Functionalization Conditions of PLGA-PEG-PLGA Copolymer with Itaconic Anhydride

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Summary: Biodegradable thermosensitive triblock copolymers based on poly(ethylene glycol) and poly(lactic-*co*-glycolic acid) (PLGA-PEG-PLGA) prepared via ring opening polymerization were modified by itaconic anhydride (ITA), which gives copolymer both reactive double bonds and functional carboxylic acid groups essential for the reaction with biological active material. Functionalization conditions comprising ITA purification, temperature, time and presence of solvent were optimized with the respect to amount of end-capped ITA. Maximum of 76.6 mol. % of bonded ITA were reached via "one pot" reaction in a bulk at 110 °C after 1.5 h. ITA functionalization thermally stabilized the original copolymer by increasing the initial degradation temperature T_d from 284 °C to 294 °C and changing the negative glass transition temperature ($T_g = -1.8$ °C) to positive one up to 2.4 °C. The novel functionalized macromonomer can be cross-linked either chemically or physically in order to produce new functionalized hydrogel network applicable as biomedical material in tissue engineering.

Keywords: biodegradable polymers; functionalization; itaconic anhydride; poly(ethylene glycol); poly(lactic-*co*-glycolic acid)

Introduction

Among known biodegradable polymers, linear aliphatic polyesters especially those derived from lactic acid (LA), glycolic acid (GA) and their copolymers poly(lactic-*co*-glycolic acid) (PLGA) are particularly attractive.[1] These materials are biocompatible and after hydrolysis at physiological conditions to lactic and glycolic acids undergo degradation via the Krebs cycle to non-toxic products (water and carbone dioxide).[2,3] Mentioned characteristics make these polymers suitable materials for numerous medical applications such as resorbable sutures, carries for the controlled release of drugs, implants for orthopedic surgery or blood vessels, which finally can be replaced by living tissues.[4] Copolymerization of poly(ethylene glycol) (PEG) with lactones and lactides has been known as a good way to obtain novel polymeric materials displaying unique physico-chemical and biological properties adaptable to specific utilization.[5]

In this work, the novel approach for synthesis of biodegradable thermosensitive poly(D,L-lactic acid-*co*-glycolic acid)-*b*-poly(ethylene glycol)-*b*-poly(D,L-lactic acid-*co*-glycolic acid) (PLGA-PEG-PLGA) copolymers both-end terminated with itaconic acid bringing both carboxyl groups (-COOH) and double bonds ($-CH=CH_2$) was utilized. After optimizing the reaction conditions, obtained well-defined ITA/PLGA-PEG-PLGA/ITA macromonomers were characterized using ^{1}H NMR, GPC, FTIR, TGA and DSC analysis.

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Materials

Poly(ethylene glycol) (PEG, $M_n = 1500$ $g \cdot mol^{-1}$, Aldrich Germany) was thoroughly degassed under vacuum for 8 hours at 130 °C. D,L-lactide (LA), glycolide (GA, Polysciences, Pennsylvania) and itaconic anhydride (ITA 97%, Fluka, Switzerland) were sublimated under reduced pressure (10 Pa) prior the use. Sn(II)2-ethylhexanoate (95%, Sigma Germany) was used as received. Tetrahydrofuran (THF, p.a., Lach-Ner, Czech Republic) was distilled under vacuum from sodium/benzophenone purple solution.

Methods

Synthesis of PLGA-PEG-PLGA Copolymer

The PLGA-PEG-PLGA triblock copolymer (ABA type) was prepared via conventional ring opening polymerization (ROP) method in a bulk under nitrogen atmosphere. PEG (4 mmol), LA (83 mmol) and GA (28 mmol) were homogenized at 130 °C followed by injecting Sn(II)2-ethylhexanoate (0.1 mmol). Reaction proceeded for 3 hours. Product was purified from unreacted monomers by dissolving in cold water and heating the solution up to 80 °C. Precipitated polymer was separated by decantation and dried in vacuum oven at 30 °C until the constant weight (for approx. 12 h). Purifying was repeated three times.

