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Core-shell Poly(D,L-lactide-*co*-glycolide)/Poly(ethyl 2-cyanoacrylate) Microparticles with Doxorubicin to Reduce Initial Burst Release

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Abstract: Monodispersed microparticles with a poly(D,L-lactide-co-glycolide) (PLGA) core and a poly(ethyl 2-cyanoacrylate) (PE2CA) shell were prepared by Shirasu porous glass (SPG) membrane emulsification to reduce the initial burst release of doxorubicin (DOX). Solution mixtures with different weight ratios of PLGA polymer and E2CA monomer were permeated under pressure through an SPG membrane with 1.9 μm pore size into a continuous water phase with sodium lauryl sulfate as a surfactant. Core-shell structured microparticles were formed by the mechanism of anionic interfacial polymerization of E2CA and precipitation of both polymers. The average diameter of the resulting microparticles with various PLGA:E2CA ratios ranged from 1.42 to 2.73 μm. The morphology and core-shell structure of the microparticles were observed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The DOX release profiles revealed that the microparticles with an equivalent PLGA:E2CA weight ratio of 1:1 exhibited the optimal condition to reduce the initial burst of DOX. The initial release rate of DOX was dependent on the PLGA:E2CA ratio, and was minimized at a 1:1 ratio.

Keywords: core-shell, poly(D,L-lactide-*co*-glycolide), ethyl 2-cyanoacrylate, membrane emulsification, doxorubicin.

Introduction

Various technologies have been proposed for the preparation of structured microparticles, which included solvent evaporation,[1,2] phase-separation,[3] and spray-drying.[4,5] The solvent evaporation method of an oil-in-water emulsion is widely used because it is a simple procedure. However, use of this method sometimes results in low encapsulation efficiency and an early rapid release of the drug. This initial release of drug from polymer matrices can be controlled by diffusion of the drug through the polymer matrix or through the water-filled pores produced by water penetration into the matrices.[6] In addition, it is influenced by many factors, including polymer molecular weight, copolymer ratio, preparation method, properties of the incorporated drug, and glass transition temperature of polymer.[7-10] In general release cases, the initial burst release arises from the drugs located on the particle surface. Such a problem reduces the effective lifetime of the devices; thus, several researchers have attempted to determine the mechanisms of the early rapid release of drugs from structured microparticles and develop a technological method of prevention.[11,12]

Monodispersity and particle size of microparticles are important properties because the size distribution of microparticles within the body affects interactions with biological cells.[13] For example, if size and monodispersity of microparticles can be controlled, drug release kinetics can be manipulated, thereby making it possible to formulate more sophisticated systems. To study the controlled-release behavior of these nano/microparticles and make them more effective for the drug delivery system (DDS), it is essential to develop monodispersed small-sized microparticles. Therefore, Shirasu porous glass (SPG) membrane emulsification was introduced to control the size and monodispersity of microparticles.[14]

The degradation rate of poly(alkylcyanoacrylate) (PACA) is known to be faster than biodegradable polymers with an ester linkage,[15] which in some cases may be more effective. However, it must be considered that a rapid degradation rate may increase toxicity with high concentrations of break down products. Moreover, PACA is not fully biodegradable, being comprised of an acrylate backbone with a pendant ester group.[16] The by-products of degradation are alkyl alcohols and poly(cyanoacrylic acid) having low molecular

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weights, which can be excreted renally. In common cases, the degradation rate of PACA is affected by physical properties of polymers such as particle size and molecular weight, the pH of the continuous water phase and enzymes.[17-22] While the major mechanism of drug release from PACA particles is bioerosion, the drug release rate from PACA particles also depends on the PACA hydrolysis rate.[23,24]

In this study, monodispersed microparticles with a poly(D,L-lactide-*co*-glycolide) (PLGA) core and a poly(ethyl 2-cyanoacrylate) (PE2CA) shell prepared by Shirasu porous glass (SPG) membrane emulsification with doxorubicin, were proposed to control initial burst release. Doxorubicin was selected as a hydrophobic drug and the release behaviors from microparticles with different ratios of PLGA and E2CA were investigated.

