Chemical enhancer induced changes in the mechanisms of transdermal delivery of zinc oxide nanoparticles

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ABSTRACT

The overlapping wavelength of photoluminescence (PL) of zinc oxide nanoparticles (ZnO NPs) and autofluorescence (AF) from the stratum corneum (SC) has for a long time held back researchers from investigating the chemically enhanced penetration pathways of ZnO NPs into the SC lipids. However, the non-linear polarization effect of second harmonic generation (SHG) may be used for ZnO NPs to be distinguished from the AF of the SC. This study combined the SHG of ZnO NPs and the AF of the SC to image the transdermal delivery of ZnO NPs under the chemical enhancer conditions of oleic acid (OA), ethanol (EtOH) and oleic acid–ethanol (OA–EtOH). In addition to qualitative imaging, the microtransport properties of ZnO NPs were quantified to give the enhancements of the vehicle-to-skin partition coefficient (K), the SHG intensity gradient (G) and the effective diffusion path length (L). The results showed that OA, EtOH and OA–EtOH were all capable of enhancing the transdermal delivery of ZnO NPs by increasing the intercellular lipid fluidity or extracting lipids from the SC.

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1. Introduction

At the present, one of the fastest growing scientific fields is that of nanoscale discovery and application. Of particular concern are the uses of nanoparticles (NPs) in applications ranging from targeted fluorescent labels in the life sciences [1], bacterial inhibitors [2], and ultraviolet radiation (UVR) protective cosmetics [3]. Specifically, inorganic ZnO NPs are popular active substances used in UVR protective substances due to their broad spectral absorption in the UVA range [4]. Overexposure of human skin to UVR may lead to sunburn and an increased risk of skin cancer [5–7]. To reduce skin damages caused by UVR, many health care practitioners have advocated UVR protective ingredients for a wide variety of dermatological products [8,9]. Application of UVR protective ingredients on top of the skin is indicated to penetrate ZnO NPs into

the SC of the skin. Based on inherent barrier properties, the SC inhibits transdermal delivery of hydrophilic and large molecular weight molecules [10,11]. The plasma membrane of the cell is mainly composed of various types of phospholipids and membrane proteins. The membrane proteins transport hydrophilic and larger uncharged polar molecules (such as glucose and ions) across membrane. However, organic compounds such as OA and EtOH are chemical enhancers that can alter the packing structure of the SC lipids to increase lipid fluidity and reduce skin resistance [12,13]. A better understanding of the transdermal delivery of ZnO NPs via chemically enhanced pathways may optimize the applications and address biocompatibility issues of NPs in the SC.

Recently, multi-photon microscopy has been used as a non-invasive method for the direct visualization of skin structures [14,15]. In multi-photon imaging, fluorophore excitation is achieved by the non-linear absorption process [16,17]. Specifically, in two-photon microscopy, two near-infrared (near-IR) photons of approximately half the one-photon excitation energy are simultaneously absorbed by the fluorescent molecule [18,19]. This approach can be used to excite intrinsic or extrinsic fluorophores, which are generally excited by ultraviolet (UV) or visible (VIS) light [20]. The near-IR radiation allows deeper penetration into the

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imaged tissues and the point-like excitation volume greatly can reduce overall specimen photodamage [21]. For skin imaging, down to tissue depths of 100–200 μm , the AF of human skin can be detected [22,23]. For transdermal delivery studies, two-photon microscopy has provided three-dimensional (3-D) images detailing the chemical enhancer- and ultrasound-induced changes in the distributions of fluorescent probes across human skin [24–26].

Despite of the advances in the study of transdermal transport of molecular dyes, the fluorescence of ZnO NPs and SC were almost overlapped that held back researchers from investigating the transdermal delivery of ZnO NPs with or without enhancers. To tackle this issue, the current study examined the non-linear polarization effect of SHG for ZnO NPs, based on its unique polarizable property as well as non-centrosymmetric structure [27,28]. The generated second-harmonic signal at twice the frequency (half the wavelength) of the excitation source was observed in ZnO NPs. After confirmation of the SC with no SHG, the combination of the SHG of ZnO NPs and AF of SC was used to image and characterize the penetration pathway and depth of ZnO NPs into nude mouse skin under three chemical enhancer conditions: OA, EtOH, and OA–EtOH. In addition to qualitative imaging, the microtransport properties of the ZnO NPs, including the K , G and L , were quantified relative to the control sample (without chemical enhancers).

