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Topical Delivery of Budesonide Emulsion Particles in the Presence of PEO-PCL-PEO Triblock Copolymers

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Abstract: This article describes the topical delivery and localization of budesonide through the hairless mouse skin. Two poly(ethylene oxide)-*block*-poly(ε-caprolactone)-*block*-poly(ethylene oxide) (PEO-PCL-PEO) triblock copolymers (T 222 and T 252) having different CL:EO ratios were added in the preparation of budesonide particles stabilized with poly(vinyl alcohol) (PVA) and Tween 80 under ultrasonication. For comparison, a commercial PEO-PPO-PEO triblock copolymer (F68) was studied under the same condition. To demonstrate the effects of the triblock copolymer, the particle size of budesonide emulsion, entrapment efficiency, and *in vitro* release were measured and compared. The budesonide particles stabilized by the triblock copolymers had a diameter of ca. 350 nm with entrapment efficiencies of 66-76%. The *In vitro* release profiles of all samples showed an initial burst followed by sustained release. The skin penetration and permeation of budesonide were analyzed by using a Frantz diffusion cell. T 222 and T 252 exhibited higher total permeation amounts, but lower budesonide penetration amounts, than F68. The results suggest that the partitioning of budesonide in each skin layer can be adjusted in order to avoid skin thinning and negative immune response arising from the penetration of budesonide in blood vessels.

Keywords: topical delivery, budesonide, triblock copolymers, permeation, penetration.

Introduction

Over the past few decades, polymeric vehicles have been investigated in drug delivery research as they could control the drug release, specific targeting, and side effects.[1-6] Many polymers have been developed for preparing particles, such as poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(ethylene glycol) (PEG or PEO) and their block copolymers.[7-9] Among these, poly (ethylene oxide)-*block*-poly(propylene oxide)-*block*-poly(ethylene oxide) triblock copolymer (PEO-PPO-PEO), known as Pluronic or Poloxamer, has been extensively studied as a drug delivery vehicle.[10,12-16] However, Pluronic could induce the toxic enhancement of plasma cholesterol and triglycerol in the body due to the non-biodegradable property. To solve the problem, there have been many attempts to replace PPO with biodegradable polymers such as PLA, poly(lactic acid-*co*-glycolic acid)(PLGA) and PCL.[11]

Budesonide ([RS]-1β, 16α 17, 21-tetrahydroxypregna-1, 4-diene-3, 20-dione cyclic 16, 17-acetal with butyraldehyde) (refer to Figure 1) is a corticosteroid used in the treatment of asthma and allergic rhinitis. It has an inhibitory activity against immune related cells and increases the synthesis of anti-inflammatory proteins such as IL-10, lipocrotin-1 and secretory leukocyte protease inhibitor. These anti-inflammatory activity means that Budesonide could be effective treatments in some inflammatory skin disease like atopic dermatitis.[17,18] However, belong to the steroid family, it has some side effects when absorbed into the blood vessels and whole body. In addition, a long period use of Budesonide causes skin atrophy dermatitis.[19,20]

Topical drug carriers such as micelles, liposomes, solid lipid nanoparticles (SLN) and polymeric nanoparticles have been developed to increase skin absorption for topical application of drugs, while causing less damage to the skin's barrier function.[21] Monika and coworkers reported that SLN could be an epidermis targeted drug delivery system, in which

