Nanotechnology in Cosmetics FDA's Perspective

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NNI Symposium
July 2015

Disclaimer

- *Any opinions expressed during this presentation are those of the presenter and should not be considered official current or future policy of the U.S. Food & Drug Administration or any other U.S. government organization.*
- *Any mention of trade names or specific products should not be considered an endorsement.*

Commercial Products Containing Nanomaterials

cosmetics

toothpaste

supplements

beer bottles

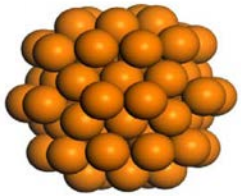
drugs

sunscreen

coatings

wound dressings

Why Nanomaterials?



Nanomaterials

Novel Properties

Specific Effects

FDA's Definition

- Nanotechnology
  - No official FDA definition
  - Agency guidance – ***Points to consider***
    - an engineered material or end product that has at least one dimension in nanoscale range (roughly 1 to 100 nm), or
    - exhibits unique properties or phenomena that enable novel applications

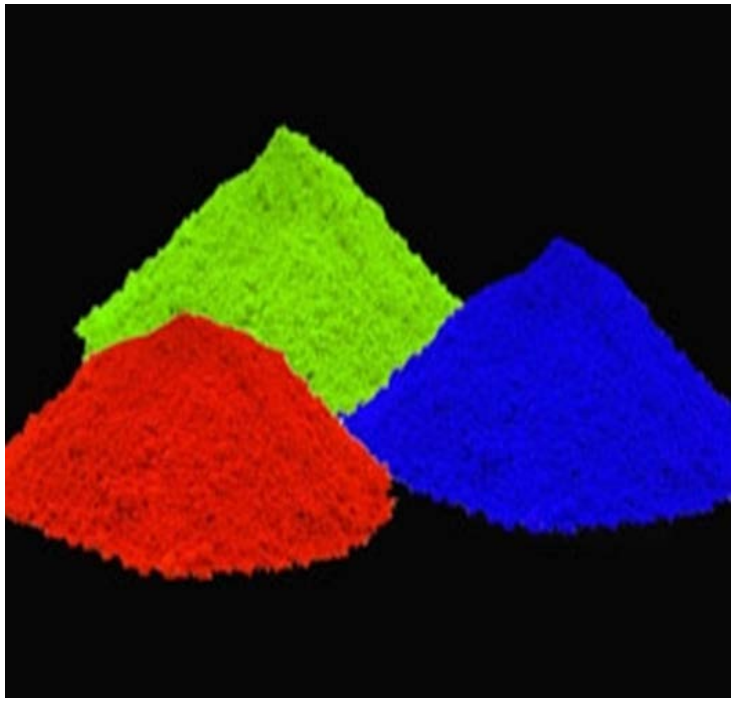
Definition – Cosmetics (201(i))

- Articles intended for:
  - Cleansing
  - Beautifying
  - Promoting attractiveness
  - Altering appearance

Excludes soaps

Definition – Color Additive (201(t))

- Dye, pigment, or other substance that, when added or applied to a food, drug, or cosmetic, is capable of imparting color



Requirements for Market

- FDA Authority Over Cosmetics
  - Under the FD&C Act, cosmetics must not be adulterated (601) or misbranded (602)
  - No pre-market approval of cosmetics, with the exception of color additives
  - Manufacturer is responsibility for safety of marketed products
  - Manufacturer or distributors should have obtained all data and information needed to substantiate the safety of the product before marketing

Regulation of Nanotechnology/Nanoparticles

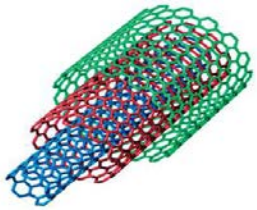
- FDA regulates products, not technology
- Cosmetics manufactured using nanotechnology are subject to the same legal requirements as any other cosmetics
- As new scientific information is presented for FDA consideration, agency policy changes may be considered

