Published in final edited form as:

J Biomed Nanotechnol. 2015 September ; 11(9): 1644–1652.

UVB dependence of quantum dot reactive oxygen species generation in common skin cell models

LUKE J. MORTENSEN¹, RENE FAULKNER¹, SUPRIYA RAVICHANDRAN¹, HONG ZHENG², and LISA A. DELOUISE^{1,2}

¹Department of Biomedical Engineering, University of Rochester

²Department of Dermatology, University of Rochester

Abstract

Studies have shown that UVB can slightly increase the penetration of nanoparticles through skin and significantly alter skin cell biology, thus it is important to understand if and how UVB may impact subsequent nanoparticle skin cell interactions. The research presented herein evaluates the effect of UVB on quantum dot (QD) uptake and reactive oxygen species (ROS) generation in primary keratinocytes, primary melanocytes, and related cell lines. QD exposure induced cell type dependent ROS responses increased by pre-exposing cells to UVB and correlated with the level of QD uptake. Our results suggest that keratinocytes may be at greater risk for QD induced ROS generation than melanocytes, and raise awareness about the differential cellular effects that topically applied nanomaterials may have on UVB exposed skin.

Keywords

Quantum dots; UVB; keratinocytes; melanocytes; nanotoxicology

1. Introduction

The explosion of nanotechnology applications in recent years has made human interaction with nanoparticles (NPs) nearly inescapable. Semiconductor quantum dots (QDs) are a technologically important group of NPs that have shown promise in the electronics and biomedical industries. In the electronics field, QDs are being investigated for use in solar cells ¹, data storage ², and consumer LED products ³. In biomedical field, they are well accepted as biological imaging probes ⁴, lymph node tracking agents ⁵, and are of interest in systemic imaging applications for medical diagnostics ⁶. Because QDs broadly absorb UVR light they have also been investigated as active ingredient formulated into sun protective consumer products that are intended to contact skin ⁷. QD have also been formulated into textiles which may contact skin ⁸. However, because of their small size, elemental composition, and their broad application concerns have been raised about their ability to penetrate epithelial tissues and their potential to cellular toxicity ^{9–12}.

Research by our group and others has investigated the ability of QDs to penetrate skin and found that under most conditions an intact skin barrier provides adequate protection^{13–17}. However, after barrier impairment by a variety of methods^{13, 18, 19, 16}, including UVB exposure^{20, 14, 21, 19}, there is an increased risk of QD skin penetration and interaction with the local epidermal cells and the body system. These results are important, as UVB damage to the skin barrier could be subsequently combined with the application of NP-containing cosmetics such as sunscreens. In fact, recent studies²² confirm that UVB skin exposure can slightly increase the penetration of TiO₂ NPs through the stratum corneum - the outermost skin layer comprised of corneocytes (terminally differentiated keratinocytes) and lipid lamellae. This potential risk motivates the need to explore the interactions of NPs with constituent skin cell types, in particular after the cells have been subjected to environmental stress like UVB exposure.

The skin epidermis is a dynamic system of several cell types that coordinate to provide a barrier between the interior and exterior of the body and to respond to stress or injury. Keratinocytes are the majority epidermal cell type. They proliferate in the basal layer along the basement membrane then gradually differentiate under an increasing calcium gradient to replenish cells in the stratum corneum that regularly slough off. Melanocytes are important skin resident pigment producing cells that provide surrounding keratinocytes (1 melanocyte supplies approximately 36 keratinocytes) with melanin that is packaged in melanosomes^{23–25}. Following UVB exposure, a flood of cytokines and prostaglandins induces a strong keratinocyte proliferative response^{26–30}. Keratinocytes are then activated to increase phagocytosis of melanosomes from neighboring melanocytes^{31–33, 24}. Keratinocytes and melanocytes are derived from different embryonic lineages^{34, 35} and they are known to have very different biology and responses to UVB exposure²³. Therefore, we anticipate that their response to NPs, especially following UVB-induced stress, may be quite different.

The toxicity and uptake mechanisms of a variety of commercially available QDs have been studied on basal-like proliferative human keratinocytes by the Monteiro-Riviere group^{36, 37, 17}. Their work has suggested toxicity limits in line with the literature on other cell types (~20 nM) for QDs with positive, negative, and neutral surface charges¹². Work in our lab has investigated the impact of keratinocyte differentiation state on QD uptake and toxicity, and discovered that keratinocytes cultured under basal-like proliferative conditions (low calcium) are more apt to associate with QDs than when cultured under differentiated conditions (high calcium)³⁸. These observations collectively suggest the potential for basal-like proliferative keratinocytes in the epidermis to be at increased risk of cell death, dysfunction, or transformation following NP uptake; as these cells remain in the basal layers and produce daughter cells for long periods of time in the epidermis³⁹. QD labeling of melanoma has been studied in the context of metastatic tumor targeting^{5, 40–42}. However, no studies exist investigating the in vitro cellular interactions or toxicity values for QDs with normal or cancerous melanocytes.

Herein, we investigate the toxicity and ROS generation of UVB-induced stress on skin cells and its impact on cellular response to subsequent addition of QDs. We quantify the relative QD cell uptake and ROS generation for primary keratinocytes and melanocytes as well as a

HaCaT keratinocyte line and a melanocytic YUSIK cell line. Our study provides a direct comparison of the toxic potential of QDs on epidermal cell types and an evaluation of the effect of a powerful UVB stressor and its impact on QD interaction.