2. Materials and methods

2.1. Preparation of ZnO nanoparticles

ZnO NPs were prepared by adding 5.280 g of zinc acetate dehydrate (reagent grade, Sigma–Aldrich) in 250 mL EtOH (99.5%, Showa) and the solution was heated until it become clear [29]. After the solution was refluxed for 1 h at 78 °C, approximate 60% of the solvent was removed by distillation and replaced by the same amount of fresh EtOH. The solution was then placed in ice bath at 0 °C and 1.392 g of lithium hydroxide (98+%, Sigma–Aldrich) was added under ultra-sonication until a transparent solution was obtained. After 60 min of reaction time, the solution consisting of small ZnO NPs with around 2 nm diameter was filtered through a 0.1 μm glass fiber filter to remove undissolved LiOH.

To avoid particle aggregation, ZnO NPs with a diameter of approximate 10 nm was achieved by continuous growth of 2 nm ZnO NPs. The solution containing 2 nm particles was heated and mixed with 10 mL of deionized water at 60 °C for about 6 min. During the cooling process, white precipitate was formed within the solution. The precipitate was separated from the supernatant solution by centrifugation at 3800 rpm for about 10 min. Next, the precipitate was washed with an EtOH–deionized water solution (19:1 by volume) and centrifuged again. The last two steps were repeated at least four times to ensure the removal of physisorbed ionic compounds. The obtained ZnO NPs have mainly the same diameter of approximate 10 nm, and are spherical in shape. The absorption and emission peaks of ZnO NPs are located at 370 nm and 525 nm, respectively.

2.2. Donor solution preparation

The donor vehicle solutions of ZnO NPs were consisted of EtOH, OA, and PBS (99%, Sigma). EtOH, OA, PBS and ZnO NPs with different weight ratio were prepared

Fig. 1. (a) UV–vis absorption and room-temperature PL spectra of ZnO NPs. Dual-channel two-photon AF (b) and SHG (c) images of ZnO NPs. Each image is $110 \times 110 \mu\text{m}^2$. (b) and (c) The two-photon AF (green) and SHG (blue) of ZnO NPs, respectively. (d) The TEM image of ZnO NPs with spherical shape.

for the chemical enhancer experiments. EtOH and OA were used as the chemical enhancer in comparison with control solution of PBS. In the control donor solution, 10% (w/w) of ZnO NPs was added to the PBS solution. The OA donor solution was prepared by adding 10% of ZnO NPs and 5% of OA (w/w) in PBS solution. The EtOH donor solution is consisted of 10% of ZnO NPs (w/w) and 45% of ethanol (w/w) in PBS solution. Finally, the OA–EtOH donor solution is composed of 10% of ZnO NPs, 5% of OA and 45% of EtOH (w/w) in PBS solution.

2.3. Preparation of the skin samples of nude mice for multi-photon microscopy imaging

The skin specimens for the ZnO NPs diffusion experiments were obtained from the dorsal region of four 10-month-old nude mice (BALB/cAnN.Cg-Foxn1nu/CrI(Nar)), purchased from Laboratory Animal Center, NLAC. After the subcutaneous fat of the skin was removed using a knife, the skin specimens were then sectioned and mounted in vertical Franz diffusion cells. The acceptor chambers of the Franz diffusion cells were filled with PBS and the donor chambers were filled with 400 μ L of donor solution. After 12 h, the skin samples were removed from Franz diffusion cells and excess formulation on the skin surface was washed 3–4 times with PBS. The circular region of the skin exposed to the donor solution was removed using a knife and then placed on a microscope slide. The skin specimen was then sealed with a glass coverslip for viewing. Note that the conditions of steady state of ZnO NPs diffusion were verified in calculating the parameters of ZnO NPs penetration. The SHG intensity profiles were measured for the diffusion times of 10 and 12 h. It was found that the gradient profiles of SHG intensity of ZnO NPs were similar under the same donor solution condition. In this study, the diffusion time of 12 h was selected for the experiment.