Preparation of ITA/PLGA-PEG-PLGA/ITA Macromonomer

In order to find the optimal conditions, the functionalization of PLGA-PEG-PLGA by ITA was done in two ways, either in THF solution (two pots reaction) or in "one pot" without the solvent. In two pots reaction, PLGA-PEG-PLGA copolymer (103 μmol) already prepared, purified and degassed, was homogenized with either sublimated or unsublimated ITA (257 μmol) and dry THF (5 mL), and the reactor was thermostated at certain temperature (23, 40, 60 or 75 °C). Reaction was started by inserting Sn(II)2-ethylhexanoate (2.3 μmol), and proceeded for 24 hours. In one pot reaction (Figure 1) the sublimated ITA (2.5 molar ratio to polymer) was added under the nitrogen atmosphere at laboratory temperature to raw (non-purified) polymerization mixture comprising ABA copolymer. No extra catalyst was added. Functionalization proceeded at 110, 115 or 155 °C for 8 hours. Prepared ITA/PLGA-PEG-PLGA/ITA macromonomers were purified in the same way like ABA copolymers mentioned above.

Characterization

Molecular weight and ITA functionalization were evaluated using proton nuclear magnetic resonance (^1H NMR) spectroscopy on 500 MHz Bruker AVANCE III instrument using 128 scans in $CDCl_3$ solvent. Number-average molecular weight (M_n) and polydispersity index (M_w/M_n) of the copolymers were determined by gel permeation chromatography (GPC) method using Agilent Technologies 1100 Series instrument equipped with autosampler, RI and UV-VIS detector and THF as the eluent at a flow rate of 1 mL · min^{-1} and calibrated by linear polystyrene standards. FTIR spectra of polymers were performed on Nicolet iS10 FTIR Spectrometer to characterize their molecular structure. The

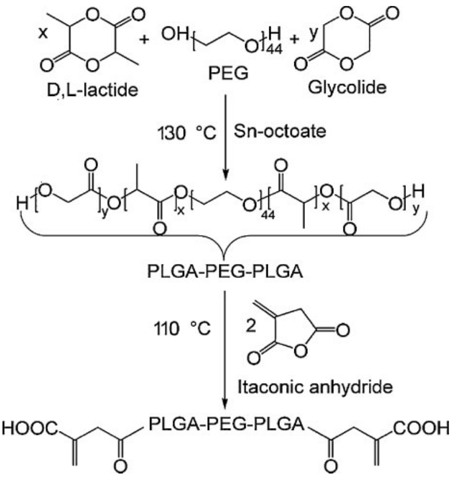

Figure 1.
"One-pot" functionalization resulting in ITA/PLGA-PEG-PLGA/ITA macromonomer.

copolymer samples were prepared by casting films from an acetone solution onto KBr plate. The spectra were measured with a resolution 6 cm^{-1} in the range of 4000–400 cm^{-1} (128 scans). The thermal degradation of selected polymers was performed by thermogravimetric analysis (TGA) on TA Instruments TGA Q500 using nitrogen as purge gas. Samples were heated from ambient temperature to 650 °C at a heating rate of 10 °C.min^{-1} and under a nitrogen flow of 60 mL · min^{-1}. Glass transition temperature (T_g) of samples were measured by using Thermal Analysis Instruments Q200 differential scanning calorimeter (DSC). Copolymers were crimped in a open aluminum pan, cooled to -40 °C and heated to 160 °C for two times. Cooling and heating rates were 10 °C · min^{-1} and steady stream of nitrogen gas was supplied at 50 mL · min^{-1}. T_g values were obtained from second cycle by Universal Analysis 2000 software.

Results and Discussion

Well-defined PLGA-PEG-PLGA triblock copolymer with molecular weight of 7.2 kg · mol^{-1}, narrow polydispersity ($M_w/M_n = 1.20$), weight ratio of PLGA/PEG = 2.5 2.5 and molar ratio of LA/GA = 3.0 was prepared via ring-opening polymerization of D,L-lactide with glycolide catalyzed by Sn-octoate and in the presence of PEG as macroinitiator. Synthesis proceeded in a bulk at 130 °C for 3 hours. Conversion increased linearly for 2.5 hours up to 88% followed by a plateau. Obtained copolymer was subsequently modified with itaconic anhydride via catalytic ROP reaction in both solution using THF as solvent (reaction no. **1** – **8** in Table 1) and in a bulk (Figure 1, reaction **9** – **12** in Table 1).