2. Experimental Section

2.1. Quantum Dot Functionalization

We functionalized 620 nm emitting CdSe/ZnS QDs purchased in toluene at a concentration of ~10 μ M (NN-Labs LLC) with dihydrolipoic acid (DHLA) for use in an aqueous environment as described previously^{21, 43}. Briefly, a 250 μ L aliquot of organic QDs were removed from their solvent by addition of 1.5 mL of a 50/50 methanol/acetone mixture and centrifugation at 12,000 rpm. The QDs were then dried using nitrogen gas, resuspended in 250 μ L tetrahydrofuran (THF), and added drop-wise to a 10,000x molar excess of pure DHLA (50 μ L) in 1 mL methanol at pH=11.0. The reaction was stirred at 60°C for 3 hours and at room temperature overnight. The functionalized QDs were then precipitated with ether, resuspended in water, and dialyzed for 72 hours. After dialyzing, the concentration was determined by measuring the absorption at the first exciton and using an extinction coefficient from the literature with Lambert-Beer's law⁴⁴.

2.2. Cell Culture

Our studies used the HaCaT and YUSIK cell lines. HaCaT cells (a gift from Dr. Alice P. Pentland, Department of Dermatology, University of Rochester Medical Center), a human keratinocyte line, were cultured at 37°C with 5% CO₂ in DMEM (Gibco, Inc.) with 5% fetal bovine serum (Gibco, Inc.) and 1% penicillin-streptomycin. YUSIK human metastatic melanoma cells (a gift from Dr. Glynis Scott, Department of Dermatology, University of Rochester Medical Center) were cultured at 37°C with 5% CO₂ in OPTI-MEM (Gibco, Inc.) with 5% fetal bovine serum (Gibco, Inc.) and 1% penicillin-streptomycin. Both cell lines were grown to confluence in BD Falcon T-75 plates (Thermo Fisher Scientific, Inc.) and plated in BD Falcon 12 well plates (Thermo Fisher Scientific, Inc.) at 10⁴ cells/cm². Cells were then grown to 80% confluence (2–3 days) and exposed to UVB and QDs as described. For primary cell controls, primary melanocytes were gifted from Dr. Glynis Scott and primary keratinocytes harvested from fresh viable human skin. We obtained abdominoplasty or mammoplasty samples from healthy adult donors (Strong and Highland Hospitals, University of Rochester, NY), stored it at 4°C, and used it within 6 h of surgery. Skin samples were approved for usage by the University of Rochester Research Subjects Review Board. To harvest basal keratinocytes, a modified version of the protocol outlined by Pentland and Needleman was used (Pentland & Needleman, 1986). Briefly, skin samples sterilized with 0.4 mL fungizone (Invitrogen) and rinsed with 1x PBS. The skin samples were thinned and incubated overnight at room temperature in 12 mL of 0.25% Trypsin-EDTA in a sterile cell culture hood. The epidermis was separated from the dermis, shaken with serum free keratinocyte growth media (KGM-SF) and 1% penicillin/streptomycin (Gibco Inc.) plus 10% fetal bovine serum (FBS), and plated in collagen coated 12 well plates (Purecol 1:5 diluted in 1x PBS). After 24 hours, the media was changed to KGM-SF without FBS, and three days later differential trypsin (~35–45 sec incubation with 0.25% Trypsin-EDTA) performed to remove residual melanocytes and fibroblasts while leaving

behind the keratinocytes. When the plates reached 80% confluence (~2 weeks), 0.3 mM calcium chloride was added to the media to simulate HaCaT culture conditions and experiments performed 48 hours later.

2.3. UV Irradiation and Quantum Dot Application

Experiments were conducted when all cells cultured reached 80% confluence. Media was harvested from each well and replaced with 300 μ L 1x PBS (for HaCaTs) or 1x DPBS (for YUSIKs to prevent cell lifting) and the cells exposed to UVB through a Schott WG 295 glass filter (BES Optics) using FS20 sunlamps (Westinghouse) as described previously⁴⁵. FS20 sunlamps emit in the UVB spectrum (290–320nm) with very low amounts of UVA. UVB lamp output was calibrated using an IL1700 light meter (International Light) with a SED 240 probe to detect light output from 255–320 nm. We irradiated three wells of each cell type to doses from 0–100 mJ/cm² and replaced their media. Cell response was measured by ROS generation as described below and mitochondrial reductase activity (MTT) 24 hours later. For viability measurements, 100 μ L MTT reagent (Invitrogen) was added to each well to achieve 1.2 mg/mL concentration and incubated for 4 hours. Then, 1 mL isopropanol was added to solubilize the Formazan crystals and the absorbance at 570 nm measured. UVB dose impact on viability using MTT was measured on 5 separate occasions with unique passages of our cell lines and the viability was statistically compared to 0 mJ/cm² controls using Student's paired t-test. To investigate the impact of DHLA QDs on cellular viability and ROS generation, HaCaT and YUSIK cells were split into two groups each, one of which received 40 mJ/cm² UVB and the other of which received 0 mJ/cm² UVB. This dose caused minor amounts of cytotoxicity for both cell types, and has been found to induce a strong biological response^{45, 46}. The cells were then incubated with 0 nM or 5 nM DHLA QD for 24 hours in media and the viability by MTT, ROS generation and QD uptake measured using flow cytometry as described below and phase contrast and fluorescence microscopy (Olympus IX70 with QImaging Retiga EXi camera) with a mercury lamp excitation source (360/30 bandpass filter) and narrow emission (620/10 bandpass filter). All images were analyzed using ImageJ.

