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Evaluation of the permeability of hair growing ingredient encapsulated PLGA nanospheres to hair follicles and their hair growing effects

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Abstract—This paper describes the process of encapsulating hair growing ingredients in the PLGA nanospheres by emulsion solvent diffusion method and investigates the feasibility of using the PLGA nanospheres as the DDS (Drug delivery System) carriers for delivering various hair growing ingredients to hair follicles. In-vitro and in-vivo tests were conducted to verify the performances of encapsulated PLGA nanospheres with three different hair growing ingredients. In the in-vitro tests, the scalp-pore permeability of hair growing ingredient encapsulated PLGA nanospheres (dispersed in the PBS solution) was examined using human scalp biopsies in a modified Bronaugh diffusion chamber in comparison to that of the control samples containing the hair growing ingredient in the PBS solution. Furthermore, the hair growing effect of the encapsulated PLGA nanospheres was evaluated with the C3H mice in the in-vivo tests. By observing the fluorescence intensity of the ingredients, as shown in the cross-section photographs of the human scalp biopsies, it was found that the dispersion liquids containing hair growing ingredient encapsulated PLGA nanospheres exerted a scalp-pore permeability 2.0- to 2.5-fold more marked than that of the control samples. Also, the hair growing activities were enhanced by using the encapsulated PLGA nanospheres, which transformed the hair growth cycle from the resting phase to the growing phase. As a result, the degree of hair growth was improved significantly. These results suggested that the PLGA nanosphere can be a new DDS carrier for delivering hair growing ingredients and drugs to the hair follicles.

Since people become more self-conscious on what others think, young men and working women are concerned of their thinning hair and hair loss problems as much as middle-age and elderly men. It is said that about 23 million Japanese are anxious about this problem.[1] In addition, diet, westernization, and greater stresses due to the changes in the social environment further add to this trend.

When the hair follicle cycle repeats, anagen becomes progressively shorter; follicles miniaturize; and, thick long hairs gradually change to thin short hairs. Ultimately, the number of follicles that no longer make the transition from telogen to anagen increases and leads to fewer soft hairs. This most often observed type of hair loss is called male baldness. There are two main treatments for it. One is to suppress the effect of male hormones by inhibiting the function of 5α reductase, a conversion enzyme that changes testosterone to dehydrotestosterone in the hair papilla cells of the bulb at the bottom of the hair follicle. The other is to improve the supply of energy to the hair papilla, which is deficient due to the poor blood circulation.

Recently researchers divided the hair loss mechanism on the molecular and genetic levels,[2–6] and discovered several effective ingredients which could stimulate hair

Keywords: PLGA nanospheres; Percutaneous hair growing ingredient delivery; Modified Bronaugh diffusion chamber; C3H mouse; Hair growing effect.
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growth by facilitating proliferation of the follicle epithelial cells, or by suppressing growth inhibition factors. These ingredients are being used as hair growing tonics. As evident from their working mechanisms, these hair growing ingredients are more effective if they act directly on the hair papillae at the bases of follicles. Thus, the performance of a hair growing tonic is determined by how well it can be delivered to the hair papillae.

Because the drug permeability through the keratin layer is very low, hair growing ingredients are delivered to the papillae mainly via the hair pores and follicles. The hair pore entrances of people having hair thinning problems are often obstructed by horny plugs and sebum plugs. The hair growing tonics now available on market consist of active ingredients dissolved in aqueous alcohol solutions, but it is difficult for them to reach the papillae through the blocked pores. As a result, their hair restoring effectiveness does not live up to the expectations of consumers. It is hoped to improve the performance of hair growing tonics by using new drug delivery techniques.

In this regard, researchers have been working on the methods applying microparticles[8] and nanoparticles[7] to improve the drug permeation to hair follicles. In particular, Lademann et al. recently found that dye-containing nanoparticles (320 nm in diameter) had better hair pore permeation than a liquid dye dispersion (dye in non-particle form) when they were massaged on the porcine skin in an in vitro experiment; and, in an in vivo evaluation using human skin, the particles had excellent retention in the hair pores.[7]

Similarly, the authors used extracted human skin to evaluate the delivery ability of vitamin C derivative (VC-IP) encapsulated PLGA nanospheres in an aqueous dispersion.[9] These biocompatible and biodegradable PLGA nanoparticles, having an average particle diameter of 215 nm, were prepared using the emulsion solvent diffusion (ESD) method. Photomicrographs showed that these PLGA nanospheres well permeated into the hair pores and delivered 10 times more reduced form vitamin C to the dermis than VC-IP liquid (O/W emulsion) 4 h after applying them on the skin samples. Furthermore, the vitamin C was still detectable in the dermis even 48 h after applying the VC-IP encapsulated PLGA nanospheres; in comparison, it lasted only 7 h with the VC-IP liquid. This was because of the sustained release characteristics of PLGA nanospheres.

