AAPS PharmSciTech (© 2013)
DOI: 10.1208/s12249-013-9943-3

Research Article

Chitosan-Modified PLGA Nanoparticles with Versatile Surface for Improved Drug Delivery

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Received 1 November 2012; accepted 22 February 2013

Abstract. Shortage of functional groups on surface of poly(lactide-co-glycolide) (PLGA)-based drug delivery carriers always hampers its wide applications such as passive targeting and conjugation with targeting molecules. In this research, PLGA nanoparticles were modified with chitosan through physical adsorption and chemical binding methods. The surface charges were regulated by altering pH value in chitosan solutions. After the introduction of chitosan, zeta potential of the PLGA nanoparticle surface changed from negative charge to positive one, making the drug carriers more affinity to cancer cells. Functional groups were compared between PLGA nanoparticles and chitosan-modified PLGA nanoparticles. Amine groups were exhibited on PLGA nanoparticle surface after the chitosan modification as confirmed by Fourier transform infrared spectroscopy and X-ray photoelectron spectroscopy. The modified nanoparticles showed an initial burst release followed by a moderate and sustained release profile. Higher percentage of drugs from cumulative release can be achieved in the same prolonged time range. Therefore, PLGA nanoparticles modified by chitosan showed versatility of surface and a possible improvement in the efficacy of current PLGA-based drug delivery system.

KEY WORDS: chitosan; drug delivery system; nanoparticles; PLGA; versatility.

INTRODUCTION

Drug delivery systems using biodegradable and biocompatible polymer poly(lactide-co-glycolide) (PLGA) have attracted increasing attention as these systems can provide a sustained and controlled drug release and reduce side effects (1). Furthermore, PLGA will be degraded into nontoxic substance and the breakdown products like lactic acid and glycolic acid are hydrophilic, diffusible, and rapidly metabolized in the human body (2). However, PLGA-based drug delivery systems are difficult to achieve functionalities such as passive targeting and active targeting because there is a deficiency of functional groups on PLGA surface. The conventional PLGA surface makes it challenging to achieve the cell affinity and immobilization of cell-targeting molecules. Therefore, decorating surface of PLGA-based drug carriers is essential for getting best ability of the system.

Drug delivery carriers with a versatile surface can improve the effectiveness of the systems. Recent studies have shown that polymeric material PLGA is a suitable vehicle that can incorporate various chemotherapeutic agents and deliver them to tumors (1,3). One of the only few drawbacks related to PLGA-based drug delivery system is the difficulty to have the targeting effects, either the passive or active ones (4), largely caused by the barren PLGA polymeric surfaces. The whole PLGA polymer chains and repeating units are mainly composed of ester groups whose activity is very low and hard to react with other functional groups. Although the uncapped PLGA with free carboxyl (COOH) termini was widely adopted, no obvious improvement in PLGA activity performance has been found (5).

Gas plasma or plasma polymerization has been used to modify and functionalize PLGA surfaces (6–8). The retention of plasma generated functional groups on PLGA surface appears quite weak (9,10), thus making it hard to have targeting molecule conjugation. Bifunctional poly(ethylene glycol) (PEG) (amine group (NH_2)-PEG-NH_2, Mw of PEG 3–5 k) has also been used as biolinker between PLGA and active targeting molecule (11–13), involving multistep reactions. But there is limited functional group for the targeting molecule to attach on as one PLGA polymer chain can only get one reactive NH_2 from the biolinker bifunctional PEG. This greatly affects the properties of PLGA surface and its following reaction with targeting molecules.

Chitosan (CS), a naturally occurring linear polysaccharide and amino polysaccharide (poly 1, 4-day-glucoamine), is biodegradable and biocompatible (14). It can be found in the shells of crustacea, the cuticles of insects, and cell walls of some fungi. The repetitive amine groups in chitosan polymer backbone can easily react with other chemical reagents. Chitosan and its derivatives have been widely used in biomedical engineering and formulation of drug delivery systems

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Published online: 06 March 2013

