Journal of
Biomedical Nanotechnology
Vol. 6, 586–595, 2010

Penetration of Quantum Dot Particles Through Human Skin

Taís Gratieri[1,2], Ulrich F. Schaefer[1], Lihong Jing[3], Mingyuan Gao[3], K.-H. Kostka[4], Renata F. V. Lopez[2], and Marc Schneider[1,5,*]

[1] *Department Biopharmaceutics and Pharmaceutical Technology, Saarland University, 66123, Saarbruecken, Germany*
[2] *Department of Pharmaceutical Sciences, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, University of São Paulo, 14040-903, Ribeirão Preto, Brazil*
[3] *Institute of Chemistry, Chinese Academy of Science, Zhong Guan Cun Bei Yi Jie 2, 100080, Beijing, China*
[4] *Department of Plastic and Hand Surgery, Caritas Hospital, D-66288 Lebach, Germany*
[5] *Department of Pharmaceutical Nanotechnology, Saarland University, 66123, Saarbruecken, Germany*

The skin is a large and accessible area of the body, offering the possibility to be used as an alternative route for drug delivery. In the last few years strong progress has been made on the developing of nanoparticulate systems for specific applications. The interaction of such small particles with human skin and their possible penetration attracted some interest from toxicological as well as from drug delivery perspectives. As size is assumed to play a key role, the aim of the present work was to investigate the penetration profile of very small model particles (~4 nm) into excised human skin under conditions chosen to mimic the *in vivo* situation. Possible application procedures such as massaging the formulation (5 to 10 minutes) were analyzed by non-invasive multiphoton- and confocal laser scanning microscopy (MPM, CLSM). Furthermore, the application on damaged skin was taken into account by deliberately removing parts of the stratum corneum. Although it was clearly observed that the mechanical actions affected the distribution pattern of the QDs on the skin surface, there was no evidence of penetration into the skin in all cases tested. QDs could be found in deeper layers only after massaging of damaged skin for 10 min. Taking these data into account, obtained on the gold standard human skin, the potential applications of nanoparticulate systems to act as carrier delivering drugs into intact skin might be limited and are only of interest for partly damaged skin.

Keywords: Quantum Dots, Human Skin, Nanoparticle Penetration, Mechanical Stress, Damaged Skin.

RESEARCH ARTICLE

1. INTRODUCTION

The skin constitutes the largest interface between the body and the environment, having several functions as thermoregulation, protection of the body against chemical, physical, and microbial injury, loss of water and other substances, among others.[1] The challenge in this field is though to overcome the inherent barrier properties of this tissue without compromising its physiology.

In recent years nanotechnology has been developing significantly. Nanomaterials with unique physical and chemical properties have been successfully employed in medical imaging, diagnoses and gene therapy for example.[2,3] Regarding skin delivery of active substances, it is stated that higher amounts of drug can be delivered through the skin when incorporated into nanoparticles.[4–6] Luengo et al. (2006) showed that nanoencapsulated flufenamic acid had enhanced transport and accumulation when compared to the drug alone. Imaging by multiphoton fluorescence microscopy found particles on the skin surface and within the dermatoglyphs, but no nanoparticles were detected within the stratum corneum (SC) or in the corneocytes.[7,8]

The ability of nanoparticulate systems to penetrate the skin is still controversial. Several works showed none or minimal nanoparticle penetration into the skin.[9–11] Other studies reported the penetration of particles. However, most of these were performed using animal models, such

*Author to whom correspondence should be addressed.

 1550-7033/2010/6/586/010 doi:10.1166/jbn.2010.1155

as porcine,[12,13] mouse[14] or rat skin.[15] Porcine skin has been widely used for *in vitro* permeation studies because of its similar histological, physiological and biochemical properties to human skin.[16–18] But when the penetration of nanoparticles is investigated porcine skin may not be the ideal model, due to the differences between porcine and human skin relevant for the particles' penetration.[7,19] Very few studies were conducted on human full thickness skin. One of them, for example, demonstrated that metallic nanoparticles with approximately 6 nm were able to penetrate the hair follicles and the SC, occasionally reaching the viable epidermis.[20]

For all penetration processes the size of the penetrant is considered to be one of the main factors. The size and nature of nanoparticles, by which the human skin may be exposed to, can vary enormously. A typical size for pharmaceutical nanosystems, such as solid lipid nanoparticles (SLN), or liposomes are in a range from 25 to 50 nm (for small unilamellar vesicles), which can be found, for example, in sunscreen formulations or cosmetic formulations, respectively.[21,22] Mineral particles, as particles composed of titanium dioxide and (TiO_2) and zinc oxide (ZnO), largely encountered in sunscreen formulations, can have smaller diameters, with typically a size range between 10 and 100 nm.[23,24] Metallic particles, as gold and silver nanoparticles, are generally even smaller in size (5–50 nm).[25,26] Other metallic particles, e.g., palladium/magnetite nano-catalysts are in the range of 20–30 nm in diameter (could be found in the environment)[27] or iron oxide particles as in contrast agents.

In this context, fluorescent quantum dots (QDs) offer favorable optical properties (for detection) combined with a very small size. Their small size makes them well suited candidates to investigate particle penetration into human skin allowing for determining a possible size cut off. Without additional surface coating, they are available in a very small size range; below 10 nm. In this way, they are smaller than the continuous phase space between the corneocytes composing the stratum corneum ($\sim$10 nm),[28,29] theoretically enabling for intercellular penetration. Besides, they could be useful in risk assessment for the typical exposure human skin may encounter from the environment. In case they do not penetrate significantly into the skin, it is presumable that bigger particles would hardly be able to penetrate as well.

A further aspect involved in particle penetration is mechanical stimulation. Some authors have suggested that mechanical stress, such as flexing, mechanical actions that occurs during repetitive motions, or even the rubbing of clothes on skin surface during walking, could alter the structural organization of skin and lead to increased penetration of nanoparticles by compromising the permeability barrier of epidermis.[12,15] FITC-dextran particles of different sizes (up to 4 μm) were investigated under mechanical stress in comparison to a reference experiment without stress.[30] A clear cut-off with respect to the size could be determined (<1 μm) but only for skin under mechanical stress. Kohli and Alpar also found latex particles (up to 500 nm) to penetrate while applying mechanical stress to the barrier.[31] Zhang and collaborators studying quantum dots penetration across intact, tape-stripped and abraded rat skin observed an increase of particles on the skin surface after flexing, but penetration into deeper layers was only observed in abraded skin.[15]

In that context, the aim of this work was to investigate ultra small fluorescent particles (Cadmium Telluride (CdTe) quantum dots) penetration into human skin. These QDs are synthesized in aqueous environment and do not need an extra modification to become water-stable.[32] Hence, the particles are smaller compared to the frequently used particles obtained by organic synthesis. Furthermore, they are also stable in aqueous media with pH values between 5 and 10. Excised human skin from abdominal plastic surgery was used for the penetration experiments.