Following the same logic, it is perceivable that the performance of hair growing ingredients can be significantly improved with longer effects if they can be encapsulated in the PLGA nanospheres.

To validate the concept, this study encapsulated commercial hair growing ingredients in the PLGA nanospheres and applied their liquid dispersion on the extracted human scalp skin to evaluate their permeation to the hair follicles. In addition, a hair growth experiment with C3H mice was also carried out to investigate the hair growth effect of encapsulated PLGA nanospheres.

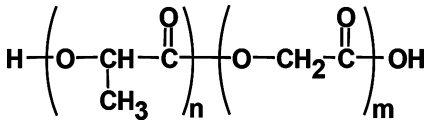


Figure 1. Structural formula of PLGA.

Co-polymer PLGA with a 75:25 ratio of lactic acid to glycolic acid and a molecular weight of 20,000 (Wako Pure Chemical Industries, PLGA7520) was used in the experiments, as shown in Figure 1. And, the model hair growing ingredients encapsulated in PLGA nanospheres were:

1. Hinokitiol (Takasago International Corporation, anti-bacterial effect).
2. Glycyrrhetinic acid (ALPS Pharmaceutical Ind. Co., Ltd, anti-inflammatory effect).
3. 6-Benzylaminopurine (Tokyo Chemical Industry Co., Ltd, hair growth promotion effect).

In addition, polyvinyl alcohol (Kuraray Co., Ltd, poval 403) was used as the dispersion stabilizer during the crystallization of PLGA nanospheres.

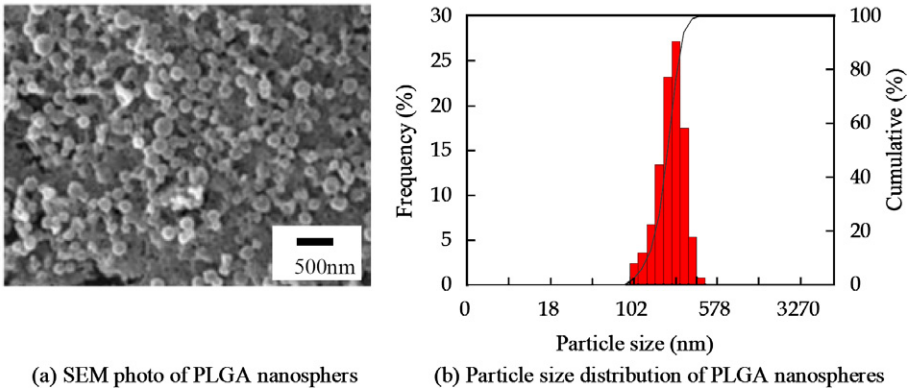
We prepared PLGA nanospheres that encapsulate hair growing ingredients with the Emulsion Solvent Diffusion Method.[10] The three hair growing ingredients to be encapsulated in PLGA were prepared separately as described in Footnote.[†]

Control PLGA nanospheres that did not contain the hair growing ingredients were prepared as described in Footnote.[‡]

As an example of nanospheres produced with this method, Figure 2a shows a photomicrograph of Hinokitiol encapsulated PLGA nanospheres, taken by a scanning electron microscope (Hitachi, S-3500N); and Figure 2b

[†] Each of them was dissolved in a 10 ml ethanol solution with the amount of 0.05 g Hinokitiol, 0.1 g glycyrrhetinic acid, and 0.1 g 6-benzylaminopurine, respectively; and then mixed with a 20 ml acetone solution containing 1.0 g of PLGA. After that, the solution mixture was dropped into an aqueous PVA solution at a constant rate of 4 ml/min. The PVA solution had 2 wt% solid concentrations and was stirred at 400 rpm, 40 °C during the operation. Acetone/ethanol and water mutually diffuse into each other and, through a self-emulsification action, the PLGA precipitates to form PLGA nanoemulsion droplets containing the hair growing ingredients in the aqueous PVA solution. Organic solvents were then removed by vacuum evaporation at 40 °C with a stir speed of 100 rpm.The PLGA nanosphere suspension obtained was centrifuged (at 41,000*g* and −20 °C for 20 min) to cause sedimentation of the nanospheres. After removing the supernatant, purified water was added to mix with the sediment. The wet mixture was then centrifuged again to remove the excess PVA that could not adsorb on the surfaces of nanospheres. Freeze drying was at last used to produce dry powders from the nanosphere suspension after two cycles of water washing and centrifugation.

