyl methacrylate) standards. The data processing was performed using the GPC LabSolutions software, version 1.25 (Shimadzu, Kyoto, Japan).

2.4. Modification of PMAG

First, 150 mg of PMAG was placed into a 10 mL Schlenk tube and dissolved in 6 mL of a DMF/H_2O mixture (1/1, *v*/*v*). The solution was purged by argon for 20 min and heated up to 40 °C with stirring using a magnetic stirrer and then 28.9 mg of $NaBH_4$ was added to the reaction mixture. After the complete dissolution of the precipitate and disappearance of the pink color of polymer, the mixture was stirred further for 1 h. Then, 155 mg of cysteamine hydrochloride was added to the reaction mixture. After that, 3.2 mL of 0.2 M solution of I_2 in DMF was introduced dropwise into the reaction mixture through a needle installed into a rubber cap of a Schlenk tube. The reaction mixture was left for 1 h with stirring. Unreacted iodine was reduced by adding ascorbic acid until the reaction mixture changed color. The solution was concentrated by the use of a rotary evaporator. Finally, the reaction mixture was placed into a dialysis bag (MWCO 1000) and dialysis was carried out for 15 h against water. The polymer product was lyophilized. To remove chloride ions from the terminal amino group of the polymer, a suspension of modified PMAG (85 mg) was placed into a 15 mL flat-bottom flask, dissolved in 1 mL of H_2O, and then 1.5 mL of NH_4OH was added. The mixture was left at 40 °C for 30 min. Then, the PMAG-NH_2 was purified by dialysis until a neutral pH (~6 h).

In order to quantify the amino groups in the polymer sample, the color reaction of the primary amine groups with TNBS was used. For that, a calibration plot was built using the solutions of cysteamine in water with concentrations from 0.005 to 0.05 mg/mL (Figure S3, Supplementary Materials). For the analysis of amino groups in PMAG-NH_2, 0.5 mL of a polymer solution (3 mg/mL) in H_2O, 1.5 mL of 0.01 M sodium borate-buffered solution (pH 9.4) and 0.15 mL of TNBS solution were added to the analysis tube. The reaction was carried out for 50 min, after which the reaction was stopped by adding 1.5 mL of acetate buffer (pH 4.8). The absorbance was measured at a wavelength of 420 nm relative to the blank sample using the Shimadzu UV-1800 (Kyoto, Japan).

2.5. Fabrication of PLA Films

PLA films were obtained by the casting of a polymer solution in chloroform (Figure S2, Supplementary Materials). To obtain 10 films, 1.0 g of PLA was dissolved in 12 mL of chloroform in a closed flask for 24 h at 20 °C. Then, the polymer solution was cast onto a cellophane substrate fixed on 30 mm diameter glass rings and left for 20 h and 25 °C to remove the solvent. To obtain films with a flat surface, a special table with height-adjustable legs was used. When performing the modification steps, for ease of manipulation, the film was not detached from the cellophane substrate and was not removed from the frame ring. After the modification was completed, the film was detached from the cellophane substrate and used for further studies.

2.6. Modification of PLA Films with PMAG-NH_2

2.6.1. PLA Surface Saponification and Determination of the Content of Carboxylic Groups

To generate the carboxylic groups on the surface of the PLA-based films, a solution of 0.1 M NaOH was poured on the surface of the film and left for 30 min. Then, the film was washed with distilled water several times for 10 min until neutralization of the pH.

Quantification of the reactive carboxylic groups that had formed on the surface of the films was performed via their reaction with glycine. First, the carboxylic groups were activated by the addition of 2 mL of EDC (1 mg/mL) and NHS (2 mg/mL) solutions in 0.01 M MES-buffered solution, pH 5.4. Activation was carried out for 30 min at 2 °C. After activation, the film was washed 5 times with distilled water for 15 s each. After that, 3 mL of glycine solution (0.1 mg/mL) in 0.025 M borate-buffered solution (pH 9.4) was placed on the surface of the activated film, which was then incubated for 24 h at 22 °C.

After the reaction was completed, 0.5 mL of reaction solution was taken and placed in a 15 mL plastic tube with a lid. Then, 2.5 mL of ninhydrin solution in ethanol (2 mg/mL) was added to the same tube, and the tube was tightly closed and placed in a glycerol bath heated to 120 °C for 40 min. Parallel to the analyzed sample, a blank experiment with 0.5 mL of borate buffer instead of the test solution was performed. After that, the test tubes were cooled in cold water for 2 min and the solutions were analyzed spectrophotometrically. The absorbance was measured relative to the blank sample at a wavelength of 400 nm. Detection of the amount of glycine unreacted with the film was carried out using a calibration plot previously built for ten standard glycine solutions in the range of 0.005 to 0.100 mg/mL (Figure S4, Supplementary Materials). The content of carboxyl groups that were accessible for modification on the film surface (*[COOH]*, mol) and their specific content ($[COOH]_{sp}$, mol/cm^2) were calculated using Equations (3) and (4):

$$[COOH] = \frac{V[C_o(Gly) - C_x(Gly)]}{M(Gly)} \quad (3)$$

$$[COOH]_{sp} = \frac{[COOH]}{\pi R^2} \quad (4)$$

where $C_o(Gly)$ and $Cx(Gly)$ are the initial concentrations of glycine and its concentration after the reaction, mg/mL, respectively; ε is the mass extinction coefficient (5.31 $\pm$ 0.14 mg·mL^{-1}·cm^{-1}); V is the sample volume (3 mL); R is the radius of the film (1.5 cm); and M is the molar mass of glycine.

2.6.2. Grafting of PMAG-NH_2 to Activated PLA Surface

First, 2.1 mL or 1.7 mL of PMAG-*s* or PMAG-*l* solution, respectively, in borate-buffered solution, pH 9.4, with concentrations of 0.75 or 1.50 mg/mL were placed on the EDC/NHS-activated surface of PLA (see Section 2.6.1). The modification was carried out overnight at room temperature with gentle shaking. The surface was washed 3 times with 0.5 M NaCl solution and then 5 times with distilled water for 5 min each to remove unbound PMAG residues.

For the quantitative determination of immobilized polymer chains, the color reaction of primary amines with TNBS was used. The PMAG solution sampled from the film after modification was analyzed according to the protocol described in Section 2.4. The amount of grafted PMAG-NH_2 chains was determined by the difference in the polymer quantities before and after modification. The corresponding quantities were calculated with the application of a calibration plot built for standard PMAG-NH_2 solutions in borate-buffered solution, pH 9.5, in the range of concentrations from 0.1 to 0.8 mg/mL (Figure S5, Supplementary Materials).

The grafting density (*GD*, nmol/cm^2) was determined as the ratio of the number of amino groups reacted to the film area (5) as follows:

$$GD = \frac{[PMAG - NH_2]}{S} = \frac{[PMAG - NH_2]}{\pi R^2} \quad (5)$$

where [*PMAG-NH*$_2$] is the quantity of the grafted PMAG-NH_2 in moles; S is the surface area of the film, cm^2; and R is the film radius (1.5 cm).

The numerical grafting density *(GD_n*, molecules/nm^2*)* was calculated as the ratio of the number of PMAG-NH_2 macromolecules to the film surface area in nm^2, as in Equation (6):

$$GD_n = \frac{\nu(PMAG - NH_2) \times N_a}{\pi R'^2} \quad (6)$$

where N_A is Avogadro's number; and R' is a radius of the PLA film expressed in nm.

The degree of surface carboxylic group substitution (*DS*—degree of substitution, %) was determined as follows:

$$DS = \frac{[PMAG - NH_2]}{[COOH]} \times 100 \quad (7)$$

where [*PMAG-NH*$_2$] is the quantity of grafted PMAG-NH$_2$ in moles; and [*COOH*] is the determined quantity of surface carboxylic groups.

2.6.3. Thermogravimetric Analysis

Thermogravimetric analysis (TGA) was used to analyze the difference in the mass loss of the neat and PMAG-modified PLA films. Neat and PMAG-modified PLA were tested to determine the difference in mass change upon heating due to the presence of PMAG chains on the surface of PLA film. TGA was carried out using a set of instruments for thermogravimetric analysis and synchronous thermal analysis with gas phase analysis by IR spectroscopy and mass spectrometry, mass measurement error ± 0.01 mg (NETZSCH TG 209 F3 Iris and STA 409 C/4/G Jupiter, Germany). The study was carried out in the temperature range of 40–510 °C under argon atmosphere.

2.7. Measurements of Contact Angles

Contact wetting angles were measured by sessile drop microphotography, followed by data processing in SCA 20 (version 1.0) software (Informer Technologies, Los Angeles, CA, USA). Distilled water (pH = 6.0) was used as the working fluid. The volume of applied liquid drops was 1.0 μL. Droplets were applied to the substrate surface using a microdoser and then sequentially photographed. Measurements were taken at several points on the film away from the edges. The resulting contact angle values were averaged, and the relative error was calculated using Student's distribution (confidence factor 0.9).