Moreover the impact of mechanical stress, massaging for 5 to 10 minutes as proposed by others,[12,15] was investigated. We restricted the impact time to values that might be acceptable for patients while applying the formulation; therefore only investigating relatively short times of mechanical stress. Furthermore, the skin barrier was damaged removing parts of the SC. In order to monitor QDs penetration under realistic conditions, the Saarbruecken penetration model[33] (SB-M) was used in which the skin itself acts as a receptor compartment. Hence strong hydration of the skin like in e.g., Franz diffusion cells is deliberately avoided because skin hydration might influence the particles invasion due to enlargement of the paracellular pathway. The QDs depth profiles were then analyzed by non-invasive multiphoton- and confocal laser scanning microscopy (MPM, CLSM) and by sectioning of the SC and subsequent fluorescence investigation.

RESEARCH ARTICLE

2. EXPERIMENTAL METHODS

2.1. QD Synthesis

CdTe stabilized with thioglycolic acid (TGA) forming stable suspension in water were prepared similar as previously described.[34,35] The synthesis yielded inter alia QDs with a size of approximately 3.5 nm equivalent to a maximum fluorescence $\lambda_{em} = 613$ nm. The core diameter was calculated from the optical properties of the particles.[36] The mean hydrodynamic size of the particles was determined to be $r_{hyd} = (9.5 \pm 0.8)$ nm by photon correlation spectroscopy. Briefly, 1.2616 g (3.008 mmol) of $Cd(ClO_4)_2 \cdot 6H_2O$ was dissolved in 160 mL Milli-Q water and mixed under stirring with 0.3713 g (3.9104 mmol) of TGA. The pH of the reaction mixture was adjusted to pH 11 using 1 M NaOH. After that, H_2Te gas (Caution: H_2Te gas is highly flammable and toxic by inhalation) carried by a N_2 flow was introduced into the reaction mixture

under stirring. Immediately, the color of the reaction mixture changed and after approximately 20 min, the resultant solution was subjected to reflux. The respective particle size was obtained by controlling the refluxing time.

2.2. Skin Preparation

Full-thickness human skin from abdominal surgery of Caucasians was used to perform the penetration experiments. The procedure was approved by the ethical committee of Saarland, Germany (Ärztekammer des Saarlandes, Dec. 2008). Adequate health and no medical history of dermatological disease were required. After excision, the skin was cut into 10×10 cm^2 pieces and the subcutaneous fatty tissue was removed from the skin specimen using a scalpel. Afterwards the surface of each specimen was cleaned with water, wrapped in aluminum foil and stored in polyethylene bags at -26 °C until use. Previous investigations have shown that no change in the penetration characteristics occurs during the storage time of 6 months.[37,38] Discs of 25 mm in diameter were punched out from frozen skin, thawed, cleaned with Ringer solution, and either transferred directly into the Saarbruecken penetration model (SB-M) or submitted to one of the mechanical treatments.

2.3. Confocal Microscopy

QDs on skin were imaged using a Zeiss Meta 510 NLO CLSM inter alia equipped with an argon-ion laser which was used for excitation of the QDs. The capability of $\lambda_{ex} =$ 800 nm for exciting the autofluorescence of the corneocytes was also exploited. The detection settings were chosen in such a way, that the signal from the skin could be easily separated from the QD signal and any interference was avoided. For stable measurements the skin samples were placed into a measurement chamber that avoids sample movement because of squeezing and drying.[8]

2.4. Experimental Procedure

The skin was either untreated or subjected to one of the following treatments:

(i) massage (for 5 or 10 min; with or without QD solution, in the last case with water),
(ii) tape-stripping (20 strips) and
(iii) tape-stripping and massage (5 or 10 min with QD solution).

It is important to stress that we deliberately chose a massage based on an individual person rubbing the suspension to simulate the *in vivo* situation of applying a formulation. The time span was also restricted to reasonable times regarding a possible application. After the mechanical procedures the skin was mounted on the Saarbruecken penetration model (SB-M) which has been developed by Professor H. Loth and his coworkers in our laboratories earlier.[33] The SB-M consists of two Teflon blocs that fit together leaving the skin with the applied formulation inside. The skin was put onto a filter paper soaked with Ringer solution and placed into the cavity of the bottom bloc. Compared to the Franz Diffusion Cell, non-physiological hydration of the skin is avoided here due to the absence of the liquid acceptor medium. If needed, 100 μL of QD solution (pH = 6.5) was applied on the skin surface before addition the second Teflon punch of 2 mm depth. A weight of 0.5 kg was placed on the punch for 2 minutes to improve the contact between the skin and the drug preparation. After 2 minutes, the Teflon punch was fixed in its position, and the gap between the two Teflon parts was sealed with Plastibase to avoid water loss from the skin. After 15 hours (due to the absence of full hydration no skin deterioration was observed) the skin was removed, washed with Milli-Q water for 10 seconds, dried and prepared for CLSM observation.[a] Five images of the skin surface at different positions (to display different representative areas of the sample) were obtained and, to compare these images, intensity values were analyzed using the freeware ImageJ (available at: http://rsbweb.nih.gov/ij/). All images were acquired with the same instrument setting to enable quantitative analysis of the fluorescence signals. The mean fluorescence intensity of the images was determined, which is a crude measure for the amount of quantum dots. Also, z-stacks with a 1 μm interval were obtained for each sample until a depth of 25 μm. Images until the depth of 10 μm were further analyzed, as described above, extracting fluorescence depth profiles.

2.5. Tape-Stripping-Strip Analyses

The skin was either untreated- no mechanical actions- or massaged for 5 or 10 min (rubbing with fingertip covered with gloves, same person, and same finger for all massages) with 100 μL of QD solution. The total contact time of the skin with the QD solution was either 10 min or 15 hours. In each case, after the experiment, the skin was washed with Milli-Q water for 10 seconds and dried. The area of the skin that was in contact with the QD solution (disc of $d = 11$ mm) was then tape-stripped under standardized conditions.[33]

Afterwards, each strip removed was analyzed by CLSM. For each strip four images at different positions were obtained. They were then analyzed as described above. All experiments were carried out in duplicates using skin from three different donors (6 samples overall).