[‡] After mixing 10 ml of ethanol into a 20 ml acetone solution containing 1.0 g of PLGA, the solution mixture was introduced into the aqueous PVA solution to make PLGA nanospheres in the same way as described in Footnote †.



(a) SEM photo of PLGA nanosphers (b) Particle size distribution of PLGA nanospheres

Figure 2. Photo of Hinokitiol loaded PLGA nanospheres and their particle size distribution.

shows the particle size distribution of the nanospheres dispersed in the pure water measured by a dynamic light scattering instrument (Nikkiso Co., Ltd, MICROTRAC UPA 150). Furthermore, the average particle diameters of the four PLGA nanospheres prepared in this experiment ranging from 182 to 210 nm are listed in Table 1. They generally had similar profile in the particle size distribution.

The encapsulation ratios of glycyrrhetinic acid and Hinokitiol in the PLGA nanospheres were quantified by using high-speed liquid chromatography (Shimadzu: UV detector SPD-20A, Inertsil ODS-3 column, 254 nm detection wavelength, acetonitrile/water 50:50 moving bed (pH 2.6) (glycyrrhetinic acid), acetonitrile/water 40:60 moving bed (pH 3.0) (Hinokitiol)). On the other hand, the 6-benzylaminopurine encapsulation rate was quantified with a UV–visible spectrophotometer (JASCO Corporation: V-530, measurement wavelength 270 nm).

First of all, permeation of hair growing ingredient encapsulated PLGA nanospheres to follicles was evaluated with the modified Bronaugh diffusion chamber[11] developed by Miwa et al. as shown in Figure 3.

Extracted human scalp skin, obtained under the dictates of informed consent and with the consent of a University Ethics Committee, was divided into long thin strips. Assuming the skin strips are representative samples from the same scalp location, we organ-cultured them in the diffusion chamber; applied a liquid dispersion of PLGA nanospheres containing the hair growing ingredients; and allowed the dispersion to naturally diffuse throughout the interior of the skin pieces.

Table 1. Properties of hair growing ingredient encapsulated PLGA nanospheres

	Type of hair growing ingredient in the PLGA nanospheres	Average particle of nanospheres size water (nm)	Content of hair growing ingredient in the nanosphere (%)
1	— (None)	196	—
2	Hinokitiol	205	3.8
3	Glycyrrhetinic acid	210	2.5
4	6-Benzyl aminopurine	182	4.7

Because different types of test skin (e.g., real human skin, artificial skin, or animal skin) and applying pressure during the evaluation have little influences on the investigation results with this test method, the in vitro evaluations can be very close to clinical tests.[11] The detailed scalp sample preparation and skin permeation evaluation procedure are described in Footnote.[§]

The hair growth effect of PLGA nanospheres on the C3H mice was the next to evaluate. In this case, a barber's clipper and a shaver were used to shave the backs

