Features of the modification of polylactide by (meth)acrylate groups in organic solvents

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Abstract. Polymerizable (meth)acrylate groups were introduced into polylactide (PLA) in the methylene chloride or toluene medium. Polymer modification was carried out both on the carboxyl and hydroxyl groups of polylactide. The efficiency of this modification was studied depending on the type of reaction. It was found that the highest degree of polylactide modification by (meth)acrylate groups (86%) is achieved through the formation of PLA urethane derivatives in the presence of diisocyanates. Samples of photocured, three-dimensionally crosslinked after such a modification polylactides were obtained, their biocompatibility was determined. It was shown that such materials are promising for use in regenerative medicine and tissue engineering.

1. Introduction

Polymers and copolymers based on lactic acid and its analogues, primarily polylactides, belong to the group of aliphatic polyesters, the interest in which is associated with properties such as biocompatibility, biodegradability and thermoplasticity [1]. Biodegradable biocompatible polymers and polylactide copolymers are widely used in medicine and various fields of technology [2-4]. Their use in medicine is due to the fact that in living organisms they are completely decomposed into non-toxic products involved in metabolism, do not cause allergic, inflammatory and other harmful reactions, and products based on polylactides can even have antimicrobial effects [5, 6]. Surgical filaments, films of various functional purposes (for example, for protection against ultraviolet radiation [7]), plates, clamps, screws, pins are made on the basis of biodegradable polymers. The application area of biodegradable polymers determines the direction of development of materials with the desired set of properties. The physicochemical properties of polylactides can vary widely due to different molecular weights, molecular weight distribution, supramolecular structure, degree of crystallinity and orientation. Depending on the application area of biodegradable materials, the requirements for them can vary greatly.

Also, a variety of biodegradable polymers, primarily polyesters, among which polylactides occupy a special place, are used as carriers for sustained release drugs. The polylactide particles themselves are characterized by accelerated decomposition of the polymer material under conditions, for example, of a
living organism. Modification of polylactide can be carried out in different ways (chemically, mechanically [8]), while it can not only control the biodegradation of the polymer matrix, but also handle the mechanical properties of the resulting material [9]. Control of the mechanical properties of the matrix, due to a variation in the degree of its crosslinking, can contribute to the creation of a material promising for implantology.

In this regard, polylactides modified with (meth)acrylate groups [10] can be a promising material for creating, on their basis, three-dimensionally cross-linked structures with a controlled degree of crosslinking for regenerative medicine, primarily obtained by photo exposure [11-14], and also act as a carrier of drug compounds with the creation of sustained release dosage forms [15].

Polylactide is an aliphatic polyester having terminal carboxyl and hydroxyl groups, which can be modified by reactable groups (including acrylates [16]). Therefore, the introduction into the polylactide of polymerization (meth)acrylic groups in this work was carried out for both functional groups to compare the effectiveness of the PLA modification depending on its method.

2. Materials and methods
To obtain acrylate derivatives, polylactide (PLA) with a molecular weight of 5 × 10³ Da, manufactured by Purac (Netherlands) was used. Modification of polylactide was carried out in organic solvents (toluene, methylene chloride) in a one-step process without isolation of intermediate products in three ways (all components were used without further purification):

According to the esterification reaction of terminal carboxyl groups with mono (meth)acrylic ethylene glycol ether (Aldrich) (AEG or MEG) by the equilibrium condensation method [17] in the presence of p-toluenesulfonic acid (Aldrich) as a catalyst in benzene or toluene with azeotropic water drainage [18]. General reaction scheme:

\[
\text{HO-CH(CH}_3\text{)CO-} (R)_n\text{-O-CH(CH}_3\text{)CO-CH}_2\text{OH} \rightarrow \text{HO-CH(CH}_3\text{)CO-} (R)_n\text{-O-CH(CH}_3\text{)CO-CH}_2\text{-CH}_2\text{-O-} \rightarrow \text{H}_2\text{O} + \text{H}_2\text{O} \quad (1);
\]

According to the esterification reaction of terminal hydroxyl groups with acrylic acid chloride (AAC, Aldrich) by the method of nonequilibrium condensation [17] in the presence of tertiary amines. The synthesis was carried out in methylene chloride, dried over calcined calcium chloride [18]. General reaction scheme:

\[
\text{HO-CH(CH}_3\text{)CO-} (R)_n\text{-O-CH(CH}_3\text{)CO-CH}_2\text{OH} \rightarrow \text{Cl-CO-CH}_2\text{H}_2 \rightarrow \text{HO-OC(CH}_3\text{)CHO(R}_n\text{CO(CH}_3\text{)CH-O-} \rightarrow \text{H}_2\text{O} + \text{HCl} \quad (2);
\]

According to the urethane formation reaction using cycloaliphatic diisocyanate-3-isocyanatomethyl-3,5,5-trimethyl-cyclohexyl isocyanate (isophorondiisocyanate, IP) and AEG (or MEG) (the reaction is similar to the procedure described in [19]). General reaction scheme:

\[
\text{HO-CH(CH}_3\text{)CO-} (R)_n\text{-O-CH(CH}_3\text{)CO-CH}_2\text{OH} \rightarrow \text{O=C-} (R)_n\text{-N=R}_2\text{-N=C=O (IP) + O=CH}_2\text{-CH}_2\text{-O-} \rightarrow \text{H}_2\text{O} \quad (3),
\]

where Ac is the remainder of acrylic acid.

