Preparation of Biocompatible Polyurethane Microcapsules containing Phospholipid-like Moieties

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1. Introduction

Microcapsules are containers surrounding functional material with a polymer membrane in the size range of about 50 nm to 2 nm [1]. The polymer wall consists of a permeable part with high porosity and a non-permeable matrix part with low porosity, which are capable or in-capable of protecting the release of interior core materials. The weight ratio between the matrix and porous parts of the polymer wall depends on the class of wall-forming materials, microen-capsulation methods and physical and chemical conditions in the process, and mean particle size of the resulting microcapsules [2].

Segmented poly(urethanes)(SPU) are linear multiblock copolymers which consists of polyols soft segments and an alternating sequence of poly(urethanes) hard segments combining of diisocyanates and low molecular chain extenders. They are extensively used in medicine, especially in the fabrication of desirable mechanical properties, as a results of their microphase separated structures, and unsurpassed patency rates, still warrants their extensive usage as blood contacting synthetic biomaterials.

On the other hand, phospholipids are the main components of the main components of the biomembrane and interesting substances in biological and biomedical field[3,4]
Recently, the phospholipid microcapsules have been used as a brug carrier, sensor, separation membrane[5]. However these phospholipid microcapsules were unstable physically, because the phospholipids constituting membranes do not bond covalently and have high mobility.

To improve the mechanical strength and the blood compatibility of the phospholipid microcapsules, we synthesized the prepolymers based on poly(butadiedneglycol)(PBG) and hexamethylene diisocyanate (HDI), and then new segmented polyurethanes with phospholipid analogues and 1,4-butanediol(BD) as chain extenders.

2.Experinental

2.1 Materials

Ethylene glycol(EG), phosphorus trichloride, dichloromethane, benzene, triethylamine(TEA), tetrahydrofuran(THF), N,N-dimethylformamide(DMF), acetone, chloroform, diethyl ether, methanol, and 1,4-butanediol were commercially obtained and purified by vacuum distillation. N-methyldiethanolamine and hexamethylene diissocyanate (HDI) were used without further purification. Poly(butadieneglycol) (PBG, Mn=2840) was served by Nippon Yushi.

2-Cholro-1,3,2-dioxaphospholane[6] was prepared by the reaction of ethylene glycol with phosphorus trichioride in dichloromethane, according to the method of Lucas et al.,(Fig.1) and oxidized to 2-chicro-2-oxo-1,3,2-dioxaphospholane with oxygen according to the procedure of Edmundson[7].

Poly(vinyl alcohol) (Mw, 1500) as a protective colloid and a perfume, migrin oil (Mw 250, Seil Perfume Co., South Korea) as a core material and dibutyl tin dilaurate (DBTDL) as catalyst were used without any further purification.

2.1.1 Synthesis of 2-[Bis(2-hydroxyethyl)methylammonio]ethyl phosphate(SPD)

Into a thorouguly dried 500cm^3 three-necked round-bottomed flask, equipped with a mechanical stirrer, drying tube, and dropping funnel, were placed 10.00g of stearyl alcohol and 4.15g of triethylamine in 150cm^3 dry THF. After cooling with ice/water (10℃), 5.27g of 2-chioro-2-oxo-1,3,2-dioxaphospholane was added slowly to the stirred solution over a period of 1hr. The reaction mixture was maintained at 10℃ for 1hr with stirring and then allowed to warm to 15~20℃.

After being kept at this temperature for 1.5hr, the precipitate formed was filtered off and washed with 30cm^{3}of dry THF. The filtrate and the THF solution were evaporated in vacuum in a stream of nitrogen. To the residue, 50cm^3 of dry THF were added. The

mixture was shaken for 30s and then filtrated with a glass filter to remove a small amount of triethylamine hydrochloride. The filtrate was evaporated in vacuum in a stream of nitrogen for about 1.5hr to give 2-stearyloxy-2-oxo-1,3,2-dioxaphospholane (3) as a white solid.

2.1.2 Oleyl-2(N-methyldiethanolammonium)ethyl phosphate (OPD)

In the similar manner of (3) 2-oleyloxy-2-oxo-1,3,2-dioxaphospholane was prepared from oleyl alcohol and 2-chloro-2-oxo-1,3,2-dioxaphospholane in THF in the presence of triethylamine at 20 to -15℃, given as a pale brown semisolid. OPD was synthesized in the manner similar to SPD.