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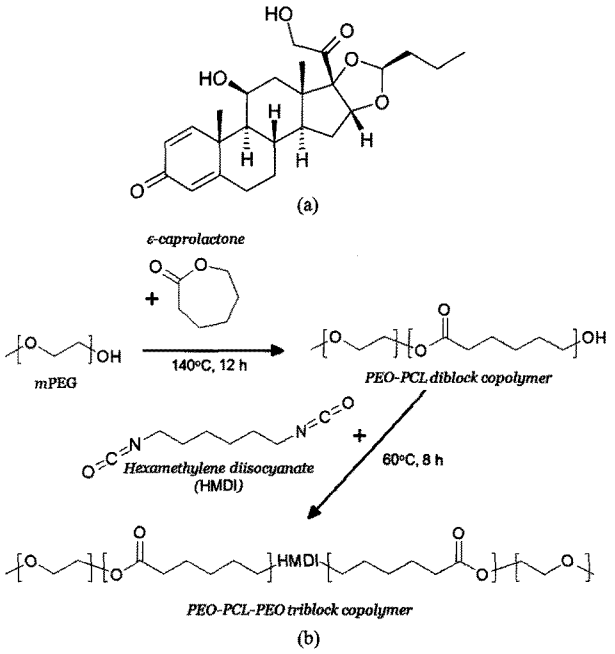

Figure 1. Chemical structures of (a) Budesonide and (b) PEO-PCL-PEO triblock copolymer.

means there is a clear decrease in systemic side effects by delivering the drug to the epidermis but not to the dermis and blood vessels.[22]

To investigate the effects of compositions and molecular weight of triblock copolymers on the topical drug delivery, we prepared two PEO-PCL-PEO triblock copolymers, i.e., T 222 and T 252. For comparative study, commercial F68 (PEO-PPO-PEO) was studied under the same condition. Budesonide emulsion particles were prepared with these triblock copolymers, and the drug entrapment efficiency and drug release profiles were investigated for the controlled drug release. The skin permeation and penetration analyses were carried out with hairless mouse skin in a Franz diffusion cell.

Experimental

Materials. Monomethoxy poly(ethylene glycol) (mPEG, M_n=2,000 g/mol) was purchased from Sigma-Aldrich and used after vacuum drying for 24 h. ε-Caprolactone (ε-CL), stannous octoate ($SnOct_2$), hexamethylene diisocyanate (HMDI), propylene glycol (PPG), anhydrous toluene, diethyl ether, methylene chloride (MC), poly(vinyl alcohol) (PVA, Fully hydrolyzed, M_n=70,000 g/mol), polysorbate-80 (Tween80), and pyrene were purchased from Sigma-Aldrich and used without further purification. F68 (PEO-PPO-PEO, M_n=8,400 g/mol, CMC=48 μM at 25 °C) was purchased from BASF Co. Methanol and acetonitrile were purchased from Merck Co. Pure water (>18.2 MΩ·cm, Millipore Co.) was used throughout the experiment.

Budesonide Emulsion Particles. PEO-PCL-PEO triblock copolymers were synthesized according to the literature.[23] In the synthesis, mPEG was used as a macro-initiator, and HMDI as a linker for PEO-PCL diblock copolymers. To vary the hydrophobicity of copolymers (the ratio of repeating units, [CL]/[EO]=0.11 and 0.31), different amount of ε-CL was added to mPEG. The chemical structure of PEO-PCL-PEO is illustrated in Figure 1. Composition of the copolymer (i.e., [CL]/[EO]) was confirmed by ^{1}H NMR (in $CDCl_3$, AVANCE digital, 400 MHz, Bruker Co.).[23] The number- and weight-average molecular weights (M_n and M_w) were measured by a GPC (OmniSEC, Viscotek Co.). Calibration was carried out using narrow polystyrene standards (EasiCal®, Polymer Laboratories Co.). Basic properties of the copolymer are summarized in Table I.

For comparison, Budesonide emulsions were prepared with three different triblock copolymers, i.e., F68, T 222, and T 252 under the same condition. In order to prepare the oil phase, 2 mg of Budesonide and 20 mg of triblock copolymers (T 222, T 252, or F68) were dissolved in 1 mL of MC. For continuous phase, 80 mg of Tween 80 and 250 mg of PVA were dissolved in 10 mL of water at 50 °C. The oil phase was then poured into the aqueous phase, vigorously stirred at 500 rpm, and ultrasonicated with a tip-type ultrasonic processor (VibraCell 750CX, 20% load) for 2 min. Subsequently, MC was removed from the emulsion in a rotary evaporator at room temperature under a reduced pressure. The average particle size of the emulsions was measured by dynamic light scattering (ELS-8000, Otsuka, Japan).