Experimental

Materials. Ethyl 2-cyanoacrylate (E2CA) was purchased from Sigma-Aldrich (USA). Poly(D,L-lactide-*co*-glycolide) (PLGA) (D,L-lactide 75: glycolide 25) with an average molecular weight of 10,000 was purchased from Wako Pure Chemicals (Japan). Methylene chloride (MC), hydrochloric acid (HCl), sodium hydroxide (NaOH) and sodium lauryl sulfate (SLS) were purchased from Duksan Pure Chemicals (Korea). Doxorubicin-HCl (DOX-HCl) was purchased from Boryung Pharm. Co. (Korea). Distilled water was prepared with a Milli-Q water purification system (Direct-Q 3, USA-Bedford, MD) with a resistance of 18 $M\Omega cm^{-1}$.

Apparatus. A miniature kit for emulsification with a microporous glass (brand name SPG) was purchased from SPG Technology Co. Ltd. (Miyazaki, Japan). A schematic diagram and details of this kit were described elsewhere.[25] A membrane with 1.9 μm pore size was used.

Preparation of Core-shell Microparticles by Membrane Emulsification. To generate HCl-free DOX, DOX-HCl was dissolved in 3 mL methanol in the presence of TEA (1.5 times the molar quantity of DOX) for 6 h and subsequently freeze-dried. The hydrophobic nature of HCl-free DOX was confirmed by its solubility in MC solvent. Typically, 0.4 g of PLGA and E2CA with different weight ratios (10:0, 7:3, 5:5, 3:7, and 0:10) was dissolved in 10 mL of MC with 4 mg of DOX. The organic solution was stored in a pressure-tight vessel, which was then slowly pressurized with nitrogen to 20 kPa. This allowed the organic solution to permeate through the SPG membrane into the continuous water phase (100 mL with 1 g of SLS at pH 2.5). The generated organic emulsions were collected in the continuous phase with gentle magnetic stirring followed by solvent evaporation for 6 h.

Characterization of Core-shell Microparticles. A field emission scanning electron microscope (FE-SEM, JSM-6500F, JEOL, Japan) and a transmission electron microscope (TEM, Zeiss EM 902, Germany) were used to observe the surface and morphology of microparticles. Gel permeation chromatography (GPC, Waters Breeze System, Waters Co., USA) was used to measure the molecular weight distribution of components in the particles after E2CA polymerization. The GPC column consisted of a series of μStyragel® columns (HR5E, HR5, HR4, HR1); tetrahydrofuran (THF) was used as an eluent at a flow rate of 1 mL/min and 1×10^3 Pa.

Encapsulation Efficiency and Release Profile of DOX from Core-shell Microparticles. For the evaluation of encapsulation efficiency of DOX within microparticles, the resulting microparticles were washed three times with DI water and freeze-dried to obtain microparticle powder. The microparticle powder (15 mg) was dissolved in MC (3 mL) and the absorbance of the solution was analyzed by UV/VIS spectrophotometer (UV-1601, Shimadzu, Japan). For the release profile, twenty milliliters of the suspension of microparticles (4 mg/mL) was sealed in a dialysis tube (MWCO 6000-8000, Membrane filtration products, Inc., USA) and incubated in 100 mL PBS with gentle shaking at 37 °C. At predetermined time intervals, 4 mL of the solution was collected from the dialysed media and replaced with fresh PBS. The DOX released was analyzed by UV/VIS spectrophotometer at 487 nm.

Results and Discussion

Mechanism of Particle Formation of Core/Shell (PLGA/PE2CA) Microparticles. The polymerization of alkyl cyanoacrylates occurred as a result of the anionic initiation of the hydroxyl group, which ionizes the cyanoacrylate monomer and in turn reacts with another monomer to form reactive chains. Polymerization is well known to be completed in several seconds.[26] In this work, the polymer (PLGA) and the monomer (E2CA) were used together to form a core-shell structure. Therefore, the mechanism of particle formation seemed to be complex compared to the simple polymer precipitation method using solvent evaporation. Figure 1 shows a schematic illustration of particle formation. While the organic solution containing PLGA, E2CA, and DOX permeated through the SPG membrane (a), the E2CA monomer on the emulsion surface, in contact with hydroxyl ions existed in the water phase and then polymerization of E2CA occurred. Polymerized E2CA was dissolved in the organic emulsion phase (b). The core-shell (PLGA-PE2CA)-structured microparticles were formed after solvent evaporation (c). The size of emulsion that permeated through the SPG membrane was generally approximately 3~6 times membrane pore size.[27] However, the size of emulsion was gradually reduced when the volatile solvent was used. The core-shell structure was attributed to the interfacial polymerization of E2CA and the difference of hydrophobicity between the two polymers. The polymerization of E2CA primarily occurred at the O/W interface because hydroxyl anions