2.4. Dual-channel multi-photon microscopy

A home-built two-photon microscope based on a commercial upright microscope (E800; Nikon, Japan) was used in this study. The output of

a titanium-sapphire pulse laser (Tsunami TM, Spectra Physics, Mountain View, CA) pumped by a diode-pumped, solid state laser (Millennia TM X, Spectra Physics, Mountain View, CA) operating at the wavelength of 780 nm was used as the excitation source. Upon reflection by the short-pass, main dichroic mirror (700dcspruv-3p, Chroma Technology, Rockingham, VT), circularly polarized laser source was then focused by an oil-immersion objective (S Fluor, NA 1.3; Nikon, Melville, NY). After the signals were generated, the broadband AF and SHG were separated by a secondary dichroic mirror (435 dcsr, Chroma Technology) and filtered respectively by two band-pass filters (MF: E435lp-700sp, SHG: HQ390/20, Chroma Technology) for the respective detection of broadband AF (435–700 nm) and SHG signals (380–400 nm). Single-photon counting photo-multiplier tubes (R7400P, Hamamatsu, Japan) were used in this system. Each optical scan was composed of 512×512 pixels. For large-area scans of the skin specimens, a two-dimensional stage scanning system (H101; Prior Scientific Instruments, Cambridge, UK) was used for specimen translation, after each x - y scan of the sample.

2.5. Data collection and analysis

ImageJ software (National Institutes of Health, Bethesda, MD) was utilized to process images. For each skin sample, four sites were examined and each of the sites was $330 \times 330 \mu\text{m}^2$ in size. The $z = 0 \mu\text{m}$ position was defined by the imaging depth where the highest average SHG count was detected. At each depth, ImageJ averages the SHG counts associated with the pixels in the 512 by 512 pixel image of the imaged field. For each site analyzed by ImageJ, a plot of the average SHG count versus the corresponding skin depth was used to generate the site-specific average SHG spatial distribution profile (SSDP).

The values for E_K , E_G and E_L were obtained following previously published methods [22–24]. The E_K value was evaluated from the average SHG intensity on the surface of each of the four sites of sample skins. Assuming that the SHG intensity of the ZnO NPs permeant, I , is directly proportional to its concentration in the skin, E_K

Fig. 2. Two-dimensional (2-D) dual-channel two-photon AF and SHG images of ZnO NPs in nude mouse skin samples under the four chemical enhancer conditions. Each image is $330 \times 330 \mu\text{m}^2$. The ZnO NPs SHG (blue) and the SC AF (green) were merged at the same imaging depth. The locations of representative corneocytes in the SC were marked with a yellow arrow.

Table 1

The weight ratios of the four donor vehicle solutions consisting of EtOH, OA, OA–EtOH and PBS.

Donor solution	ZnO NPs	PBS	Oleic acid	Ethanol
Control	1	9	–	–
OA	1	8.5	0.5	–
EtOH	1	4.5	–	4.5
OA–EtOH	1	4	0.5	4.5

can be approximated from the average SHG intensity profiles using the following equation:

$$E_K = \frac{I_{Enhancer}(z=0)}{I_{Control}(z=0)} \quad (1)$$

where $I_{Enhancer}(z=0)$ is the average value of the SHG intensity of ZnO NPs on the surface of the chemically enhanced skin samples and $I_{Control}(z=0)$ is the average value of the SHG intensity of ZnO NPs on the surface of the control (PBS) skin samples.

The SHG intensity gradient, G , in the SC was evaluated based on the average intensity values and calculated by performing a linear regression for the data points comprising the first 11 axial scans of the skin. In calculating the enhancement in the ZnO NPs permeant intensity gradient, E_G , the following equation was used:

$$E_G = \frac{(dl/dz)_{Enhancer}}{(dl/dz)_{Control}} \quad (2)$$

where $(dl/dz)_{Enhancer}$ and $(dl/dz)_{Control}$ are the ZnO NPs permeant intensity gradients corresponding to the intensity profiles of the enhanced skin samples and that of the control skin samples, respectively.

The enhancement in the effective diffusion path length, E_L , for enhanced skin samples relative to the control skin samples was evaluated from the values of E_K and E_G as follows:

$$E_L = \frac{E_K}{E_G} \quad (3)$$

where the values of E_K and E_G were evaluated from the average SHG intensity profiles using the two-photon microscopy.