The amount of Budesonide in the emulsion particles was determined after the solvent extraction of Budesonide with acetonitrile. The measurements were performed 3 times. The entrapment efficiency of Budesonide in nanoparticles

Table I. Properties of the PEO-PCL-PEO Triblock Copolymers and F68[18]

Triblock Copolymers	[CL]/[EO][a] (-)	M_n[a] (g/mol)	M_n[b] (g/mol)	M_w[b] (g/mol)	M_w/M_n (-)	CMC[c] (μM)
T 222 (EO_{45}-CL_{10}-EO_{45})	0.11	6,013	4,356	4,913	1.13	40
T 252 (EO_{45}-CL_{28}-EO_{45})	0.31	8,426	7,939	10,640	1.34	14
F68 (EO_{80}-PO_{30}-EO_{80})	0.19[d]	-	8,400[e]	-	-	48[e]

[a]Determined by measuring the relative areas of the methylene peak at 2.30 ppm (CL unit) and the peak at 3.65 ppm (EO unit) in ^{1}H NMR (in $CDCl_3$) analysis. [b]Determined by GPC (THF, narrow polystyrene standard of 580~7,500,000 g/mol). [c]Determined by pyrene-UV absorption analyses at 372 nm wavelength. [d]The ratio of propylene oxide to ethylene oxide unit, [PO]/[EO]. [e]Referred from the website, http://www2.basf.us/performancechemical/bcperfpluronic_ grid .html.

Table II. Grading Scores Used in the Draize Method

Description	Score
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formations (injuries in depth)	4
Edema	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edge of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4

was calculated as below:

$$\text{Entrapment efficiency}(\%) = \frac{\text{Amount of measured Budesonide (mg)}}{\text{Amount of total Budesonide (mg)}} \times 100 \quad (1)$$

Skin Irritation of PEO-PCL-PEO. In order to investigate the skin irritation of PEO-PCL-PEO triblock copolymer itself, Draize method was performed with New Zealand White Rabbits (Sam:NZW, Samtako Bio, Korea) according to the Korean Good Laboratory Practice (KGLP). After the 24 h application of copolymer onto the skin, degree of erythema (redness value) and edema of the skin surface were classified according to the visual score as described in Table II. The primary irritation index (P.I.I.) was obtained by calculating the average scores of the erythema and edema in conformity with the Draize method.

Quantitative Analysis of Budesonide. The concentration of Budesonide was measured in a high performance liquid chromatography (HPLC, Waters Co., USA) at ambient temperature. The HPLC system was equipped with an analytical column (Xterra C18, 150 mm × 3.9 mm, 300 E, Waters Co., USA) at the wavelength of 210 nm. The 50% methanol aqueous solution was used as a mobile phase and its flow rate was 1 mL/min. There was a linear relationship in the range from 0.5 to 100 μg/mL ($y = 88034x - 80.632$, $R^2 = 0.99$).

In vitro release of Budesonide from the emulsion particles was observed by using the HPLC for 72 h. The Budesonide emulsion was enclosed in a dialysis tubing (GeBA, cellulose membrane, molecular cut-off = 30 kDa) and incubated in a 50 mL of PBS solution (50 μM phosphate buffer, pH = 7.4, 0.5 wt% sodium dodecyl sulfate). While sampling, the release medium was retained with the constant volume of fresh PBS solution at a regular time-interval.

Critical Micelle Concentration. Critical micelle concentrations (CMCs) were determined from the UV-visible absorption intensity of pyrene ($[Py]_w^{sat} = 10^{-7}$ M at 25 °C) as a function of the block copolymer concentration.[24]

Topical Delivery of Budesonide. We investigated the topical delivery of Budesonide from the emulsion particles through hairless mouse skin in the modified Franz diffusion cell. The skin penetration (or permeation) of Budesonide supersaturated solutions prepared with propylene glycol (PPG) or pure water was also carried out for comparative study of enhancement effect. A section of mouse skin was mounted in between donor and receptor chambers. The actual permeation area was 2.14 cm^2. The temperature of the receptor chamber was maintained at 37±0.5 °C.

