Preparation and Cytocompatibility of Chitosan-Modified Polylactide

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ABSTRACT: Chitosan-modified PLA (CMPLA) was fabricated to improve cytocompatibility of polylactide (PLA). PMAA-grafted PLA (PMAA-PLA) was obtained through α-methacrylic acid (MAA) grafted polymerization on PLA surface with photooxidization and UV irradiation. Steady PMAA-PLA microparticle suspension with an average size as 172.8 ± 3.6 nm and zeta potential as −95.0 ± 0.6 mV was prepared through solvent volatilization. By static electricity interaction and other interactions between PMAA-PLA microsparticles and chitosan molecules, CMPLA was obtained. FTIR, XPS, SEM, and zeta potential analyses indicated that CMPLA was modified with chitosan molecules uniformly. Compared with the PLA control, CMPLA adapted to supporting the attachment and proliferation of L929 cells better. The obtained CMPLA was expected to be used as perfect biomaterial for tissue regeneration. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 110: 408–412, 2008

Key words: polylactide; chitosan; microspheres; surface modification; cytocompatibility

INTRODUCTION

Biomaterials were required to possess not only suitable mechanical properties closely matched to the target tissues but also good biological interactions with cells and/or hosts when implanted.[1] Polylactide (PLA) is one of typical polymers widely used in biomedical field due to its advantages such as nontoxicity, flexibility, and biodegradability. However, the cytocompatibility of PLA needs to be improved because its hydrophobicity retards cell attachment.[2,3] Biomacromolecules such as collagen, chitosan, and so forth can effectively accelerate cell attachment and spreading, so one approach to improve cytocompatibility of PLA is to obtain biomacromolecules-modified PLA.[4] Many researchers have applied some methods to introduce biomacromolecules onto PLA surface for eliciting specific cellular responses.[1,4–11]

In this study, to improve cytocompatibility of PLA, chitosan was used as a surface modifier. First, poly(α-methacrylic acid)-grafted PLA (PMAA-PLA) was prepared via photooxidization and UV-induced polymerization. Subsequently, chitosan-modified PLA (CMPLA) was obtained by dropped chitosan solution into PMAA-PLA microsphere suspension prepared through solvent volatilization. The cytocompatibility *in vitro* of the CMPLA was carried out by L929 fibroblast culture.

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MATERIALS AND METHODS

Preparation of CMPLA

α-methacrylic acid (MAA) was introduced onto the PLA surface using a grafting polymeric method as described previously.[12,13] The PLA was immersed in hydrogen peroxide solution (30%) and irradiated with UV light for 50 min. The photooxidized PLA was rinsed with deionized water to remove excess hydrogen peroxide and dried. The photooxidized PLA were then immersed into MAA aqueous solution (15%) and poly(α-methacrylic acid)-grafted PLA (PMAA-PLA) was obtained by UV irradiation for 30 min. PMAA-PLA was then rinsed with deionized water at 65°C and dried.[2]

The PMAA-PLA acetone solution was dropped into Na_2HPO_4 aqueous solution (pH = 7–8) with stirring. After acetone in the solution was volatilized, PMAA-PLA microparticle suspension with pH = 7 was obtained. Then, chitosan solution was dropped into PMAA-PLA microparticle suspension until much flocculent precipitates appeared. Chitosan-modified PLA (CMPLA) powder was obtained after the precipitates were washed by centrifuge with deionized water thoroughly, dried at room temperature, and ground. Chitosan solution was added into Na_2HPO_4 aqueous solution (pH = 7) as control. CMPLA disk (10 mm in diameter, 1-mm thick) was made by pressing CMPLA

powder under the pressure of 300 MPa for 5 min to be used for cell culture.

Characterization of CMPLA

The average size and zeta potential of the PMAA-PLA microparticles were measured by a Nano ZS instrument (Malvern, UK). Data for each sample were obtained from three measurements. The zeta potential of PMAA-PLA microparticles along with change of pH value was measured through dropping acidic solution into the PMAA-PLA suspension. The flocculent PMAA-PLA deposit appeared at pH = 2.7 was rinsed with deionized water and dried. Scanning electron microscopy (SEM, JSM5900) was used to observe the surface morphology of obtained materials. FTIR spectra of materials were obtained employing Fourier-transform infrared spectroscopy (PE spectrum one (B)). X-ray photoelectronic spectra (XPS) were recorded employing Mg Kα excitation radiation by an X-ray photoelectronic spectroscopy (XSAM 800).