[§] The scalp sample preparation and skin permeation evaluation were carried out as follows. (a) Scalp sample was extracted from the forehead of a 40-year-old woman and its subcutaneous tissue was removed until only about 1 mm left. The scalp sample was then vertically divided into eight strips (3×5 mm). Cut edges of each sample strip were covered with biocompatible TG polymer to prevent damage. (b) The sample strips were placed inside a modified Bronaugh diffusion chamber, which had been disinfected with γ radiation. (c) After putting the chamber on a 24-well microplate, serum-free DHEM culture (Dulbecco's modified Eagle medium) was introduced to submerge the skin sample and supplied nutrients underneath. The chamber was ventilated with 5% CO_2 to maintain the culture medium at a pH value of 7.2–7.3. Relative humidity in the chamber was 95–100%; and, the chamber was kept dark. (d) Test liquids (pH 7.4) were made by dispersing the Hinokitiol-encapsulated PLGA nanospheres and the glycyrrhetinic acid-encapsulated PLGA nanospheres (as prepared in 2.2.) in a phosphoric acid buffer solution (PBS), respectively. Controls were PBS solutions containing either Hinokitiol or glycyrrhetinic acid. Test liquids were applied onto the skin sides of the samples at 20.0 $\mu L/cm^2$ and naturally diffused to the epidermis. Concentration of Hinokitiol or glycyrrhetinic acid in each test liquid was 0.036 wt%. Because both Hinokitiol and glycyrrhetinic acid had low solubility, we prepared and used solutions at a concentration lower than their saturation, so that material dissolution would not limit the permeation rate in question. The PBS solution is a solvent with poor permeability. It was used to emphasize the permeability of PLGA nanospheres to follicles in human scalp skin tests. (e) The scalp skin samples were removed 4 h after applying test liquids; and, a cryostat was used to cut vertically 4-μm-thick cross-sections of follicles. (f) These cross-sections were photographed with a confocal laser fluorescent scanning microscope (Bio-Rad, MRC-600, excitation wavelength 480 nm). The fluorescent intensity of hair growing ingredients distributed from the scalp surface toward the follicle bases was measured, and the integrated distribution amount was evaluated.

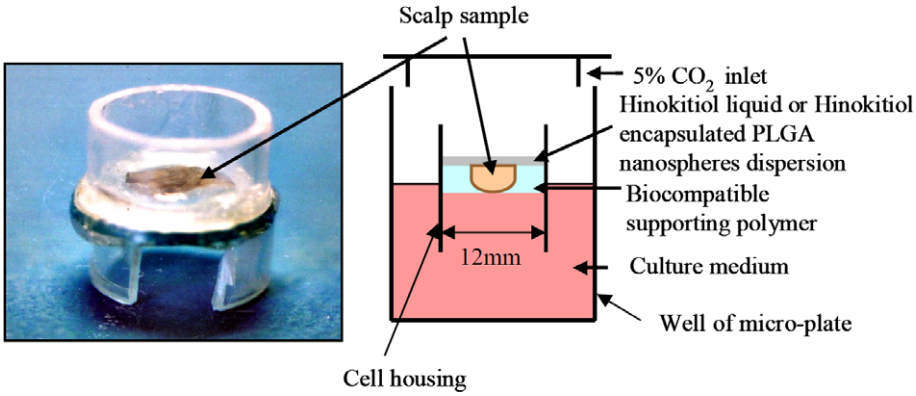


Figure 3. Modified Bronaugh diffusion chamber.

of C3H mice to prepare the testing area. Also, ethanol solution was used instead of PBS solution, because the PBS had a very poor wettability on the oily mouse skin, which caused the test liquid to run off from the edge of the shaved area. During the tests, while inducing anagen, PLGA nanosphere dispersions were applied on the testing area to observe its fur growing effect.

To make the test more precise, factors causing variations in the fur growth such as species, age, and sex were standardized. Also, test conditions including culturing conditions (number of mice per cage, food) and how the mice were being shaved were determined based on preliminary studies. The procedure for preparing the mice for experiments and applying the test liquids are described in Footnote.[¶]

Six different test liquids were used in the experiments (Runs 2–7) as indicated in Table 2, and 100 μL of each test liquid was applied once a day on the mouse for 14 consecutive days. Specifically, the test run conditions are as follows.

1. Run 1: Blank test, in which nothing was applied.
2. Run 2: Applied only the 30% ethanol aqueous solution, which was the main solvent used in the rest of test liquids.
3. Run 3: Applied the 30% ethanol dispersion of empty PLGA nanospheres (0.5 wt% PLGA nanosphere concentration), which was the control for hair growing ingredients encapsulated in nanospheres.
4. Runs 4 and 5: Compared the fur growth effect of Hinokitiol by itself and encapsulated in the PLGA nanospheres. The amount of Hinokitiol applied was kept 19.0 μg for both test runs. The concentration of PLGA nanospheres administered in this case was equivalent to 0.5 wt%. The solvent chosen was 30% aqueous ethanol solution, which was found capable of uniformly dispersing nanospheres in the preliminary tests and it was the lowest concentration that could completely dissolve Hinokitiol.
5. Runs 6 and 7: Investigated the fur growth effect of 6-benzylaminopurine, the hair growing ingredient available over-the-counter, by itself and encapsulated in the PLGA nanospheres. The amount of 6-benzylaminopurine applied was kept 23.5 μg for both test runs. The concentration of PLGA nanospheres administered in this case was equivalent to 0.5 wt%. The solvent chosen for dispersing PLGA nanospheres was 30% ethanol; and the solvent for 6-benzylaminopurine was a 35% aqueous ethanol solution, which was the lowest ethanol concentration that could dissolve the 6-benzylaminopurine.