In the case of penetration analysis, 1 mL of sample was taken from the receptor solution (12 mL, 50 μM PBS solution) at a designated time interval for 12 h and the penetrated amount of Budesonide was measured by using the HPLC. The volume of each sample was replaced with the same volume of PBS solution.

In the case of permeation analysis, the skin was detached from the diffusion cell and was rinsed with methanol to remove Budesonide residue. A cellophane adhesive tape (CuDerm, USA) was used to strip the stratum corneum layer completely (more than 10 times) and Budesonide was extracted by methanol followed by the filtration with a 0.22 μm membrane filter. The rest of the skin, i.e., viable epidermis, was homogenized and Budesonide was extracted by methanol. The permeation amount of Budesonide in each layer was determined by using the HPLC. The data were measured 5 times, and the average value and standard deviation were recorded. In order to confirm the permeation of triblock copolymer, confocal laser scanning microscopy was performed with the hairless mouse skin after diffusion cell analysis. In this case, 0.1 wt% aqueous solution of fluorescein isothiocyanate (FITC)-labeled T 252 was added into the donor chamber and kept for 12 h at 32 °C.[25] After that, the skin was taken from the cell and embedded in OCT compound (Optimal Cutting Temperature, Tissue-Tak®) to prepare a cryostat microtome sample. The fluorescence image of the FITC-labeled T 252 in the mouse skin was observed by CLSM technique at 488 nm wavelength.

Results and Discussion

Characteristics of PEO-PCL-PEO. PEO-PCL-PEO triblock copolymers were successfully synthesized by the ring opening polymerization with biodegradable ε-CL and biocompatible mPEG. As shown in Table I, the experimental values of M_n of the PEO-PCL-PEO from ^{1}H NMR agreed well with the expected values. The values of [CL]/[EO] obtained from ^{1}H NMR were comparable to the calculated values based on the formula. The CMC of T 252 was lower

than that of T 2-2-2 due to the high ratio of [CL]/[EO]. From the results, it was found that the CMC of the triblock copolymers decreased from 40 to 14 μM as the PCL chain length increased. The M_n value of F68 was comparable to T 252; however, [CL]/[EO] value was in between those of T 222 and T 252, and the CMC was higher than that of T 222.

Skin Irritation of PEO-PCL-PEO. In order to utilize the PEO-PCL-PEO block copolymers in topical delivery, they should be compatible with the skin without any side reactions. To evaluate the compatibility, we investigated skin irritation of the block copolymers and the results were listed in Table III. After the 24 h application negligible erythema was observed only in the abraded skin, but it disappeared after 72 h. Nothing was observed in the intact skin. In both skins, P.I.I. values were evaluated as 0.25, indicating no irritations at all.

Particle Sizes of Budesonide Emulsion. It has been well known that the size of drug emulsion significantly affects the rate of skin penetration.[26] The average diameter of the Budesonide emulsion stabilized by F68 or PEO-PCL-PEO triblock copolymers are plotted in Figure 2. In this work, the large amounts of PVA (250 mg) and Tween 80 (80 mg) were used as main stabilizer. For this reason, we expect that the emulsion size would not be changed even if the amphiphilicity and molecular weight of triblock copolymers are different. In the cases of T 222 and T 252, the diameters of Budesonide emulsions were almost the same, 350 nm and 348 nm, respectively.