The degree of hydrophilization (*HD*) was calculated using Equation (8); it shows how many times the value of the edge wetting angle decreased.

$$HD = \left(1 - \frac{\varphi'}{\varphi^0}\right) \times 100\% \quad (8)$$

where φ' and φ^0 are the contact angles of the modified and neat films, respectively.

2.8. Ellipsometry

The film specimens were studied by ellipsometry using the Ellipse-1891 SAG spectral ellipsometric complex with a wavelength range of 350–1000 nm (Scientific Production Company "Center of Nanotechnologies", Novosibirsk, Russia). The films under study were cut into squares (with sides of approximately 1 cm) and attached to the polished surface of a silicon slide. With this aim, 50–80 μL of water was applied dropwise to the slide, after which the films were placed over them such that there were no air bubbles between the film and the silicon surface. The slides with the films were left for several hours to dry completely.

Ready slides with films were placed on the working table of the ellipsometer and aligned using the autocollimator; then Ψ were measured at wavelengths from 350 to 100 nm. Each film was examined at 3–4 points away from the edges. The resulting Ψ – λ dependences were averaged. The angle of incidence/reflection of the beam from the film surface was 70°.

2.9. AFM

The morphology of PLA-based films was studied by atomic force spectroscopy (AFM) on the analytical probe microscopy module of the Nanolab research platform with an integrated Omicron VT AFM XA 50/500 scanning probe microscope (Omicron NanoTechnology GmbH, Germany) operating under ultrahigh vacuum conditions (p = 1–2 × 10^{-10} mbar).

For the study, the film was dried and trimmed to obtain a square sample with a side of approximately 1 cm. The sample was placed in the working chamber of the probe microscopy platform, the cantilever was set up, and then air was evacuated until an ultrahigh vacuum was reached. Scanning was performed in automatic mode.

Regions of 0.5 × 0.5 μm, 1.0 × 1.0 μm, and 3.0 × 1.9 μm were scanned. The resolution of the resulting image was 10 nm. Data processing was performed using the freely distributed non-commercial software WxSM (ver. 5.0) [40]. The roughness coefficient was defined as the ratio of the real area of the curvilinear surface to the conditional flat area (Equation (9)). The area of the curvilinear surface was calculated in Origin 9.1 software using the built-in command for finding the area of the curvilinear surface given by a two-dimensional matrix of height values (coord. Z) from X and Y coordinates.

$$Roughness = \frac{S^0}{S_r} \quad (9)$$

where S^0 and S_r are the calculated conditional flat area and the real area of the curvilinear surface, respectively.

2.10. RGD Peptide Conjugation

Conjugation of the peptide with PMAG bound to PLA films was carried out similarly to a previously developed protocol [29,41]. PMAG on the surface of PLA was oxidized with $NaIO_4$ solution in deionized water at 4–6 °C in the dark for 24 h. With this aim, 1.5 mL of aqueous $NaIO_4$ solution was added onto the film surface. The amount of $NaIO_4$ was calculated considering the following molar ratio: [$NaIO_4$]:[MAG] = 0.7. This ratio led to the generation of 30 mol% of aldehyde groups. After the generation of aldehyde groups in PMAG, 1.5 mL of a solution of GRGDSP with a concentration of 150 μg/mL in 0.01 M sodium phosphate solution (pH 8.0) was added to the films. The system was left for 2 h at 22 °C for conjugation to take place. The solution was then collected for the TNBS assay of the unbound peptide. The surface of films was washed three times with deionized water. The washing fractions were combined with the peptide solution after the reaction and lyophilized. The dry residue was dissolved in 0.5 mL of water and analyzed using the TNBS assay as described in Section 2.4.

The reduction of formed aldimine bonds (Schiff base) and unreacted aldehyde groups was carried out with sodium borohydride solution. The reaction was performed using a 3-fold molar excess of $NaBH_4$ in water relative to the -CHO groups at 22 °C for 1 h with gentle shaking. Finally, the films were washed 3 times with water and stored at 4 °C.

2.11. Cell Adhesion

Prior to use in cell culture, all the PLA films were sterilized under UV light exposure for 10 min. From one 30 mm film, films of 6 mm in diameter were cut and glued to the wells of non-adhesive 96-well plate using BF-6 medical glue (Tula Pharmaceutical Factory, Tula, Russia) from the unmodified side. Next, 5×10^3 MSCs in 100 μL of medium were seeded on the surface of each testing specimen. The plastic of the wells of the 96-well adhesive plate was involved in the study as the positive control. Cells were cultivated in a humidified atmosphere for 48 h at 37 °C in alpha-MEM containing 10% human serum. After cultivation, some of the specimens (n = 4) were subjected to an MTT assay, while others were treated with 4% neutral formalin solution for 30 min to fix the cells. The MTT assay was performed using the standard protocol described earlier [42]. Cell adhesion on the surface of the samples was calculated as a percentage relative to the positive control (adhesive cultural plastic surface), whose normalized optical density was taken as 100%. Cells fixed on the surface of film specimens were stained with calcein-AM solution (4 μg/mL) to visualize the cells. The stained films were examined using fluorescence microscopy (CELENA S Digital Imaging System) at an emission wavelength of 530 nm (an excitation wavelength is 480 nm).

The results were expressed as mean ± standard deviation (SD). A normality test and a test for equal variances were performed before running Student's *t*-test to compare results between the two groups.

3. Results and Discussion

3.1. Fabrication and Pre-Treatment of PLA Films

PLA used for the preparation of the films was synthesized via ring-opening polymerization using tin (II) octoate as a catalyst. The obtained sample had the viscosity–average molecular weight of about 198,000 ([η] = 1.49 dL/g).

PLA-based films were fabricated using the solution casting approach (Figure S2, Supplementary Materials). For that, 5.3% PLA solution in chloroform was cast onto the cellophane substrate, followed by solvent evaporation within 20 h at 25 °C. The morphology of the surface of the PLA films obtained using this method was evaluated by atomic force microscopy (AFM) (Figure 2). The obtained images indicate a low surface roughness. The average height difference is approximately 1.29, 1.35, and 2.16 nm for the scanned areas of 0.5 × 0.5 μm, 1.0 × 1.0 μm, and 3.0 × 1.9 μm, respectively. Computer processing of AFM data allowed for the evaluation of the real curved surface area and roughness (for area 3.0 × 1.9 μm). The latter was calculated as the ratio of curved surface area to flat surface area. The calculated curved surface area was found to be 5.732 ± 0.003 μm^2, while the theoretical flat surface area of the scanned region was 5.70 μm^2. Thus, the roughness for the obtained PLA films was 0.56 ± 0.05%.

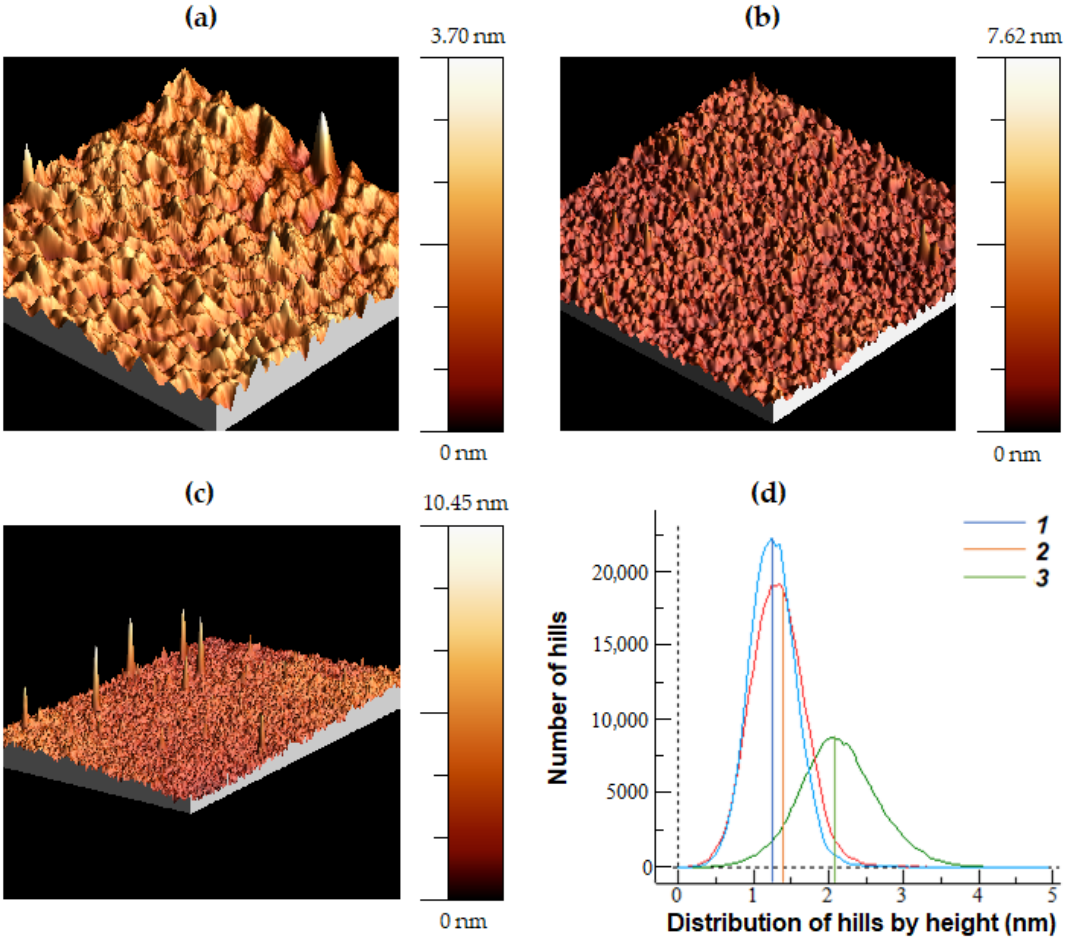

Figure 2. AFM scans of the regions of 0.5 × 0.5 μm (**a**), 1.0 × 1.0 μm (**b**), and 3.0 × 1.9 μm (**c**), as well as distribution of hills by height (**d**) for the PLA-based films obtained. In (**d**): ***1***—distribution for a region of 0.5 × 0.5 μm; **2**—distribution for a region of 1.0 × 1.0 μm; **3**—distribution for a region of 3.0 × 1.9 μm.