Table 2. Administered materials to C3H mice

Run	Type of hair growing ingredients in the PLGA nanospheres	Type of hair growing ingredients in solvent	Content of ethanol in water (%)
1	—	—	—
2	—	—	30
3	— (None)	—	30
4	—	Hinokitiol	30
5	Hinokitiol	—	30
6	—	6-Benzyl aminopurine	35
7	6-Benzyl aminopurine	—	30

During the experiments, test liquids with the PLGA nanospheres (Runs 3, 5, and 7) were prepared every 7 days and kept refrigerated to minimize PLGA hydrolysis.

Fifteen days after starting the tests, the mice were immobilized by inhalation anesthesia; and their shaved areas were photographed. Visual evaluation was made to judge the extent of fur re-growth on each mouse according to five levels, that is, 0–4 (0: no blackening at all; 1: blackening visible; 2: fur starting to grow; 3 and 4: fur growth in two levels depending on the extent of growth). The statistic score (mean ± SD) of the mice in each group (n = 6–9) was calculated and used as an index to determine the fur growth extent.

[¶] After 1 week of acclimatization, a barber's clipper was used to cut the fur from the lower back areas (2 × 4 cm) of C3H/HeN Slc mice (SHIMIZU Laboratory Supplies Co. Ltd, males, 8 weeks old, n = 6–9 for each sample, kept in same cage). Special care was taken for not injuring or irritating their skins. One day later, a shaver was used to shave the areas and expose the skin. After that, 100 μL test liquid was applied to each mouse's shaved area with a pipette and thinly spread the liquid over the entire area using the side of the pipette for not irritating their skins.

Figure 4 shows cross-section photographs of follicles taken 4 h after applying the Hinokitiol solution (Fig. 4a), and the liquid dispersion containing Hinokitiol encapsulated PLGA nanospheres (Fig. 4b). The photos on the left were the graphs of fluorescence intensity from Hinokitiol's self-luminescence when it was vertically scanned. This graph revealed that a very weak fluorescence was concentrated about 1.0 mm from the vicinity of the scalp surface when applying the Hinokitiol solution. It indicated that Hinokitiol was not sufficient in the follicles. On the other hand, when applying the liquid dispersion containing encapsulated PLGA nanospheres with the same amount of Hinokitiol, very strong fluorescence deep into the follicles, 0.66–1.39 mm from the scalp surface, was observed. Furthermore, comparing total area covered under the fluorescence intensity profile, the liquid dispersion containing Hinokitiol encapsulated PLGA nanospheres was 2.5 times of the Hinokitiol solution.

Normally hair follicles are not formed vertically in relation to the scalp surface as shown in the photograph, but rather slanted. It was caused by the vertical cutting used in preparing the skin strips from the scalp sample. In the strip sample preparation, it was difficult to create sections that accurately captured the papillae, which were situated about 2.0 mm from the scalp surface and controlled the hair growth.

The fluorescence scanning micrographs taken after applying PLGA nanospheres revealed strong Hinokitiol fluorescence down to a depth of about 1.2 mm from the scalp surface, which suggested that high concentration of Hinokitiol could be at places down to the papillae. And, its actual integrated fluorescence intensity area would have been even higher.

Although not shown here, when applying PLGA nanospheres that contain glycyrrhetinic acid, we also observed 100% more intense fluorescence from the scalp surface down to the follicles than using the glycyrrhetinic acid solution.

These results demonstrated that applying PLGA nanospheres encapsulation technique could deliver more Hinokitiol and glycyrrhetinic acid to the follicles.

