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Assessment of penetration of quantum dots through *in vitro* and *in vivo* human skin using the human skin equivalent model and the tape stripping method

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ABSTRACT

Quantum dots (QDs) are rapidly emerging as an important class of nanoparticles (NPs) with potential applications in medicine. However, little is known about penetration of QDs through human skin. This study investigated skin penetration of QDs in both *in vivo* and *in vitro* human skin. Using the tape stripping method, this study demonstrates for the first time that QDs can actually penetrate through the stratum corneum (SC) of human skin. Transmission electron microscope (TEM) and energy diverse X-ray (EDX) analysis showed accumulation of QDs in the SC of a human skin equivalent model (HSEM) after dermal exposure to QDs. These findings suggest possible transdermal absorption of QDs after dermal exposure over a relatively long period of time.

1. Introduction

Use of nanoparticles (NPs) increased in both commercial and biomedical fields, including cosmetics, microelectronics, drug delivery, cancer detection, and therapeutics. Quantum dot (QD) NPs have potential for medical applications due to optical characteristics that enable them to overcome the limitations of previously used organic fluorophores [1]. Findings from recent studies suggest that the QD may be an interesting candidate for use in development of medical applications involving drug or gene delivery, while at the same time use of QDs has resulted in increased concern over their potential toxicity [2].

Because the skin is the main target tissue for NPs exposure, assessment of skin penetration of QDs has attracted a great deal of attention. Ryman-Rasmussen et al. [3] showed extensive penetration of QDs through *ex vivo* porcine skin. In contrast, recent papers have reported that skin penetration of QDs did not occur in intact mouse skin [4], was minimal in a murine model [5], and was limited primarily to the stratum corneum (SC) layer of pig skin [6].

In general, skin permeability of animals is greater than that of humans [7]. Some researchers have suggested that in the context of transdermal penetrability to nanoparticles, porcine skin represents a poor model of human skin [8]. However, little is known about penetration of QDs through human skin. In this study, we assessed percutaneous absorption of QDs in both *in vivo* and *in vitro* human skin.

2. Materials and methods

Materials. QD 565-PEG-amine was obtained from Invitrogen (Hayward, CA, USA). QD 565-PEG-amine was positively charged as supplied and at physiological pH. We have used the EpiDerm™ model as a human skin equivalent model (HSEM). The EpiDerm™ model was purchased from MatTek (EPI-200, Ashland, MA, USA).

Transmission electron microscope (TEM) and energy diverse X-ray (EDX). TEM micrographs were taken for characterization of QDs. TEM specimens of QDs were prepared by placement of one drop onto a 200 mesh carbon-coated copper grid, followed by dehydration in an oven at 60 °C for 15 min. TEM images of specimens were observed with a Tecnai 20 (FEI Co., Eindhoven, Netherlands) at an acceleration voltage of 200 kV.

After the last receptor fluid sampling in the Franz-type diffusion cells (FDC), the EpiDerm™ was removed from the system and punch biopsy was taken from the center. The tissue was fixed in 2.5% glutaraldehyde, 0.1 M phosphate buffer and post-fixed in 1% osmium tetroxide. After dehydration with an ascending series of ethanol dilutions, ultrathin sections were cut. The specimens were embedded in Embed-812, and observed with TEM. EDX spectra

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were also acquired for determination of the elemental composition of particles assumed to be QDs.

Tape stripping. This study was approved by the Institutional Review Board, and volunteers provided written consent. Two healthy male volunteers (average age 32 years) participated in the experiment. Twenty microliter of QDs at 0.8 μM concentration were applied in occlusion with Tegaderm® (Transparent Film Dressing, 3M, St. Paul, MN, USA) on the internal surface of the upper arms for 4 h. Because they will not be immediately absorbed into the skin, application of NPs under occlusion is recommended [9]. Adhesive tape (Scotch Transparent Tape 600, 3M, St. Paul, MN, USA) was applied and pressed onto the skin using a roller. The skin was stripped with 15 pieces of adhesive tape. Negative control strips were obtained from a non-treated area of the upper arms. Tape strips removed from skin were analyzed using the LSM 510 confocal microscope (Carl Zeiss, Yena, Germany).