One of the drawbacks of PLA-based materials, in addition to relative hydrophobicity, is the absence of the necessary number of reactive groups on the surface for modification. In order to increase the amount of surface carboxyl groups, the films were exposed to an alkali solution to hydrolyze part of the ester linkages of the PLA main chain. The degree of hydrolysis and the amount of carboxyl groups generated on the surface can be varied by changing the concentration of the alkali solution and the saponification time. The content of carboxyl groups accessible for the reaction on the surface of PLA-based films was determined by quantifying the amount of bound glycine. For this purpose, the carboxyl groups on the surface of the PLA film were activated to obtain N-hydroxysuccinimide (NHS) ester and then treated with the solution of a glycine (Section 2.6.1). Glycine was selected since it is a small molecule containing an amino group for covalent binding and does not provide steric hindrance during immobilization. At the same time, glycine is highly hydrophilic and also contains a carboxyl group. These factors minimize the possibility of glycine nonspecific interactions with the surface and allow for the quantification of COOH groups on the surface of the films.

It was observed that increased alkali concentrations and/or saponification time resulted in visible film disintegration during saponification or during subsequent modification (Table 1). A downward change in alkali concentration and/or saponification time led to the generation of a reduced number of carboxyl groups on the surface. The following was determined as the optimal saponification conditions: 0.1 M sodium hydroxide solution and saponification time of 30 min. The quantity of the accessible for reaction carboxylic groups determined on the surface of PLA-based films after saponification under optimized conditions was found to be 802 ± 25 nmol/film (R_{film} = 1.5 cm) which corresponds to a density of 114 nmol/cm^2 (Table 1).

Table 1. Effect of sodium hydroxide concentration on the amount of introduced COOH groups and film stability.

#	[NaOH] (mol/L)	Saponification Time (min)	[COOH] (nmol/cm^2)	Visible Film Destruction
1	0.01	30	21 ± 3	no
2		60	34 ± 4	no
3	0.05	30	65 ± 7	no
4		60	83 ± 10	no
5	0.10	10	95 ± 12	no
6		30	114 ± 15	no
7		60	305 ± 34	yes
8	0.25	10	438 ± 52	yes
9		30	492 ± 54	yes
10		60	547 ± 62	yes
11	0.50	10	688 ± 81	yes
12		30	775 ± 90	yes

The results obtained for the surface treatment of PLA-based materials with alkali are consistent with the recent results. In particular, Chen et al. found that surface treatment with alkali solutions (pH 12.5) for 6 h affects the mechanical properties of PLA-based 3D-printed materials. Specifically, the stiffness and Young's modulus were reduced for alkali-treated 3D-printed materials under compression conditions compared to the neat PLA matrix [43]. At the same time, surface treatment for 1 h did not have such a significant effect.

3.2. Synthesis of PMAG with Terminal Amino Group

Polymer belonging to the glycopolymer class, namely PMAG was selected in this study as a hydrophilic polymer for the covalent surface modification of PLA-based materials. PMAG was synthesized by RAFT polymerization and modified according to the scheme shown in Figure 3.

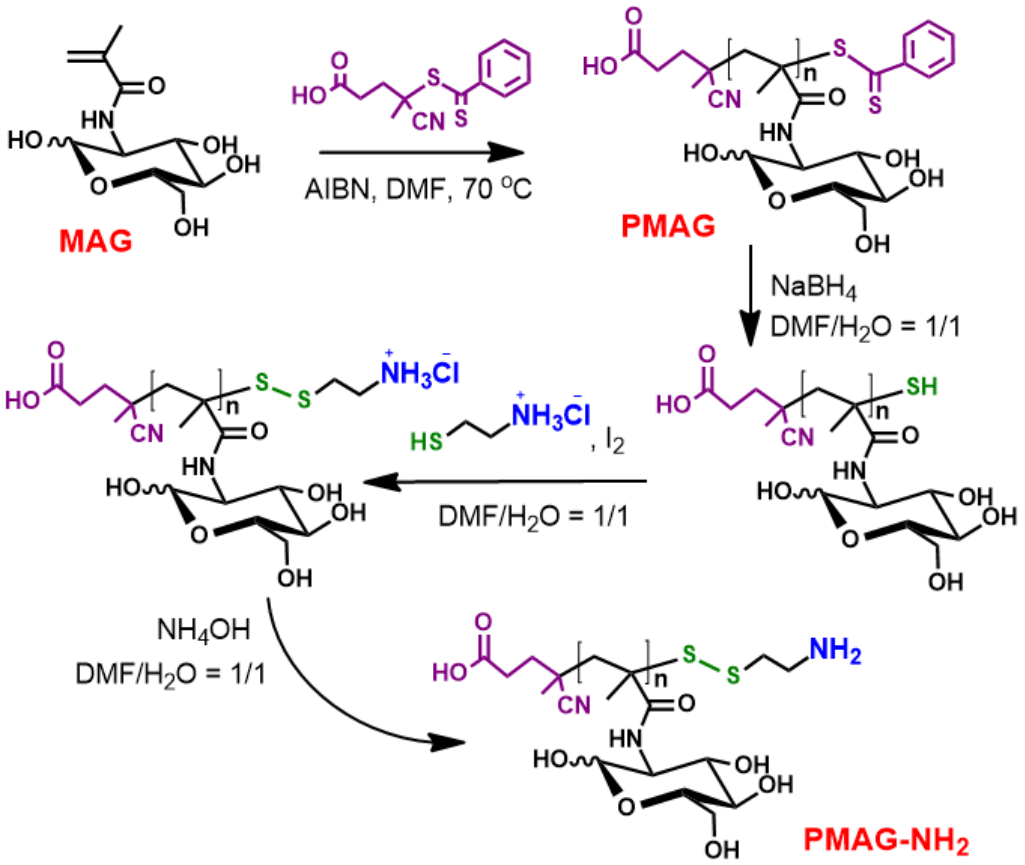

Figure 3. Schemes for synthesis of PMAG and its modification to produce a terminal amino group for further covalent modification of PLA-based materials.

MAG, CTA, and AIBN were used as monomer, RAFT-agent, and initiator, respectively. Variations in the [MAG]/[CTA] ratio allowed for the synthesis of two samples with different lengths. The ratios of the initial components and the characteristics of the synthesized samples are presented in Table 2. Two PMAG samples, with their respective degrees of polymerization of 21 and 38 units (^{1}H NMR spectroscopy) corresponding to the number-average molecular weight (M_n) of 5470 and 9670, were synthesized and designated as PMAG-s and PMAG-l, respectively. The molecular weights calculated from the ^{1}H NMR spectra were in agreement with the results obtained by the SEC method (Table 2). Both samples were characterized by low dispersity, which is typical for polymers obtained by RAFT polymerization under optimal conditions [38].

Table 2. Ratios of initial components and characteristics of the obtained PMAG samples.

Sample	Molar Ratio of Components			Conversion (%) [a]	Molecular Weight [b]		Đ [b]	*DP* [a]	M_n [a]
	MAG	CTA	AIBN		M_n	M_w			
PMAG-*s*	20	1	0.25	81	5000	5440	1.08	21	5470
PMAG-*l*	50	1	0.25	66	8900	9440	1.06	38	9670

[a] Determined by ^{1}H NMR spectroscopy. [b] Determined by SEC.

In order to perform single-point attachment of PMAG to the surface of carboxylated PLA-based films, the terminal dithiobenzoate group of PMAG was converted to a thiol group via reduction with $NaBH_4$ [44], followed by modification with cysteamine hydrochloride to introduce the required amino group (Figure 3) [45]. Quantification of the amino groups in the modified polymer sample was carried out using the TNBS assay (Section 2.6.2). Comparison of the theoretical amount of introduced amino groups,

known from polymerization conditions, with the obtained experimental data allowed us to determine the modification efficacy (Table 3).