PLGA nanospheres are sufficiently small and can deliver hair growing ingredients to follicles via hair pores, which is about 2 μm in diameter. It is also speculated that because the surfaces of PLGA nanospheres can deform and swell due to their surrounding moistures, it helped them to diffuse to follicles. In addition, it is speculated that liquid dispersion of ingredient encapsulated PLGA nanospheres had a lower surface tension than the

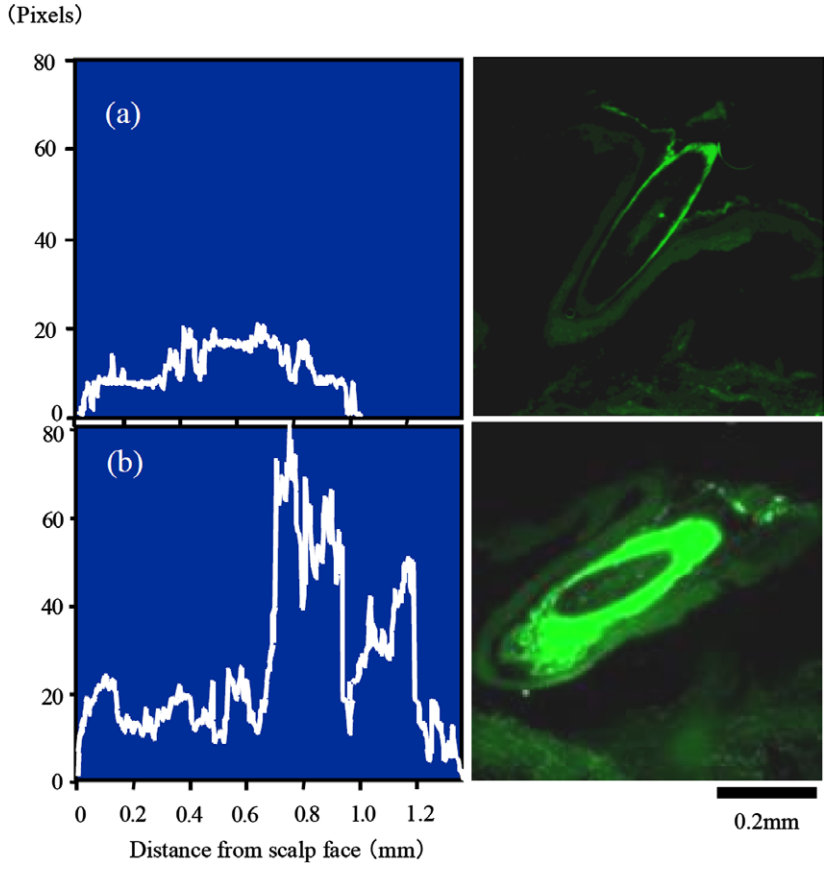


Figure 4. Cross-section photographs of human scalp biopsies and follicles of a 40-year-old woman taken 4 h after applying (a) the hinokitiol solution; and (b) the liquid dispersion of Hinokitiol encapsulated PLGA nanospheres.