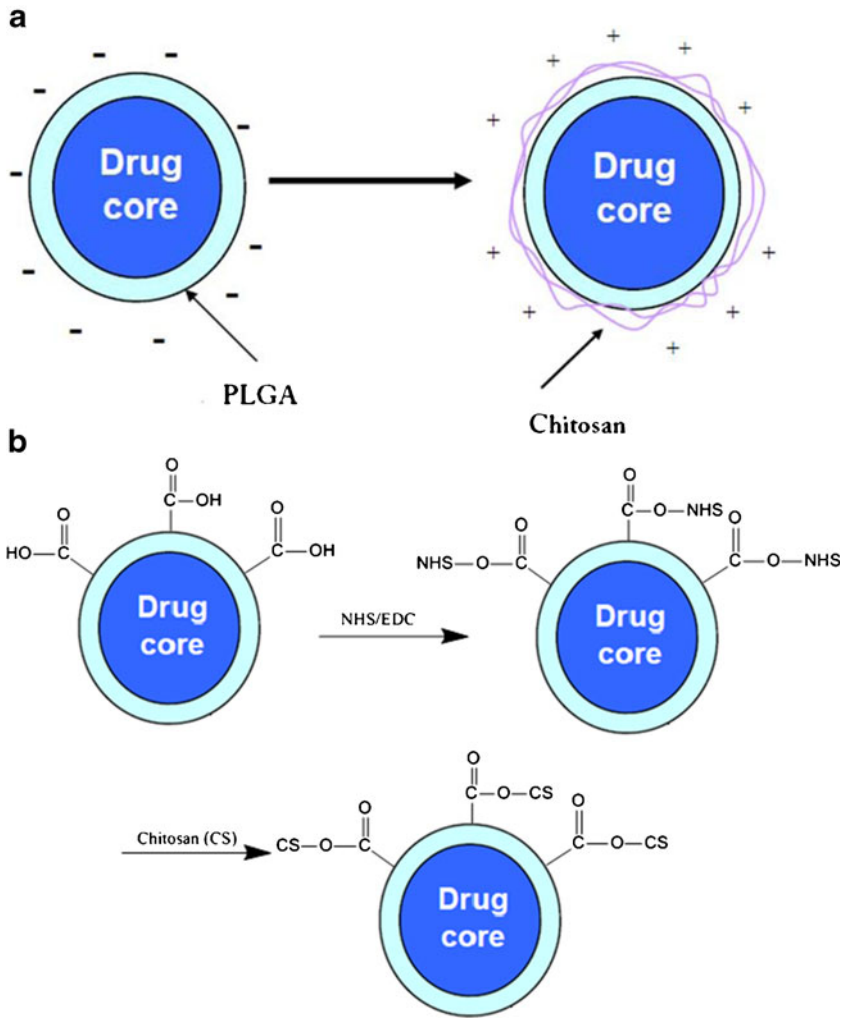

Fig. 1. Schematic maps of chitosan modified PLGA nanoparticles by **a** physical adsorption method, **b** chemical binding method

(15). Chitosan has been formulated into microspheres, tablets, and membranes (16,17) as drug delivery carriers. As chitosan can form films easily, it was used to coat the microspheres including poly(lactic acid)–poly(caprolactone) blend. The prepared microspheres exhibited a very high potential for the targeted delivery of therapeutic agents to treat restenosis (18). Moreover, some chitosan derivatives like lactosaminated and galactosylated ones were used to conjugate with drugs (19).

Therefore, the purpose of this study was to functionalize the surface of PLGA-based drug delivery carriers with chitosan to exhibit versatility, as chitosan has a very high positive zeta potential. This property enables chitosan to have *in vitro* cytotoxicity against different kinds of human cancer cell lines. There are repeating amine (NH_2) units in the chitosan polymer chains, which makes PLGA surface more easily to involve into chemical reactions. Chitosan was used to modify PLGA nanoparticle surface using physical adsorption and chemical binding methods. Change of surface charge and surface composition was tested by zeta potential, FTIR, and X-ray photoelectron spectroscopy (XPS). Drug release profiles were also tested to examine the effect of chitosan modification.

MATERIALS AND METHODS

Materials

PLGA-COOH with a lactide to glycolide ratio of 50/50 and an average molecular weight of 15 k was purchased from Ji'nan Daigang Biological Co. Ltd (Shandong, China). CS (medium viscosity; deacetylation degree, >84%) was purchased from

Table I. Nanoparticles Characterizations

	CS/PLGA (*w*/*w*)	Zeta potential (mV)	Particle size (nm)	EE (%)
Pure PLGA	0:1	−22.5	197.8	28.6
Physical adsorption	0.2:1	14.4	233.4	24.3
	0.4:1	19.9	255.7	22.6
	0.6:1	23.9	268.3	20.5
	0.8:1	24.1	279.5	22.9
Chemical binding	0.2:1	5.7	298.3	22.5
	0.4:1	18.7	308.4	25.3
	0.6:1	22.3	322.5	23.8
	0.8:1	22.0	335.7	24.1

Zhanjiang Xinmao Chemical & Glass Company (Zhanjiang, China). The 5-fluorouracil (5-FU), methylene chloride (DCM), and polyvinyl alcohol (PVA) with 86.7–88.7% hydrolysis degree and molecular mass 31 k were ordered from Sigma Chemical Co, USA. All chemicals were analytical grade and used without further purification.