2.4. ROS Staining and Flow Cytometric Analysis

To determine the cellular ROS response to UVB and QD exposure, 24 hours after exposure YUSIKs and HaCaTs were both stained for ROS production. Dichlorofluorescein diacetate (DCFDA) (Sigma Aldrich) was diluted from its methanol stock concentration of 50 mM and added to the media to yield a final concentration of 5 μ M. The cells were incubated for 30 minutes at 37°C. To prepare for microscopy, the media was removed and cells were rinsed with 1x DPBS two times and incubated with 3% formalin for 15 minutes and the cells imaged as described above. For flow cytometric evaluation, after incubation time the media containing non-adherent cells was removed and the cells were lifted from the plate with 0.25% Trypsin-EDTA (Gibco, Inc.) at room temperature and quenched with FBS. The lifted cells were combined with non-adherent cells and the solution was centrifuged and washed with 1x PBS. The cells were resuspended in 3% formalin (VWR International) for analysis. The appropriate single stain compensation controls were prepared using HaCaT cells. The samples were finally analyzed using an 18-color BD LSRII flow cytometer with filters for DCFDA (488 nm ex. 515/20 nm em.) and QDs (405 nm ex. 660/40 nm.), and results

processed using Flow Jo (Version 8.8.6) software. We gated the FSC/SSC scatter plot to eliminate debris and multi-cellular events, and evaluated the percentage of the 50,000 total cells counted that were positive for QD and/or DCFDA fluorescence, with gating based on unstained controls. For ease of comparing histograms we normalized each point to the maximum intensity in each histogram, multiplied by 100 and labeled the y-axis % of the max. Results displayed are a representative experiment that has been repeated five separate times.

3. Results

3.1. UVB Influence on ROS and Viability

To determine the impact of UVB exposure on QDs cellular ROS generation and cytotoxicity, we first investigated the ROS and viability impact of increasing doses of UVB on HaCaT and YUSIK cells. The MTT assay was used to assess cytotoxicity. The average of 5 separate studies with unique passages of our cell lines are presented and the viability was statistically compared to 0 mJ/cm² controls using Student's paired t-test. For both cell types, UVB had a quantifiable impact on viability. With HaCaTs, a 20 mJ/cm² caused a statistically significant decrease in viability (Figure 1, left axis, $p < 0.05$ t-test with Bonferroni correction). Increasing doses continued to affect HaCaT viability, with the 100 mJ/cm² dose causing a 50% decrease in viability of the cells. For YUSIKs, a 40 mJ/cm² dose UVB was required to find statistical significance, and at 100 mJ/cm², 80% viability remained (Figure 1, left axis). With ROS, the HaCaTs evidenced a strong response, as measured by DCFDA relative fluorescence intensity (normalized to the 0 mJ/cm² UVB dose), at 20 mJ/cm² that continued to rise until 60 mJ/cm² (Figure 1, right axis). ROS then fell gradually with increasing UVB dose. If the relative ROS generation values were further normalized to viability yielding a per cell measure, the response reached a peak at 60 mJ/cm² and remained at close to the same level with increasing UVB (data not shown), which may suggest a maximum ROS level sustainable before the cells transition into a cytotoxic state. For YUSIKs, 20 mJ/cm² and 40 mJ/cm² UVB provided statistically significant increases in ROS over the control but levels were respectively lower than the HaCaT ROS response (Figure 1, right axis). From 60 mJ/cm² to 100 mJ/cm², no statistical significance was observed. The same trend in per cell ROS generation was observed, with the YUSIKs exhibiting a lower maximum ROS production. Since both cell types exhibited statistically significant differences in ROS generation and viability with a 40 mJ/cm² UVB dose, this dosage was used in subsequent experiments.

3.2. QD Cellular Interaction

The viability impact (MTT assay) of a range of QD concentrations (0.5 to 50.0 nM) was tested using HaCaTs and YUSIKs, with and without UVB pre-exposure. A dose of 5.0 nM QDs yielded the greatest difference between no UVB and UVB, and was chosen for subsequent experiments. To visualize the cellular association in culture with HaCaT and YUSIK cells, wide field fluorescence microscopy was used (Figure 2). The fluorescent images were captured using identical exposure and gain conditions for both cell types. The HaCaT cells demonstrated a healthy morphology in the presence of 5.0 nM QDs that corresponded to the limited amount of toxicity by MTT (Figure 2). Low amounts of QDs

were present on the plate, showing little non-specific deposition. With no UVB exposure, the HaCaT cells had a number of QDs present in almost all cells (Figure 2A). This trend appeared after UVB exposure as well, with multiple QDs present in all cells (Figure 2B). Minimal difference in QD cell association was observable between the UVB exposed and non-irradiated cells. In the YUSIK cells, little QD association was observable with and without UVB (Figure 2, C and D). With very long integration times, some QD fluorescence could be observed, but overall levels were quite low compared to the HaCaTs. Phase contrast and wide field fluorescence microscopy provide information about QD cell association, but difficulty in quantification and challenges in visualizing ROS generation with in vitro cell culture underscore the need for flow cytometry.

3.3. QD Uptake by Flow Cytometry

When HaCaT cells were incubated with 5.0 nM QDs for 24 hours, a large portion of the cells became QD positive (59.8%) as determined by flow cytometry (Figure 3). With UVB pre-exposure, the percent of QD positive cells increased to 68.1% (Figure 3B). Both values were higher than those for YUSIKs (17.1% QD positive), and when YUSIKs were pre-exposed to UVB (18.3% QD positive) (Figure 3C,D), which corresponded well to the data observed by fluorescence microscopy in the plate (Figure 2). Fluorescence intensity histograms are representative of 5 independent experiments. This result implies that there was relatively lower QD cell association by the YUSIK versus the HaCaT cells and the UVB pre-exposure stimulated QD cell association for HaCaT but not YUSIK cells.