[a]A slight change of hydration state might occur during this procedure but looking at theoretical estimations regarding penetration fluxes this is not likely in the short time span water is present.[39] Hereafter the skin was dried and there should be only a minor effect concerning hydration.

2.6. Statistical Analyses

The data obtained from the images' analysis were submitted to unpaired t-test. Values with $P < 0.05$ were considered statistically different (Prisma, Graphpad Software, La Jolla, US).

3. RESULTS

Figure 1 depicts the pattern of QDs distribution on the surface of human skin after 15 hours incubation in the SB-M. Normal human skin exhibited no fluorescence in the range of the band pass of 560–615 nm, which was used for the QDs detection when excited with $\lambda_{ex} = 458$ nm (red channel, Fig. 1(A)) and the respective settings. In contrast, the excitation of two-photon or multiphoton fluorescence excited with $\lambda_{ex} = 800$ nm excludes the QDs signal and is depicted in green (false colors) (Fig. 1(B)). The autofluorescence image indicates the arrangement of the corneocytes on the skin's surface. Figure 1(C) displays the merged channels overlaying the QDs and the skin signal. The distribution of the QDs on skin reflects clearly the skin's morphology as the shape of the corneocytes is visible. The QDs tend to assemble along the edges of these cells.

The pattern of distribution of QDs on the surface of human skin after mechanical impaction as well as the respective z-stack obtained after 5 min massage and incubation for 15 h in the SB-M is shown in Figure 2.

After 5 min massage the mean fluorescence intensity on the skin surface is higher than without massage (Fig. 2 vs. Fig. 1(C)) and the quantum dots are more homogeneously distributed. They can be seen on the whole surface and no longer aligned only on the corneocytes edges (Fig. 2 xy-section). As the instrument settings are unchanged, the clear increase in the mean fluorescence can be attributed to a higher amount of QDs on the surface. The z-profiles of the merged images reveal the localization of the QDs only on the surface; no penetration into the SC could be visualized by the optical sections.

The analysis of the images of the skin surface after different mechanical treatments (Fig. 3) revealed that the fluorescence intensity of the control, where no mechanical actions were applied, was significantly smaller than the fluorescence observed on the surface of the skin submitted to any mechanical treatment. The massage with water alone, before the addition of the solution containing the QDs, had an effect on the distribution of the QDs on the skin surface. After such a treatment more QDs were distributed on the surface when compared to the control. Nonetheless, the highest fluorescence was still observed on the edges, between the corneocytes. There was no statistical difference between the fluorescence intensity massaging the skin with water alone for 5 or 10 min before applying the QDs and that massaging the skin with QD solution for 5 minutes, even though, after the latter, the

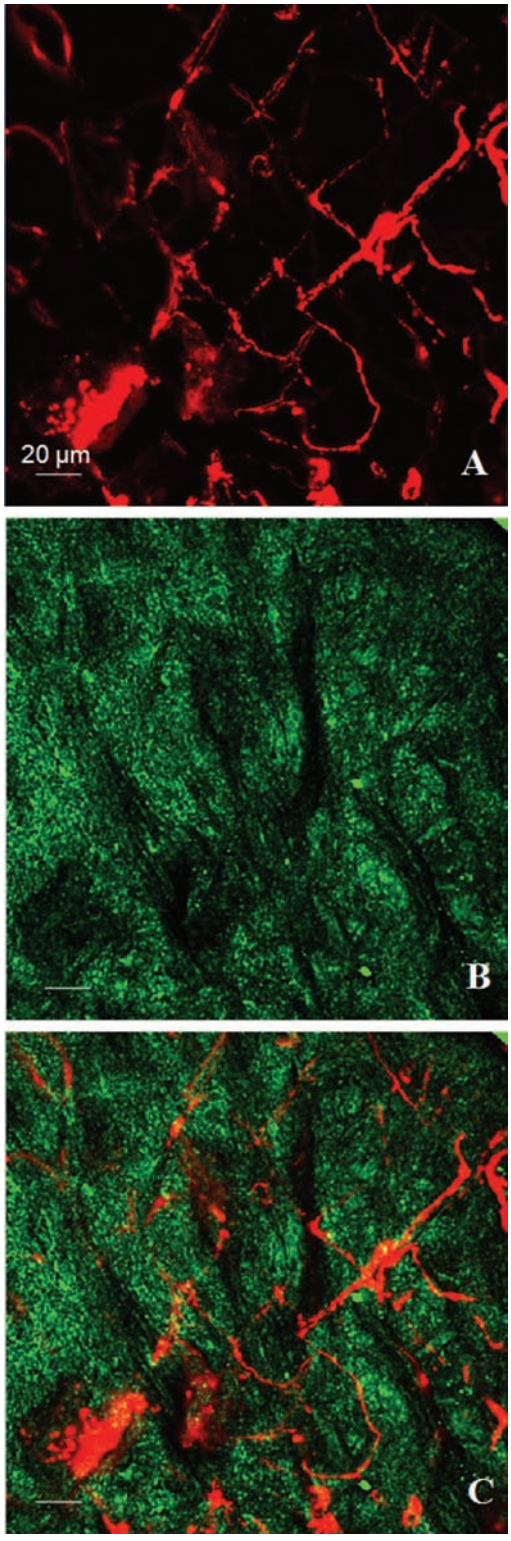

Fig. 1. Confocal scanning microscopy image of skin surface after 15 hours incubation with 100 μL of quantum dot solution in Saarbruecken penetration Model. (A) Quantum dots distribution pattern, channel 1, excitation at 458 nm band pass of 560–615 nm; (B) Skin auto-fluorescence, channel 2, excitation at 800 nm, detection range from 400 to 500 nm; (C) Overlapped images of channel 1 and 2. Normal human skin (blank) without the QDs application exhibited no fluorescence at the band pass of 560–615 nm used for the QDs detection.