Table III. Primary Irritation Index of PEO-PCL-PEO Triblock Copolymer

Triblock Copolymers			P.I.I.
T 222	Applied site	Abraded skin	0.25
		Intact skin	0.00
	Control site	Abraded skin	0.25
		Intact skin	0.00
T 252	Applied site	Abraded skin	0.25
		Intact skin	0.00
	Control site	Abraded skin	0.25
		Intact skin	0.00

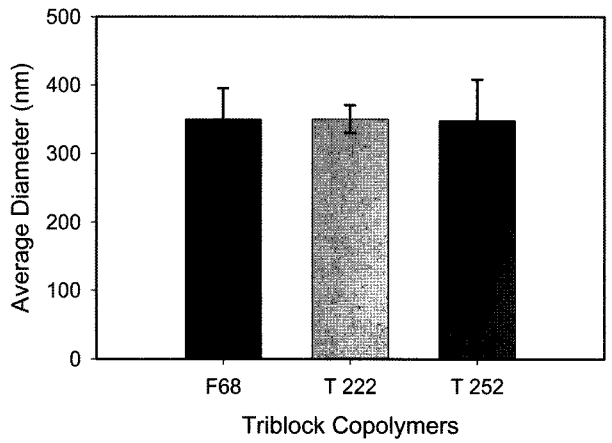

Figure 2. The average particle size of the Budesonide emulsions stabilized by F68 or PEO-PCL-PEO triblock copolymers (n=3).

Drug Entrapment Efficiency. The entrapment efficiencies of Budesonide by triblock copolymers are shown in Figure 3. In general, the entrapment efficiency of hydrophobic drug stabilized by amphiphilic copolymers is dependent on the size of emulsion, concentration and solubilization ability of the copolymer.[27,28] In this work, the similar particle sizes were obtained regardless of triblock copolymers. As shown in Figure 3, however, the highest efficiency (76%) is observed in T 252 due to its hydrophobic character, compared with F68 (66%) and T 222 (71%). The results can be rationalized by two governing factors, CMC and [CL](or [PO])/[EO]. For T 252 and T 222, the CMC values are 14 and 40 μM, respectively, and which are lower than that of F68. The lower CMC, the more hydrophobic drug can be solubilized. However, the ratios of [CL](or [PO])/[EO] are 0.11, 0.19, and 0.31 for T 222, F68, and T 252, respectively, and which does not coincide with the previous results. We could not find the reason for this inconsistency, since the interaction between Budesonide and CL or PO units are not clear.

***In-vitro* Release of Budesonide.** The *in vitro* release profiles of Budesonide emulsion stabilized by triblock copolymers are presented in Figure 4. The results were plotted in terms of the relative and cumulative amount (%) of Budesonide in the PBS solution versus release time for 72 h. All samples showed the similar release pattern having an initial burst followed by a sustained release. The most significant initial burst was observed in T 222 among the copolymers. In all samples, more than 50% of Budesonide was released from the emulsion particles within the first 3 h (refer to the inset of Figure 4). For T 252, the initial slope of release

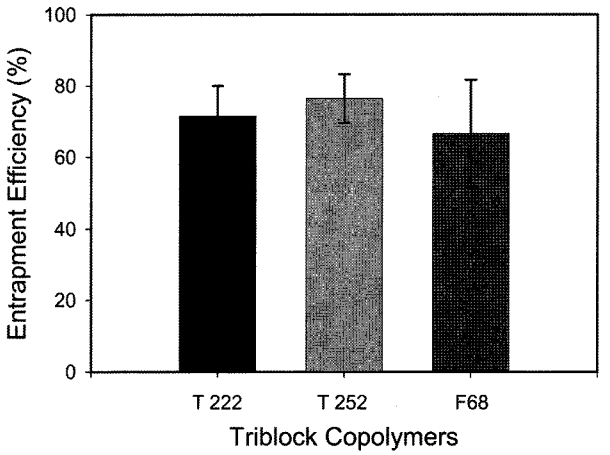

Figure 3. The entrapment efficiency of Budesonide by F68 or PEO-PCL-PEO triblock copolymers (n=3).