Percutaneous penetration test. The EpiDerm™ model was used for epidermal membranes of FDC (Permegear, Riegelsville, PA, USA). According to manufacturer's instructions, the EpiDerm™ model was removed from cell culture inserts and placed in FDC. As described elsewhere [10], the EpiDerm™ was mounted onto FDC, with the top layer of the EpiDerm™ facing the donor chamber. FDC were maintained at 37 °C with thermostated water in the jacket surrounding the cells. Receptor chambers were filled with 4 ml phosphate buffered saline (PBS) and 40 μl of QDs at 0.8 μM concentration were applied to donor chambers. Samples of receptor fluid were taken at various time intervals (8, 12, and 24 h) and were assessed for permeant concentration. The receptor solution was replaced with fresh solution at each time point. The concentration of permeating particles was investigated by detection of the fluorescence spectra using a fluorescence microscopic system in each of the samples withdrawn. In the fluorescence microscopic

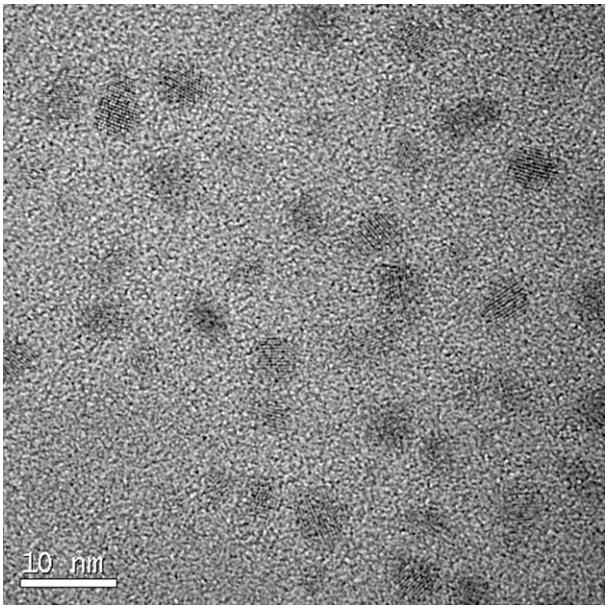

Fig. 1. TEM image of QD nanoparticles used in this study. Nanoparticles were deposited on TEM grids and directly observed. TEM images showed that QDs were uniform spherical shape and measured 6 ± 2 nm in diameter. Scale bar represents 10 nm.

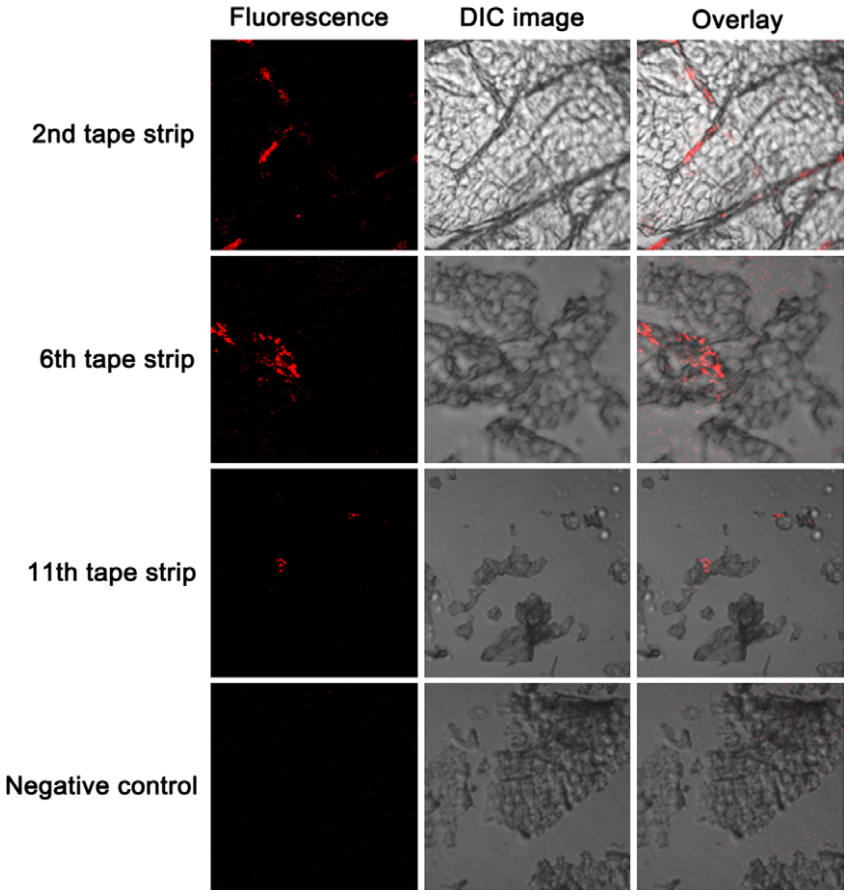

Fig. 2. Confocal images for the tape strips of skin applied for 4 h with QDs. Left of row: Fluorescence indicating QDs 565. Center of row: Differential interference contrast (DIC) image depicting the stratum corneum. Right of row: Overlay of DIC and fluorescence. Fluorescence intensity showed a progressive decrease with depth.