Preparation of Chitosan-Modified PLGA Encapsulated 5-FU Nanoparticles

The surface of PLGA nanoparticles was modified with chitosan (PLGA-CS) using two different methods, i.e., physical adsorption and chemical binding. The schematic procedures of these two processes are described in Fig. 1a and b, respectively. The electrostatic adhesion takes effect as the chitosan surface is positively charged while the PLGA with carboxyl groups have a negative surface charge.

The physical adsorption uses a modified aqueous drug solution (W_1)/organic phase (O)/external phase (W_2) multiple emulsion and solvent evaporation technique (20). Briefly, 100 mg of PLGA was dissolved in 6 ml DCM. 5-FU was dissolved into a water solution to get the inner aqueous phase. Into the O, W_1 was emulsified using a probe sonicator (Shengxi Instrument Company, Shanghai, China) for 2 min with a 30% of amplitude to form a W_1/O emulsion. The first emulsion was added to the W_2 which contains the surfactant PVA and different concentration of chitosan and sonicated for 1 min. The pH of outer aqueous phase was adjusted from 3.0 to 9.0 by progressive addition of HCl and NaOH. Resulting $W_1/O/W_2$ emulsion was stirred with a magnetic stirrer for 5 h to allow solvent evaporation and particle hardening. The nanoparticles were then separated by ultracentrifugation at 12 krpm for 20 min, washed with distilled water three times to remove 5-FU on the surface of particles and excessive surfactant, and followed by a further centrifugation to eliminate the washing solution. Finally, the nanoparticles were collected with a freeze-dryer and preserved in a desiccator for evaluation and analysis.

For chemical binding, PLGA nanoparticles were prepared using the same procedures as physical adsorption without addition of chitosan in the W_2. After the freeze-dried PLGA nanoparticles were obtained, they were dispersed in PBS solution (pH6.0) under bath sonication. *N*-hydroxysuccinimide (NHS) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl) as activating agent and dehydrating agent were fed into the above solution to activate the carboxyl group of PLGA. Different concentrations of chitosan were added into the obtained solution and reaction lasted for 24 h. The excess EDC, NHS, and unreacted chitosan were eliminated by centrifugation.

Characterization of Nanoparticles

Particle size and zeta potential were measured by dynamic light scattering (DLS) spectrometer using Zetasizer Nano ZS (Malvern Instruments, UK). The surface elements of different polymer samples were determined using XPS (VG310F Scanning Auger Microprobe). The samples' infrared spectra were tested with FTIR (Bruker Vetex-70 Spectrometer). Average 32 scans of spectra were recorded in the standard wavenumber range of 400–4,000 cm^{-1} at a resolution of 4 cm^{-1}.

To determine the percentage of drug 5-FU entrapped in PLGA nanoparticles before and after chitosan modification an indirect method was carried out by measuring the drugs that were not encapsulated. The prepared nanoparticle solution ($W_1/O/W_2$) was centrifuged at 12 krpm for 20 min. Then the supernatant was collected and tested by ultraviolet–visible spectrometer (Cary 300 Varian) at a wavelength of 265 nm. The encapsulation efficiency (EE) was calculated with the following equation:

$$\mathrm{EE}(\%) = \frac{A - B}{A} \times 100 \tag{1}$$

where A is total feeding drug and B is the drug in the supernatant solution after centrifugation. During the test, all measurements were made in triplicate and the mean values are shown in the results.

In Vitro Drug Release Profiles

The *in vitro* drug release from PLGA and PLGA-CS nanoparticles was measured in triplicate in phosphate buffered saline (pH=7.4). First, the as-prepared nanoparticles were placed in a dialysis bag whose molecular weight cutoff was 8,000 Da. The tests were performed in a constant-temperature shower mixer at 100 rpm at 37°C with 50 mL dissolution