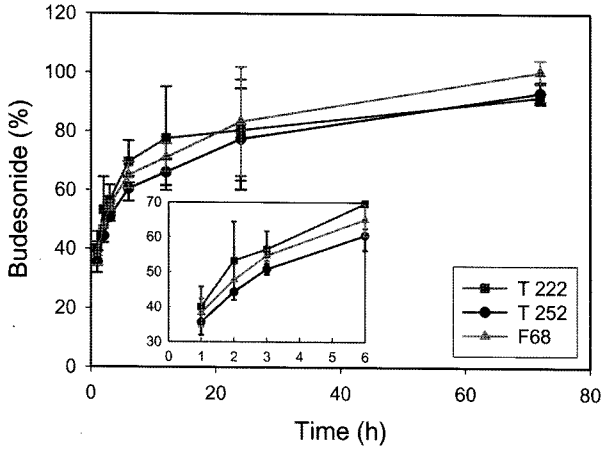

Figure 4. Release profile of Budesonide from the emulsion particles prepared with F68, T 222, and T 252 in PBS solution at 37±0.5 °C (n=3). The initial Budesonide concentration was 2 mg/mL. The inset shows the initial release profiles.

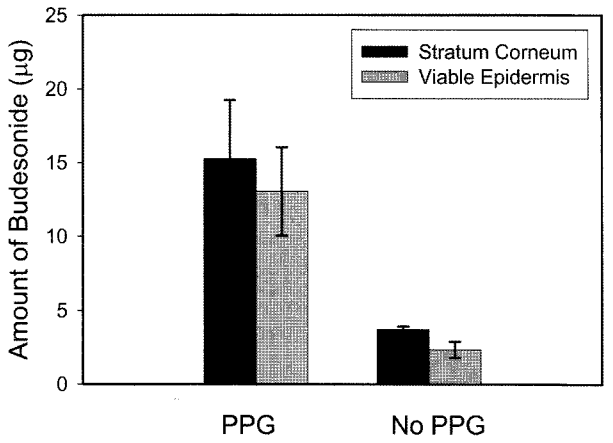

Figure 6. The cumulative permeation amount of Budesonide taken from the each layer of the hairless mouse skin after 12 h at 37±0.5 °C (n=3). PPG or pure water was used as an enhancer and the initial supersaturation concentrations of Budesonide in PPG and pure water were 20 and 5 mg/mL, respectively.

curve was slightly lower than those of F68 and T 222, and in which such a minimal initial burst may be closely attributed to the stronger hydrophobic interaction between PCL domain of T 252 and Budesonide.

Topical Delivery of Budesonide. PPG can be often used as an additive to enhance the permeation of hydrophobic drugs. In order to investigate the enhanced topical delivery by triblock copolymers, the skin permeation and penetration studies were carried out with the supersaturated Budesonide in PPG or pure water prior to the topical delivery with Budesonide emulsions. As shown in Figure 5, it was observed that the topical delivery of Budesonide itself could be possible in the presence of PPG or pure water without any chemical enhancers. The initial supersaturation concentrations of

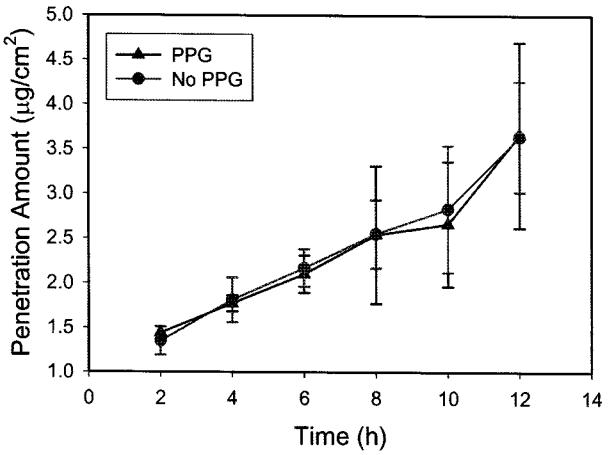

Figure 5. The cumulative penetration amount of Budesonide dissolved in PPG or pure water through the hairless mouse skin for 12 h at 37±0.5 °C. The initial supersaturation concentrations of Budesonide in PPG and pure water were 20 and 5 mg/mL, respectively.