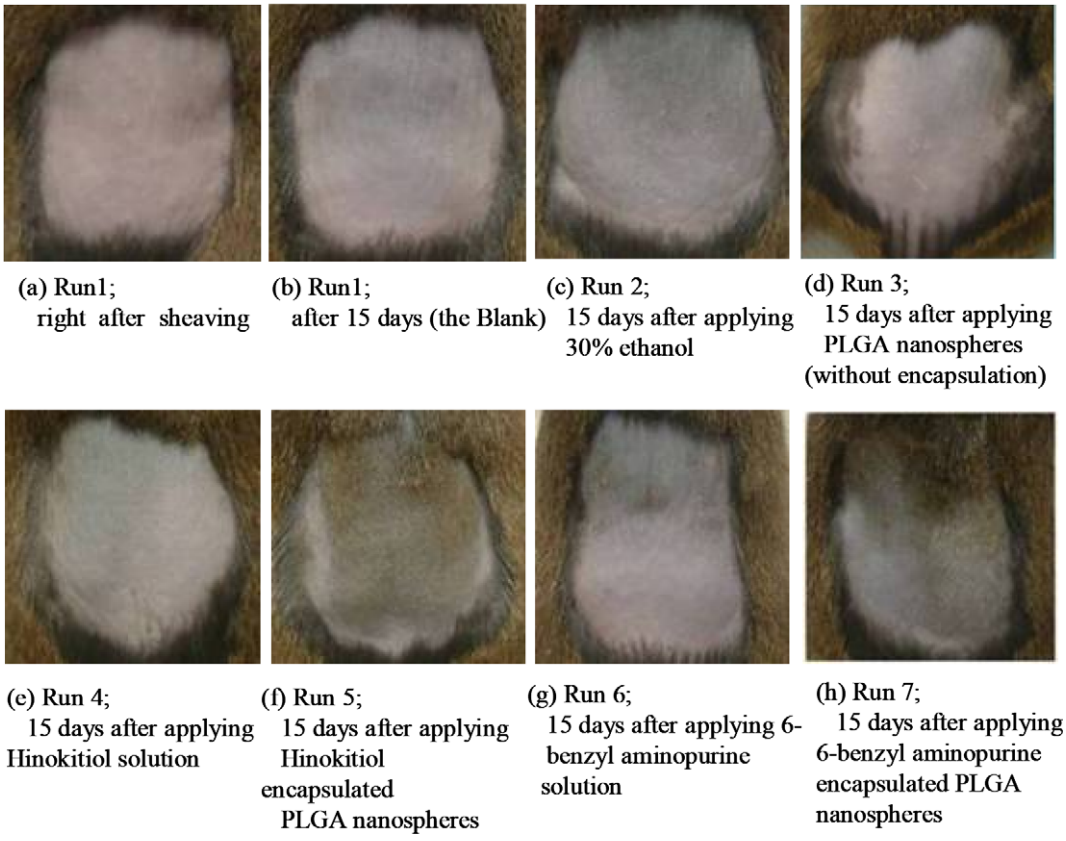


Figure 5. Photos showing the skins of C3H mice.

Table 3. Evalution of results from hair growth tests with C3H mice

Run	Material/mouse number	1	2	3	4	5	6	7	8	Fur growth index (mean ± SD)
1	None-administered (the blank)	1	2	1	1	1	1	1	1	1.1 ± 0.33
2	Ethanol solution	1	1	2	2	2	1	—	—	1.5 ± 0.76
3	PLGA nonospheres (without encapsulation)	1	1	2	2	3	1	—	—	1.7 ± 0.75
4	Hinokitiol solution	1	1	2	2	1	2	—	—	1.5 ± 0.50
5	Hinokitiol encapsulated PLGA nonospheres	2	3	2	2	2	3	—	—	2.3 ± 0.47
6	6-Benzyl aminopurine solution	2	0	2	2	1	2	—	—	1.5 ± 0.76
7	6-Benzyl aminopurine encapsulated PLGA nonospheres	1	1	4	3	2	3	—	—	2.3 ± 1.11

ingredient solution on the scalp (see Footnote[l]), which facilitated diffusion of the nanospheres to follicles.

Therefore using PLGA nanospheres made it possible to improve the delivery of hair growing ingredients to follicles.

Figure 5 shows the C3H mice (a) just after shaving; (b) without applying any test liquid for 15 days (the blank); and (c)–(h) 15 days after applying various test liquids, respectively. Table 3 presents the evaluation values of fur growth extent in each test group.

Figure 5b shows the results of Run 1, in which nothing was applied to the mice. In this case, fur growth index was 1.1 ± 0.33. Compared to Figure 5a, a just-shaved mouse, there was slight blackening sign on the skin; and, the follicle cycle of the C3H mice used in this experiment had a fairly stable 15-d telogen.

Figure 5c shows the results after applying the 30% ethanol solution in Run 2. Compared to the blank Figure 5b, fur started growing on some mice, and fur growth index was 1.3 ± 0.47, which signified the improvement in the fur growth.

Figure 5d shows the results of Run 3, in which empty PLGA nanospheres were dispersed in the 30% ethanol solution as used in Run 2. Fur growth index was found

[l] Applying the platinum torus method for surface tension measurement (Hosokawa Micron, Peneto Analyzer™, $n = 5$), it was found that the surface tension of Hinokitiol PBS solution was 70.7 dyn/cm, slightly different from that of PBS solution (i.e., 72.0 dyn/cm); but, the PBS dispersion containing Hinokitiol, encapsulated PLGA nanospheres had a surface tension of 49.3 dyn/cm. It appeared that the PLGA nanospheres could considerably reduce the surface tension of solvents.

to be 1.7 ± 0.75, an improvement better than that of Run 2. This might be a result of ethanol permeation enhancement due to the hair pore diffusion effect of PLGA nanospheres, which stimulated bulbs more and accelerated their transition from telogen to anagen.