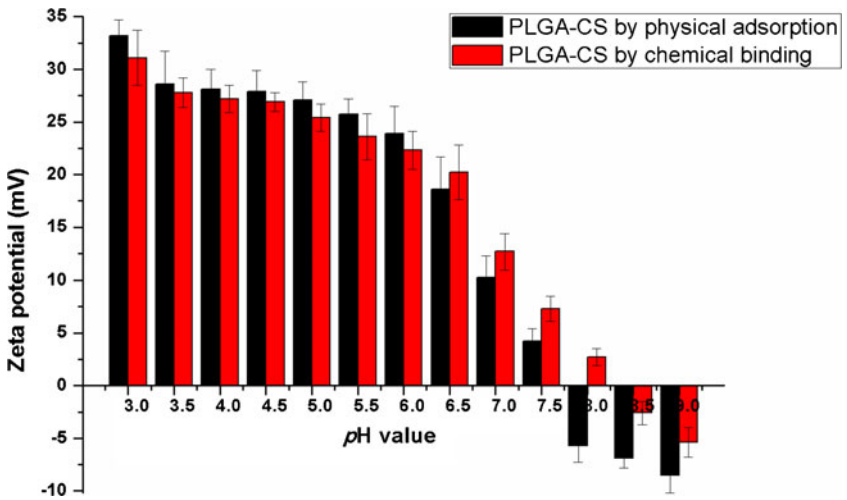

Fig. 2. Effect of pH value on the zeta potential of PLGA-CS nanoparticles

solution. After a fixed time interval, 3.5 ml of the solution was taken out and diluted to 30-fold volume. Then the ultraviolet absorbance at 265 nm was tested. After that, 3.5 ml of fresh buffer solution was added to the release medium to maintain a constant solution volume. After n samples were taken, the drug that was released could be calculated as $C=C_n\times50+(C_1+C_2+\ldots..+C_{n-1})\times3.5$, where C_i is the concentration of the solution of the nth sample.

RESULTS AND DISCUSSION

Zeta Potentials of Nanoparticles

The zeta potential is a key index to identify the surface charge of nanoparticles. And the surface charge in the form of zeta potential is related to the property of drug delivery system and stabilization of the prepared sample solutions. For most of the cancer cell membranes, surfaces usually show negative charge (21), so nanoparticles fabricated by PLGA have less affinity to cancer cells due to the expression of negatively charged carboxyl groups on PLGA surface. Therefore, chitosan was introduced to modify the charge of PLGA surface by physical adsorption and chemical binding methods in this study.

From Table I, it is found that the unmodified PLGA nanoparticles have a zeta potential of −22.5 mV which could be ascribed to the presence of end carboxyl groups on the polymeric nanoparticle surfaces (22). Obviously, after the chitosan treatments, all samples show a positive zeta potential which is an indication of the amine groups in the chitosan structure, suggesting that chitosan is successfully coated onto the PLGA nanoparticle surfaces using both physical and chemical methods. As most of the cancer cell membrane