The initial reagents, the reaction mixture and the resulting products, as well as the sol fraction of the cured polymer films, were analyzed by gel permeation chromatography (GPC), Waters Breeze chromatograph, using as detectors variable wavelength refractometer and UV module and standard columns Ultrastyrogel 100, 500, and 1000 A, the elution rate of 1 ml/min. The molecular weights of the products were evaluated by the corresponding calibration curves.

The formation of the (meth)acrylate derivative of PLA was also controlled by the appearance of the double bond absorption band (1629-1638 cm⁻¹) in the IR spectrum of the modified PLA, purified from by-products and starting reagents (IR Fourier spectrometer, Varian).

To study the reactivity of unsaturated groups introduced into the PLA, we used photo curing with light of a DRT-1000 mercury lamp for 3 min to obtain a solid transparent film up to 1 mm thickness.
For photocuring, the resulting reaction mixture containing the modified polylactide was dissolved in methylene chloride, followed by the addition of Darocur-4265 photoinitiator (a 1:1 weight mixture of diphenyl (2,4,6-trimethylbenzoyl) phosphate oxide and 2-hydroxy-2-methylpropio-phenone). The samples obtained after photocuring were kept in tetrahydrofuran (THF) for a day to remove uncrosslinked polymer and modification by-products from the samples, washed with water to remove tetrahydrofuran residues during the day, and then dried in air at room temperature. The extract in THF was also used to analyze the degree of polylactide modification. GPC analysis of such a solution made it possible to estimate the amount of unmodified PLA, as well as the amount of acrylate polylactide derivative contained in the reaction mixture, which was determined by the difference between the amount of polylactide that entered into the reaction and the amount of unmodified PLA contained in the extract of the photocured reaction mixture.

3. Results and discussions

3.1. Modification of polylactide at the terminal carboxyl groups by ethylene glycol mono(meth)acrylic ester

The simplest one-step process for the modification of carboxyl groups of PLA with the introduction of polymerization-capable groups is the esterification by unsaturated derivatives of diatomic alcohols. In this work, ethylene glycol monoacrylate was used as the esterifying agent (reaction (1) from the methodological part). However, it should be borne in mind that several adverse reactions can occur simultaneously in the system. This is, first of all, the reactions of intermolecular interaction of terminal groups (carboxyl and hydroxyl) of polylactide:

$$2\{\text{HO-CH(CH3)CO-(R)n-O-CH(CH3)CO-OH}\} \rightarrow \text{HO-CH(CH3)CO-(R)n-O-CH(CH3)CO-OH} + \text{H}_2\text{O}.$$  

In the case of this process, we could expect a noticeable increase in the molecular weight (MW) of the reaction product. On the other hand, partial destruction of PLA is possible under conditions of esterification at high temperature in the presence of p-toluenesulfonic acid, which causes an acidic reaction medium. Finally, a highly reactive AEG is addicted to a disproportionation reaction to form ethylene glycol diacrylate and a dimerization reaction.

After distillation of the solvent — toluene — a chromatographic analysis of the reaction products was carried out; it was found that the original AEG, taken in excess, is completely absent. However, it was noted the appearance of two new products with MW = 250 (ethylene glycol diacrylate) and MW = 400 corresponding to AEG dimerization products, which confirms the occurrence of a side process of AEG disproportionation. In this case, a decrease in MW of polylactide from 5066 introduced into the reaction to 3670 of the final product should be noted. This indicates a partial destruction of the PLA in the presence of an acidic catalyst and an elevated reaction temperature.

The data of IR spectroscopy indicate the presence of a double bond (1637 cm⁻¹) in the PLA esterification product purified from impurities by reprecipitation in hexane, which confirms the preparation of the acrylate derivative of PLA by the esterification of the terminal carboxyl groups of polylactide with ethylene glycol monoacrylate. Only ~ 20-25% used polylactide had been modified. However, even this amount of modified polymerization-based PLA, if there are by-products in the reaction mixture — diacrylates obtained as a result of AEG disproportionation and acting as cross-linking agents, leads to the formation of a network structure (partially soluble in THF) during photocuring (fully cross-linked three-dimensional structure of the modified polylactide does not dissolve in tetrahydrofuran).

3.2. Modification of polylactide at the terminal hydroxyl groups by acrylic acid chloride

After polylactide modification according to reaction (2), the chromatogram of the reaction product is similar to the chromatogram of the initial PLA and indicates the almost complete absence of by-
products. The presence of a double bond in the modification product is confirmed by infrared spectroscopy (1629 cm⁻¹).