In addition, we evaluated the hair growth effect by encapsulating Hinokitiol in PLGA nanospheres. Hinokitiol is known for its antibacterial and anti-inflammatory effects; but, it is also often used in the over-the-counter hair restorers, because it can normalize the metabolism of matrix and papilla cells.[11] Figure 5e shows the results of the Hinokitiol solution application in Run 4. Its fur growth index was 1.5 ± 0.50. However, applying liquid dispersion of Hinokitiol encapsulated PLGA nanospheres, the fur growth index could reach 2.3 ± 0.47, as shown in Figure 5f. The effect of applying PLGA nanospheres as the Hinokitiol carrier was obvious.

Furthermore, to study the dissolution rate of Hinokitiol from PLGA nanospheres, Hinokitiol encapsulated PLGA nanospheres were dispersed in a 20% ethanol aqueous solution at 32 ± 1 °C and HPLC was used to measure the Hinokitiol that dissolved in the solution as a function of time. As the PLGA hydrolyzed, Hinokitiol was gradually released. About 55% of it dissolved out from the nanospheres after 12 h; and about 80% after 96 h as shown in Figure 6. In addition, the permeation of Hinokitiol through the human scalp was improved by encapsulating it in the PLGA nanospheres as described.

Although there were differences in the hair follicles of humans and mice; solvents used to properly wet the test skins; and skin thickness (the thickness of mouse skin is about one-sixth of human skin), PLGA nanospheres seemed also able to diffuse to the follicles of mouse skin. Furthermore, the Hinokitiol could slowly be released from the nanospheres, which invigorated the mouse's matrix and papillae cells on a sustained release basis and accelerated its follicle cycle transition from telogen to anagen.

We also evaluated hair growth promotion by encapsulating 6-Benzylaminopurine in PLGA nanospheres. In another test example, an over-the-counter hair growing tonic, a solution of 6-benzylaminopurine, was applied in the Run 6. Test result is shown in Figure 5g. Its fur growth index was 1.5 ± 0.76. On the other hand, applying liquid dispersion of 6-benzylaminopurine encapsulated PLGA nanospheres could raise fur growth index to 2.3 ± 1.11, as shown in Figure 5h. Just as with Hinokitiol, the use of PLGA nanospheres enhanced the effectiveness of the hair growing tonic. It is known that 6-benzylaminopurine limits the enzyme activity that inhibits nutrient absorption by matrix cells and other cells in response to a depilation signal secreted by papilla cells.[7] It appeared that applying 6-benzylaminopurine encapsulated PLGA nanospheres expedited nutrient absorption by matrix cells and other cells on a sustained release basis.

In summary, this study evaluated the permeation of hair growing ingredients encapsulated in PLGA nanospheres to the follicles of extracted human skin, and their effects on the fur growth of C3H mice.

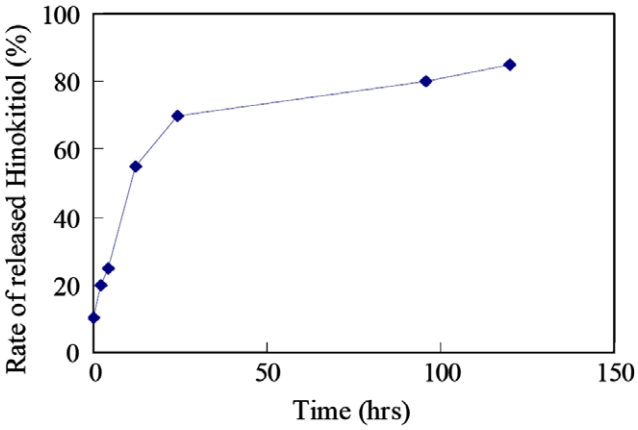


Figure 6. Evalution of Hinokitiol released from the encapsulated PLGA nanospheres Hinokitiol encapsulated PLGA nanosphere concentration: 1 wt% solvent: 20 wt% ethanol concluding 10 wt% PPG-4-Ceteth-20 (surafce active agent) temperature: 32 ± 1 °C.

1. From photographic observations of the cross-section of extracted human scalp skin, it was found that encapsulating hair growing ingredients in the PLGA nanospheres helped to deliver the ingredients from the scalp surface to follicles (1.2 mm from the scalp surface) 2–2.5 times in comparison to applying hair growing tonic solution alone.
2. Fur growth evaluation using C3H mice found that encapsulating hair growing ingredients in PLGA nanospheres enhanced the follicle cycle transition from telogen to anagen more than merely applying the solution of hair growing ingredients and thus improved the degree of fur growth in the experiments.

These results suggested that PLGA nanospheres are useful carriers to deliver hair growing ingredients to follicles.

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