3.4. ROS Generation

Some research has suggested that the endocytosis of NPs impacts ROS generation⁴⁷. To determine whether this was the case in our system, the ROS generation capacity of the cells was tested with UVB alone, with QDs alone, and with QD and UVB pre-exposure. For HaCaTs, QDs alone induced a significant increase of ROS generation (from 4.9% ROS+ to 19.8% ROS+) (Figure 4A). As expected, exposure of HaCaTs to UVB substantially increased the percent of cells that were ROS+ (26.3%). The subsequent addition of QDs then further increased the ROS+ cell population to 34.5% (Figure 4B). For YUSIKs, QD addition raised the ROS+ portion from 3.3% to 8.6% (Figure 4C). When exposed to UVB, a substantial portion of the population became ROS+ (27.9%), with a small increase to 30.3% with the addition of QDs (Figure 4D). Intensity histograms are representative of 5 independent experiments.

3.5. Cell Model Comparison

Thus far, this study has assumed that the HaCaT keratinocytes and YUSIK cells could serve as models for investigating the interaction of QDs with primary keratinocyte and melanocyte cells. Model cell lines are quite useful due to their increased proliferation ability, ease of acquisition, and decreased inter-sample variance as compared to primary cells, but it is important to determine the suitability of the cell line for the study at hand. HaCaT cells are a well-accepted keratinocyte model that has been used in a large number of UVB-related studies^{48–51}. YUSIK cells are also a highly studied cell line, with known mutations that increase proliferation and diminish cellular adhesion as compared to primary melanocytes^{52–55}. To confirm the aptness of our selected model cells for this study, we

compared QD association for the cell lines versus primary keratinocytes and melanocytes at a 5.0 nM dose without and with UVB exposure as described above. For HaCaTs, QD association levels compared well with primary keratinocytes, with a decrease in the average association in non-irradiated and irradiated samples, but no statistically significant difference discernible (Figure 5A). With YUSIKs, the levels were even closer together with very little association present with and without UVB irradiation (Figure 5B). Since association levels appeared to correspond to the QD ROS generation in our data and in other literature, we concluded that the cell lines chosen were appropriate for comparison.

4. Discussion

UVB is a potent skin carcinogenic agent⁵⁶ that is known to produce ROS in cells^{57, 58}. UVB has also been shown in recent literature to increase the risk of NP skin penetration^{20, 14, 21}. This bespeaks a need to understand the impact of UVB on NP epidermal skin cell interactions. Our study examined the differences in effects of UVB on the interaction of QDs with primary keratinocyte, primary melanocytes, and related cell lines. Based on similarities in QD cell association and UVB response (Figure 5) we found that HaCaT and YUSIK cell lines could serve as model cell lines for primary keratinocytes and melanocytes.

Our work first investigated the impact of UVB alone. We found that ROS generation and cell viability are dependent on cell type, with the HaCaT keratinocytes exhibiting a higher relative ROS generation after UVB exposure compared to the melanocytic YUSIK cells (Figure 4). This is expected based on toxicity levels found using MTT. This result is also supported by the literature, with melanocyte cells demonstrating cytotoxic resistance to UVB due to strong melanin UV light absorption and scattering⁵⁹ and melanin ROS scavenging⁶⁰.

Next we investigated the effect of QDs on ROS generation with and without UVB pre-exposure. Our study performed a direct comparison of skin cell types and found a difference in ROS induced by cell exposure to QDs. Induction of ROS in HaCaT cells by QDs compares well to that found in similar HaCaT studies using coated and uncoated Ag NPs²³, amorphous silicon NPs⁶¹, and carbon nanotubes⁶². With QDs added after UVB pre-exposure, the HaCaT cells again were found to have a significant increase in ROS generation versus the UVB no QD condition, as well as the QD no UVB sample. These results suggest that the ROS generated by UVB photoproducts and QD mediated processes have additive effects in HaCaTs. For YUSIKs, lower association could be observed with QDs by fluorescence microscopy and flow cytometry. When UVB irradiated cells were exposed to QDs, there was not a significant increase in QD association versus the no-UVB condition. This relatively limited QD association was likely at least in part responsible for the relatively lower impact of QD exposure on ROS generation and is perhaps consistent with their known function as secretory cells (i.e. transferring melanin pigment to surrounding cells).

Results of our study provide insight into what might occur after the exposure of skin to UVB and subsequent permeation and interaction of NPs within the local epidermal environment.

We see an increase in cellular ROS with UVB and QDs that suggests independent ROS induction mechanisms for HaCaTs but not for YUSIKs. This difference is most likely due to the low levels of QD association observed in the YUSIKs. The increase in QD association observed in keratinocytes after UVB is supported by the known role UVB to increase the phagocytic capacity of keratinocytes to uptake melanosomes. In keratinocytes, UVB activates KGFR^{31–33} and MyoX mediated phagocytosis²³. HaCaT uptake of melanosomes (~0.5 μm $\times$ 0.25 μm)⁶³ is also known to be increased with UVB exposure^{32, 33, 25}. Some studies have found a substantial increase in HaCaT uptake of latex beads with sizes of 0.1 μm , 0.5 μm , and 1.0 μm after UVB exposure^{33, 63}. Although our results are consistent with this literature, the size difference between our ~20 nm hydrodynamic diameter QDs and the latex particles likely contributes to any difference in uptake rate. Smaller particles (25–50 nm) have been experimentally and mathematically determined to have the most efficient endocytosis through pathways like clathrin coated pits with short lag times^{64, 65, 37}, whereas particles in the size range of latex beads or melanosomes require a slower phagocytic/filopodial mechanism^{33, 23, 63}. Recent work by the Monteiro-Riviere group investigating the uptake of QDs in keratinocytes has supported this hypothesis by suggesting that low density lipoprotein/scavenger receptor mediated uptake is responsible for the endocytosis of carboxylated QDs in keratinocytes³⁷, which are commonly accepted to be forms of clathrin-related endocytic pathways, supporting literature in other cell lines⁶⁶. The fact that QDs generated a significantly higher ROS response in keratinocytes with UVB pre-exposure also suggests the possibility to incur substantial keratinocyte stress in vivo compared to melanocytes, which despite their dendricity and their multi-keratinocyte interactions, appear less susceptible to the effects of QD exposure. Future studies will seek to validate these in vitro findings using human skin models.