Table 3. The content of amino groups introduced into PMAG during modification of terminal dithiobenzoate moieties with cysteamine.

Sample	[-NH_2] (nmol per 1 mg of PMAG)		Modification Efficacy (%)
	Theoretical	TNBS Assay	
PMAG-NH_2-*s*	183	173 ± 4	94.5 ± 1.6
PMAG-NH_2-*l*	103	98 ± 3	95.2 ± 2.3

The results obtained show that the molar fraction of amino groups determined in the PMAG-NH_2-s sample is 1.76 times higher than in the PMAG-NH_2-l sample, which is comparable to the ratio of M_n values for these polymers (1.77, see Table 2). Thus, it can be concluded that, in the selected range of molecular weights, the kinetics of the redox reaction is independent of the PMAG macromolecules' degree of polymerization.

3.3. Modification of PLA-Based Films with PMAG-NH_2

Grafting of glycopolymer to the surface of PLA-based films was carried out by the reaction of the terminal amino groups of PMAG-NH_2 with the surface carboxylic groups of the PLA activated to NHS esters (Figure 4). Modification was carried out in 0.01 M borate buffered-solution, pH 9.4, at room temperature with gentle stirring for 24 h.

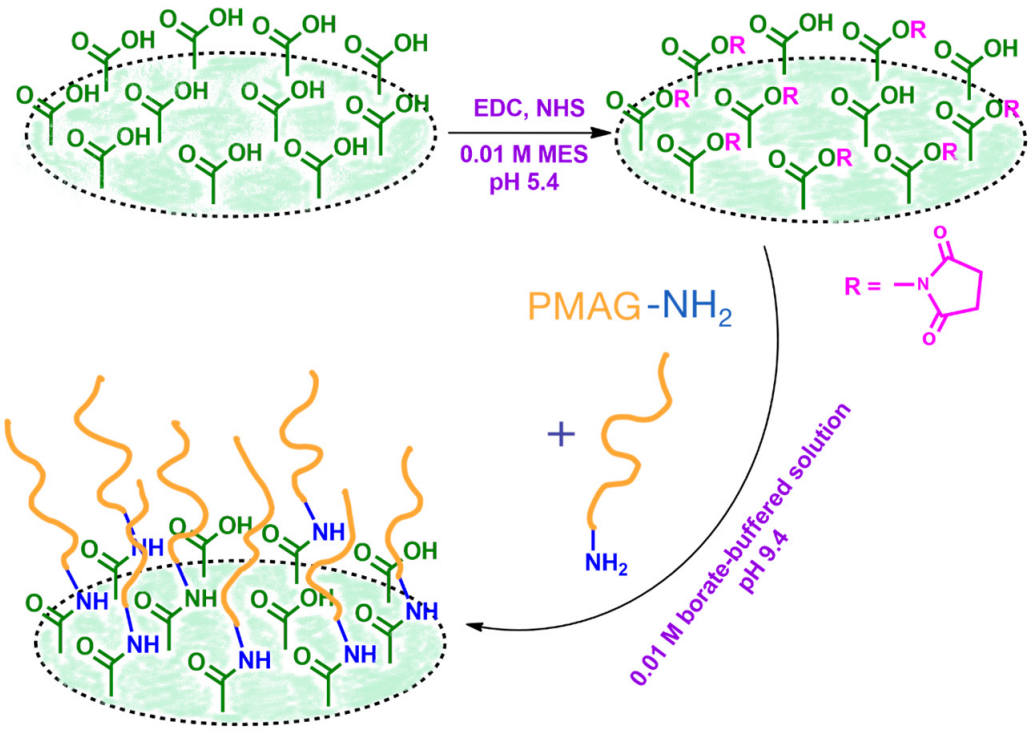

Figure 4. Scheme of PMAG-NH_2 grafting to the surface of PLA-based films.

The content of PMAG-NH_2 used for the modification of the PLA films was set as 233 or 466 nmol in the case of PMAG-NH_2-s, which corresponds to the [−COOH]/[−NH_2] ratio equal to 3.4 and 1.7, respectively. Considering the longer polymer chain of PMAG-NH_2-l, which can cause the steric hindrance for the reaction, this modifier was taken in smaller quantities of 163 and 326 nmol ([-COOH]/[-NH_2] ratio equal to 4.9 and 2.5, respectively). The results of the modification reaction are summarized in Table 4. As can be seen, the use of a higher initial amount of the polymer resulted in a higher quantity of grafted PMAG

and, consequently, higher grafting efficacy and surface carboxylic groups substitution. The quantity of the grafted polymer and the efficacy of grafting increased with increases in both the initial amount of PMAG-NH_2 and PMAG-NH_2 chain length. At the same time, the grafting density was higher for the shorter PMAG-NH_2 chain (PMAG-NH_2-s) at selected amounts of polymers.

Table 4. Results of the PLA films surface grafting with PMAG-NH_2 of different molecular weights.

Sample	Grafted [PMAG-NH_2] (nmol)		Efficacy of Grafting (%)	Grafting Density		Degree of Substitution of Surface Carboxyl Groups, (%)
	Theoretical	Determined [a]		nmol/cm^2	Molecules/nm^2	
PMAG-NH_2-*s*	233	48 ± 2	20.6 ± 0.9	6.8	41	6.0
	466	162 ± 7	34.8 ± 1.5	22.9	138	20.2
PMAG-NH_2-*l*	163	35 ± 2	21.5 ± 1.0	5.0	30	4.4
	326	134 ± 6	41.1 ± 1.7	19.0	114	16.7

[a] TNBS assay was applied to determine unreacted PMAG-NH_2.

In our previous work, we applied a five-step modification of PLA films with PMAG through the reaction of aldehyde groups, which had been formed in PMAG upon the oxidation of vicinal glucose diol, as well as amino groups introduced on the PLA surface. The amount of grafted PMAG in that study was evaluated to be 12 nmol/film, which corresponded to 1.7 nmol/cm^2 [37]. One can observe that such a grafting density value is much lower than the same parameter in the presented research (5–23 nmol/cm^2). This could be explained by the multipoint interaction of the oxidized PMAG with the surface in the previous work, in which the PMAG macromolecules were supposed to flatten out on the film in the form of a layer which was adjacent to the surface. Such flattening blocked the possibility for other oxidized PMAG macromolecules to interact with the surface amino groups. In contrast to the previous work, in this study, the developed protocol guaranteed the single-point brush-like attachment of the polymer, allowing more macromolecules to interact with the surface. Thus, the approach developed in this study not only reduced the number of modification steps from five to three but also allowed for an increased quantity of grafted polymer (Table 4).

It is known that the thermal degradation of a polymer is quite sensitive to the types of bonds in the backbone and the side chains of the macromolecule. Thus, thermogravimetric analysis (TGA) was used to confirm the success of the modification of the PLA-based films with PMAG. According to the TGA results, the main degradation processes in the samples under investigation occurred between 215 and 315 °C (Figure 5). It could be concluded from the analysis of the previously published studies that PMAG, being a carbon-backbone polymer, has higher thermal stability than PLA, which is a polyester [46,47]. This is in line with the observed results, showing that the PMAG-modified PLA film has a slower rate of mass loss than the neat PLA film. The observed TGA curves are smooth, without any steps (Figure 5a).

The DTG curves (Figure 5b) show single major peaks distinguished by their extrema. In addition, the extremum of the modified sample is in the higher temperature region. Overall, these findings confirm the formation of covalent bonds between PLA and PMAG segments and the generation of a uniform chemical structure and not just a polymer blend.

To monitor the changes in the properties of the PLA films after modification, neat, saponified, and PMAG-modified films were analyzed by the ellipsometry method. In our case, the Ψ-λ dependences were recorded to qualitatively observe the differences in the various films (Figure 6). The dependence of Ψ on wavelength (λ) correlates with the thickness of the polymer films, with the film being thicker if the Ψ-λ dependence is lower. The analysis of the obtained plots allows us to form the conclusion that film treated with alkali solution is the thinnest. This result confirms the surface hydrolysis of PLA film followed by a loss of thickness. At the same time, the film modified with PMAG has the largest thickness. Moreover, the type of the plot for the PLA-PMAG film differs from the

pure and saponified PLA films, which indicates not only the large thickness of this film but also the different optical properties of this film. This, in turn, suggests the presence of a different surface composition compared to other films and indirectly confirms the success of modification.