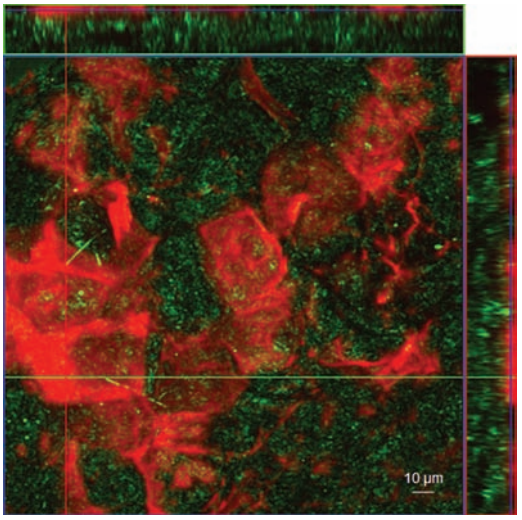

Fig. 2. Microscopy image of skin surface submitted to 5 min massage with QD solution and 15 hours incubation in SB-M. The main window displays a xy-section of the skin's surface where as the other two frames present the xz- and yz-section, respectively. In these sections the fluorescence of the QDs can only be observed on the top of the sample. The images depict the overlay image of two channels: channel 1, excitation at 458 nm band pass of 560–615 nm; channel 2, excitation at 800 nm and detection in a range of 400–500 nm.

QDs were slightly more homogeneously distributed on the whole surface of the sample, as seen in Figure 2.

Furthermore, the distribution of the QDs after massage was dependent on the time of the mechanical action—the fluorescence intensity was significantly higher ($P < 0.05$) after 10 min massage when compared to the 5 min massage with water or QDs and also when compared to the 10 min massage with water alone.

In comparison to the control, a significant higher fluorescence ($P < 0.05$) on the sample's surface was obtained after tape-stripping, and higher fluorescence was observed after massaging the tape-stripped skin. There was no statistically significant difference between the fluorescence intensity massaging the tape-stripped skin for 5 or 10 min with quantum dot solution.

Z-stacks of the skin from the surface to 10 μm in depth resulted only in varying fluorescence intensities on the surface, according to the mechanical treatment applied. Deeper layers were also investigated but no fluorescence was found, hence no significant penetration of QDs into the viable epidermis could be concluded (Fig. 4). The quantitative analysis of fluorescence intensity of the images obtained by the *z*-stacks revealed that the fluorescence drops significantly after 3 μm in depth (Fig. 4). More than 80% of the whole fluorescence observed on the skin after each treatment is localized in the first 3 μm of the optical sections.

The results obtained for the tape-stripping analysis (Fig. 5) also revealed that the majority of QDs present in the stratum corneum after each treatment could be observed only in the first three strips. However, the difference between the fluorescence with and without massage is relatively large. The fluorescence intensities are more than 2 fold higher as compared to the samples without mechanical impact.

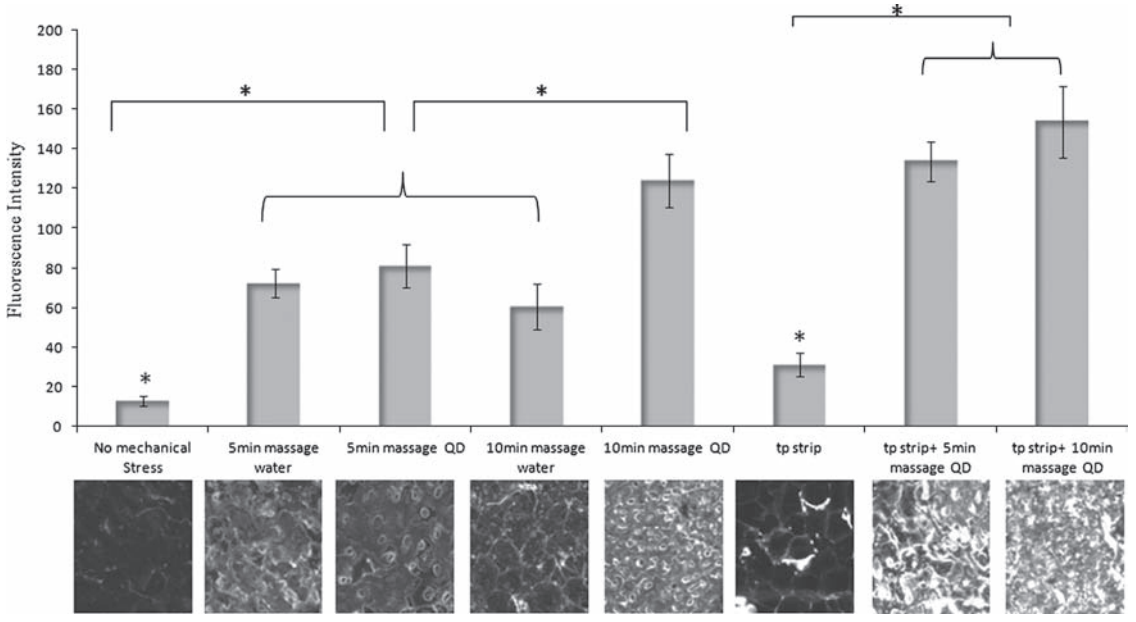

Fig. 3. Fluorescence intensity of QD distribution collected on the skin surface after QDs application onto the skin and different mechanical actions. Each column represents the average and standard deviation of five images of each sample. Values were corrected by background fluorescence. The experiment was performed in triplicate with skin proceeding from different individuals. One image of the surface of each sample is represented below the respective column. The connected columns represent treatments without statistical difference between them ($P > 0.05$). The asterisks ($*$) represent statistical difference ($P < 0.05$).

RESEARCH ARTICLE

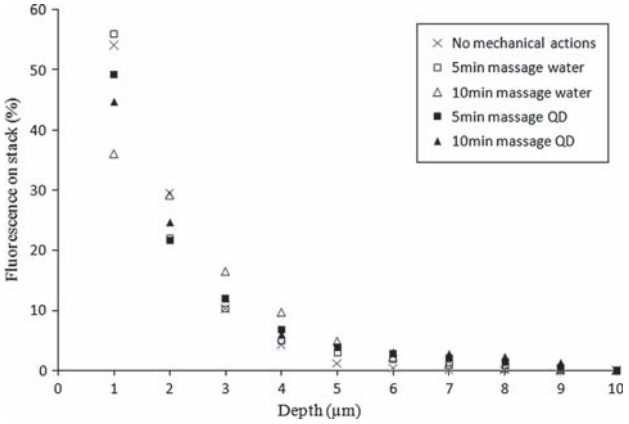

Fig. 4. Percentage of fluorescence found on each image of the *z*-stack of human skin submitted to different mechanical treatments. The sum of all stacks for each treatment represents 100%. Values were corrected from background fluorescence. Images proceeding from the z-stacks were analyzed using ImageJ. Results are the means of four measurements. Error bars are omitted for clarity purpose.