Cell culture

Cell lines L929 mouse fibroblasts were used for the cell culture study. L929 cells were routinely grown and maintained in RPMI-1640 medium containing 10% FBS and 100 U/mL penicillin and 100 μg/mL streptomycin. The PLA disks and CMPLA disks sterilized by UV-radiation for 8 h were placed on the bottom of the 24-well tissue culture plates. And blank culture plates used as negative controls. Cells were seeded with the density of 5×10^4/mL and maintained in a humidified atmosphere with 5% CO_2 at 37°C.

After cultured for 24 h, the CMPLA disks were fixed by 2.5% glutaraldehyde and treated in a series of ethanol solution. Then, critical point drying was performed, and cell morphology on the CMPLA disks was observed under SEM.

MTT method was used to measure cell proliferation after adding 100 μL MTT solution (5 mg/mL) to every well and culturing for 4 h at 37°C. After removal of the medium, the formazan pigment by reducing MTT was dissolved with acidic isopropanol (0.1*N* HCl in absolute isopropanol). To a 96-well ELISA plate 150 μL pigment solution was added and the absorbance was measured at 570 nm.

RESULTS AND DISCUSSION

FTIR spectra analysis

Figure 1 showed the FTIR spectra of PLA, PMAA-PLA, and CMPLA. The peaks at 2995 and 2947 cm^{-1} were ascribed to stretching vibration of C—H of PLA and PMAA-PLA. The peaks at 3583 and 1755 cm^{-1} attributed to stretching vibration of O—H and C=O in PMAA-PLA were stronger than those of PLA, and the width of C=O peak of PMAA-PLA was bigger than that of PLA, which indicated that PMAA was grafted onto the PLA surface [Fig. 1(a,b)]. The characteristic absorbable peaks of chitosan appeared in FTIR spectrum of CMPLA [Fig. 1(c)]. The peak at about 3440 cm^{-1} arose from *N*—H and O—H stretching vibration. The peak that appeared about 1650 cm^{-1} was attributed to the bending vibrations of *N*—H in chitosan.[10] These results indicated the presence of chitosan in CMPLA microparticles.

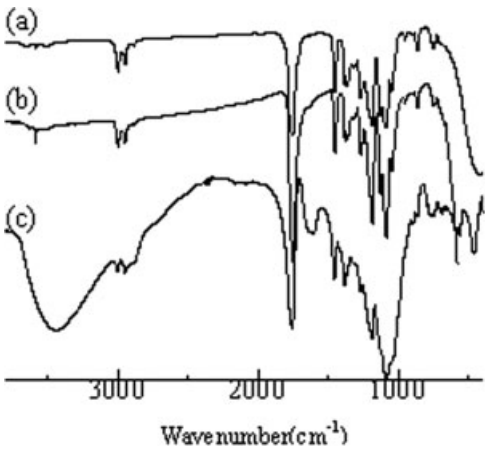

Figure 1 FTIR spectra of (a) PLA, (b) PMAA-PLA, (c) CMPLA.

The measurement of particle size and zeta potential

The size distribution of PMAA-PLA microparticles obtained with Nano ZS instrument was from 59 to 615 nm and the average size was 172.8 ± 3.6 nm. The zeta potential of PMAA-PLA microparticle surface was −95.0 ± 0.6 mV, which meant that PMAA-PLA microparticles in suspension had high surface negative charge and were not readily conglomerated.[14] The hydrophilicity of PMAA-PLA was enhanced after MAA was grafted polymerization on PLA surfaces due to MAA molecule containing hydrophilic carboxyl group. The carboxyl groups of PMAA-PLA surfaces could ionize into COO^- with negative charge in alkaline solution. Then, when PMAA-PLA acetone solution was dropped into Na_2HPO_4 aqueous solution with stirring, PMAA-PLA would cause sedimentation and form microparticles with hydrophilic groups (carboxyl groups) on their surfaces. Because carboxyl groups ionized into COO^-, the PMAA-PLA microparticle surfaces possessed high negative charge which would prevent congregating between microparticles. Then the PMAA-PLA microparticles had nanometer size and high negative zeta potential.