Budesonide in PPG and pure water were 20 and 5 mg/mL, respectively. The flux of Budesonide in each medium was calculated as 0.50 and 0.40 mg/cm^2·h, respectively. Figure 6 shows the cumulative permeation amount of Budesonide in the stratum corneum and viable epidermis with different medium, i.e., PPG and pure water. The cumulative penetration amount of Budesonide through the hairless mouse skin showed no significant difference between PPG and pure water; however, it was found that the permeation of Budesonide in the skin layer in the presence of PPG showed a higher concentration compared with to those of pure water. The permeation amounts of Budesonide in the stratum corneum layer were higher than those in the viable epidermis regardless of the medium. PPG could enhance the permeation of Budesonide due to the hydrophobic nature of Budesonide.

The cumulative permeation amount of Budesonide in the stratum corneum and viable epidermis is plotted in Figure 7. It has been well-known that the stratum corneum consists of hydrophilic "bricks" (bundles of keratins) and hydrophobic "mortar" (mixed lamellar structure of ceramides, cholesterols, fatty acids, etc.). Therefore, amphiphilic nature should be required for effective transdermal penetration through the stratum corneum. In our system, an intercellular route of the mortar seems more favorable than a transcellular route of the bricks, since the molecular weights of block copolymer are higher than 500 Da. Therefore, the hydrophile-lipophophile balance (HLB) of block copolymers is important.

As seen in Figure 7, the total permeation amount of Budesonide was 5.59, 5.90, and 5.95 μg for F68, T 222, and T 252, respectively. The total amounts were similar in T 222 and T 252; however, the permeation amount of F68 was about 0.36 μg lower than T 252. In the stratum corneum layer, T 222 and F68 showed ca. 3.7 μg of Budesonide. For

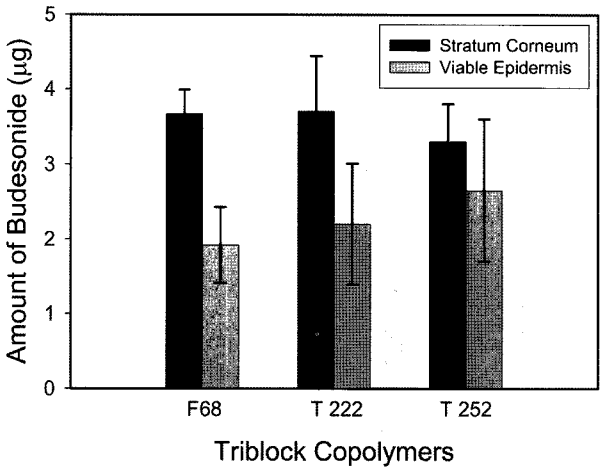

Figure 7. The cumulative permeation amount of Budesonide from emulsions prepared with F68, T 222, and T 252 in through each layer of the mouse skin at 37±0.5 °C for 12 h (n=5). The initial concentration of Budesonide was 2 mg/mL.

T 252, however, the amount of Budesonide was 3.3 μg, which was lower than those of T 222 and F68. The amount of Budesonide in the viable epidermis increased from F68, T 222, to T 252. The results imply that the partitioning of Budesonide in each layer can be adjusted in terms of the HLB of triblock copolymers even though the representative HLB value of each layer has not been known yet. From the total amount of Budesonide permeated, we can expect that F68 can penetrate through the stratum corneum and viable epidermis layer.