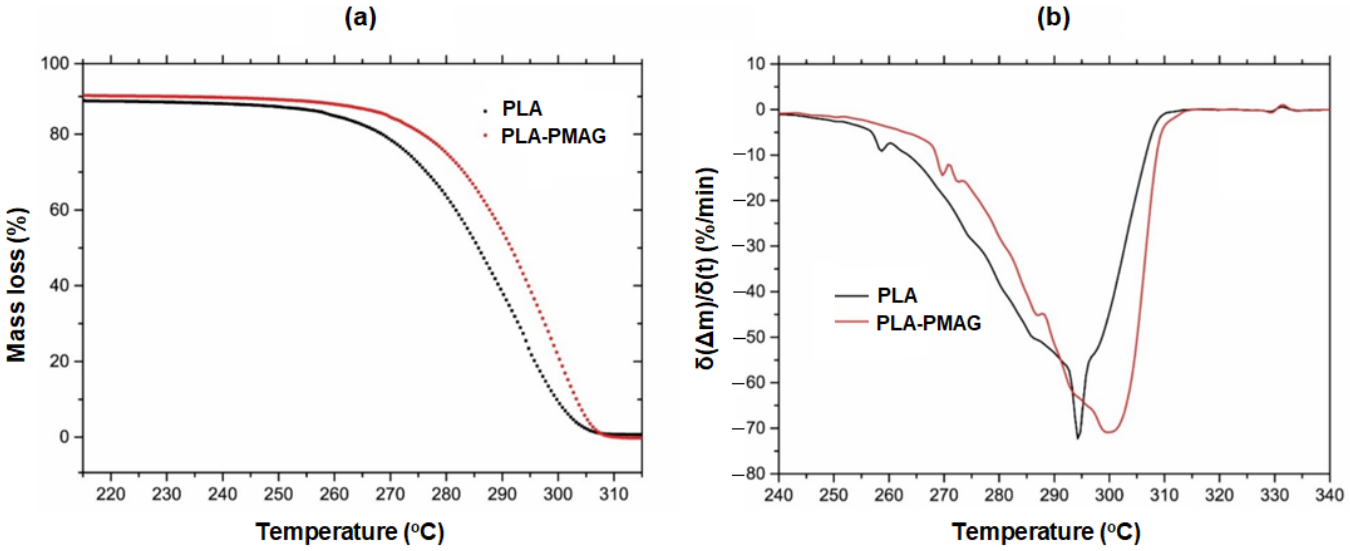

Figure 5. TGA (**a**) and DTG (**b**) plots for the neat PLA and PLA-PMAG-*l* (low content of PMAG) films.

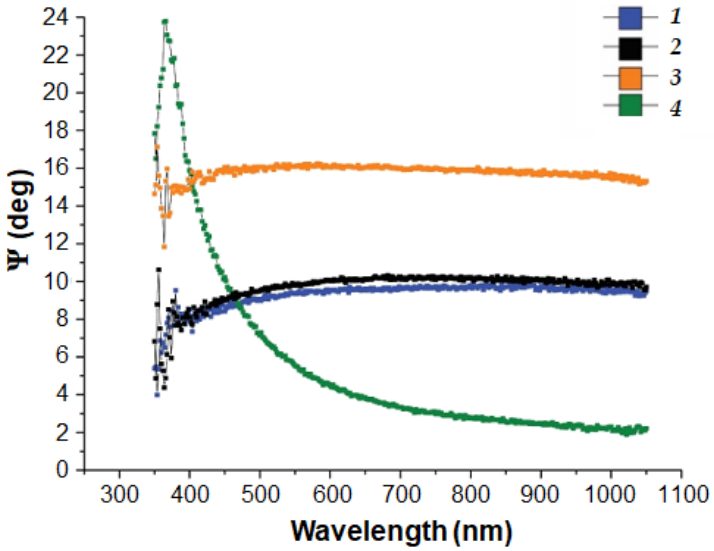

Figure 6. Ψ-λ plots for the neat, saponified and modified with PMAG-*l* PLA films: ***1***, **2**—neat PLA films (two scans were carried out for equipment performance testing purpose), ***3***—saponified PLA film and, ***4***—PLA-PMAG film.

Since hydrophilization of the surface of PLA-based materials was one of the major tasks of this study, the contact angles of wetting were measured on the surface of the functionalized PLA films. The results obtained are presented in Figure 7a. In general, a decrease in contact angle values was detected for all the PLA films, which were modified by PMAG. However, analysis of the results reveals several trends regarding the influence of PMAG chain length and grafting density on the degree of hydrophilization of the PLA surface. One can observe that hydrophilization (Figure 7b) increased with decreasing molecular weight from 9670 to 5470, while lowering the grafting density. The observed results may be explained as follows. With increasing molecular weight and grafting density, the number of intrapolymer and interpolymer interactions appears to increase

due to multiple hydrogen bonds [48], which decreases the participation of the saccharide moiety in solvation but increases the role of the hydrophobic main chain. The effect of chain length and grafting density have recently been shown for some other hydrophilic copolymers, e.g., mPEG and poly(2-hydroxyethyl methacrylate) (PHEMA) [49,50]. For example, increasing the thickness of the PHEMA-grafted layer led to an increase in the contact angle values.

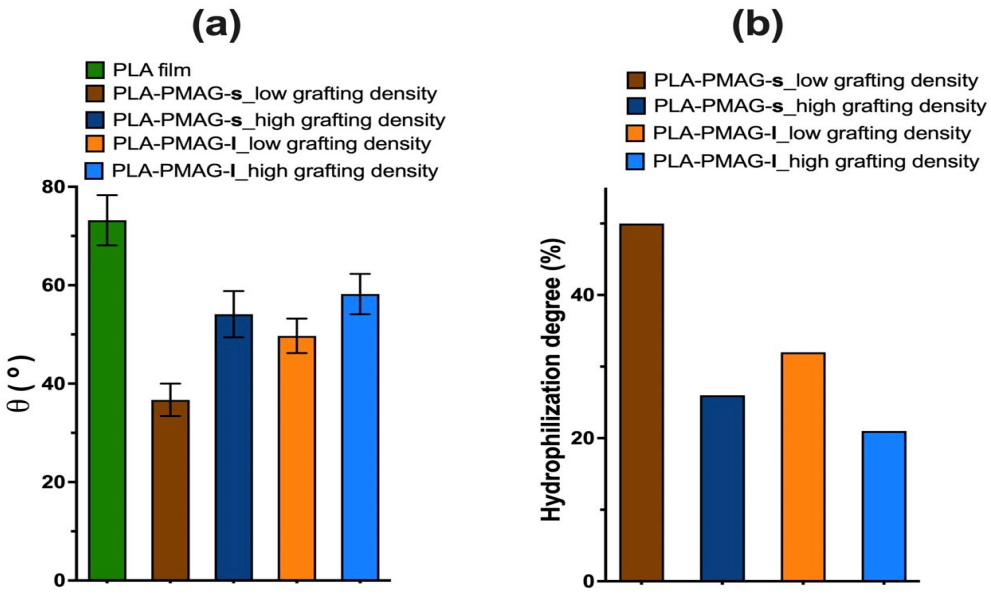

Figure 7. Results of contact angle measurements (**a**) and hydrophilization degree (**b**) for the modified films as compared to the neat PLA film (see Table 2 for PMAG-*s* and PMAG-*l* characteristics and Table 4 for grafting density parameter).

3.4. Functionalization of PLA-PMAG Films with Biospecific Ligands

PMAG selected for hydrophilization of PLA films can be easily used for covalent biofunctionalization. In particular, the glucose moieties presented in each monomer unit can easily undergo oxidation with sodium periodate, with the generation of aldehyde groups which are highly reactive towards the primary amino groups of biomolecules [41]. This approach can be used for conjugation to the PMAG of both proteins [29] and peptides [51].

Since aliphatic polyesters in general, and in particular PLA-based materials, are widely used for the development of scaffolds of various designs and compositions, in this study, an RGD peptide, i.e., GRGDSP, was selected as a model bispecific molecule. The scheme of covalent attachment of GRGDSP to the PMAG bound to the PLA films is shown in Figure 8. Since the RGD peptide used has only one terminal α-amino group, only the single-point attachment of the peptide to the polymer is possible.

In order to avoid possible steric hindrances during peptide immobilization, PLA films containing short chains of PMAG at low grafting density were used for the modification with GRGDSP. The results of peptide immobilization are presented in Table 5. As can be seen, the reaction proceeded with high efficiency of peptide immobilization.

In addition, the adhesion of MSCs on the surface of the neat PLA film, PLA-PMAG-*s* films and PLA-PMAG (RGD) films was examined using MTT assay and fluorescence microscopy (Figure 9). It is known that neat PLA and PCL materials demonstrate low cell adhesion [6,42,52]. Indeed, the obtained results indicate about 60% cell adhesion compared to the control (plastic of the adhesive cell culture plate). In turn, hydrophilization via the glucose units present on the PLA-PMAG surface improved the adhesion of the cells. Both these factors, namely hydrophilization and the presence of glucose, are known to enhance the cell adhesion properties of the material alone [53,54]. As expected [55,56], the additional immobilization of the RGD peptide considerably improved the attractiveness of the PLA material for the MSCs, which are often used in tissue engineering.