Furthermore, these studies demonstrated as well that the mechanical treatment depends on the duration of the massage. Higher fluorescence intensity was observed in the tape strips analyzed after 10 min massage, either when the skin was immediately tape-stripped or when it was left for 15 h incubation in the SB-M and then tape-stripped. After 15 hours of incubation the amount of QDs present on the first 3 to 4 strips was higher than the amount found after 10 min exposure, but for the further strips, from the fifth strip on (analyses were made until the 20th strip, data not shown) there was no difference between treatments and incubation times and the values are very close to the blank, i.e., the background.

After tape-stripping with 20 strips followed or not by 5 minutes massage, the majority of quantum dots were also found on the first 3 μm of the sample (82.66 and 85.29%, respectively) (Fig. 6). After the first micrometer

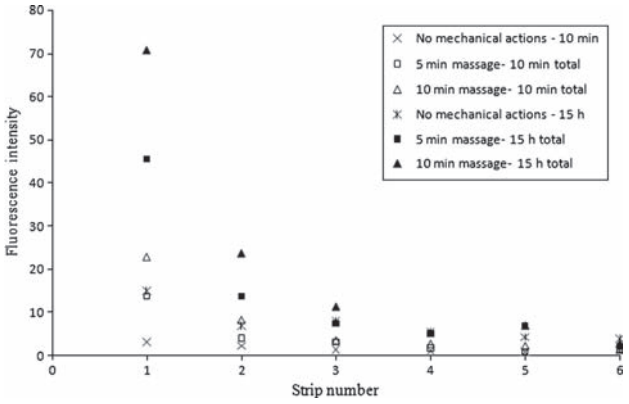

Fig. 5. Fluorescence intensity on each strip after tape-stripping the skin submitted to different mechanical treatments. The results represent the average of 24 images (the experiments were done in duplicates and four images per sample were acquired, three skins- proceeding from three different individuals were used). Error bars are omitted for clarity purpose.

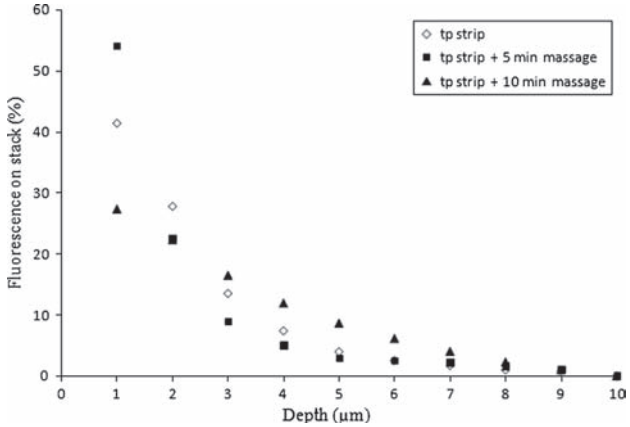

Fig. 6. Percentage of fluorescence found on each *z*-stack of human skin submitted to different mechanical treatments. The sum of the intensities of all stacks for each treatment represent 100%. Values were corrected from background fluorescence. Images proceeding from the *z*-stacks were analyzed using an ImageJ. Results are the means of four measurements. Error bars are omitted for clarity purpose.

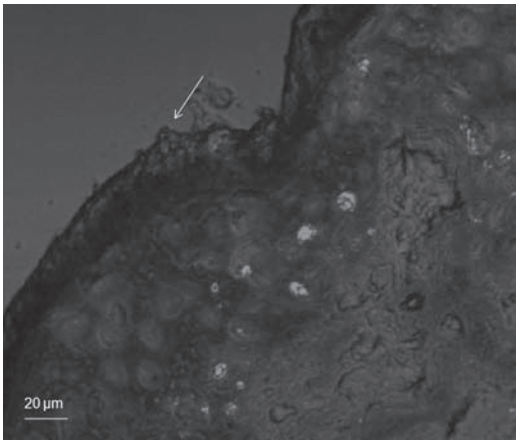

Fig. 7. Multiphoton image ($\lambda_{ex} = 800$ nm) of transversal cut of skin after tape-stripping and five minutes massage. The arrow indicates the dark structures on the top of the sample coming from remaining parts of the SC.

the amount of fluorescence seems to decay exponentially following the same pattern as when the stratum corneum was damaged. A cross section of the skin submitted to tape-stripping followed by 5 min massage revealed that even after such treatment there is still some stratum corneum remaining (Fig. 7). Nonetheless, after tape-stripping and 10 min massage the quantum dots seem to penetrate into deeper layers. At a depth of 7 μm QDs could still be found (Fig. 6).

4. DISCUSSION

The stratum corneum, the outermost layer of the skin, exerts the main barrier function of the skin, controlling percutaneous absorption of dermally applied substances and regulating fluid homeostasis. The distances between

RESEARCH ARTICLE

the corneocytes composing the SC are in the range of ~100 nm, whereas the continuous phases only offer a width of ~10 nm.[28, 29] Taking these facts into consideration the nanoparticles prepared are small enough ($Ø_{core}$ = 3.6 nm, r_{hyd} = 9.5 nm) to fit into the continuous phases and allow for an intercellular penetration.

From the results presented (Fig. 1) it was possible to observe that the QDs distributed themselves on the skin surface mainly along the edges of the corneocytes. For porcine skin Zhang et al. (2008) also found great amounts of QDs within the intercellular spaces of the outermost SC layers.[11] Evaluating the *z*-stacks no fluorescence (QDs) could be visualized in deeper layers of the SC (Fig. 2). These results are also in accordance with the findings of Zhang and Monteiro-Riviere.[15] Using rat skin as an *in vitro* model they reported that the QD penetration was limited to the uppermost stratum corneum layer. However, the fluorescence intensities could be quantitatively investigated and the distribution pattern along the corneocytes might indicate a preference of the QDs for possible gates for invasion. However, the thioglycolic acid-stabilized particles did not penetrate far into the SC.