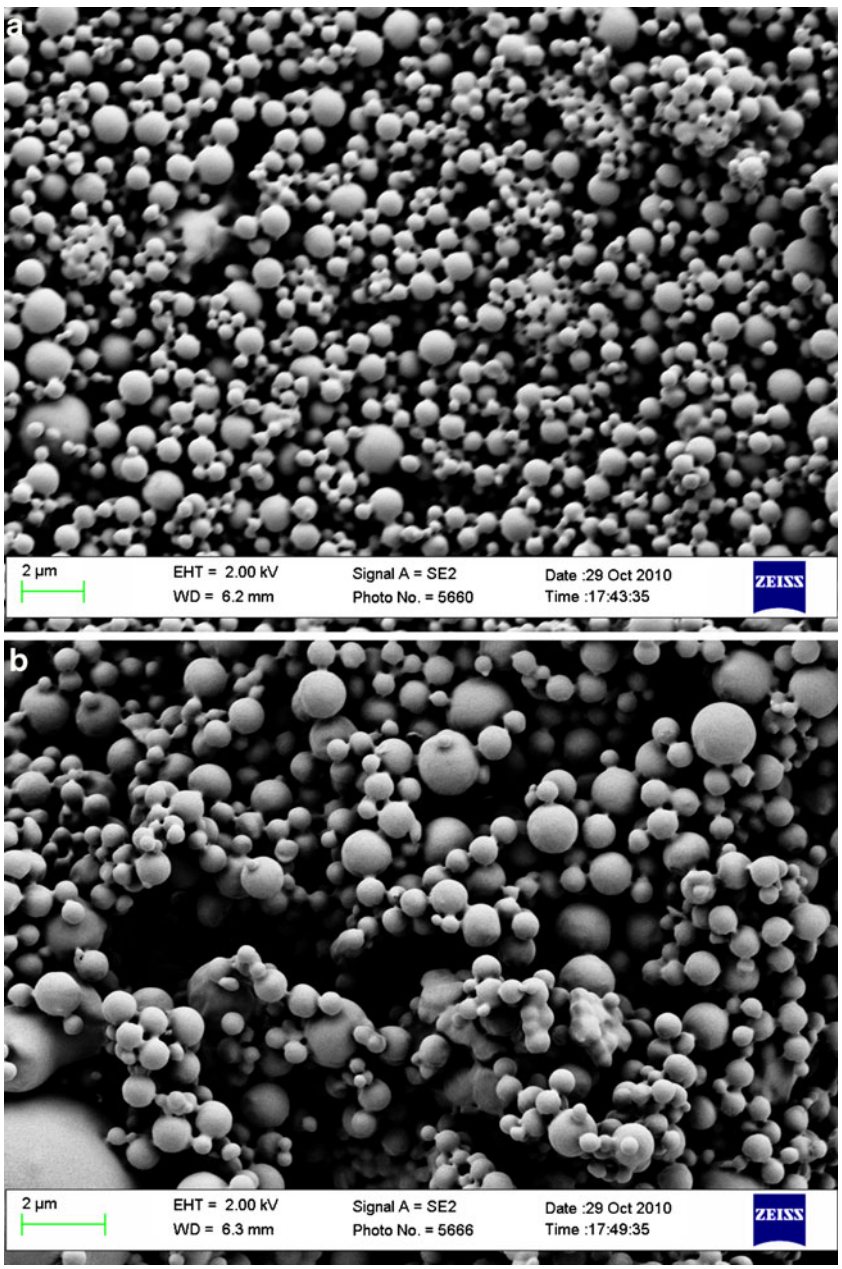

Fig. 3. SEM images of PLGA-CS prepared by **a** physical adsorption and **b** physical binding

surfaces are negatively charged, it is expected to the positively charged chitosan on the PLGA surface will significantly enhance the interaction between the nanoparticles and cancer cells and facilitate the cellular update.

A higher zeta potential leads to a larger repulsive force between different particles in a single solution and can stabilize the particle suspension (23). The CS decorated PLGA nanoparticles with a larger amount of fed chitosan exhibit a higher zeta potential (Table I). When the ratio of chitosan to PLGA is 0.2:1, the zeta potential of the nanoparticles prepared by physical adsorption is higher than those prepared by chemical binding. Therefore, the physical adsorption is more effective than the chemical binding when chitosan concentration is low. When the ratio of chitosan to PLGA is increased to 0.6:1, the zeta potential for physical adsorption and chemical binding is very close with a value of 23.90 and 22.32 mV, respectively. When the ratio of chitosan to PLGA further increases, the zeta potential do not change greatly. It is believed that the amount of chitosan has a saturation value that can be coated on PLGA surface (24). When the ratio of chitosan to PLGA is 0.6:1 or higher, the zeta potential seems to become a plateau and no more chitosan can be attached to PLGA, either by physical adsorption or chemical binding.

Effect of pH Value on the Surface Charge

The pH value of the chitosan solution has a significant influence on the surface charge of nanoparticles. The samples fabricated by both physical adsorption and chemical binding methods with a chitosan to PLGA ratio of 0.6:1 were used in the study to examine the pH effect. At pH6.0, the zeta potentials are 23.9 and 22.3 mV as indicated in Table I. Then the pH in W_2 of fabrication solution was adjusted by progressive addition of HCl and NaOH to cover a pH range from 3.0 to 9.0. All the zeta potentials were positive when the pH value was 7.5 and below (Fig. 2). For PLGA-CS samples prepared by physical adsorption, zeta potential is in the range of 4.2–33.2 mV. For PLGA-CS nanoparticles fabricated by the chemical binding method, zeta potential changes from 7.3 to 31.1 mV. Zeta potential increases as the decrease of pH value, which is due to the degree of protonation of amino groups in chitosan (25). When the pH value is higher than 8, most of surface charges of PLGA-CS nanoparticles becomes negative and the hydroxyl groups (OH) have more effect than the protonation of amino groups in chitosan solution.