If the particles in suspension had a large negative or positive zeta potential then they would tend to repel each other and there was no tendency to flocculate. However, if the particles had low zeta potential values then there was no force to prevent the particles coming together and flocculating. The general dividing line between stable and unstable suspensions was generally taken at either + 30 mV or −30 mV.[14] When chitosan solution with positive surface charges was added into PMAA-PLA microparticle suspension, the negative charges on PMAA-PLA microparticle surfaces would be neutralized due to electrostatic interaction and other weak interactions between PMAA-PLA microparticles and chitosan molecules. If the zeta potential of PMAA-PLA microparticles decreased to larger than −30 mV, PMAA-PLA microparticles would occur flocculating. Chitosan molecules that congregated on the PMAA-PLA microparticle surfaces would cause precipitation along with PMAA-PLA microparticle sedimentation. In this way, PMAA-PLA microparticle surfaces should be coved by chitosan partly or completely and chitosan-modified PLA material (CMPLA) was obtained. In our study, flocculent CMPLA deposit appeared at pH ≈ 5. However, there was no deposit appearing at any time when chitosan solution was dropped into the Na_2HPO_4 aqueous solution (pH = 7). This proved there were interactions between chitosan and PMAA-PLA microparticles.

The high negative charge of PMAA-PLA microparticle surfaces in suspension might mainly arise from ionized carboxyl groups, so the most important factor that affects the stability and zeta potential of PMAA-PLA microparticles is pH. Figure 2 showed the effect of zeta potential of PMAA-PLA microparticles on the pH value. From Figure 2 it could be seen that the absolute value of zeta potential of PMAA-PLA microparticles diminished with decline of pH value. However, the zeta potential of PMAA-PLA microparticles still remained higher value at pH ≥ 5 which implied that the carboxyl groups were ionized completely. Only when pH value of the system diminished to 3.0, the absolute value of zeta potential of PMAA-PLA microparticles was less than + 30 mV and flocculent

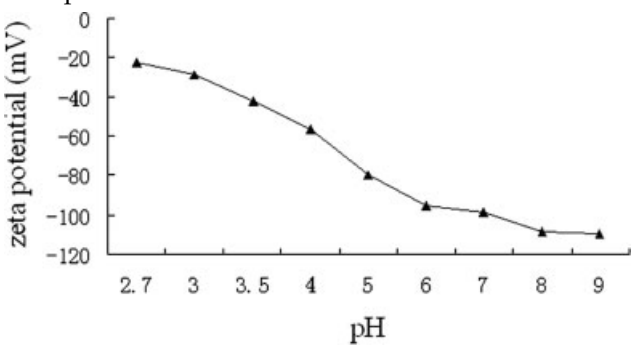

Figure 2 The effect curve of zeta potential on the pH value.

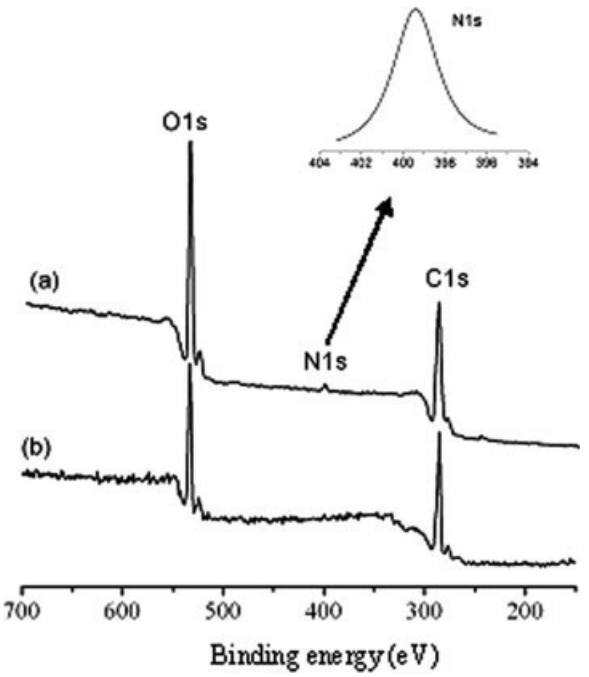

Figure 3 XPS spectra of (a) CMPLA and (b) PLA.

PMAA-PLA deposit could be observed. When the pH value decreased to 2.7, all PMAA-PLA microparticles occurred conglomerating and obtained PMAA-PLA deposit. Compared the condition of PMAA-PLA and CMPLA deposit, it could be seen that there were strong interactions other than simple blend between two phases in CMPLA material.

XPS analysis

A convincing evidence of chitosan along with PMAA-PLA microparticles occurred conglomerating was the appearance of the peak of nitrogen in the XPS observation of CMPLA because nitrogen atom was presented in chitosan molecules but not in PLA and PMAA.[3,5] The XPS spectra (Fig. 3) indicated that no peak of nitrogen was found in PLA control while a peak of nitrogen was detected from the CMPLA surface. And the N/C of CMPLA determined by XPS was 3.34%. After CMPLA deposit produced in acidic solution (pH ≈ 5) was washed with deionized water thoroughly, chitosan was still found in CMPLA material. These results further showed that there was chitosan present and interactions between two phases in CMPLA material.

