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Synthesis of the Biomimetic Polymer: Aliphatic Diamine and RGDS Modified Poly(*d,l*-lactic acid)

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Abstract: A novel poly(*d, l*-lactic acid) (PDLLA) based biomimetic polymer was synthesized by grafting maleic anhydride, butanediamine and arg-gly-asp-ser (RGDS) peptides onto the backbone of PDLLA, aiming to overcome the acidity and auto-accelerating degradation of PDLLA during degradation and to improve its biospecificity and biocompatibility. The synthetic copolymer was characterized by FTIR, ^{13}C NMR and amino acid analyzer (AAA).

Keywords: Poly(*d, l*-lactic acid), maleic anhydride, butanediamine, arg-gly-asp-ser, modification.

Weak hydrophilicity and poor cell affinity of poly(*d,l*-lactic acid) (PDLLA) are major drawbacks that restrict its wide applications in medical areas despite its good biodegradability and biocompatibility[1-3]. Chemical modifications are often used to overcome these drawbacks and some good results were obtained[4-7]. In our previous work, we successfully synthesized a novel maleic anhydride modified PDLLA (MPLA) by melt free radical copolymerization using benzoyl peroxide (BPO) as an initiator[3]. The anhydrides in MPLA could provide highly reactive groups for further chemical modification. Here, on this basis, we graft butanediamine (BDA) and adhesive peptide arg-gly-asp-ser (RGDS) onto the backbone of MPLA to overcome the acidity and auto-accelerating degradation of MPLA and to improve its hydrophilicity and cell affinity.

Experimental

The synthetic route of BDA and RGDS modified MPLA was shown in **Scheme 1**.

The MPLA was prepared according to the literature 3. 10 g MPLA (containing 2.36% maleic anhydride in weight) and 0.32 g BDA were dissolved by 50 mL and 5 mL tetrahydrofuran (THF) respectively, and then dropped MPLA solution into BDA solution with stirring, controlling the temperature below 20℃. The reaction lasted for 10 min below 20℃ and 30 min at room temperature. After reaction, the mixture was precipitated with excessive distilled water and yielded BDA modified MPLA (BMPLA). The resulting copolymer was freeze dried and characterized *via* FTIR and ^{13}C NMR.

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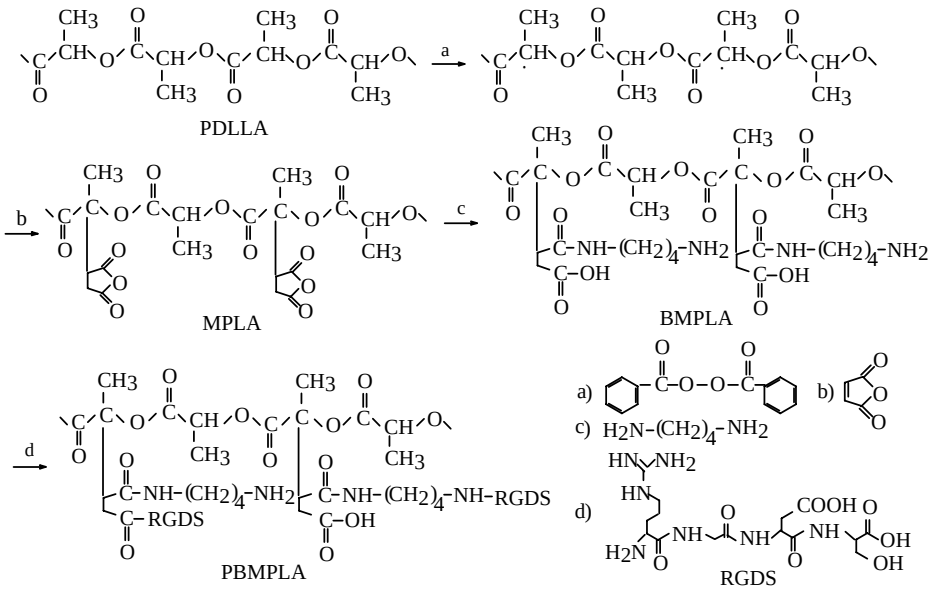
Scheme 1 Synthesis of BDA and RGDS modified poly(*d, l*-lactic acid)



BMPLA was used for further modification. 10 mg RGDS was dissolved in 50 mL THF and then added 1 g BMPLA into this peptide solution. The pH of the mixture was adjusted to 7-8 with N-methylmorpholine. 0.15 g N, N-dicyclohexylcarbodiimide (DCC) was dissolved in 5 mL THF and then dropped into the polymer/peptide solution with stirring in lower temperature (0℃). After 24 h, the solution was filtered to remove the product N, N′-dicyclohexylurea (DCU). Then, precipitating the solution with excessive distilled water yielded peptide modified BMPLA (PBMPLA). The intermediate product was freeze dried. The peptide concentration was determined from amino acid analyzer (AAA).

Results and Discussion

The characterization of MPLA was reported in literature 3. Here, we put emphasis on the characterization of BMPLA and PBMPLA. In the IR spectrum of BMPLA (**Figure 1b**), there were two new absorption peaks apparently at 1680 cm^{-1} and 1540 cm^{-1}, which were attributed to the C-O stretching vibration and the N-H bending vibration in amido bonds (-NHCO-) respectively, indicating that BDA has successfully bonded to the MPLA backbone. Comparing the ^{13}C NMR spectra of BMPLA (**Figure 2b)** with MPLA (**Figure 2a)**, we found that there were two new types of carbon (66.677 ppm and 67.906 ppm, methylene carbons next to primary amino groups and amido bonds, respectively) appeared. Besides, due to the N-acylation of anhydride groups by BDA into amido bonds and carboxyl groups, anhydride carbons (167.788 ppm) disappeared. This confirmed that anhydride groups in MPLA have totally reacted with BDA.