Conclusions

Characteristics of NPs are well-accepted to cause differences in cellular uptake and stress, and our results presented here underline the importance of comparison with cell types at risk to interact with NPs. Our results find that UVB can increase the ROS generative potential of QDs in HaCaT keratinocytes, but have a lower impact on the less phagocytic YUSIK cells. Our results suggest work to investigate the impact of other model cell types and QD surface coatings on uptake and ROS generation, as well as work to decrease NP cellular uptake for cosmetics or increase NP uptake for specific targeted therapeutics. We have raised important questions about epidermal cellular interactions of QDs and the impact of UVB, which is of increasing interest with the growing potential for NPs to interact with human skin.

Acknowledgements

Flow cytometry experiments were performed at the University of Rochester Medical Center Flow Core, which is headed by Dr. Timothy Bushnell. This work was supported by the National Science Foundation (CBET 0837891) and the National Institute of Health (NIAID K25AI060884).

REFERENCES

1. Koleyil GI, Levina L, Shukla H, Myrskog SH, Hinds S, Pattantyus-Abraham AG, Sargent EH. Efficient, stable infrared photovoltaics based on solution-cast colloidal quantum dots. *ACS Nano*. 2008; 2:833–840. [PubMed: 19206479]

2. Geller M, Marent A, Nowozin T, Bimberg D. Self-organized quantum dots for future semiconductor memories. *Journal of Physics: Condensed Matter*. 2008; 20:454202.
3. Zhao J, Bardecker JA, Munro AM, Liu MS, Niu Y, Ding IK, Luo J, Chen B, Jen AK, Ginger DS. Efficient CdSe/CdS quantum dot light-emitting diodes using a thermally polymerized hole transport layer. *Nano Lett*. 2006; 6:463–467. [PubMed: 16522043]
4. Walling MA, Novak JA, Shepard JRE. Quantum dots for live cell and in vivo imaging. *Int J Mol Sci*. 2009; 10:441–491. [PubMed: 19333416]
5. Ballou B, Ernst LA, Andreko S, Harper T, Fitzpatrick JAJ, Waggoner AS, B. M Jr. Sentinel lymph node imaging using quantum dots in mouse tumor models. *Bioconjugate Chem*. 2007; 18:389–396.
6. Medintz IL, Mattoussi H, Clapp AR. Potential clinical applications of quantum dots. *Int J Nanomed*. 2008; 3:151–167.
7. Hollingsworth, J.; Klimov, V.; Anikeeva, P. Google Patents. 2005.
8. Lee DY, Pham JT, Lawrence J, Lee CH, Parkos C, Emrick T, Crosby AJ. Macroscopic nanoparticle ribbons and fabrics. *Advanced materials*. 2013; 25:1248–1253. [PubMed: 23143819]
9. Hardman R. A Toxicological Review of Quantum Dots: Toxicity Depends on Physico-Chemical and Environmental Factors. *Environ Health Persp*. 2006; 114:165–172.
10. Lewinski NA, Colvin VL, Drezek RA. Cytotoxicity of nanoparticles. *Small*. 2008; 4:26–49. [PubMed: 18165959]
11. Rzigalinski BA, Strobl JS. Cadmium-containing nanoparticles: perspectives on pharmacology and toxicology of quantum dots. *Toxicol Appl Pharm*. 2009; 238:280–288.
12. Winnik FM, Maysinger D. Quantum dot cytotoxicity and ways to reduce it. *Accounts of chemical research*. 2013; 46:672–680. [PubMed: 22775328]
13. Gopee NV, Roberts DW, Webb P, Cozart CR, Siitonen PH, Latendresse JR, Warbitton AR, Yu WW, Colvin VL, Walker NJ, Howard PC. Quantitative determination of skin penetration of PEG-coated CdSe quantum dots in dermabraded but not intact SKH-1 hairless mouse skin. *Toxicol Sci*. 2009; 111:37–48. [PubMed: 19574408]
14. Mortensen LJ, Oberdorster G, Pentland AP, DeLouise LA. In vivo skin penetration of quantum dot nanoparticles in the murine model: the effect of UVR. *Nano Lett*. 2008; 8:2779–2787. [PubMed: 18687009]
15. Prow TW, Monteiro-Riviere NA, Inman AO, Grice JE, Chen X, Zhao X, Sanchez WH, Gierden A, Kendall MA, Zvyagin AV, Erdmann D, Riviere JE, Roberts MS. Quantum dot penetration into viable human skin. *Nanotoxicology*. 2012; 6:173–185. [PubMed: 21456897]
16. Zhang LW, Monteiro-Riviere NA. Assessment of quantum dot penetration into intact, tape-stripped, abraded and flexed rat skin. *Skin Pharmacol Appl*. 2008; 21:166–180.
17. Zhang LW, Yu WW, Colvin VL, Monteiro-Riviere NA. Biological interactions of quantum dot nanoparticles in skin and in human epidermal keratinocytes. *Toxicol Appl Pharm*. 2008; 228:200–211.
18. Paliwal S, Menon GK, Mitragotri S. Low-frequency sonophoresis: ultrastructural basis for stratum corneum permeability assessed using quantum dots. *J Invest Dermatol*. 2006; 126:1095–1101. [PubMed: 16528354]
19. Ravichandran S, Mortensen LJ, Delouise LA. Quantification of human skin barrier function and susceptibility to quantum dot skin penetration. *Nanotoxicology*. 2011; 5:675–686. [PubMed: 21142716]
20. Mortensen LJ, Jatana S, Gelein R, De Benedetto A, De Mesy Bentley KL, Beck LA, Elder A, Delouise LA. Quantification of quantum dot murine skin penetration with UVR barrier impairment. *Nanotoxicology*. 2013; 7:1386–1398. [PubMed: 23078247]
21. Mortensen LJ, Zheng H, Faulknor R, De Benedetto A, Beck L, DeLouise LA. Increased in vivo skin penetration of quantum dots with UVR and in vitro quantum dot cytotoxicity. *Proceedings SPIE*. 2009; 7189:718919.
22. Monteiro-Riviere NA, Wiench K, Landsiedel R, Schulte S, Inman AO, Riviere JE. Safety Evaluation of Sunscreen Formulations Containing Titanium Dioxide and Zinc Oxide Nanoparticles in UVB Sunburned Skin: An In Vitro and In Vivo Study. *Toxicological Sciences*. 2011; 123:264–280. [PubMed: 21642632]