Physicochemical Properties of Prepared Nanoparticles

The morphologies and sizes of PLGA-CS nanoparticles prepared by physical and chemical methods are characterized with both electron microscopy and dynamic light scattering. The samples prepared with a chitosan to PLGA ratio of 0.6:1 were selected in the test. The prepared particles have a spherical shape and smooth surface (Fig. 3) with an average size between 200 and 300 nm for particles prepared by physical adsorption while the particles prepared with chemical binding method have a diameter of over 300 nm. DLS was also used to measure nanoparticle size at room temperature after the samples were diluted 10 times with distilled water. The *Z*-average particle size was calculated from the intensity weighted distribution. The samples corresponding to the ones of SEM have a *Z*-average particle size of 268.3 and 322.5 nm (Table I), respectively which is in agreement with the SEM measurement. Both of the modified PLGA-CS nanoparticles are overly larger than that of the PLGA nanoparticles (197.8 nm; Table I) due to the adsorption and binding of chitosan molecules. The size of the nanoparticles prepared with both physical adsorption and chemical binding will be reduced in future studies for improved efficacy as particles smaller than 200 nm are preferential in targeted drug delivery (26).

The PLGA nanoparticles have an encapsulation efficiency of 28.6% (Table I). For chitosan-coated PLGA nanoparticles, the encapsulation efficiency decreases. The higher the amount of the chitosan in the fabrication, the lower the encapsulation efficiency. For PLGA-CS nanoparticles prepared by physical adsorption, this is because chitosan is positively charged and model therapeutic drugs 5-FU is negatively charged. The chitosan absorbs some of the 5-FU drugs to the surface of the PLGA nanoparticles during the fabrication and hardening process, which significantly affects the encapsulation efficiency. For PLGA-CS nanoparticles prepared with chemical binding method, chitosan reacts with PLGA nanoparticles in dispersed solution for 24 h, which leads to drug release from the PLGA nanoparticles. A high chitosan in the solution accelerates this process.

Chemical Compositions of the Surface

Chemical compositions of the nanoparticle surface were tested to check the formation of PLGA-CS nanoparticles. Figure 4 shows the FTIR spectra of different polymeric nanoparticles investigated in this study. From the PLGA spectrum (curve a in Fig. 4), the peak of C–O–C stretching is at 1,088 cm^{-1}, C–H stretching in methyl groups at 1,460 cm^{-1}, C=O at 1,750 cm^{-1}, CH, CH_2, and CH_3 stretching vibrations between 2,850 and 3,000 cm^{-1}, and OH stretching around 3,500 cm^{-1}. For chitosan (curve b in Fig. 4), the intense peaks at 1,654 and 1,597 cm^{-1} confirm the presence of amide I and amide II in the chemical structure of chitosan. The peak of C–H stretch is at 2,900 cm^{-1}, C–H bend is at 1,360–1,440 cm^{-1}. The chitosan's peak at 3,500 cm^{-1} corresponds to the N–H stretch which is overlapped with the O–H stretch. Chitosan peaks at 3,500 and

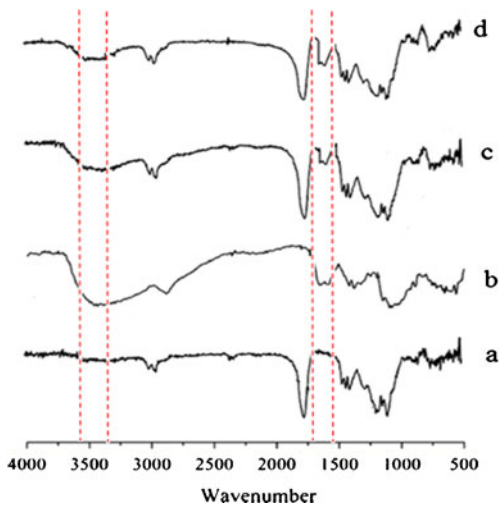

Fig. 4. FTIR spectra of nanoparticles (*a* PLGA nanoparticles, *b* chitosan, *c* CS-modified PLGA nanoparticles, *d* CS-modified PLGA by covalent binding)