Figure 1 IR spectra of MPLA(a) and BMPLA(b)

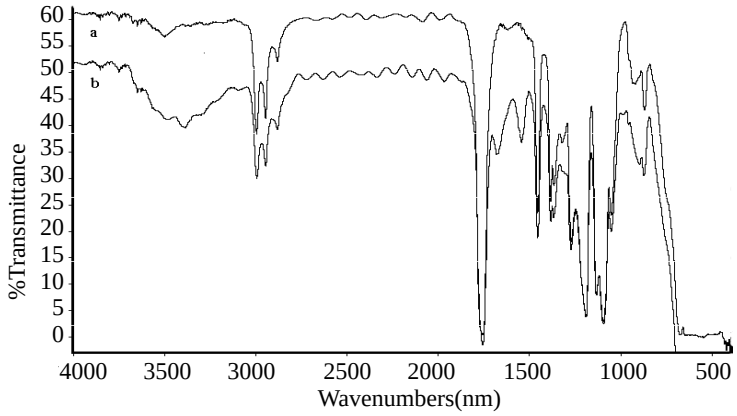


Figure 2 ^{13}C NMR spectra of MPLA(a) and BMPLA(b)

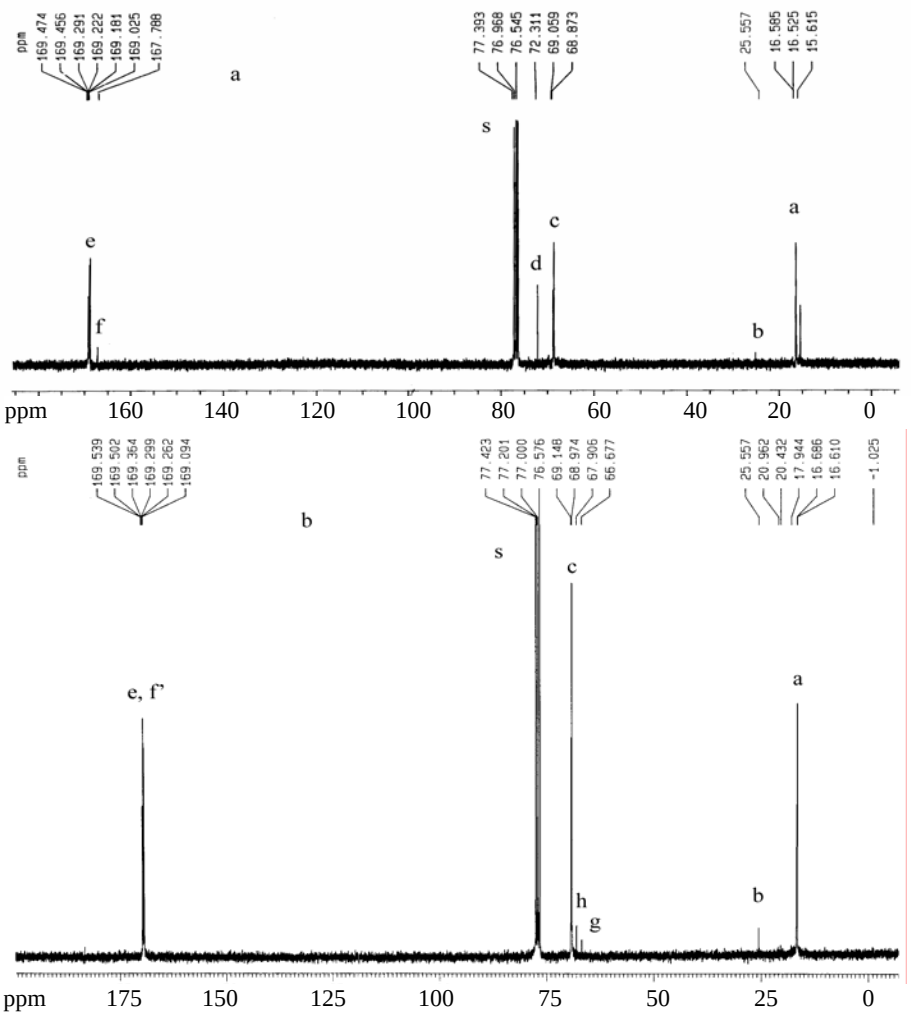


Table 1 The result of amino acid analysis (AAA)

Amino Acid	Content (mg/g)	Molar Weight (g/mol)	Mole Number (μ mol/g)
Arg	0.821	174	4.72
Gly	0.401	75	5.35
Asp	0.688	133	5.17
Ser	0.550	105	5.24
Mean Value			5.12

(Note: Arg mole number lower than others may be due to hydrolysis losses during analysis.)

The results of AAA (**Table 1)** demonstrated that RGDS was coupled to the copolymer at a concentration of 5.12 μ mol/g. Very small concentrations of active peptides can have dramatic biological affects. According to the results of Massia[8], a surface density of only 1 fmol/cm^2 of an RGD peptide effectively promoted cell adhesion to an otherwise nonadherent surface. Assuming a density of 1 g/cm^3 and a 1 nm access layer, a film containing 5.12 μ mol/g of peptide has a surface concentration of 512 fmol/cm^2 . Therefore, by carefully selecting processing conditions, this new biomimetic copolymer can overcome the acidity and auto-accelerating degradation of MPLA during degradation. At the same time, it can improve cell adhesion and other responses through specific adhesion receptors in the cell membrane.

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