The cumulative penetration amount of Budesonide is shown in Figure 8. At 12 h, one can see that the penetration amount of Budesonide stabilized with F68 is approximately 0.4 μg higher than those with T 222 and T 252. This result is in good agreement with Figure 7. This may be attributed

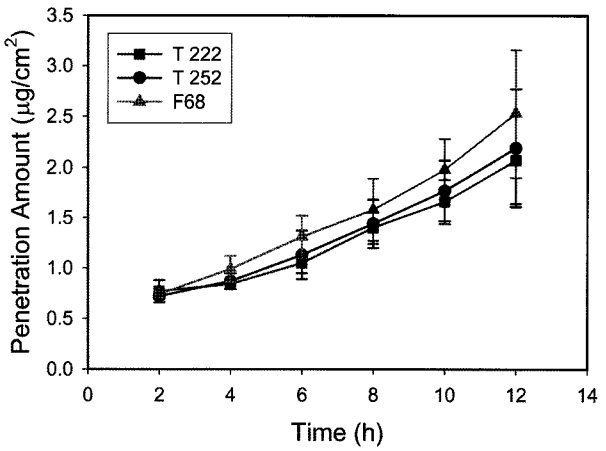

Figure 8. The cumulative penetration amount of Budesonide from the Budesonide emulsion particles prepared with F68, T 222, and T 252 through the hairless mouse skin for 12 h at 37± 0.5 °C (n=5). The initial concentration of Budesonide was 2 mg/mL.

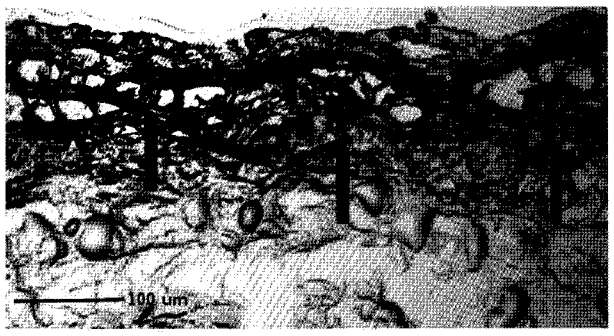

Figure 9. A CLSM image of the cross-sectional mouse skin treated with FITC-labeled T 252 triblock copolymer. The uppermost layer with green color indicates the stratum corneum (thickness ~10 μm). The arrows indicate the FITC-labeled T 252 of green color.

to the less hydrophilic nature of T 222 and T 252 as compared with F68 (refer to Table I, CMC value). We could not get any concrete evidences for the penetration routes; however, it would be suggested that most of the Budesonide would penetrate through hair follicles or stratum corneum with adsorption of the particles stabilized by the triblock copolymers, since the particle sizes are too big to penetrate directly through the intercellular route of stratum corneum. In addition, the relative penetration amounts of Budesonide with the triblock copolymers are higher than those with PPG or pure water because the initial concentration of Budesonide in the emulsion state was 2 mg/mL.

In order to confirm the permeation of T 252 through stratum corneum, a CLSM study was carried out with FITC-labeled T 252 and the cross-sectional image of the hairless mouse skin was illustrated in Figure 9.[25] As seen in Figure 9, most of FITC-labeled T 252 was located in the layer of stratum corneum. However, some of FITC-labeled T 252 was observed in the viable epidermis layer. From the data, it can be suggested that that the triblock copolymers having appropriate amphiphilicity and molecular weight can permeate through the stratum corneum and enhance the permeation of hydrophobic drugs via the stratum corneum. More detailed study on the design and synthesis of triblock copolymers is ongoing for better permeation efficacy at the moment.

Conclusions

Budesonide has a higher affinity for glucocorticoid receptors and shows better topical anti-inflammatory results than cortisol. Being a steroid, however, it has several side effects such as, skin thinning and negative immune responses. Therefore, we explored the potential of amphiphilic triblock copolymers to permeate and localize Budesonide in the epidermis layer. The skin irritation of PEO-PCL-PEO triblock copolymers was evaluated and the results were found to be positive without any skin problems. The drug entrapment efficiency was from 66% to 76%, which was strongly affected

by the hydrophobic nature of the copolymers, regardless of particle size. T 252 showed less significant initial burst than F68 or T 222 and it could enhance the permeation of Budesonide in the viable epidermis layer. These results suggest that Budesonide particles prepared with T 252 can be utilized in the treatment of atopic dermatitis with reduced side effects of Budesonide absorbed in the blood vessels.

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