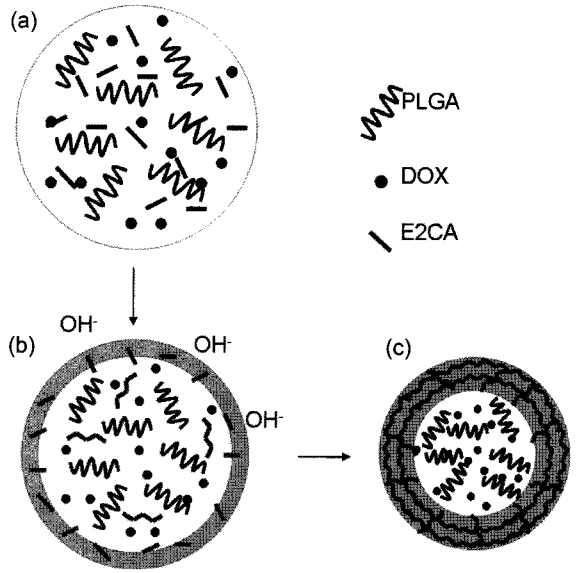

Figure 1. Schematic illustration of the PLGA-PE2CA microparticle formation.

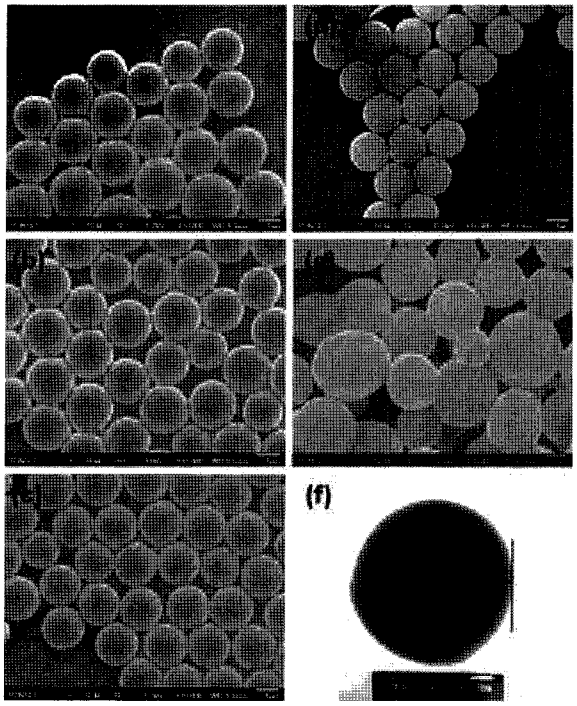

Figure 2. (a-e) SEM and (f) TEM images of core-shell (PLGA-PE2CA) microparticles: (a) L10C0, (b) L7C3, (c, f) L5C5, (d) L3C7, and (e) L0C10.

existed in the water phase, which induced the shell formation of PE2CA. In addition, PE2CA has a more hydrophilic nature than PLGA; this can be proven by the solubility parameters (PLGA: 23.24 $MPa^{1/2}$ and PE2CA: 31.77 $MPa^{1/2}$).[28,29] Finally, hydrophilic PE2CA migrated to the shell and then PLGA formed in the core.

Characteristics of Core-shell Microparticles. The average particle size and DOX loading efficiency of microparticles with different weight ratios of PLGA and E2CA are given in Table I. Monodispersed microparticles were prepared under all conditions except L0C10. The average particle size of microparticles decreased from 1.92 to 1.42 μm as the weight ratio of E2CA increased, even if the initial emulsion droplet size was assumed to be slightly different due to the viscosity difference between the polymer (PLGA) and the monomer (E2CA). As the E2CA ratio increased, the initial emulsion droplet size decreased and the encapsulation efficiency of DOX also decreased from 77.5 to 60.9%. The hydrophobic-DOX molecule is concluded to have a higher affinity to the hydrophobic PLGA than the relatively hydrophilic PE2CA. For this reason, the hydrophobic-DOX molecule preferred to be located in the PLGA core. Therefore, the decrease of the PLGA ratio has resulted in reduced encapsulation efficiency.