3. Results and discussion

3.1. Optical properties, dual-channel two-photon and electron microscopic images of ZnO NPs

The optical properties of ZnO NPs were first examined by UV–vis absorption and PL spectroscopy. The absorption onset of 380 nm and the broad blue–green emission band at ~571 nm were observed (Fig. 1a). They were agreed with the previous reports [30–32]. Because the fluorescence of ZnO NPs and SC were almost overlapped, in this work, we utilized the non-linear optical characteristic of ZnO NPs to observe their transport with chemical enhancers in SC. Fig. 1b and c shows the dual-channel two-photon images with

Fig. 3. The magnified images of selected regions of interest in Fig. 2. (a), (b), (c) and (d) represent the donor solution conditions of control, OA, EtOH and OA–EtOH respectively.

the size ($110 \times 100 \mu\text{m}^2$) of ZnO NPs. Displayed in Fig. 1b and c are, respectively, the two-photon AF (bandwidth 435 to 700 nm) and SHG (bandwidth 380–400 nm) images of ZnO NPs. The dual-channel two-photon images show that ZnO NPs can be effectively imaged by two-photon microscopy. Besides the optical images, the ZnO NPs were also characterized by transmission electron microscopy (TEM). The electron microscopic image (Fig. 1d) indicated that the averaged diameter of the NPs was ~ 10 nm.

3.2. Imaging the chemical enhanced transdermal delivery of ZnO NPs

To analyze the transdermal transport of ZnO NPs with different chemical enhancers, the representative dual-channel two-photon images of ZnO NPs distribution in the nude mouse SC are shown in Fig. 2, while the formulations of four donor solutions, control and enhancers OA, EtOH and OA–EtOH, are presented in Table 1. In Fig. 2, the SHG images (blue pseudocolor) show the distributions of ZnO NPs and AF (green pseudocolor) is used to indicate the SC of the skin. Where appropriate, the locations of representative corneocytes in the SC are marked with a yellow arrow in each image, as the corneocytes can be clearly identified. Moreover, Fig. 3 shows the magnified images of selected regions of interest as marked in Fig. 2. The SHG of ZnO NPs and the AF of SC are merged at the same imaging depth. The images are presented in column at $z = 0 \mu\text{m}$

(the skin surface), $z = 15 \mu\text{m}$ and $z = 30 \mu\text{m}$, to provide qualitative information on the amount and locations of ZnO NPs with chemical enhancers.

From the dual-channel two-photon images, the ZnO NPs are observed to transport between the intercellular domains of the SC and distribute heterogeneously within the SC. In addition, the majority of the second harmonic generation of ZnO NPs is found to locate at the uppermost layers of the skin at approximate depths around $30 \mu\text{m}$, with insignificant SHG detected beyond that depth. The result suggests that the SC acts as the primary barrier to the transdermal transport of ZnO NPs and it is through the multi-lamellar lipid regions between the corneocytes that the primary ZnO NPs transport across the mouse skins. Furthermore, the tendency of ZnO NPs to distribute in the intercellular domains suggests that the intracellular pathways are not effective for ZnO NPs delivery. These suggestions correspond to the accumulation of ZnO NPs on the surface of human skin and around corneocytes presented by electron micrographs [33].

3.3. 3-D SHG images of ZnO NPs distribution in the SC under different chemical enhancer conditions

To illustrate the extent of ZnO NPs penetration, axial-scans using SHG microscopy was applied to show the 3-D distribution of ZnO

Fig. 4. 3-D SHG images of ZnO NPs in the upper 60 μm of the nude mouse skin samples. Each 3-D image corresponds to a field size of $330 \times 330 \mu\text{m}^2$ and a depth of 60 μm from the surface of the nude mouse skin. The color scale is a relative indication of the SHG intensity, where regions in white (corresponding to 255) reflect high SHG intensity and regions in black (corresponding to 0) reflect low SHG intensity. (a–d) illustrate the ZnO NPs spatial distributions under the four donor solution conditions (Table 1). The white arrow indicates the penetration pathway of ZnO NPs into the SC.

NPs. In Fig. 4, each 3-D image stack represents a field of view $330 \times 300 \mu\text{m}^2$ in size and at a depth of $60 \mu\text{m}$ from the surface of the skin. Fig. 4a–d illustrates the SHG spatial distributions of ZnO NPs across the skin sample ($60 \mu\text{m}$) with four donor solutions, control and enhancers OA, EtOH and OA–EtOH, respectively. The attached color scales were used for these images.