23. Lu W, Senapati D, Wang S, Tovmachenko O, Singh AK, Yu H, Ray PC. Effect of surface coating on the toxicity of silver nanomaterials on human skin keratinocytes. *Chemical Physics Letters*. 2010; 487:92–96.
24. Seiberg M. Keratinocyte-melanocyte interactions during melanosome transfer. *Pigment Cell Res*. 2001; 14:236–242. [PubMed: 11549105]
25. Van Den Bossche K, Naeyaert JM, Lambert J. The quest for the mechanism of melanin transfer. *Traffic*. 2006; 7:769–778. [PubMed: 16787393]
26. Black AK, Fincham N, Greaves MW, Hensby CN. Time course changes in levels of arachidonic acid and prostaglandins D2, E2, F2 alpha in human skin following ultraviolet B irradiation. *Br J Clin Pharmacol*. 1980; 10:453–457. [PubMed: 7437257]
27. Epstein JH, Fukuyama K, Fye K. Effects of ultraviolet radiation on the mitotic cycle and DNA, RNA and protein synthesis in mammalian epidermis in vivo. *Photochem Photobiol*. 1970; 12:57–65. [PubMed: 5498527]
28. Holleran WM, Uchida Y, Halkier-Sorensen L, Haratake A, Hara M, Epstein JH, Elias PM. Structural and biochemical basis for the UVB-induced alterations in epidermal barrier function. *Photodermatol Photoimmunol Photomed*. 1997; 13:117–128. [PubMed: 9453079]
29. Kupper TS, Chua AO, Flood P, McGuire J, Gubler U. Interleukin 1 gene expression in cultured human keratinocytes is augmented by ultraviolet irradiation. *J Clin Invest*. 1987; 80:430–436. [PubMed: 3497177]
30. Pentland AP, Scott G, VanBuskirk J, Tanck C, LaRossa G, Brouxhon S. Cyclooxygenase-1 deletion enhances apoptosis but does not protect against ultraviolet light-induced tumors. *Cancer Res*. 2004; 64:5587–5591. [PubMed: 15313895]
31. Belleudi F, Purpura V, Scrofani C, Persechino F, Leone L, Torrisi MR. Expression and signaling of the tyrosine kinase FGFR2b/KGFR regulates phagocytosis and melanosome uptake in human keratinocytes. *FASEB J*. 2011; 25:170–181. [PubMed: 20844240]
32. Cardinali G, Bolasco G, Aspite N, Lucania G, Lotti LV, Torrisi MR, Picardo M. Melanosome transfer promoted by keratinocyte growth factor in light and dark skin-derived keratinocytes. *J Invest Dermatol*. 2008; 128:558–567. [PubMed: 17882267]
33. Cardinali G, Ceccarelli S, Kovacs D, Aspite N, Lotti LV, Torrisi MR, Picardo M. Keratinocyte growth factor promotes melanosome transfer to keratinocytes. *J Invest Dermatol*. 2005; 125:1190–1199. [PubMed: 16354189]
34. Biggs, LC.; Mikkola, ML. *Seminars in cell & developmental biology*. 2014. Early inductive events in ectodermal appendage morphogenesis.
35. Cichorek M, Wachulska M, Stasiewicz A, Tyminska A. Skin melanocytes: biology and development. *Postepy dermatologii i alergologii*. 2013; 30:30–41. [PubMed: 24278043]
36. Ryman-Rasmussen JP, Riviere JE, Monteiro-Riviere NA. Variables influencing interactions of untargeted quantum dot nanoparticles with skin cells and identification of biochemical modulators. *Nano Lett*. 2007; 7:1344–1348. [PubMed: 17408303]
37. Zhang LW, Monteiro-Riviere NA. Mechanisms of quantum dot nanoparticle cellular uptake. *Toxicol Sci*. 2009; 110:138–155. [PubMed: 19414515]
38. Mortensen LJ, Ravichandran S, DeLouise LA. The impact of UVB exposure and differentiation state of primary keratinocytes on their interaction with quantum dots. *Nanotoxicology*. 2013; 7:1244–1254. [PubMed: 22998293]
39. Clayton E, Doupé DP, Klein AM, Winton DJ, Simons BD, Jones PH. A single type of progenitor cell maintains normal epidermis. *Nature*. 2007; 446:185–189. [PubMed: 17330052]
40. Choi HS, Liu W, Liu F, Nasr K, Misra P, Bawendi MG, Frangioni JV. Design considerations for tumour-targeted nanoparticles. *Nat Nanotechnol*. 2010; 5:42–47. [PubMed: 19893516]
41. Li Z, Huang P, Lin J, He R, Liu B, Zhang X, Yang S, Xi P, Zhang X, Ren Q, Cui D. Arginine-glycine-aspartic acid-conjugated dendrimer-modified quantum dots for targeting and imaging melanoma. *J Nanosci Nanotechnol*. 2010; 10:4859–4867. [PubMed: 21125820]
42. Voura EB, Jaiswal JK, Mattoussi H, Simon SM. Tracking metastatic tumor cell extravasation with quantum dot nanocrystals and fluorescence emission-scanning microscopy. *Nat Med*. 2004; 10:993–998. [PubMed: 15334072]