Based on the first results it was clear that different treatments will be difficult to be distinguished based on the depth of penetration, since no penetration was observed. But it was also clear that mechanical stimulation, a 5 min massage for example could influence the QDs' distribution on the skin surface (Fig. 2). In this way, in order to obtain objective results about the different distribution profiles, the skin was imaged in five different representative regions and the fluorescence in each picture was quantified. This method showed to be simple and reproducible, even though skin provided by different individuals was used. The detection parameters were set to properly analyze the control, where no mechanical stimulation is applied, and the fluorescence is smaller. In order to establish a comparison among treatments these parameters had to be kept constant. They were chosen to assure enough sensitivity in deeper layers, this way, avoiding underestimations. As a result, some over-exposition was observed but only for the two most superficial images in cases where the skin have been previously damaged (last two treatments). As the images were used to quantify the fluorescence it may have caused an underestimation of the total fluorescence on the surface of the skin after these treatments. This underestimation gives smaller values for the top layers but it does not affect the final conclusions or the proper quantification in deeper layers, which is of much higher importance.

Some authors have suggested that mechanical stimulation, such as flexing, could alter the structural organization of skin and lead to increased penetration of nanoparticles by compromising the permeability barrier of epidermis.[12, 15] To verify these effects the skin was submitted to a massage with or without QDs. When QDs were not present, water alone was used, in order to maintain comparable hydration levels. When compared to the control, the massage with water alone had already a significant impact on the tissue, as a higher amount of fluorescence was detected (Fig. 3). But this impact did not seem to be time dependent. There was no statistical difference between massaging with water for 5 or 10 min. However, when the quantum dot suspension was added before the massage, a time-dependence was observed with a statistically higher fluorescence after 10 min massage. One possible explanation for this phenomenon is that the main effect promoted by the massage with water is to remove the top loosely bound layers of the stratum corneum. The stratum corneum, as discussed above, the top layer of the epidermis and the main barrier for penetration, can be also divided in two parts, the stratum disjunctum and the stratum conjunctum. The first one has about 3 to 5 μm of depth and is composed of loosely packed corneocytes. These corneocytes have lost the hemidesmosomes so that they can desquamate.[40] The packing of the corneocytes on the superficial layer is lipid-mediated and has greater susceptibility and vulnerability to mechanical or chemically induced disruption.[41] The adjacent deeper layer, the stratum conjunctum, is the most tightly packed region in the horny layer, and has been described as the "bottle neck" for material transport across the skin.[42] Based on this hypothesis, the removal of the uppermost layers of the stratum corneum can be achieved within 5 min massage. Increasing the mechanical stress duration does not change the results, as the stratum disjunctum was already removed. On the other hand, when QDs are present together with the massage, with longer mechanical stress durations the particles are continuously being pushed against the tissue, and even if it did not promote the penetration, the QDs could continuously be distributed on the surface. Previous work has suggested a time dependence of the mechanical action on the particle penetration.[12] Rouse et al. (2007) studied the effects of mechanical flexion on the penetration of fullerene amino acid-derivatized peptide nanoparticles through dermatomed porcine skin. The authors reported that flexing times of 15–30 minutes showed no evidence of increasing penetration in comparison to unflexed samples, whereas dermal penetration could be observed after flexing times of 60–90 minutes. In the present work the massage was manually performed by the same person. No residues of the suspension could be observed on the gloves after the massage. Even though a different mechanical impact (more directly) was applied in comparison to the flexing described in literature no penetration of the nanoparticles was observed. In future works an automated system will be constructed to evaluate the impact of longer mechanical stimulation times even though this would only be relevant for general understanding as such long impaction might not be relevant for therapeutic purposes.

Due to its special structure, the skin provides the main barrier between the body and the environment, at the same time limiting the drug delivery through this route.[1] A damage to the stratum corneum barrier is followed by transepidermal water loss (TEWL), which is representative of the water evaporation rate from the skin surface.[43] Scratching behavior in patients with atopic dermatitis can cause physical damage to the skin, and a relationship between higher TEWL values and increased severity of atopic dermatitis symptoms has been reported.[44] In the present work tape-stripping was used as a model for investigation of QDs penetration behavior when the main barrier of the skin is damaged. This can drive to conclusions regarding the potential applications of nanoparticulate systems as drug delivery systems for damaged skin, for patients with skin diseases, for example or an increased risk regarding particle exposition.

After tape-stripping significantly more quantum dots were observed on the skin surface when compared to the control (Fig. 3). After tape-stripping the stratum disjunctum is mostly removed, and the surface of the skin remaining is more flat, what could have facilitated the contact with the QDs, hence increasing the fluorescence intensity but not altering the distribution pattern. There was no statistically significant difference in fluorescence intensity on the skin surface after massaging the tape-stripped skin for 5 or 10 min. It was discussed above that the 5 min massage had already an impact on the tissue because it could remove or at least loosen the stratum disjunctum. On the tape-stripped skin this layer was already absent, therefore, it was already expected that the duration time of the mechanical stress would not have a strong impact on the amount of QDs accumulated on the surface. Indeed, the fluorescence intensity obtained and the distribution pattern of QDs, were equivalent to the 10 min massage treatment on intact skin. The duration of the mechanical stimulation on tape-stripped skin, however, had an impact on the penetration profile of the QDs. These results, presented in Figure 6, will be discussed later.

In order to confirm the low or absent QD penetration into deeper layers of the skin two techniques were used. First, optical sections of the skin were performed and thereafter analyzed regarding the fluorescence intensity profiles. Second the skin was submitted to 5 or 10 minutes massage with QD solution, tape-stripped and the strips were analyzed hereafter. These methods can be complementary, since analyzing the strips it is possible to determine with good resolution and low background if QDs are present in the removed layer of the stratum corneum. Using the same instrument setting for image acquisition, quantitative comparisons could be made. This allows for a reconstruction of the fluorescence profiles with the strip number. However, it should be taken into consideration that it is not easy to precisely determine the amount of SC removed. Combining the results allowed comparing the penetration depth and to obtain knowledge about the penetration profile of the particles.

When no mechanical treatment was applied and after 5 or 10 min massage, more than 80% of QDs were already removed by the first three strips (Fig. 5). These results could be related to the optical z-stacks (Fig. 4), where also approximately 80% of the QDs were found in the top 3 μm of the SC. It can be then assumed that more than 80% of the QDs were present on the first 3 μm of stratum corneum, and therefore, even after 5 or 10 min of mechanical stimulation it was confirmed that quantum dots do not show significant penetration.