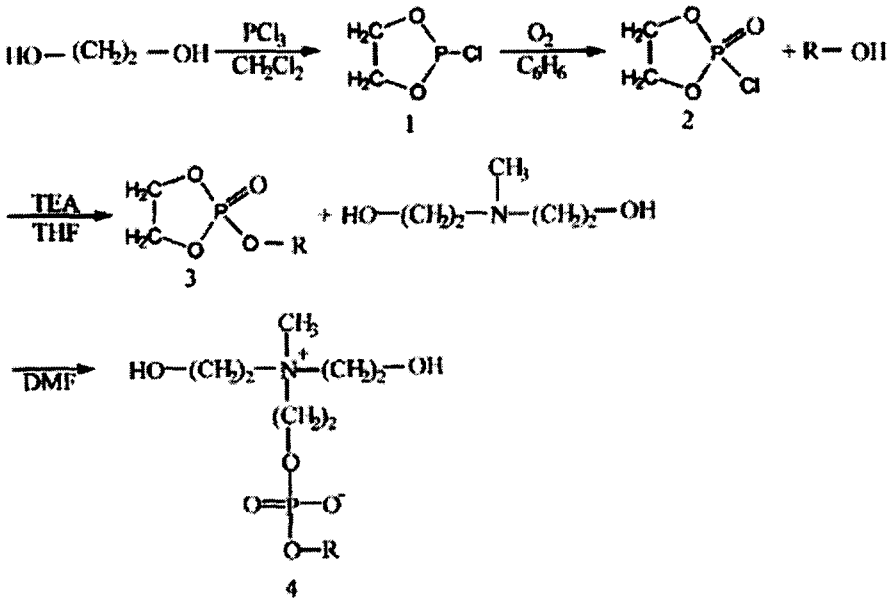


Fig. 1. Synthesis of phosphonelipid diols

2.2 Preparation of microcapsules

In this study, a typical interfacial polymerization method was employed for the microencapsulation of polyurethane (Fig.2). Oily solution with 0.23 M HDI and PBG and 0.5 M perfume, migrin oil as core material was prepared by an adequate mixture. O/W emulsion was formed by pouring 150g of the organic solution into 1000ml of aqueous solution with 0.01 M PVA as a protective colloid and immersed vigorously using an emulsifying apparatus. The resulting emulsion was stirred under atmospheric pressure at

25℃ for 5 min. DBTDL as a catalyst and polyether polyol with high or low molecular weight were added into the O/W emulsion. After going up to 90℃, 1,4-butadiene and phospholipid analogues as a chain extender was added into the resultant solution. A reaction time of the subsequent 60 min formed polyurethane microcapsules containing migrin oil as a penetrator with molecular weight similar to the core materials. The microcapsule slurry was decanted and washed with 30% ethanol aqueous solution to remove unreacted HDI and PBG and free migrin oil on their surfaces. The filtered microcapsules were dried at room temperature for 24h.

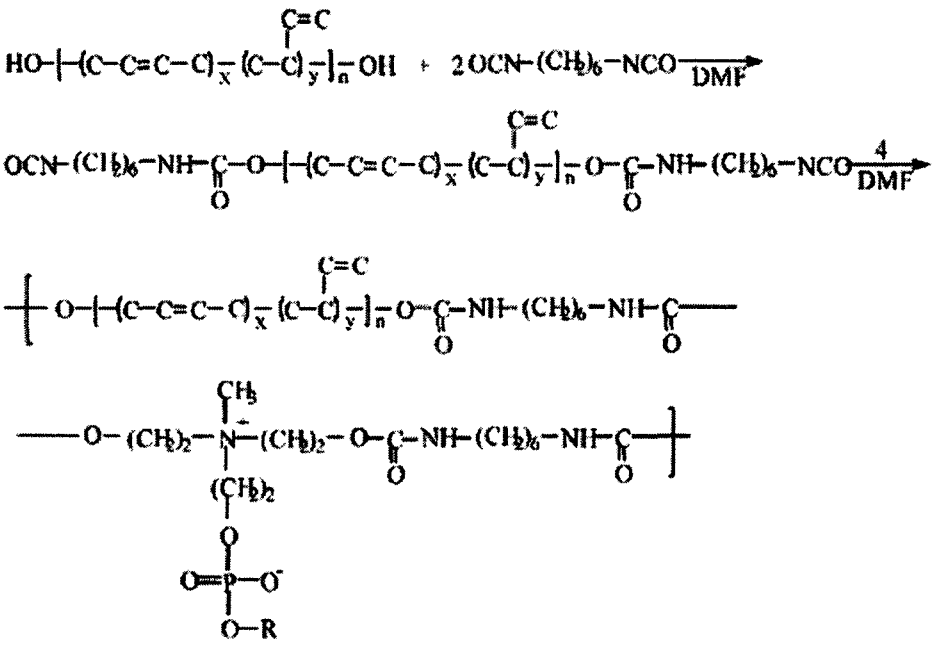


Fig. 2. Synthesis of Segmented Polyurethanes

2.3 Characterization of the microcapsules

Infrared spectra of core material and microcapsules were obtained with a computerized Nicolet Impact 400D Fourier Transform infrared spectrophotometer.

Scanning electron microscopy was performed using a JSM-5400 (JEOL Co. Ltd., Japan). Microcapsules were sprinkled onto a double sided tape, sputter-coated with gold and examined in the microscope.

X-ray photoelectron spectroscopy (XPS) was carried out for surface analysis. All samples were vacuum-dried for 24hr at room temperature and stored in a desiccatior.