As the Table 1 shows, the amount of end-capped ITA was significantly affected by using a solvent (THF), ITA purification prior the use and temperature of modification reaction. In the case of synthesis in THF solution, a very low amount or even no linked ITA was detected (reactions **1** – **7**). The highest content of bonded ITA (13.8 mol.%) was obtained for solution reaction no. **8** carried out at 75 °C with sublimated ITA. It is known that itaconic anhydride passes on to itaconic acid by hydration in the presence of moisture, which might evoke undesired side reactions (e.g. acidolysis, esterolysis, hydrolysis etc.) and thus suppress polymer modification. Sublimation of ITA under reduced pressure at 65 °C both removed water and enabled possible itaconic acid to turn back to anhydride by dehydration (confirmed by FTIR). Thus other reactions were carried out only with sublimated ITA, in bulk (without THF) in order to increase the concentration of polymer end-groups in

Table 1.
Properties of ITA/PLGA-PEG-PLGA/ITA prepared macromonomers.

No.	Solvent	Temp.	$M_n$$^{a)}$	$M_w/M_n$$^{a)}$	$M_n$$^{b)}$	$M_{n(theor)}/M_{n(GPC)}/M_{n(NMR)}$	Bonded ITA$^{b)}$
		°C	kg · mol^{-1}		kg · mol^{-1}		mol.%
1	THF	23	6.8	1.27	5.2	1/0.77/1.01	N.D.
2^{S}	THF	23	6.8	1.27	5.2	1/0.77/1.02	N.D.
3	THF	40	6.9	1.25	5.2	1/0.76/1.02	N.D.
4^{S}	THF	40	6.9	1.27	5.1	1/0.76/1.02	1.4
5	THF	60	6.6	1.27	5.1	1/0.79/1.02	N.D.
6^{S}	THF	60	6.9	1.27	5.3	1/0.79/1.05	1.4
7	THF	75	4.9	1.37	5.6	1/1.13/0.99	N.D.
8^{S}	THF	75	4.4	1.37	5.8	1/1.27/0.95	13.8
9^{S}	No	110	5.9	1.38	5.5	1/0.90/0.96	76.6
10^{S}	No	115	4.9	1.42	5.9	1/1.07/0.88	72.0
11^{S}	No	155	6.2	1.38	5.0	1/0.84/1.05	12.5
12^{S*}	No	155	3.3	1.46	5.9	1/1.58/0.89	30.0

^{S}sublimated ITA,*synthesis under vacuum, runs without mark were carried out with unsublimated ITA,$^{a)}$measured by GPC,$^{b)}$obtained from 1H NMR, N.D. not detected.

reaction mixture and at higher temperature to increase the ITA reactivity.

The content of end-capped ITA at samples prepared in a bulk (reactions no. **9** - **12**) was significantly higher. The highest amount of bonded ITA (76.6 mol.%) was obtained at 110 °C (no. **9**) with polymer polydispersity index of $M_w/M_n = 1.38$ and a very good correlation between theoretically calculated molecular weight and those observed experimentally by GPC and NMR ($M_{n(theor)}/M_{n(GPC)}/M_{n(NMR)} = 1/0.90/0.96$). Temperatures lower than 110 °C were not possible to apply for bulk reaction due to the difficulty in stirring of the high density polymerization mixture. At temperature similar like boiling point of ITA (115 °C) amount of bonded ITA decreased to 72 mol.% (no. **10**) and at temperature of 155 °C (when both ABA copolymer and ITA are still stable, proved by TGA) only very low amount of bonded ITA (12.5%) was obtained by reason that ITA occurs in condensation phase. 30 mol.% of bonded ITA, high polydispersity index ($M_w/M_n = 1.46$) and lower molecular weight ($M_n = 3.3\ \mathrm{kg \cdot mol^{-1}}$) together with change in product color (from yellowish to dark brown) in case of reaction no. **12** could resulted from the degradation process of ITA (to itaconic acid or methylmaleic anhydride) and modified ABA copolymer occurred at 155 °C under vacuum.