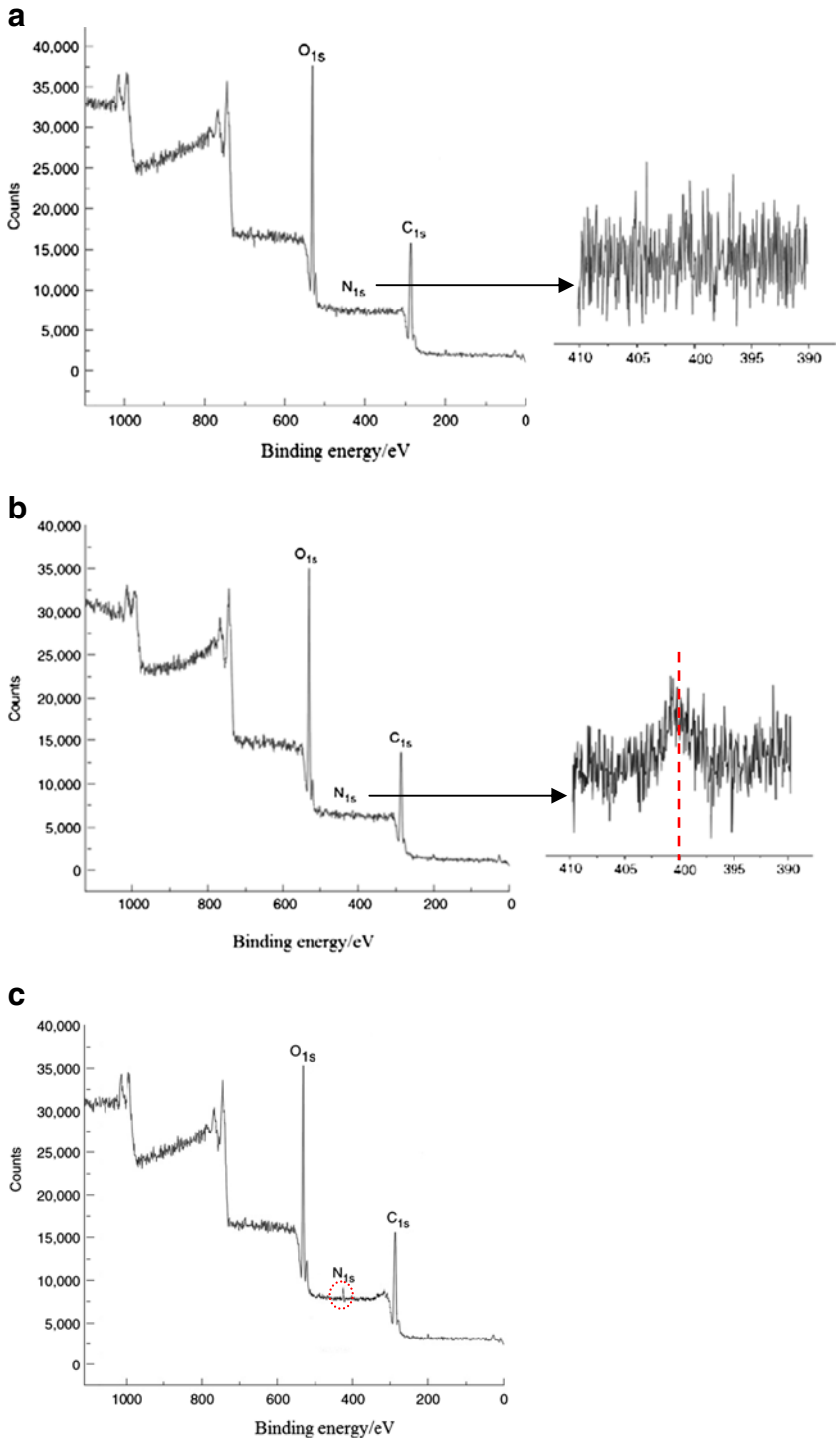

Fig. 5. XPS spectra of **a** PLGA, **b** PLGA-CS nanoparticles prepared by physical adsorption method, and **c** PLGA-CS nanoparticles prepared by chemical binding method

1,654 cm^{-1} are clearly shown in the PLGA-CS nanoparticles (curves c and d in Fig. 4), indicating that the chitosan has been successfully coated onto the PLGA nanoparticle surface by physical adsorption and chemical binding. The amine group (NH_2) expressed from the chitosan on PLGA nanoparticle surface makes PLGA-based drug delivery carriers active and versatile, which will largely facilitate the conjugation of cell-targeting molecules onto the systems and delivery of the drugs to specific target sites (27).

High-resolution XPS was also employed to further check the surface chemical elements and chemical compositions. XPS can provide qualitative and quantitative information on different elements on the particles surface and test the chemical compositions on the top layer of the polymer surface (28).