The penetration pathways of ZnO NPs are visible in the 3-D images of Fig. 4. A significant quantity of ZnO NPs accumulation indicated by white arrows reveals the transdermal pathway of ZnO NPs with chemical enhancers. Such result correlates with the previous discussion that ZnO NPs with chemical enhancers transport through intercellular pathway to penetrate into SC and do not enter the corneocytes. Moreover, the axial distribution of ZnO NPs with OA enhancer appears to increase at channel-like area, compared with control solution. The increase in axial distribution reflects the increased intensity of SHG signals. For EtOH enhancer, the SHG signal intensity of ZnO NPs is apparently spread out across the SC. The essence of the axial distribution for ZnO NPs with OA–EtOH enhancer is closer to the axial distribution of ZnO NPs with EtOH enhancer, rather than that of ZnO NPs with OA enhancer.

3.4. Axial SHG intensity profiles of ZnO NPs

In addition to qualitative imaging, we also determined the average ZnO SHG intensity profiles under different chemical enhancer conditions and the results are shown in Fig. 5. The average SHG intensity of each donor solution was plotted on the y axis and the depth (z) from the skin surface (at $z=0 \mu\text{m}$) was plotted on the x axis in μm scale. The error bars represent standard deviation (SD) of the average value of the ZnO NPs SHG intensity.

From the profiles, the SHG intensities of ZnO NPs with OA, EtOH and OA–EtOH enhancers on the skin surface appear to be higher than that of ZnO NPs with control donor solution. As the depth of the skin increases, the SHG signals of ZnO NPs with OA enhancer become to decay slower than those of ZnO NPs with the individual EtOH and OA–EtOH enhancers. In regard to EtOH and OA–EtOH enhancers, the increases in the SHG intensity gradient are almost the same. With noise signals defined by less than one-tenth of the SHG intensity, the penetration depths of ZnO NPs with control, enhancers OA, EtOH and OA–EtOH were 10, 30, 25 and $20 \mu\text{m}$ from the skin surface.

Fig. 5. Axial (z) SHG intensity profiles of ZnO NPs in nude mouse skins (skin surface at $z=0 \mu\text{m}$). The average SHG intensity profiles of ZnO NPs under the four chemical enhancer conditions (Table 1) are shown.

3.5. Chemical enhancements in the vehicle-to-skin partition coefficient, the intensity gradient and the effective diffusion path length for ZnO NPs

To quantify our imaging results, we calculated from the average SHG intensity profiles in Fig. 5 and listed, in Table 2, the E_K values of the ZnO NPs at the skin surface, $I(z=0)$, for enhancers OA, EtOH and OA–EtOH, respectively. To calculate the E_G values, the intensity gradients were determined from the average SHG intensity profiles and the results are -3.26 ± 1.73 , -2.02 ± 1.36 , -4.74 ± 3.04 and -4.81 ± 3.67 , for control, enhancers OA, EtOH and OA–EtOH respectively. Linear regression yields R^2 values of control, enhancers OA, EtOH and OA–EtOH to be 0.92, 0.94, 0.99 and 0.99, respectively. Based on the slope values determined, the E_G values for enhancers OA, EtOH and OA–EtOH are 0.62 ± 0.86 , 1.45 ± 0.83 and 1.48 ± 0.93 , respectively. Utilizing the equation $E_L = E_K/E_G$, the values of enhancers OA, EtOH and OA–EtOH are 1.85 ± 1.44 , 1.25 ± 0.65 and 1.30 ± 0.93 , respectively. For reference, the enhancement values calculated for sulforhodamine B (SRB) with oleic acid enhancer are: $E_K = 4.56 \pm 2.05$, $E_L = 3.66 \pm 1.67$, and $E_G = 1.24 \pm 0.09$ [25].

From the E_K value of 1.15 ± 0.46 for OA enhancer, the accumulation of ZnO NPs on the skin surface is slightly increased by 15%. OA is capable of altering SC lipid ordering to change the transdermal permeability of the permeant. The increase of ZnO NPs on the skin surface can thus be explained by the formation of separate domains of OA pools within the SC lipids. Such explanation corresponds to $E_G = 0.62 \pm 0.86$ for OA enhancer. $E_G (0.62 \pm 0.86) < 1$ reflects the fact that the delivery of ZnO NPs into SC is through the phase-separated OA domains. After ZnO NPs accumulate into OA pools, the decreasing rate of the SHG intensity gradient is 62% for OA enhancer, compared with the control. The observed increase in $E_L = 1.85 \pm 1.44$ for OA enhancer verifies the creation of a micro-transport environment in the SC.