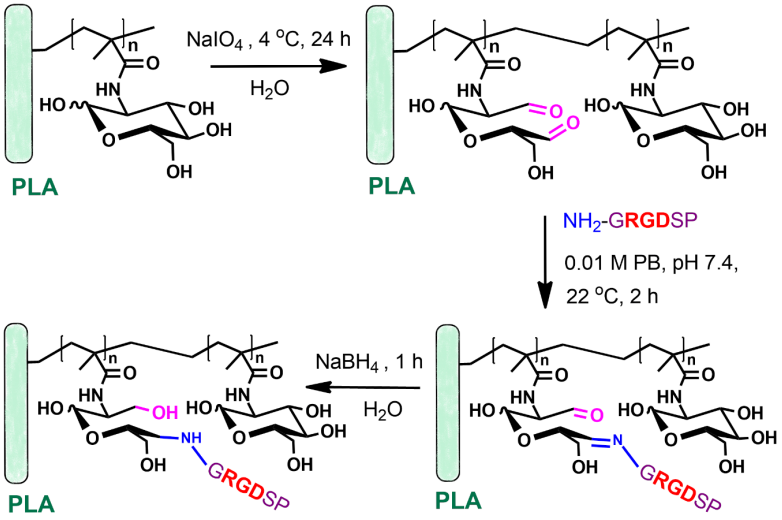

Figure 8. Scheme for immobilization of GRGDSP peptide to PLA-PMAG films.

Table 5. Results of the covalent immobilization of GRGDSP onto the surface of PLA-PMAG films.

Sample [a]	Quantity of GRGDSP (nmol)		Conjugation Efficacy (%)	Immobilization Density (nmol/cm^2)	Degree of Substitution in PMAG [b] (%)
	Initial	Immobilized			
PLA-PMAG-NH_2-*s*	340	252 ± 9	74.1 ± 2.7	35.6	25

[a] The sample with low grafting density was used; [b] Calculated regarding all MAG units in the polymer chain (*DP* = 21).

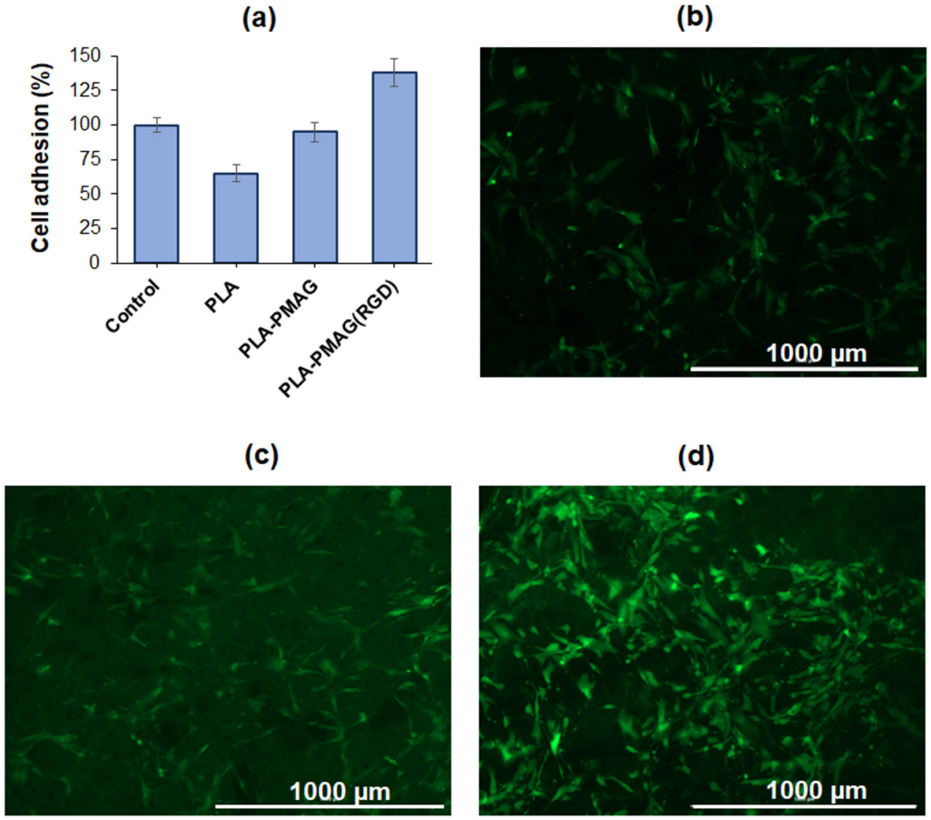

Figure 9. Adhesion and proliferation of MSCs on the surface PLA-based films (48 h): (**a**) MTT assay ($p < 0.05$); (**b**–**d**) fluorescence microscopy images of: (**b**) neat PLA film, (**c**) PLA-PMAG film and (**d**) PLA-PMAG(RGD) film. Scale bar is 1000 µm.

4. Conclusions

In this study, a novel method for modifying PLA-based materials with a hydrophilic bioinspired polymer, namely PMAG, was developed. For this purpose, a well-defined glycopolymer with two different polymerization degrees and containing a terminal amino group (PMAG-NH_2) was synthesized. Single-point grafting of PMAG-NH_2 to PLA surface was carried out via the reaction of the terminal amino group of glycopolymer with activated carboxyl groups pre-generated on the surface of PLA-based films. Under the selected grafting conditions, no drastic difference in the grafting of glycopolymers with the degrees of polymerization of 21 and 38 to the PLA surface was observed. Depending on the initial content of the glycopolymer in the reaction medium and polymer chain length, the grafting efficiency varied from 20.6 to 41.1%, and the grafting density was 5–23 nmol/cm^2. The developed approach outperformed the known multi-point PMAG immobilization technique in terms of grafting efficiency. Grafting PMAG onto the surface allowed for an increase in the degree of surface hydrophilization by up to 50% as compared to the neat PLA film. The attached PMAG can be further applied for surface biofunctionalization with bioactive amino-bearing compounds, for example, peptides. In this study, we have performed the conjugation of the RGD peptide α-amino group with the aldehyde groups in PMAG, which were generated by oxidation. The PMAG-modified hydrophilic PLA surface showed improved cell adhesion as compared to the neat PLA, while the best MSCs adhesion and proliferation was observed for the films containing PMAG with the conjugated RGD peptide. Although the current study was devoted to the surface of PLA films as model materials, the developed approach can be also applied to other aliphatic polyester-based materials of various designs, such as 3D scaffolds, nano- and microparticles, etc.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/surfaces7040067/s1, Figure S1: ^{1}H NMR spectrum of PMAG-*s* (D_2O, 60 °C); Figure S2: Scheme of preparation of PLA-based films by solution casting (a) and the construction from a glass ring and cellophane substrate used for solution casting (b): 1—before solution casting, 2—after solution casting; 3—a ready film (turned with the film upwards). Figure S3: Calibration curve built for cysteamine-TNBS adduct for the determination of the concentration of amino groups in PMAG-NH_2 solution by TNBS assay. Mass extinction coefficient is: $\varepsilon = 17.6 \pm 0.4$ $(mg/mL)^{-1} \cdot cm^{-1}$. Figure S4: Calibration curve for glycine-ninhydrin adduct for the determination of glycine concentration in solution by ninhydrin assay after PLA film modification. Mass extinction coefficient is $\varepsilon = 5.31 \pm 0.14$ $(mg/mL)^{-1} \cdot cm^{-1}$. Figure S5: Calibration curve built for PMAG-NH_2-TNBS adduct for the determination of the concentration of PMAG-NH_2 in solution by TNBS assay after PLA film modification: (a) PMAG-*l*-NH_2; (b) PMAG-*s*-NH_2. Mass extinction coefficients are as follows: (a) $\varepsilon = 0.132 \pm 0.002$ $(mg/mL)^{-1}$ cm^{-1}; (b) $\varepsilon = 0.204 \pm 0.007$ $(mg/mL)^{-1} \cdot cm^{-1}$.

Author Contributions: Conceptualization, V.K.-V.; methodology, E.S., M.L., E.K.-V. and V.K.-V.; formal analysis, V.K.-V., E.S., M.L. and E.K.-V.; investigation, K.A., E.S., M.L. and V.K.-V.; resources, V.K.-V. and T.T.; data curation, V.K.-V., K.A. and E.K.-V.; writing—original draft preparation, V.K.-V. and E.K.-V.; writing—review and editing, V.K.-V. and T.T.; visualization, V.K.-V., K.A. and E.K.-V.; supervision, V.K.-V.; project administration, V.K.-V.; funding acquisition, V.K.-V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Russian Science Foundation, grant number 24-15-00185.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data are available within the article and its Supplementary Materials.

Acknowledgments: The work was performed with the use of equipment of the Research Park of SPbU (Magnetic Resonance Research Centre, Thermogravimetric and Calorimetric Research Centre, Centre for Innovative Technologies of Composite Nanomaterials).