The XPS spectra from the PLGA nanoparticles clearly show that the O1s peak appears at 533 eV and the C1s peak is at 287 eV (Fig. 5a), which confirms the carbon and oxygen elements in the PLGA polymer matrix. The peak around the N1s is not obvious

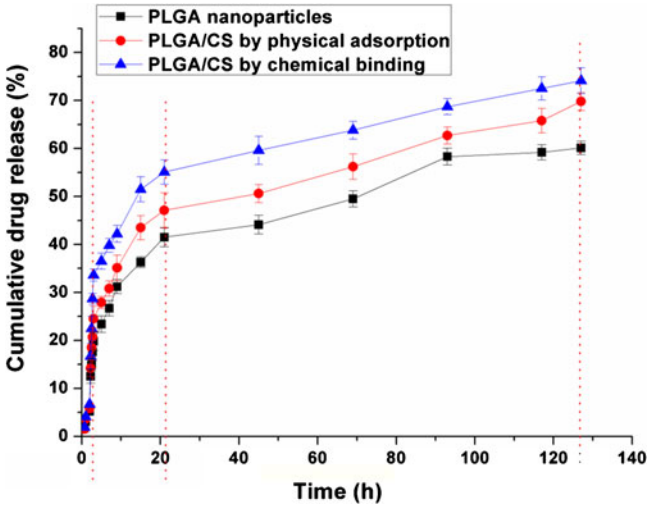

Fig. 6. Cumulative drug release from PLGA nanoparticles, PLGA-CS nanoparticles by physical adsorption and PLGA-CS nanoparticles by chemical binding method

and no peak is shown in this area when the range is zoomed in. The XPS spectra of PLGA-CS nanoparticles prepared by physical adsorption also show distinct oxygen and carbon peaks at 533 and 287 eV (Fig. 5b), respectively, which means the main structure of PLGA does not change during chitosan modification. The zoomed in view of the nitrogen area showed a signal at 399 eV which corresponds to the amino group (NH_2) in chitosan (29). For XPS spectra of PLGA nanoparticles prepared by the chemical binding method, O1s and C1s peaks are located nearly at the same binding energy as those of PLGA nanoparticles and PLGA-CS nanoparticles prepared by the physical adsorption method. A small N1s peak can be detected in the binding energy of 408 eV, due to the generation of CO–NH binding after the chemical reaction between PLGA and chitosan. For covalent bonding, high polarity results in high binding energy. The binding CH_2–NH_2 in chitosan structure has less polarity than CO–NH binding in PLGA-CS nanoparticles, so N1s binding energy from PLGA-CS nanoparticles prepared by chemical binding is higher than that from PLGA-CS nanoparticles prepared by physical adsorption. The existence of nitrogen in PLGA-CS nanoparticles confirmed from XPS (Fig. 5) indicates that the chitosan has successfully been coated onto the PLGA surface since chitosan contains the nitrogen element while no nitrogen element appears in the PLGA polymer structure.

In Vitro Drug Release Profile

In vitro drug release profiles of 5-FU from the treated PLGA-CS nanoparticles and untreated PLGA nanoparticles are shown in Fig. 6. All three samples, PLGA nanoparticles, PLGA-CS nanoparticles by physical adsorption, and PLGA-CS nanoparticles by chemical binding show a burst release in the first 4 h. The two modified PLGA-CS nanoparticles exhibit heavier burst release than the untreated PLGA nanoparticles because the chitosan and 5-FU have opposite surface charge and some of the drug 5-FU is adsorbed to PLGA nanoparticle surface in the fabrication and particle hardening process. Drug releases from PLGA and PLGA-CS nanoparticles experience the processes of swelling of PLGA polymer, pore diffusion, polymer erosion, and degradation (30). Therefore, after the burst release, all three nanoparticles show moderate and sustained release. Chitosan is more hydrophilic than PLGA, which results in PBS solution more easily penetrated into the nanaoparticle matrix and more drugs released during the same time. Also the positively charged chitosan on the nanoparticle surface can accelerate the release of negatively charged 5-FU drug due to electrostatic actions. Both factors lead to a higher cumulative drug release from PLGA-CS nanoparticles.

CONCLUSIONS

This study fabricates a novel PLGA-based drug delivery system with active and versatile surfaces. Chitosan was successfully coated to PLGA nanoparticles surface by both physical adsorption and chemical binding methods, which was confirmed by zeta potential, FTIR, and XPS. Chitosan-modified PLGA nanoparticles exhibited a positive charge surface and the zeta potential dramatically decreased as pH value increased. The chitosan on PLGA nanoparticles surface affected the drug release profile. The positive charge and hydrophilic property induced a moderate and prolonged drug release and a high cumulative drug release. Therefore, this study provided a method for extending the utilization of PLGA-based drug delivery system and a possible high cell-targeting molecule conjugation in future investigation.

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