To assess the reactivity of unsaturated groups introduced into the PLA, a photopolymerization reaction of the modified PLA was carried out. After photopolymerization, the film was kept in THF. The solid, transparent film was completely dissolved in tetrahydrofuran, which indicates the absence of network structures. GPC analysis of the solution made it possible to estimate the amount of unmodified PLA. Modification was ~ 65% in the absence of any side processes. Also, when analyzing the chromatogram of the solution of the photocured reaction mixture, it was noted the presence of high molecular weight fractions of PLA formed as a result of the interaction of the acrylate groups of the modified polylactide. At the same time, the formation of a network structure during the photocure of the modified PLA obtained by this method turned out to be possible when an additional crosslinking agent, such as oligourethane (meth)acrylate (OUA or OUM), was added to the reaction mixture in an amount of 10-20% by weight of the polymer.

3.3. Modification of polylactide through the formation of intermediate urethane derivatives of PLA

Modification of polylactide was also carried out by mono(meth)acrylic ester of ethylene glycol in the presence of diisocyanates according to reaction (3) of the methodological part. The completeness of the reaction was monitored by the disappearance of the absorption band of isocyanate groups (2268 cm⁻¹) in the IR spectrum of the reaction mixture and by the appearance of the absorption band of a double bond (1637 cm⁻¹) of acrylate groups in the IR spectrum of the reprecipitated reaction mixture that did not contain unreacted monomethacrylic ethylene glycol ether according to GPC data.

Usually, the equimolar ratio of reactants is used to introduce urethane groups into acrylic or methacrylic oligomers. Herewith, the completeness of the reaction is usually achieved with a 10% excess of isocyanate and (meth) acrylate reagents. In this case, however, it should be borne in mind that due to the low reactivity of the hydroxyl groups of PLA with an equimolar ratio of reagents (PLA: IP: AEG = 1: 1: 1.1), the reaction can also occur without the participation of PLA between diisocyanate and AEG with the formation of oligourethandi(meth)acrylate.

To establish the reaction mechanism and increase the efficiency of PLA modification, the effect of excess IP and AEG on the composition of the formed products was studied (table 1).

| Ratio of components PLA: IP: AEG | PLA | Excerpted after 20 hours |
|---------------------------------|-----|-------------------------|
| 1 : 1 : 1                       | 88  | 70                      |
| 1 : 10 : 10                     | 45  | 5 – 8                   |

It turned out that with an increase in the content of isophorondiisocyanate and ethylene glycol acrylate in the reaction mixture to a molar ratio PLA: IP: AEG = 1: 10: (10 ... 19) the content of the modified PLA increases (from 30 to 65 ... 86%), but it also increases amount of by-products, in particular urethane derivatives of di(meth)acrylates, including OUM (OUA), which obviously play the role of crosslinking agents in the photopolymerization of modified PLA, increasing the degree of crosslinking. It was shown that an increase in the content of reagents (IP and AEG) leads to an increase in the content of modified PLA and diacrylates, which allows one to obtain a three-dimensionally cross-linked polymer with a given degree of crosslinking. Other impurities and by-products did not affect either the depth of the modification of the PLA or the degree of crosslinking of the modified polylactide for all considered cases.
At the final stage, three-dimensional structures were formed using the two-photon polymerization method [20] (figure 1). The photopolymerization of the modified PLA was carried out in the presence of 1% Michler ketone (as a photoinitiator) using installation described in [21], the obtained three-dimensional structures were washed with dichloromethane to remove unreacted material.

![Figure 1. Three-dimensional crosslinked structures (scaffolds) obtained by two-photon laser polymerization of a composition consisting of methacrylate-containing polylactide, 10% by weight, OUM as a crosslinking agent, as well as a photoinitiator.](image)

For the scaffolds shown in figure 1, biocompatibility was determined. Biological tests were carried out by planting osteoblasts on the obtained scaffolds in Hanks saline solution (HBSS) in Dulbecco's medium (DPBS) [22]. Cytotoxicity was determined by the number of living cells planted on the surface of the scaffolds studied, compared with the model toxic environment (figure 2, red x-shaped dots). Preservation of the living cell population in the studied samples was shown (blue triangular dots in figure 2).

4. Conclusions

Thus, the modification of polylactide (meth) acrylate-containing compounds was carried out both on the carboxyl and hydroxyl groups of the polymer. The degree of modification of the PLA was determined for each case. It was shown that the best way of introducing polymerization-capable (meth) acrylate groups into polylactide is the reaction of urethane formation during the interaction of terminal hydroxyl groups of the polymer with diisocyanates and ethylene glycol mono(meth)acrylic ester (the degree of modification was 86%). Based on such a modified PLA, three-dimensionally crosslinked structures were obtained by two-photon polymerization; wherein the control of crosslinking is possible by varying the ratio of components during the modification reaction. Biologically tests have shown the safety of samples of cross-linked modified polylactide for living cells, which makes it a promising material for the manufacture of implants.

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