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Brannigan, R.P.; Dove, A.P. Synthesis, properties and biomedical applications of hydrolytically degradable materials based on aliphatic polyesters and polycarbonates. *Biomater. Sci.* **2017**, *5*, 9–21. [CrossRef] [PubMed]
2. Hussain, M.; Khan, S.M.; Shafiq, M.; Abbas, N. A review on PLA-based biodegradable materials for biomedical applications. *Giant* **2024**, *18*, 100261. [CrossRef]
3. Malikmammadov, E.; Tanir, T.E.; Kiziltay, A.; Hasirci, V.; Hasirci, N. PCL and PCL-based materials in biomedical applications. *J. Biomater. Sci. Polym. Ed.* **2018**, *29*, 863–893. [CrossRef] [PubMed]
4. Pulingam, T.; Appaturi, J.N.; Parumasivam, T.; Ahmad, A.; Sudesh, K. Biomedical Applications of Polyhydroxyalkanoate in Tissue Engineering. *Polymers* **2022**, *14*, 2141. [CrossRef] [PubMed]
5. Liu, S.; Qin, S.; He, M.; Zhou, D.; Qin, Q.; Wang, H. Current applications of poly(lactic acid) composites in tissue engineering and drug delivery. *Compos. Part B Eng.* **2020**, *199*, 108238. [CrossRef]
6. Castañeda-Rodríguez, S.; González-Torres, M.; Ribas-Aparicio, R.M.; Del Prado-Audelo, M.L.; Leyva-Gómez, G.; Gürer, E.S.; Sharifi-Rad, J. Recent advances in modified poly(lactic acid) as tissue engineering materials. *J. Biol. Eng.* **2023**, *17*, 21. [CrossRef]
7. Rasal, R.M.; Janorkar, A.V.; Hirt, D.E. Poly(lactic acid) modifications. *Prog. Polym. Sci.* **2010**, *35*, 338–356. [CrossRef]
8. Karthikeyan, A.; Girard, M.; Dumont, M.-J.; Chouinard, G.; Tavares, J.R. Surface Modification of Commercially Available PLA Polymer Mesh. *Ind. Eng. Chem. Res.* **2022**, *61*, 17297–17305. [CrossRef]
9. Baran, E.H.; Erbil, H.Y. Surface Modification of 3D Printed PLA Objects by Fused Deposition Modeling: A Review. *Colloids Interfaces* **2019**, *3*, 43. [CrossRef]
10. Atthoff, B.; Hilborn, J. Protein adsorption onto polyester surfaces: Is there a need for surface activation? *J. Biomed. Mater. Res. Part B Appl. Biomater.* **2007**, *80B*, 121–130. [CrossRef]
11. Yoon, J.-Y.; Kim, J.-J.; El-Fiqi, A.; Jang, J.-H.; Kim, H.-W. Ultrahigh protein adsorption capacity and sustained release of nanocomposite scaffolds: Implication for growth factor delivery systems. *RSC Adv.* **2017**, *7*, 16453–16459. [CrossRef]
12. Zhu, Q.; Chang, K.; Qi, L.; Li, X.; Gao, W.; Gao, Q. Surface Modification of Poly(l-lactic acid) through Stereocomplexation with Enantiomeric Poly(d-lactic acid) and Its Copolymer. *Polymers* **2021**, *13*, 1757. [CrossRef] [PubMed]
13. Laput, O.A.; Vasenina, I.V.; Korzhova, A.G.; Bryuzgina, A.A.; Khomutova, U.V.; Tuyakova, S.G.; Akhmadeev, Y.H.; Shugurov, V.V.; Bolbasov, E.N.; Tverdokhlebov, S.I.; et al. Effect of Nitrogen Arc Discharge Plasma Treatment on Physicochemical Properties and Biocompatibility of PLA-Based Scaffolds. *Polymers* **2023**, *15*, 3381. [CrossRef] [PubMed]
14. Donate, R.; Alemán-Domínguez, M.E.; Monzón, M. On the Effectiveness of Oxygen Plasma and Alkali Surface Treatments to Modify the Properties of Polylactic Acid Scaffolds. *Polymers* **2021**, *13*, 1643. [CrossRef] [PubMed]
15. Narushima, K.; Yamashita, N.; Fukuoka, M.; Inagaki, N.; Isono, Y.; Islam, M.R. Surface Modifications of Polyester Films by Ammonia Plasma. *Jpn. J. Appl. Phys.* **2007**, *46*, 4238. [CrossRef]
16. Yang, J.; Shi, G.; Bei, J.; Wang, S.; Cao, Y.; Shang, Q.; Yang, G.; Wang, W. Fabrication and surface modification of macroporous poly(L-lactic acid) and poly(L-lactic-co-glycolic acid) (70/30) cell scaffolds for human skin fibroblast cell culture. *J. Biomed. Mater. Res.* **2002**, *62*, 438–446. [CrossRef]
17. Yang, J.; Bei, J.; Wang, S. Enhanced cell affinity of poly (d,l-lactide) by combining plasma treatment with collagen anchorage. *Biomaterials* **2002**, *23*, 2607–2614. [CrossRef]
18. Wang, S.; Cui, W.; Bei, J. Bulk and surface modifications of polylactide. *Anal. Bioanal. Chem.* **2005**, *381*, 547–556. [CrossRef]
19. Cai, K.; Yao, K.; Cui, Y.; Lin, S.; Yang, Z.; Li, X.; Xie, H.; Qing, T.; Luo, J. Surface modification of poly(D,L-lactic acid) with chitosan and its effects on the culture of osteoblasts in vitro. *J. Biomed. Mater. Res.* **2002**, *60*, 398–404. [CrossRef]
20. Zhu, Y.; Gao, C.; Liu, X.; He, T.; Shen, J. Immobilization of Biomacromolecules onto Aminolyzed Poly(L-lactic acid) toward Acceleration of Endothelium Regeneration. *Tissue Eng.* **2004**, *10*, 53–61. [CrossRef]
21. Xu, F.J.; Yang, X.C.; Li, C.Y.; Yang, W.T. Functionalized Polylactide Film Surfaces via Surface-Initiated ATRP. *Macromolecules* **2011**, *44*, 2371–2377. [CrossRef]
22. Steffens, G.C.M.; Nothdurft, L.; Buse, G.; Thissen, H.; Höcker, H.; Klee, D. High density binding of proteins and peptides to poly(d,l-lactide) grafted with polyacrylic acid. *Biomaterials* **2002**, *23*, 3523–3531. [CrossRef] [PubMed]
23. Korzhikov-Vlakh, V.; Krylova, M.; Sinitsyna, E.; Ivankova, E.; Averianov, I.; Tennikova, T. Hydrogel Layers on the Surface of Polyester-Based Materials for Improvement of Their Biointeractions and Controlled Release of Proteins. *Polymers* **2016**, *8*, 418. [CrossRef] [PubMed]
24. Leonovich, M.; Korzhikov-Vlakh, V.; Lavrentieva, A.; Pepelanova, I.; Korzhikova-Vlakh, E.; Tennikova, T. Poly(lactic acid) and Nanocrystalline Cellulose Methacrylated Particles for Preparation of Cryogelated and 3D-Printed Scaffolds for Tissue Engineering. *Polymers* **2023**, *15*, 651. [CrossRef]
25. Ninaya, Z.H.A.; Hamid, Z.A.A.; Ahmad, Z.; Jaafar, M.; Yahaya, B.H. Development and Evaluation of Surface Modified Poly (lactic acid) Microsphere via Irradiation Techniques for Drug Delivery System. *Procedia Chem.* **2016**, *19*, 373–380. [CrossRef]
26. Korzhikov-Vlakh, V.; Mikhailova, A.; Sinitsyna, E.; Korzhikova-Vlakh, E.; Tennikova, T. Gradient Functionalization of Poly(lactic acid)-Based Materials with Polylysine for Spatially Controlled Cell Adhesion. *Polymers* **2024**, *16*, 2888. [CrossRef]
27. Mehmood, R.; Amaldoss, M.J.N.; Mofarah, S.S.; Zheng, Q.; Kaushik, N.K.; Ariotti, N.; Rawal, A.; Yang, J.-L.; Koshy, P.; Sorrell, C.C. Green Stealth Engineering of Lifetime-Biocatalytic Nanocatalyst for Neuroblastoma Therapy. *Appl. Surf. Sci.* **2022**, *572*, 151464. [CrossRef]