For EtOH enhancer, the E_K value of 1.81 ± 0.56 shows that chemical enhancer of EtOH can enhance the accumulation of ZnO NPs on the skin surface by 81%. EtOH is a potent solvent for both polar and non-polar species. Therefore, it is possible that the presence of EtOH in the donor solution is capable of leaching significant quantities of non-covalently bound amphiphilic SC lipids, causing the modulation of the skin barrier. However, the enhancement of E_G value for EtOH enhancer is $1.45 \pm 0.83 > 1$, indicating that the decreasing rate of the SHG intensity gradient is faster than control by 45%. Therefore, the effect of the modulation of skin barrier caused by EtOH enhancer is constrained, allowing ZnO NPs to accumulate merely on the skin surface with a minimum penetration. The E_L value of the EtOH enhancer, 1.25 ± 0.65 , reflects the accumulation of ZnO NPs on the skin surface associated with the fast decreasing rate of the SHG intensity gradient.

As regard to OA–EtOH enhancer, the E_K value of 1.93 ± 0.62 demonstrates the significant ZnO accumulation on the skin surface by 93%. Compared with EtOH donor solution, the additional presence of OA in OA–EtOH donor solution appears to increase the ZnO NPs accumulation by only 12%. Whereas, compared to OA solution, adding EtOH enhancer to OA donor solution, the accumulation of ZnO NPs increases less than 81%. This phenomenon suggests the

Table 2

Calculated values of E_K , E_G , and E_L , for transdermal delivery of ZnO NPs.

Donor solution	$I(z=0)$	$dI/dz(g)$	E_K	E_G	E_L
Control	31.78 ± 13.86	-3.26 ± 1.73	–	–	–
OA	35.44 ± 5.80	-2.02 ± 1.36	1.15 ± 0.46	0.62 ± 0.86	1.85 ± 1.44
EtOH	57.42 ± 20.15	-4.74 ± 3.04	1.81 ± 0.56	1.45 ± 0.83	1.25 ± 0.65
OA–EtOH	61.31 ± 27.15	-4.81 ± 3.67	1.93 ± 0.62	1.48 ± 0.93	1.30 ± 0.71

mutual interference of OA and EtOH effects on the SC lipids. The phase-separated domains formed by OA enhancer to decrease the skin barrier are disturbed by the effect of EtOH enhancer in modulating the SC lipid fluidity. The decreasing rate of the SHG intensity gradient for OA–EtOH enhancer is shown by the E_G value of $1.48 \pm 0.93 > 1$, with 86% faster than OA enhancer and with 3% faster than EtOH enhancer. Consequently, the E_L value of 1.30 ± 0.71 for OA–EtOH enhancer indicates that the SC lipid fluidity increased by OA enhancer is offset by the leaching effect of EtOH enhancer.

4. Conclusion

In this work, we succeed in combining the second harmonic generation of ZnO nanoparticles and autofluorescence of the stratum corneum to image the transdermal pathway of ZnO nanoparticles with oleic acid, ethanol and oleic acid–ethanol enhancers, using two-photon microscopy. To quantify dual-channel two-photon microscopy images, the enhancement values of vehicle-to-skin partition coefficient and second harmonic generation intensity gradient were calculated from the second harmonic generation signal intensity of ZnO nanoparticles. The ratio of the enhancement values of vehicle-to-skin partition coefficient and second harmonic generation intensity gradient reflects the enhancement values of effective diffusion path length. The results suggested that the multilamellar lipid regions between the corneocytes were the pathways for ZnO nanoparticles delivery. With oleic acid enhancer, the transport of ZnO nanoparticles into the stratum corneum is endorsed by the phase-separated oleic acid domains, while ethanol enhancer leaches significant amount of non-covalently bound amphiphilic stratum corneum lipids to modulate the skin barrier. As regards oleic acid–ethanol donor solution, the increase of stratum corneum lipid fluidity associated with oleic acid enhancer was offset by the effect of ethanol in loosening the stratum corneum lipid structure. Among our choices of the different chemical enhancer conditions, the oleic acid–ethanol combination can be regarded as the most effective donor solution in transdermal ZnO nanoparticles into the stratum corneum.

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Appendix

Figures with essential color discrimination. Certain images in the majority of figures in this article are difficult to interpret in black and white. The full color images can be found in the on-line version, at doi:10.1016/j.biomaterials.2009.02.003.

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