Table I. Characteristics of Microparticles with Different Weight Ratios

Sample	Weight Ratio (PLGA:E2CA)	Average Particle Size (μm)[a]	DOX Loading Efficiency (%)[b]
L10C0	10 : 0	1.92 ± 0.09	77.5 ± 3.5
L7C3	7 : 3	1.74 ± 0.11	72.3 ± 3.3
L5C5	5 : 5	1.51 ± 0.09	71.1 ± 2.9
L3C7	3 : 7	1.42 ± 0.12	66.7 ± 4.3
L0C10	0 : 10	2.73 ± 0.98	60.9 ± 4.2

[a]The number-average particle size was measured from the SEM images directly. The size of 500 particles were counted and averaged. [b]Measured triple and calculated.

Figure 2 shows SEM and TEM images of core-shell structured microparticles. The microparticles were monodispersed with a mean diameter of 1.92 (Figure 2(a)) to 1.42 μm (Figure 2(e)), and aggregated particles were observed in the L0C10 sample. The core-shell morphology can be confirmed by TEM (Figure 2(f)) where the core and shell material was assumed to consist of PLGA and PE2CA, respectively. The low pH in the continuous phase could be considered to be one factor affecting the size distribution of the resulting particles. Actually, it was reported that the pH of the continuous media could have an effect on the mechanism of polymer degradation.[30] At pH 7.4, the PLGA microspheres followed the usual morphological changes, such as surface erosion and pore formation, whereas at pH 2.4 the microspheres maintained smooth surfaces throughout the degradation process. However, the effect of the pH of the continuous phase seems to be negligible since the time for solvent evaporation at the low pH is very short compared to the other degradation experiments.

The molecular weights of the microparticles prepared with different weight ratios were determined by GPC. Fig-

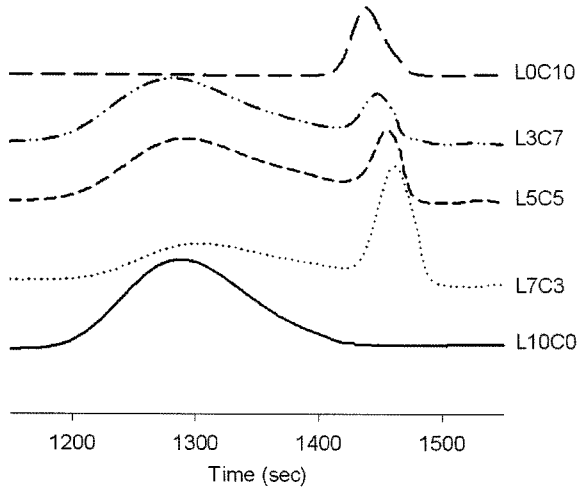

Figure 3. GPC curves for microparticles with different PLGA-PE2CA ratios.

ure 3 shows the GPC profiles over time. The elution time in each profile was normalized with that in the PLGA profile as a standard on the assumption that the molecular weight of PLGA is not changed. While a single peak was observed in L10C0 and L0C10, two peaks (one broad and the other sharp) appeared in the other samples. The broad peak corresponded with PLGA and the narrow peak was PE2CA. The PE2CA peak shifted gradually. The molecular weight of PE2CA increased from 2,546 to 2,985 with respect to the increase in the weight ratio of E2CA. The molecular weight of PE2CA is suggested to increase by the increase in the monomer content with a constant concentration of hydroxyl anion. Similar results previously reported indicated that the increase of pH in the water phase lead to a decrease in the molecular weight of PACA polymers. Use of a large amount of initiator (in higher pH conditions) lowered the molecular weight, which is typical of emulsion polymerization.[31]

Release Profile of DOX from Core-shell Structured Microparticles. In our concept of particle design, the hydrophobic PLGA polymer was selected as a container for DOX and PE2CA served as a barrier against drug diffusion to reduce the initial burst effect. The sustained release and the loading efficiency were limited in the single PE2CA composition because the degradation rate of PE2CA is very fast. Therefore, both PLGA and PE2CA polymers were involved in the design of the core-shell structured particle. The initial burst release was minimized by the PE2CA shell layer and the drug was released slowly over the desired period by the PLGA core. Figure 4 shows the cumulative release profiles of DOX from microparticles with different weight ratios of PLGA and E2CA. The L10C0 sample exhibited a typical drug release profile, and the slope of the release decreased as the content of E2CA increased up to fifty percent. Therefore, it was concluded that the PE2CA shell layer effectively reduced the initial burst of DOX. However, at E2CA contents greater than fifty percent, the release slope increased.