Kinetics of PLGA-PEG-PLGA functionalization with ITA was evaluated only for the reaction no. **9** (in a bulk at 110 °C) when undesired side reactions did not take place as in the case of higher temperatures. The amount of ITA bonded to the original PLGA–PEG–PLGA copolymer (calculated from ^{1}H NMR) increased within 1.5 hours up to 76.6 mol. % then plateau was observed. Prolonged time of modification for following 6.5 hours did not allow for linking more ITA to the end of polymer, probably due to the low concentration of ITA in a viscous polymer mixture.

The amount of end-capped ITA and real M_n was determined by ^{1}H NMR spectroscopy (Figure 2) from integrals of characteristic proton intensities of itaconic acid double bond ($OC(CH_2)CCH_2COOH$) at $\delta = 5.7$ - 5.8 ppm (g) and $\delta = 6.35$ - 6.5 ppm (f), lactic acid ($C\text{-}C\underline{H_3}(C{=}O)$) at $\delta = 1.5$ - 1.65 ppm (e), glycolic acid ($OC\underline{H_2}O$) at $\delta = 4.6$ - 4.9 ppm (b) and PEG ($OC\underline{H_2}\text{-}C\underline{H_2}O$) at $\delta = 3.55$ - 3.75 ppm (d).

The ITA functionalization was confirmed by changing absorptions in FTIR spectra of –OH bands in PLGA-PEG-

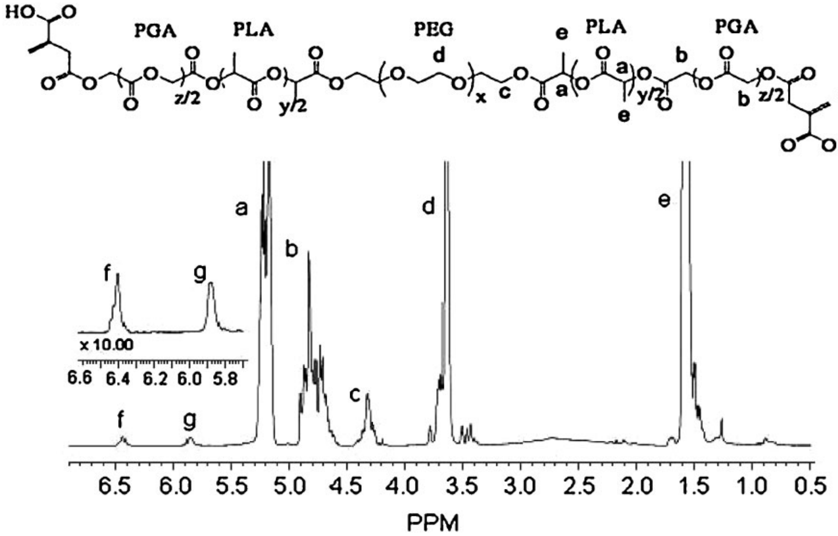

Figure 2.
^{1}H NMR of ITA/PLGA-PEG-PLGA/ITA copolymer prepared in a bulk, at 110 °C for 1.5h.

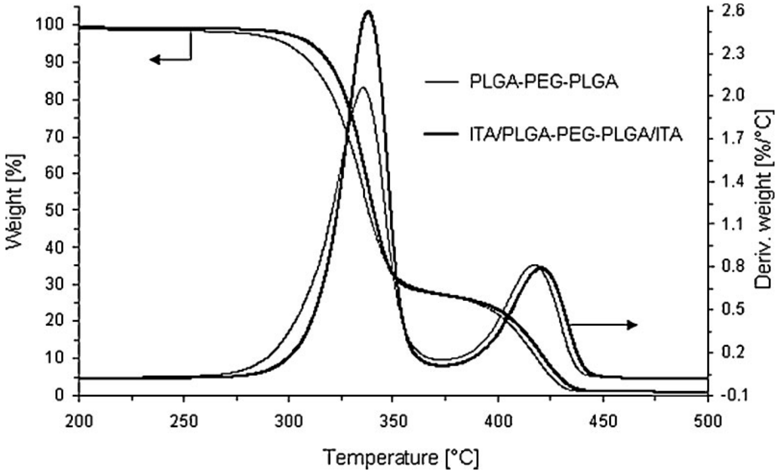

Figure 3.
TGA of PLGA-PEG-PLGA and ITA/PLGA-PEG-PLGA/ITA copolymers.