SEM analysis

Figure 4 showed the SEM photos of PMAA-PLA microparticles, PMAA-PLA deposit (obtained at pH = 2.7), and CMPLA material. The diameter of PMAA-PLA microparticles with a spherical structure ranged from 50 to 600 nm according to the result of

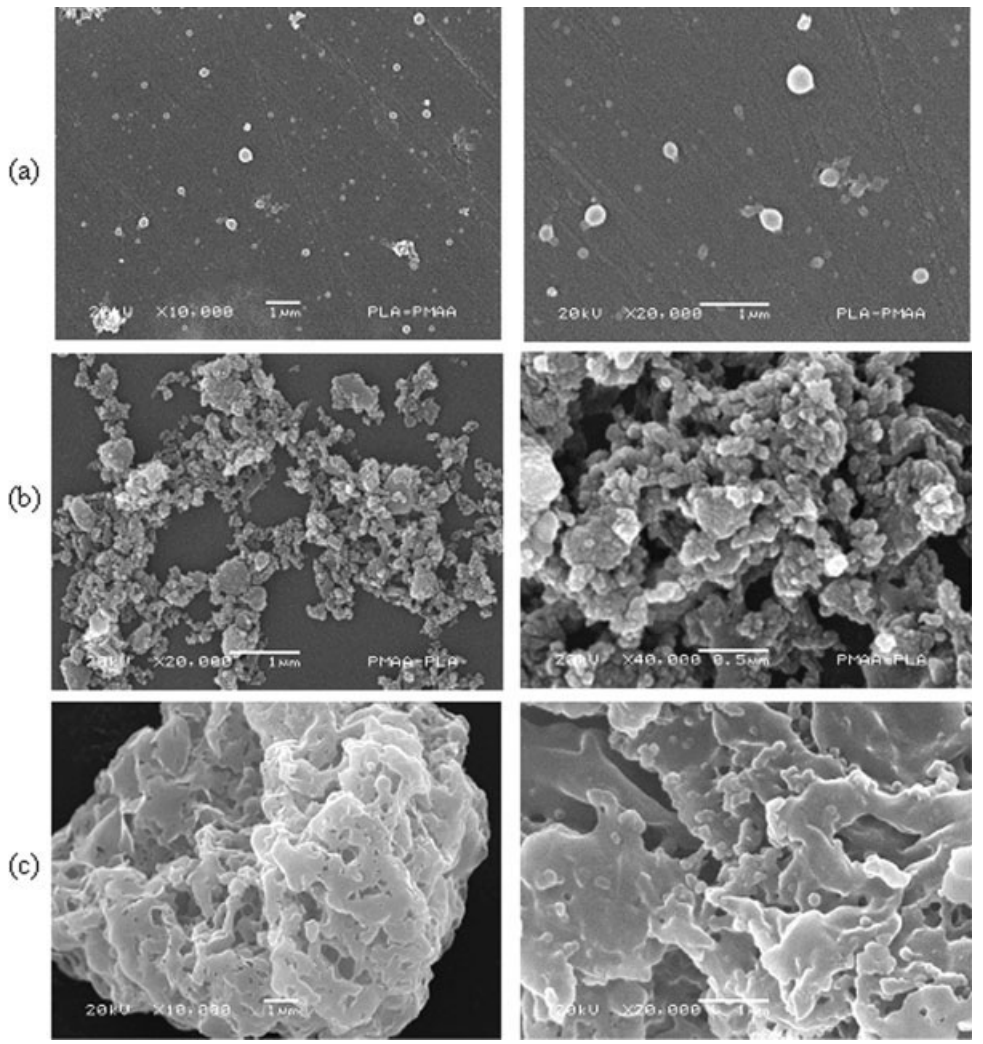

Figure 4 Surface morphology of (a) PMAA-PLA microspheres, (b) PMAA-PLA deposit, and (c) CMPLA.

particle size measurement [Fig. 4(a)]. And PMAA-PLA deposit was composed of many PMAA-PLA microspheres or anamorphic PMAA-PLA microspheres [Fig. 4(b)]. The surface morphology of CMPLA material was completely different from that of PMAA-PLA deposit although a few microspheres were observed on the CMPLA [Fig. 4(c)]. All these results further proved that CMPLA material was not a simple mixture of PMAA-PLA microparticles and chitosan molecules but chitosan-modified PLA material with interactions between two phases.