Looking at the partly damaged (tape-stripped) skin QDs could be only observed on the surface (Fig. 6). The z-stacks revealed that the highest amount of QDs remained in the first 3 μm of the damaged skin sample, following the same profile than the other treatments. This means that even skin with a thinner stratum corneum is still an efficient protective barrier. The thin layer left on the surface after 20 strips (Fig. 7) resulted in a similar penetration behavior than with the full stratum corneum. However, 10 min massage of tape-stripped skin resulted in QDs penetrating into deeper skin layers (Fig. 6). Thus, massaging the tape-stripped skin for 10 min had no significant impact on the distribution of QDs on the surface, but it promoted the penetration of low amounts into deeper layers.

5. CONCLUSION

In conclusion, although it was clearly observed that the mechanical actions affected the distribution pattern of the QDs on the skin surface, there was no evidence of penetration into the skin in all cases tested. The fluorescence intensity varied according to type and duration of mechanical treatment. After 10 min massage the fluorescence intensity on the skin surface was higher. In this case, the QDs were also distributed more homogeneously on the surface. QD penetration was observed only after 10 min massage on partly damaged (tape-stripped) skin. Taking these data into account, the potential applications of nanoparticulate systems to act as carrier delivering drugs into intact skin may be limited and only slightly more interesting for partly damaged skin.

Acknowledgments: Taís Gratieri would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil, for partially supporting this research. Mingyuan Gao thanks NSFC project 20820102035 for financial support. Furthermore, the authors thank Hagar I. Labouta for assistance.

References and Notes

1. U. F. Schaefer, S. Hansen, M. Schneider, J. C. Luengo, and C. M. Lehr, Models for skin absorption and skin toxicity testing, Drug Absorption Studies: *In Situ*, *In Vitro* and *In Silico* Models, edited by C. Ehrhardt and K. J. Kim, AAPS Press, Springer, US (**2008**).

2. W. Chen, Nanoparticle fluorescence based technology for biological applications. *J. Nanosci. Nanotechnol.* 8, 1019 **(2008)**.

3. J. He, H. F. VanBrocklin, B. L. Franc, Y. Seo, and E. F. Jones, Nanoprobes for medical diagnosis: Current status of nanotechnology in molecular imaging. *Current Nanoscience* 4, 17 **(2008)**.

4. H. H. Joo, H. Y. Lee, J. C. Kim, H. J. Lee, and H. Y. Kang, Integrities and skin permeation-enhancing characteristics of behenic acid nanoparticles. *J. Dispersion Sci. Technol.* 30, 149 **(2009)**.

5. J. Li, Y. L. Zhai, B. Zhang, L. D. Deng, Y. S. Xu, and A. J. Dong, Methoxy poly(ethylene glycol)-block-poly(D,L-lactic acid) copolymer nanoparticles as carriers for transdermal drug delivery. *Polym. Int.* 57, 268 **(2008)**.

6. S. Miyazaki, A. Takahashi, W. Kubo, J. Bachynsky, and R. Lobenberg, Poly *n*-butylcyanoacrylate (PNBCA) nanocapsules as a carrier for NSAIDs: *In vitro* release and *in vivo* skin penetration. *Journal of Pharmacy and Pharmaceutical Sciences* 6, 238 **(2003)**.

7. J. Luengo, B. Weiss, M. Schneider, A. Ehlers, F. Stracke, K. Konig, K. H. Kostka, C. M. Lehr, and U. F. Schaefer, Influence of nanoencapsulation on human skin transport of flufenamic acid. *Skin Pharmacol. Physiol.* 19, 190 **(2006)**.

8. F. Stracke, B. Weiss, C. M. Lehr, K. Konig, U. F. Schaefer, and M. Schneider, Multiphoton microscopy for the investigation of dermal penetration of nanoparticle-borne drugs. *J. Invest. Dermatol.* 126, 2224 **(2006)**.

9. S. E. Cross, B. Innes, M. S. Roberts, T. Tsuzuki, T. A. Robertson, and P. McCormick, Human skin penetration of sunscreen nanoparticles: *In-vitro* assessment of a novel micronized zinc oxide formulation. *Skin Pharmacol. Physiol.* 20, 148 **(2007)**.

10. L. J. Mortensen, G. Oberdorster, A. P. Pentland, and L. A. Delouise, *In vivo* skin penetration of quantum dot nanoparticles in the murine model: The effect of UVR. *Nano Lett.* 8, 2779 **(2008)**.

11. L. W. Zhang, W. W. Yu, V. L. Colvin, and N. A. Monteiro-Riviere, Biological interactions of quantum dot nanoparticles in skin and in human epidermal keratinocytes. *Toxicol. Appl. Pharmacol.* 228, 200 **(2008)**.

12. J. G. Rouse, J. Yang, J. P. Ryman-Rasmussen, A. R. Barron, and N. A. Monteiro-Riviere, Effects of mechanical flexion on the penetration of fullerene amino acid-derivatized peptide nanoparticles through skin. *Nano Lett.* 7, 155 **(2007)**.

13. J. P. Ryman-Rasmussen, J. E. Riviere, and N. A. Monteiro-Riviere, Penetration of intact skin by quantum dots with diverse physicochemical properties. *Toxicol. Sci.* 91, 159 **(2006)**.

14. M. Q. Chu, Q. Wu, J. X. Wang, S. K. Hou, Y. Miao, J. L. Peng, and Y. Sun, *In vitro* and *in vivo* transdermal delivery capacity of quantum dots through mouse skin. *Nanotechnology* 8, 1 **(2007)**.

15. L. W. Zhang and N. A. Monteiro-Riviere, Assessment of quantum dot penetration into intact, tape-stripped, abraded and flexed rat skin. *Skin Pharmacol. Physiol.* 21, 166 **(2008)**.

16. G. M. Gelfuso, F. V. Figueiredo, T. Gratieri, and R. F. Lopez, The effects of pH and ionic strength on topical delivery of a negatively charged porphyrin (TPPS(4)). *J. Pharm. Sci.* 97, 4249 **(2008)**.

17. H. Herai, T. Gratieri, J. A. Thomazine, M. V. Bentley, and R. F. Lopez, Doxorubicin skin penetration from monoolein-containing propylene glycol formulations, *Int. J. Pharm.* 329, 88 **(2007)**.

18. R. F. Lopez, J. H. Collett, and M. V. Bentley, Influence of cyclodextrin complexation on the *in vitro* permeation and skin metabolism of dexamethasone. *Int. J. Pharm.* 200, 127 **(2000)**.