PLGA absorption spectrum to –COOH vibration bands in ITA/PLGA-PEG-PLGA/ITA macromonomer. Carboxylic functional groups absorbance bands corresponding to the C=O stretching vibrations of dimer form were found in range of 1700 – 1725 cm^{-1}, the characteristic line of OH stretching for –COOH appeared in range of 2500 – 2700 cm^{-1} and peak of carboxylic functional groups at the end of polymer showed up at 3450 cm^{-1}.

The thermal stability of copolymers studied by TGA is shown in Figure 3. Thermograms of both copolymers exhibited similar two-stage changes in the weight loss corresponding to decomposition of ester bonds in PLGA (total weight loss at the end of first stage $\triangle W^1 = 72$ wt.%) and ether bonds in PEG (total weight loss at the end of second stage $\triangle W^2 = 26$ wt.%). As can be seen, ITA/PLGA-PEG-PLGA/ITA copolymer is more stable and starts degradation later (initial degradation temperature $T_\mathrm{d} = 294\,^\circ\mathrm{C}$) but faster (maximum weight loss rate $\mathrm{d}W^1 = 2.56$ wt.%·°C^{-1}) in first degradation step (with the temperature at max. rate $T_\mathrm{dm} = 338\,^\circ\mathrm{C}$) then PLGA-PEG-PLGA copolymer having $T_\mathrm{d} = 284\,^\circ\mathrm{C}$, $\mathrm{d}W^1 =$ 2.03 wt.%·°C^{-1} and $T_\mathrm{dm} = 336\,^\circ\mathrm{C}$ proving improved thermal stability of PLGA ester bonds by ITA linking. Degradation of PEG in second stage was not significantly affected. Both temperatures at maximum rates are similar ($T^2_\mathrm{dm} = 417$ and $420\,^\circ\mathrm{C}$ for modified and unmodified polymer, respectively) as well as the maximum weight loss rate of both polymers ($\mathrm{d}W^1 = 0.78$ wt.%·°C^{-1}).

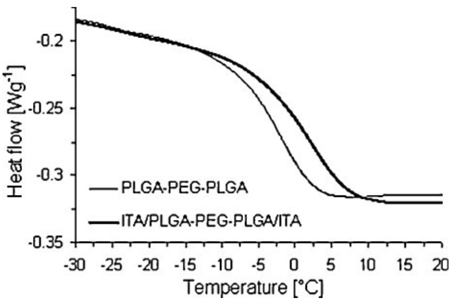

Figure 4.
DSC of PLGA-PEG-PLGA and ITA/PLGA-PEG-PLGA/ITA copolymers.

Glass transition temperature (T_g) calculated from DSC analysis (Figure 4) was -1.8 °C and 2.4 °C for PLGA-PEG-PLGA and ITA/PLGA-PEG-PLGA/ITA copolymer, respectively. The higher T_g of ITA functionalized polymer corresponds well to its to better thermal stability.

Conclusion

The highest amount of ITA (76.6 mol.%) bonded to PLGA-PEG-PLGA copolymer was obtained with ITA purified by sublimation in a bulk at 110 °C after 1.5 hours. Higher temperature of modification resulted in broader polydispersity along with lower molecular weight and change in color predicated both ITA and polymer degradation. Prolonged reaction time had no effect on the better end-capping, lower temperature and solvent addition significantly reduced or even suppressed the ITA functionalization. Purification of ITA by sublimation prior the use increased the yield of end-capped ITA. Based on thermal analyses, ITA improves thermal properties of original PLGA-PEG-PLGA copolymer. Resulting functionalized macromonomer might become suitable biomaterial as the novel hydrogel in the field of tissue engineering.

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