Cell culture

MTT assay was used to evaluate cell viability on biomaterials. Figure 5 showed the cell viability result of the L929 cells cultured on the PLA disks, CMPLA disks and negative controls, respectively. From Fig-

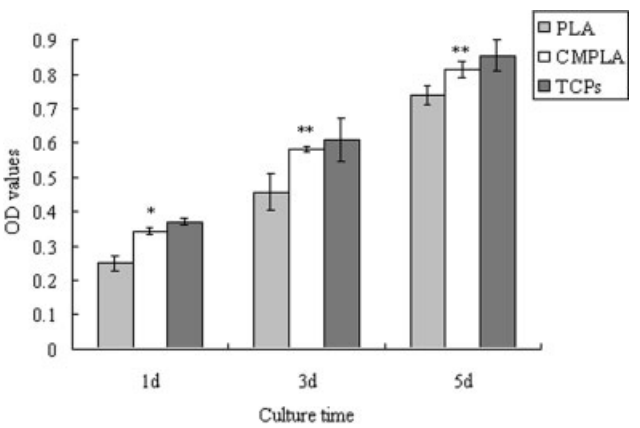

Figure 5 MTT viability of L929 cell on PLA, CMPLA disks, and TCPs at culture time of 1, 3, 5 days. Cell seeding density was 8×10^4/mL. $^*P < 0.05$ (compared to cell density on PLA disks on the respective day), $^{**}P < 0.01$ (compared to cell density on PLA disks on the respective day).

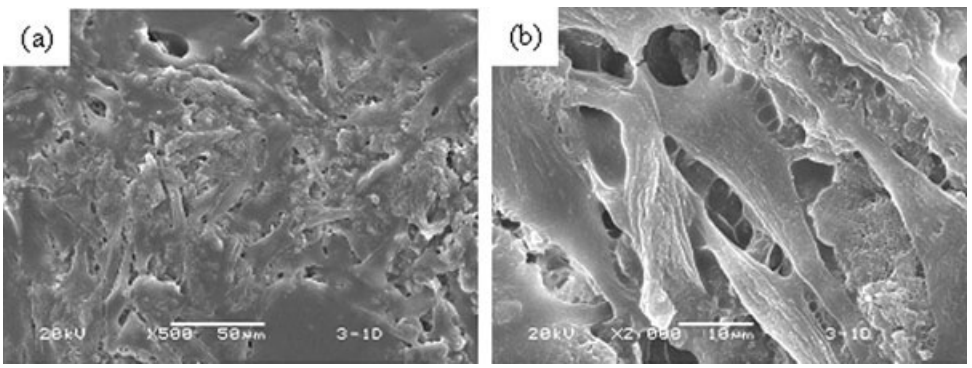

Figure 6 Surface morphology of cell culture for one day on CMPLA disk.

ure 5 it could be seen that the proliferation of the cells on CMPLA disk surfaces was significantly ($P < 0.05$) enhanced compared with the control PLA disk surfaces after culture for 1 day. This trend continued as cell proliferation was greater ($P < 0.01$) on CMPLA disk surfaces than that of control PLA disk surfaces after 3- and 5-day culture. These results gave the evidence that chitosan-modified PLA had prominent effect on the L929 cells proliferation.

Figure 6 showed the surface morphology of L929 cells on CMPLA disks under SEM after 1-day culture. The L929 cells on the CMPLA disks had attached and spread very well, leading to the difficulty to distinguish mono cell at some area of disk surface. And all cells stretched out many pseudopods and closely caught hold of the surfaces of CMPLA disk. Together with the cell proliferation results, it could be concluded that the chitosan-modified PLA was more favorable for L929 cells attachment and growth.

CONCLUSIONS

MAA was grafted polymerization onto inert PLA surface through UV irradiation and PMAA-PLA microsphere suspension was successfully prepared through solvent volatilization. The stability of PMAA-PLA microsphere suspension was related to the pH value of system and the absolute value of zeta potential of PMAA-PLA microspheres diminished with decline of pH value. CMPLA material obtained through interactions between PMAA-PLA microspheres and chitosan molecules was chitosan-modified PLA but not a simple mixture. Compared with the PLA control, CMPLA material was favorable to supporting the attachment and proliferation of L929 cells better. The obtained CMPLA material was potential to be used as biomaterial for tissue regeneration.

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