28. Moreno, A.; Lligadas, G.; Adamson, J.; Maurya, D.S.; Percec, V. Assembling Complex Macromolecules and Self-Organizations of Biological Relevance with Cu(I)-Catalyzed Azide-Alkyne, Thio-Bromo, and TERMINI Double "Click" Reactions. *Polymers* **2023**, *15*, 1075. [CrossRef]
29. Korzhikov, V.; Roeker, S.; Vlakh, E.; Kasper, C.; Tennikova, T. Synthesis of Multifunctional Polyvinylsaccharide Containing Controllable Amounts of Biospecific Ligands. *Bioconjug. Chem.* **2008**, *19*, 617–625. [CrossRef]
30. Roeker, S.; Böhm, S.; Diederichs, S.; Bode, F.; Quade, A.; Korzhikov, V.; van Griensven, M.; Tennikova, T.B.; Kasper, C. A study on the influence of biocompatible composites with bioactive ligands toward their effect on cell adhesion and growth for the application in bone tissue engineering. *J. Biomed. Mater. Res. Part B Appl. Biomater.* **2009**, *91B*, 153–162. [CrossRef]
31. Kumar, V.B.; Tiwari, O.S.; Finkelstein-Zuta, G.; Rencus-Lazar, S.; Gazit, E. Design of Functional RGD Peptide-Based Biomaterials for Tissue Engineering. *Pharmaceutics* **2023**, *15*, 345. [CrossRef] [PubMed]
32. Zhu, L.; Liu, Y.; Wang, A.; Zhu, Z.; Li, Y.; Zhu, C.; Che, Z.; Liu, T.; Liu, H.; Huang, L. Application of BMP in Bone Tissue Engineering. *Front. Bioeng. Biotechnol.* **2022**, *10*, 810880. [CrossRef] [PubMed]
33. Kouhi, M.; Fathi, M.; Prabhakaran, M.P.; Shamanian, M.; Ramakrishna, S. Poly L lysine-modified PHBV based nanofibrous scaffolds for bone cell mineralization and osteogenic differentiation. *Appl. Surf. Sci.* **2018**, *457*, 616–625. [CrossRef]
34. Cha, M.; Jin, Y.-Z.; Park, J.W.; Lee, K.M.; Han, S.H.; Choi, B.S.; Lee, J.H. Three-dimensional printed polylactic acid scaffold integrated with BMP-2 laden hydrogel for precise bone regeneration. *Biomater. Res.* **2021**, *25*, 35. [CrossRef] [PubMed]
35. Kung, F.-C.; Kuo, Y.-L.; Gunduz, O.; Lin, C.-C. Dual RGD-immobilized poly(L-lactic acid) by atmospheric pressure plasma jet for bone tissue engineering. *Colloids Surf. B Biointerfaces* **2019**, *178*, 358–364. [CrossRef] [PubMed]
36. Zheng, S.; Guan, Y.; Yu, H.; Huang, G.; Zheng, C. Poly-L-lysine-coated PLGA/poly(amino acid)-modified hydroxyapatite porous scaffolds as efficient tissue engineering scaffolds for cell adhesion, proliferation, and differentiation. *New J. Chem.* **2019**, *43*, 9989–10002.
37. Korzhikov-Vlakh, V.; Averianov, I.; Sinitsyna, E.; Nashchekina, Y.; Polyakov, D.; Guryanov, I.; Lavrentieva, A.; Raddatz, L.; Korzhikova-Vlakh, E.; Scheper, T.; et al. Novel Pathway for Efficient Covalent Modification of Polyester Materials of Different Design to Prepare Biomimetic Surfaces. *Polymers* **2018**, *10*, 1299. [CrossRef]
38. Levit, M.; Zashikhina, N.; Dobrodumov, A.; Kashina, A.; Tarasenko, I.; Panarin, E.; Fiorucci, S.; Korzhikova-Vlakh, E.; Tennikova, T. Synthesis and characterization of well-defined poly(2-deoxy-2-methacrylamido-d-glucose) and its biopotential block copolymers via RAFT and ROP polymerization. *Eur. Polym. J.* **2018**, *105*, 26–37. [CrossRef]
39. Schindler, A.; Harper, D. Polylactide. II. Viscosity–molecular weight relationships and unperturbed chain dimensions. *J. Polym. Sci. Polym. Chem. Ed.* **1979**, *17*, 2593–2599. [CrossRef]
40. Horcas, I.; Fernández, R.; Gómez-Rodríguez, J.M.; Colchero, J.; Gómez-Herrero, J.; Baro, A.M. WSXM: A software for scanning probe microscopy and a tool for nanotechnology. *Rev. Sci. Instrum.* **2007**, *78*, 13705. [CrossRef]
41. Stepanova, M.; Levit, M.; Egorova, T.; Nashchekina, Y.; Sall, T.; Demyanova, E.; Guryanov, I.; Korzhikova-Vlakh, E. Poly(2-Deoxy-2-Methacrylamido-D-Glucose)-Based Complex Conjugates of Colistin, Deferoxamine and Vitamin B12: Synthesis and Biological Evaluation. *Pharmaceutics* **2024**, *16*, 1080. [CrossRef]
42. Solomakha, O.; Stepanova, M.; Dobrodumov, A.; Gofman, I.; Nashchekina, Y.; Nashchekin, A.; Korzhikova-Vlakh, E. Chemical Modification of Nanocrystalline Cellulose for Manufacturing of Osteoconductive Composite Materials. *Polymers* **2024**, *16*, 1936. [CrossRef] [PubMed]
43. Chen, W.; Nichols, L.; Brinkley, F.; Bohna, K.; Tian, W.; Priddy, M.W.; Priddy, L.B. Alkali treatment facilitates functional nano-hydroxyapatite coating of 3D printed polylactic acid scaffolds. *Mater. Sci. Eng. C* **2021**, *120*, 111686. [CrossRef] [PubMed]
44. Chernikova, E.V.; Kudryavtsev, Y.V. RAFT-Based Polymers for Click Reactions. *Polymers* **2022**, *14*, 570. [CrossRef] [PubMed]
45. Levit, M.; Zashikhina, N.; Vdovchenko, A.; Dobrodumov, A.; Zakharova, N.; Kashina, A.; Rühl, E.; Lavrentieva, A.; Scheper, T.; Tennikova, T.; et al. Bio-Inspired Amphiphilic Block-Copolymers Based on Synthetic Glycopolymer and Poly(Amino Acid) as Potential Drug Delivery Systems. *Polymers* **2020**, *12*, 183. [CrossRef] [PubMed]
46. Levit, M.; Vdovchenko, A.; Dzhuzha, A.; Zashikhina, N.; Katernyuk, E.; Gostev, A.; Sivtsov, E.; Lavrentieva, A.; Tennikova, T.; Korzhikova-Vlakh, E. Self-assembled nanoparticles based on block-copolymers of poly(2-deoxy-2-methacrylamido-d-glucose)/poly(n-vinyl succinamic acid) with poly(o-cholesteryl methacrylate) for delivery of hydrophobic drugs. *Int. J. Mol. Sci.* **2021**, *22*, 11457. [CrossRef]
47. Kervran, M.; Vagner, C.; Cochez, M.; Ponçot, M.; Saeb, M.R.; Vahabi, H. Thermal degradation of polylactic acid (PLA)/polyhydroxybutyrate (PHB) blends: A systematic review. *Polym. Degrad. Stab.* **2022**, *201*, 109995. [CrossRef]
48. Oh, T.; Hoshino, Y.; Miura, Y. Aggregation of a double hydrophilic block glycopolymer: The effect of block polymer ratio. *J. Mater. Chem. B* **2020**, *8*, 10101–10107. [CrossRef]
49. Wang, L.-L.; Wu, J.-J.; Zhang, Z.-B.; Zhou, J.; He, X.-C.; Yu, H.-Y.; Gu, J.-S. Methoxypolyethylene glycol grafting on polypropylene membrane for enhanced antifouling characteristics–Effect of pendant length and grafting density. *Sep. Purif. Technol.* **2016**, *164*, 81–88. [CrossRef]
50. Yang, W.; Zhang, R.; Wu, Y.; Pei, X.; Liu, Y.; Zhou, F. Enhancement of graft density and chain length of hydrophilic polymer brush for effective marine antifouling. *J. Appl. Polym. Sci.* **2018**, *135*, 46232. [CrossRef]
51. Dvoretckaia, A.; Egorova, T.; Dzhuzha, A.; Levit, M.; Sivtsov, E.; Demyanova, E.; Korzhikova-Vlakh, E. Polymyxin B Conjugates with Bio-Inspired Synthetic Polymers of Different Nature. *Int. J. Mol. Sci.* **2023**, *24*, 1832. [CrossRef] [PubMed]

52. Biagini, G.; Senegaglia, A.C.; Pereira, T.; Berti, L.F.; Marcon, B.H.; Stimamiglio, M.A. 3D Poly(Lactic Acid) Scaffolds Promote Different Behaviors on Endothelial Progenitors and Adipose-Derived Stromal Cells in Comparison With Standard 2D Cultures. *Front. Bioeng. Biotechnol.* **2021**, *9*, 700862. [CrossRef]
53. Jacobs, T.; Morent, R.; De Geyter, N.; Dubruel, P.; Leys, C. Plasma Surface Modification of Biomedical Polymers: Influence on Cell-Material Interaction. *Plasma Chem. Plasma Process.* **2012**, *32*, 1039–1073. [CrossRef]
54. Shin, H.; Nichol, J.W.; Khademhosseini, A. Cell-adhesive and mechanically tunable glucose-based biodegradable hydrogels. *Acta Biomater.* **2011**, *7*, 106–114. [CrossRef] [PubMed]
55. Cai, S.; Wu, C.; Yang, W.; Liang, W.; Yu, H.; Liu, L. Recent advance in surface modification for regulating cell adhesion and behaviors. *Nanotechnol. Rev.* **2020**, *9*, 971–989. [CrossRef]
56. Jose, J.; Sultan, S.; Kalarikkal, N.; Thomas, S.; Mathew, A.P. Fabrication and functionalization of 3D-printed soft and hard scaffolds with growth factors for enhanced bioactivity. *RSC Adv.* **2020**, *10*, 37928–37937. [CrossRef]