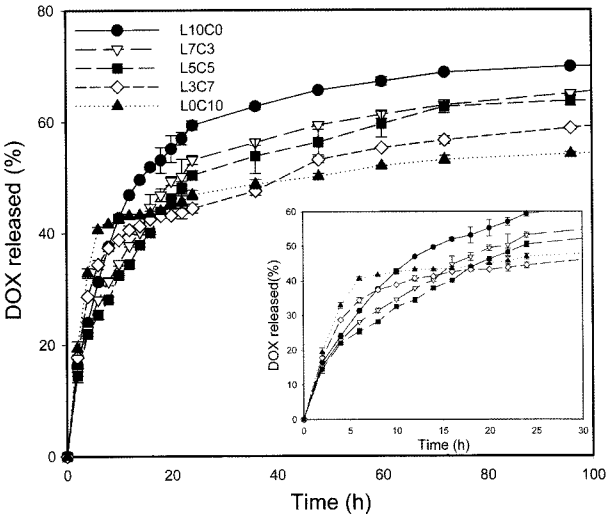

Figure 4. Release profiles of DOX from microparticles with different composition ratio (n=3).

The influence of different weight ratios can be explained by considering the distribution of the drug within microparticles. If the E2CA content of the microparticles is less than fifty percent, DOX is assumed to be located in the PLGA core and not in the shell layer. When the E2CA content of the microparticle is greater than fifty percent, the small amount of PLGA cannot assimilate all of the DOX molecules, and therefore, the rest of the DOX molecules are located in the PE2CA shell layer. The latter phenomenon triggers the initial burst of DOX. Within the scope of our experiment, it was concluded that microparticles with the weight ratio of PLGA 50% and PE2CA 50% minimized the initial burst of DOX. Additionally, greater PLGA content is required for effective reduction of the initial burst if a greater amount of DOX is involved.

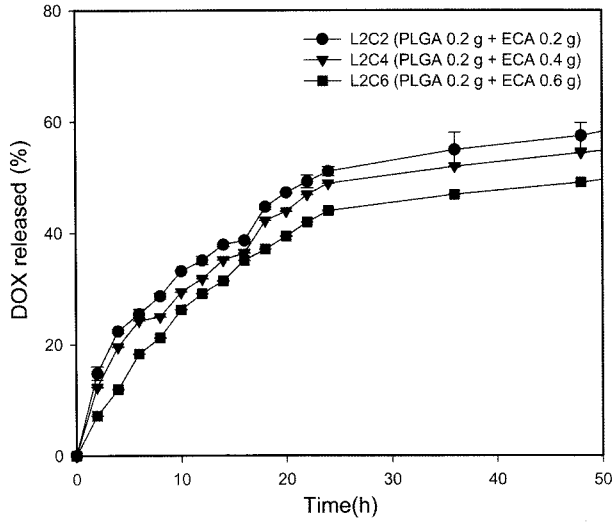

Figure 5. Release profiles of DOX from microparticles with different shell contents (n=3).

Figure 5 shows the DOX release profiles from microparticles. The L5C5 sample showed the typical drug release profiles, and the slope of release pattern slightly decreased as the content of E2CA was increased up to three times. The increase in the content of the PE2CA shell effectively reduced the release rate of DOX. Up to three times the E2CA content, the slope of drug release can be lowered by increasing shell content. Consequently, it was demonstrated that the slope of the DOX release profile could be slowed down by increasing the content of the PE2CA shell in the core-shell structured microparticles.

Conclusions

In this study, the initial release behavior of DOX was controlled by core-shell structured microparticles composed of a PLGA core and a PE2CA shell, which were prepared by SPG membrane emulsification. Core-shell morphology of microparticles was spontaneously formed by the mechanism of interfacial polymerization of E2CA and by the difference in solubility between the two polymers. The slope of initial drug release was decreased as the PE2CA ratio increased up to fifty percent, which is the optimum condition to reduce the initial burst by controlling the ratio and content of the shell layer. It was suggested that DOX molecules tended to be located in the PLGA core due to their affinity to PLGA. In conclusion, we have demonstrated the reduction of the initial burst of DOX by employing the shell layer.

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