2.4 Evaluation of Thrombogenicity

The polymer microcapsules were washed with saline and incubated at 37℃ for 1hr with freshly prepared, platelet rich plasma(PRP) which was obtained from the centrifugation of rabbit blood. The samples were rinsed with saline and treated with 2.5% glutaraldehyde in saline at refrigerated temperature overnight. This treament fixes all platelets attached to the surface of the polymers. The samples were rinsed with saline and dehydrated by systematic immersion in a series of ethanol-water solutions; 60, 70, 80, 90 and 100%v/v. Following critical point drying with CO_2, the samples were coated with gold for the analysis by scanning electron microscopy(SEM).

3. Results and discussion

3.1 preparation and characterization of phosphonolipid diols

The new diols and segmented polyurethanes were characterized according to NMR spectror.

The ^1H NMR spectrum of SPD($CDCl_3$) has following chemical shift : δ= 0.88($C-CH_3$, 3H), 1.26($-(CH_2)_{16}-$, 32H), 1.7~2.1($PO-CH_2-C$, 2H), 3.6~4.4($-OCH_2CH_2-$, 4H) and the 1HNMR spectrum of OPD : δ=0.88($-CH_3$, 3H), 1.26($-CH_2$, 28H), 3.12(N^+-CH_3, 3H), 3.70~4.55($-OCH_2-$, N^+-CH_2, 14H), 5.2(-CH=CH-, 2H), 5.8~6.1(-OH, 2H). The structures of SPD and OPD were indentified by these characteristic peaks.

3.2 preparation and characterization of polyurethane microcapsules

The polymerizations of segmented polyurethanes were characterized by the IR spectra. The polymers were indentified by the characteristic peaks 1700~1710cm^{-1} (-CONH-), at 1260cm^{-1}(-P=O-), at 1080cm^{-1}($-PO-CH_2-$), and at 965 and 905 cm^{-1} (-CH=CH-).

3.3 Surface elemental analysis

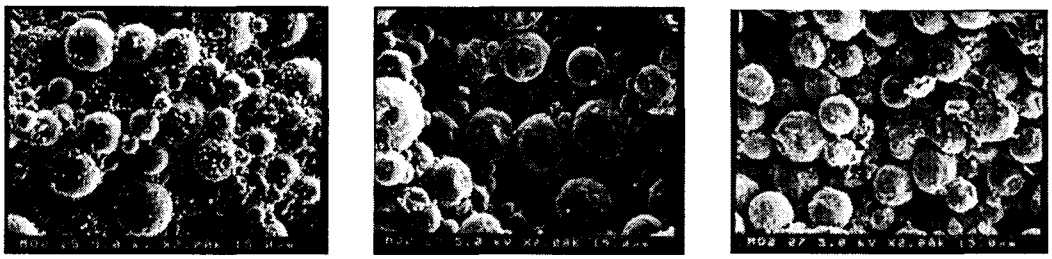
The XPS surface elemental analysis of polyurethane films was carried out. The

air-side surface of PBG-HDI-SPD and PBG-HDI-BD films shows peaks from phosphorus at 133eV, which was not present at PBG-HDI-BD. The glass side surface of PBG-HDI-SPD films shows the same peak of phosphorus as the air sied surface of the film. On the other hand the peak of phosphorous was not present at the glass sied surface of PBG-HDI-OPD film. It means that phospholipid chains of PBG-HDI-OPD was present or near the surface of the polymer film.

3.4 Nonthrombogenicity of the microcapsules surfaces

Thrombogencicity of the microcapsules surface was evaluated with PRP by in vitro adhesion test. The results of platelets retention at the surface with varying segmented polyurethanes are shown in Fig.3 Platelet adhesion was minimized at the surfaces of segmented polyurethanes. On the contrary no such suppressive effect on platelet adhesion was observed for PVA film. This significant differences in platelet adhesion behavior between PVA and segmented polyurethanes films strongly suggest that the formation of microdomain structure is a determinative factor for the antithrombogenic feature of segmented polyurethanes.

Futhermore platelet adhesion was effectively reduced at the segmented polyurethanes surfaces with containing phospholipid-like moieties. In case of the film surface of polyurethane with OPD platelets were less adhered to the surface. It suggests that platelets recognize the pattern of phospholipid structure and the adhesion is effectively at the surface of the segmented polyurethane microcapsules.



(a) (b) (c)

Fig. 3. SEM photographs of microcapsule surfaces after contacting with PRP
(a) PBG-HDI-BD (b) PBG-HDI-SPD (c) PBG-HDI-OPD

4. Conclusion

New segmented polyurethanes with phospholipid-like moieties were synthesized and their blood compatibilities were investigated. The platelet adhesions were effectively suppressed at the surface of PBG-HDI-OPD film. This study suggests that synthesized segmented polyurethanes with phospholipid-like moieties are very exciting as new biomaterials and their prospective imfortance for biomedical applications.

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