43. Zheng H, Mortensen LJ, DeLouise LA. Thiol antioxidant-functionalized CdSe/ZnS quantum dots: synthesis, characterization, cytotoxicity. *Journal of biomedical nanotechnology*. 2013; 9:382–392. [PubMed: 23620993]
44. Yu WW, Qu L, Guo W, Peng X. Experimental Determination of the Extinction Coefficient of CdTe, CdSe, and CdS Nanocrystals. *Chemistry of Materials*. 2003; 15:2854–2860.
45. Brouxhon S, Kyranides S, O'Banion MK, Johnson R, Pearce DA, Centola GM, Miller J.-n. H. McGrath KH, Erdle B, Scott G, Schneider S, VanBuskirk J, Pentland AP. Sequential Down-regulation of E-Cadherin with Squamous Cell Carcinoma Progression: Loss of E-Cadherin via a Prostaglandin E2-EP2 Dependent Posttranslational Mechanism. *Cancer Res*. 2007; 67:7654–7664. [PubMed: 17699770]
46. Chaturvedi V, Qin JZ, Stennett L, Choubey D, Nickoloff BJ. Resistance to UV-induced apoptosis in human keratinocytes during accelerated senescence is associated with functional inactivation of p53. *J Cell Physiol*. 2004; 198:100–109. [PubMed: 14584049]
47. Chang E, Thekkekk N, Yu WW, Colvin VL, Drezeck R. Evaluation of quantum dot cytotoxicity based on intracellular uptake. *Small*. 2006; 2:1412–1417. [PubMed: 17192996]
48. Aoki-Yoshida A, Aoki R, Takayama Y. Protective effect of pyruvate against UVB-induced damage in HaCaT human keratinocytes. *Journal of bioscience and bioengineering*. 2013; 115:442–448. [PubMed: 23219088]
49. Kim SB, Kang OH, Joung DK, Mun SH, Seo YS, Cha MR, Ryu SY, Shin DW, Kwon DY. Anti-inflammatory effects of tectroside on UVB-induced HaCaT cells. *International journal of molecular medicine*. 2013; 31:1471–1476. [PubMed: 23588209]
50. Park YK, Jang BC. UVB-induced anti-survival and pro-apoptotic effects on HaCaT human keratinocytes via caspase- and PKC-dependent downregulation of PKB, HIAP-1, Mcl-1, XIAP and ER stress. *International journal of molecular medicine*. 2014; 33:695–702. [PubMed: 24356997]
51. Vitale N, Kisslinger A, Paladino S, Procaccini C, Matarese G, Pierantoni GM, Mancini FP, Tramontano D. Resveratrol couples apoptosis with autophagy in UVB-irradiated HaCaT cells. *PloS one*. 2013; 8:e80728. [PubMed: 24260465]
52. Aziz SA, Jilaveanu LB, Zito C, Camp RL, Rimm DL, Conrad P, Kluger HM. Vertical targeting of the phosphatidylinositol-3 kinase pathway as a strategy for treating melanoma. *Clin Cancer Res*. 2010; 16:6029–6039. [PubMed: 21169255]
53. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA. Mutations of the BRAF gene in human cancer. *Nature*. 2002; 417:949–954. [PubMed: 12068308]
54. McClelland L, Chen Y, Soong J, Kuo I, Scott G. Plexin B1 inhibits integrin-dependent pp125FAK and Rho activity in melanoma. *Pigment Cell Melanoma Res*. 2011; 24:165–174. [PubMed: 21029396]
55. Scott GA, McClelland LA, Fricke AF, Fender A. Plexin C1, a receptor for semaphorin 7a, inactivates cofilin and is a potential tumor suppressor for melanoma progression. *J Invest Dermatol*. 2009; 129:954–963. [PubMed: 18987670]
56. de Gruijl FR, Sterenborg HJ, Forbes PD, Davies RE, Cole C, Kelfkens G, van Weelden H, Slaper H, van der Leun JC. Wavelength dependence of skin cancer induction by ultraviolet irradiation of albino hairless mice. *Cancer Res*. 1993; 53:53–60. [PubMed: 8416751]
57. Cadet J, Sage E, Douki T. Ultraviolet radiation-mediated damage to cellular DNA. *Mutat Res*. 2005; 571:3–17. [PubMed: 15748634]
58. Zhang X, Rosenstein BS, Wang Y, Lebwohl M, Wei H. Identification of possible reactive oxygen species involved in ultraviolet radiation-induced oxidative DNA damage. *Free Radic Biol Med*. 1997; 23:980–985. [PubMed: 9358240]