19. I. P. Dick and R. C. Scott, Pig ear skin as an *in-vitro* model for human skin permeability. *J. Pharm. Pharmacol.* 44, 640 **(1992)**.

20. B. Baroli, M. G. Ennas, F. Loffredo, M. Isola, R. Pinna, and M. A. Lopez-Quintela, Penetration of metallic nanoparticles in human full-thickness skin. *J. Invest. Dermatol.* 127, 1701 **(2007)**.

21. J. de Leeuw, H. C.de Vijlder, P. Bjerring, and H. A. M. Neumann, Liposomes in dermatology today. *Journal of the European Academy of Dermatology and Venereology* 23, 505 **(2009)**.

22. J. Pardeike, A. Hommoss, and R. H. Muller, Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. *Int. J. Pharm.* 366, 170 **(2009)**.

23. A. O. Gamer, E. Leibold, and B. van Ravenzwaay, The *in vitro* absorption of microfine zinc oxide and titanium dioxide through porcine skin. *Toxicol. In Vitro* 20, 301 **(2006)**.

24. A. Mavon, C. Miquel, O. Lejeune, B. Payre, and P. Moretto, *In vitro* percutaneous absorption and *in vivo* stratum corneum distribution of an organic and a mineral sunscreen. *Skin Pharmacol. Physiol.* 20, 10 **(2007)**.

25. N. R. Panyala, E. M. Pena-Mendez, and J. Havel, Silver or silver nanoparticles: A hazardous threat to the environment and human health? *Journal of Applied Biomedicine* 6, 117 **(2008)**.

26. G. Sonavane, K. Tomoda, A. Sano, H. Ohshima, H. Terada, and K. Makino, *In vitro* permeation of gold nanoparticles through rat skin and rat intestine: Effect of particle size, *Colloids Surf., B—Biointerfaces* 65, 1 **(2008)**.

27. H. Hildebrand, D. Kuhnel, A. Potthoff, K. Mackenzie, A. Springer, and K. Schirmer, Evaluating the cytotoxicity of palladium/magnetite nano-catalysts intended for wastewater treatment. *Environ. Pollut.* 158, 65 **(2010)**.

28. A. Naegel, S. Hansen, D. Neumann, C. M. Lehr, U. F. Schaefer, G. Wittum, and M. Heisig, *In-silico* model of skin penetration based on experimentally determined input parameters. Part II: Mathematical modelling of *in-vitro* diffusion experiments. Identification of critical input parameters. *Eur. J. Pharm. Biopharm.* 68, 368 **(2008)**.

29. T. F. Wang, G. B. Kasting, and J. M. Nitsche, A multiphase microscopic diffusion model for stratum corneum permeability. I. Formulation, solution, and illustrative results for representative compounds. *J. Pharm. Sci.* 95, 620 **(2006)**.

30. S. S. Tinkle, J. M. Antonini, B. A. Rich, J. R. Roberts, R. Salmen, K. DePree, and E. J. Adkins, Skin as a route of exposure and sensitization in chronic beryllium disease. *Environ. Health Perspect.* 111, 1202 **(2003)**.

31. A. K. Kohli and H. O. Alpar, Potential use of nanoparticles for transcutaneous vaccine delivery: Effect of particle size and charge. *Int. J. Pharm.* 275, 13 **(2004)**.

32. A. L. Rogach, A. Kornowski, M. Y. Gao, A. Eychmuller, and H. Weller, Synthesis and characterization of a size series of extremely small thiol-stabilized CdSe nanocrystals. *J. Phys. Chem. B* 103, 3065 **(1999)**.

33. H. Wagner, K. H. Kostka, C. M. Lehr, and U. F. Schaefer, Drug distribution in human skin using two different *in vitro* test systems: Comparison with *in vivo* data. *Pharm. Res.* 17, 1475 **(2000)**.

34. M. Y. Gao, S. Kirstein, H. Mohwald, A. L. Rogach, A. Kornowski, A. Eychmuller, and H. Weller, Strongly photoluminescent CdTe nanocrystals by proper surface modification. *J. Phys. Chem. B* 102, 8360 **(1998)**.

35. Y. H. Yang, L. H. Jing, X. L. Yu, D. D. Yan, and M. Y. Gao, Coating aqueous quantum dots with silica via reverse microemulsion method: Toward size-controllable and robust fluorescent nanoparticles. *Chem. Mater.* 19, 4123 **(2007)**.

36. W. W. Yu, L. Qu, W. Guo, and X. Peng, Experimental determination of the extinction coefficient of CdTe, CdSe, and CdS nanocrystals. *Chem. Mater.* 15, 2854 **(2003)**.

37. R. L. Bronaugh, R. F. Stewart, and M. Simon, Methods for *in vitro* percutaneous absorption studies. VII: Use of excised human skin. *J. Pharm. Sci.* 75, 1094 **(1986)**.

38. H. Wagner, K. H. Kostka, W. Adelhardt, and U. F. Schaefer, Effects of various vehicles on the penetration of flufenamic acid into human skin, *Eur. J. Pharm. Biopharm.* 58, 121 **(2004)**.

39. M. Schneider, F. Stracke, S. Hansen, and U. F. Schaefer, Nanoparticles and their interactions with the dermal barrier. *Journal of Dermato-Endocrinology* 1, 197 **(2009)**.

40. H. Loth, Vehicular influence on transdermal drug penetration. *Int. J. Pharm.* 68, 1 **(1991)**.

41. M. Fartasch, The nature of the epidermal barrier: Structural aspects. *Advanced Drug Delivery Reviews* 18, 273 **(1996)**.
42. A. Schatzlein and G. Cevc, Non-uniform cellular packing of the stratum corneum and permeability barrier function of intact skin: A high-resolution confocal laser scanning microscopy study using highly deformable vesicles (Transfersomes). *British Journal of Dermatology* 138, 583 **(1998)**.
43. Y. Honma, I. Arai, T. Sakurai, N. Futaki, Y. Hashimoto, M. Sugimoto, Y. Nakanishi, and S. Nakaike, Effects of indomethacin and dexamethasone on mechanical scratching-induced cutaneous barrier disruption in mice. *Exp. Dermatol.* 15, 501 **(2006)**.
44. M. Watanabe, H. Tagami, I. Horii, M. Takahashi, and A. M. Kligman, Functional analyses of the superficial stratum corneum in atopic xerosis. *Arch. Dermatol.* 127, 1689 **(1991)**.

Received: 29 March 2010. Revised/Accepted: 12 May 2010.