59. Leeuw SMD, Smit NPM, Veldhoven MV, Pennings EM, Pavel S, Simons JWIM, Schothorst AA. Melanin content of cultured human melanocytes and UV-induced cytotoxicity. *Journal of Photochemistry and Photobiology B: Biology*. 2001; 61:106–113.
60. Bustamante J, Bredeston L, Malanga G, Mordoh J. Role of Melanin as a Scavenger of Active Oxygen Species. *Pigment Cell Research*. 1993; 6:348–353. [PubMed: 8302773]
61. Nabeshi H, Yoshikawa T, Matsuyama K, Nakazato Y, Tochigi S, Kondoh S, Hirai T, Akase T, Nagano K, Abe Y, Yoshioka Y, Kamada H, Itoh N, Tsunoda S, Tsutsumi Y. Amorphous nanosilica induce endocytosis-dependent ROS generation and DNA damage in human keratinocytes. *Part Fibre Toxicol*. 2011; 8:1–1. [PubMed: 21235812]
62. Manna SK, Sarkar S, Barr J, Wise K, Barrera EV, Jejelowo O, Rice-Ficht AC, Ramesh GT. Single-walled carbon nanotube induces oxidative stress and activates nuclear transcription factor-kappaB in human keratinocytes. *Nano Lett*. 2005; 5:1676–1684. [PubMed: 16159204]
63. Virador VM, Muller J, Wu X, Abdel-Malek ZA, Yu ZX, Ferrans VJ, Kobayashi N, Wakamatsu K, Ito S, Hammer JA, Hearing VJ. Influence of alpha-melanocyte-stimulating hormone and ultraviolet radiation on the transfer of melanosomes to keratinocytes. *FASEB J*. 2002; 16:105–107. [PubMed: 11729101]
64. Chithrani BD, Ghazani AA, Chan WC. Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. *Nano Lett*. 2006; 6:662–668. [PubMed: 16608261]
65. Jiang W, Kim BYS, Rutka JT, Chan WCW. Nanoparticle-mediated cellular response is size-dependent. *Nat Nanotechnol*. 2008; 3:145–150. [PubMed: 18654486]
66. Kelf TA, Sreenivasan VKA, Sun J, Kim EJ, Goldys EM, Zvyagin AV. Non-specific cellular uptake of surface-functionalized quantum dots. *Nanotechnology*. 2010; 21:285105. [PubMed: 20585157]

Figure 1.

Summary of UVB dose impact on relative cell viability as measured by MTT (bar chart on left axis) and ROS production as determined using flow cytometry (line chart on right axis). UVB causes a detriment to cell viability starting at 20 mJ/cm² for HaCaT keratinocytes, but its toxicity is delayed until 40 mJ/cm² for YUSIK melanocytes with a much greater effect of UVB on the HaCaTs than the YUSIKs at high UVB doses. ROS generation peaks at 60 mJ/cm² for HaCaTs and 40 mJ/cm² for YUSIKs, with the more limited number of cells at higher doses limiting the total amount of ROS. HaCaTs have a statistically significant increase in ROS at all UVB doses, while the YUSIKs no longer generate a significant difference after 40 mJ/cm² (* = $p < 0.05$, ** = $p < 0.01$, $n=4$ for all experimental conditions, paired t-test with Bonferonni correction).

Figure 2.

Phase contrast and widefield fluorescent microscopy of the QD-cell interaction for HaCaTs and YUSIKs. Cells were incubated with 5.0 nM DHLA QDs for 24 hours without and with 40 mJ/cm² UVB. Cells were rinsed 2x with PBS, fixed and imaged at 40× magnification. UVB decreases cell density for the irradiated HaCaTs and irradiated YUSIKs. The media was removed and replaced with 1x DPBS to allow imaging in the plate. HaCaTs have a strong QD signal without UVB (A) and with UVB (B). A UVB-induced difference in uptake is not discernible for the HaCaTs or the YUSIKs. With YUSIK cells, little QD fluorescence can be seen in non-irradiated (C) and irradiated (D) cells.

Figure 3.

QD cell uptake quantified using flow cytometry. For ease of comparison the data points are normalized to the highest intensity value within each histogram and plotted on the y-axis as a % of the maximum. HaCaT cells exhibit higher levels of QD uptake compared to the YUSIK cells and exhibit significant increases in median fluorescence intensity over the no-QD controls both in non-irradiated (A and B) and UV-irradiated (C and D) samples. Graphs are representative of 5 independent experiments.

Figure 4.

Relative ROS generation from UVB and the incubation of cells with QDs. For ease of comparison the data points are normalized to the highest intensity value within each histogram and plotted on the y-axis as a % of the maximum. (A) The % of ROS+ HaCaTs increases following 24 hr exposure to 5 nM QDs (19.8%) relative to control cells (4.9%) with no QD exposure. (B) UVB alone also increases the ROS+ HaCaT cells to 26.3% relative to unirradiated cells (4.9%), and addition of QD post UVB exposure further increases the ROS+ HaCaT cells to 34.5%. (C) In contrast, for YUSIK cells a lower percent ROS+ cells were found following exposure to 5 nM QDs (8.6%). (D) UVB exposure similarly increased the percent of ROS+ cells but only a slight increase in ROS+ cells occurred following exposure to 5 nM QDs. Reported values are representative of 5 independent experiments.

Figure 5.

QD uptake exhibits similar levels to their primary cell corollary with and without UVB for the HaCaT and YUSIK cell lines. HaCaTs and primary keratinocytes exhibit similar levels of QD uptake with and without UVB exposure, which suggests that HaCaT cells are a suitable model for investigating NP keratinocyte interactions (A). Primary cells have a slightly higher average level of QD uptake, but no statistical difference is discernible. When the YUSIKs were compared to primary melanocytes with and without UVB, the same levels of QD uptake were observed as well (B). The data indicates that keratinocytes more readily phagocytose QDs than melanocytes.