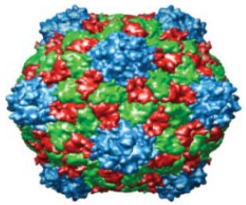
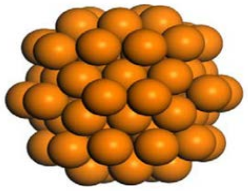
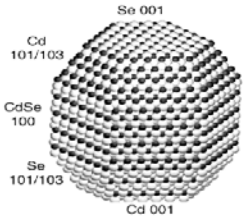
Current Reported Uses of Nanotechnology in Cosmetics

- Excellent dispersability
- Alter optical properties
- Deliver water or lipid soluble ingredients
- Protect light or oxygen sensitive ingredients
- Improve stability of chemically unstable ingredients
- Controlled release of ingredients
- Improve skin hydration
- Transparent on skin
- Increase protection against both UVA and UVB rays

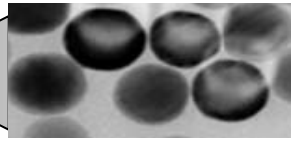
Diversity of Nanomaterials

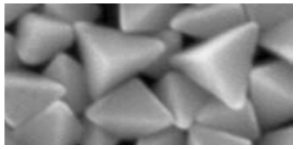
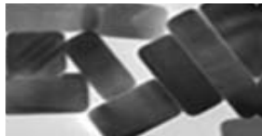
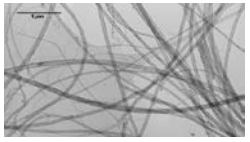
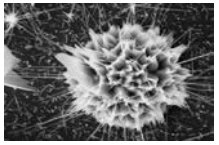
Organic **Inorganic**



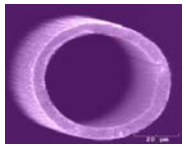
Shapes



Structures





Size
Surface
Formulation

Cobley et al., Plasmonics 2009, 4, 171; http://www.itap.physik.uni-stuttgart.de/~gkig/neu/english/schurr.htm; http://www.herrera.unt.edu.ar/nano/research.html
http://eosl.gtri.gatech.edu/Default.aspx?alias=eosl.gtri.gatech.edu/newnano; http://lo.epfl.ch/plasmonicSHRIMP

Guidance for Industry
Safety of Nanomaterials in Cosmetic Products

Guidance document addressing ***points to consider*** in assessing the safety of nanomaterials in cosmetic products

Issued June 24, 2014

Guidance for Cosmetic Products, cont.

- Nanomaterials are engineered material or end product that have at least one dimension in the nanoscale range (roughly 1 nm to 100 nm)
- This extremely small size leads to unique properties different from those of conventional product
- These unique properties could affect the function, quality, safety, and/or effectiveness of a product

Guidance for Cosmetic Products, cont.

Nanomaterial characterization

- *Physico-Chemical Properties*
  - Type/Size
  - Aggregation/Agglomeration
  - Morphology (shape, surface area, etc.)
  - Surface chemistry (zeta potential, surface coating, etc.)
  - Solubility
  - Stability
  - Composition
  - Impurities

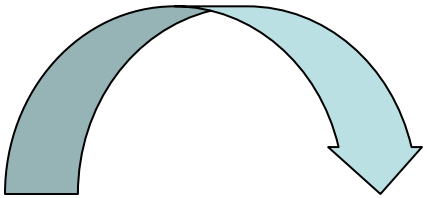
Guidance for Cosmetic Products, cont.

Physico-chemical Properties

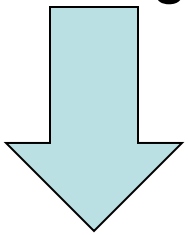
Modify cellular uptake

Protein binding

Translocation



Interaction with biological systems



Ability to distribute throughout the body

Guidance for Cosmetic Products, cont.

Toxicity Concerns

- Similar to that of other FDA regulated products
- Potential for toxicity related to type of nanoparticle used
- Exposure through new routes
- Absorption into and through skin
- Uptake and bioavailability
- New impurities during manufacturing
- New toxicology issues not seen in their traditional counterparts

Guidance for Cosmetic Products, cont.

Toxicity Testing Methods, cont.

- Traditional testing methods may need to be modified to address
  - absorption
  - biodistribution
  - accumulation
  - clearance
- Testing methods used may need to address
  - short-term toxicity
  - long-term toxicity of nanomaterials

Guidance for Cosmetic Products, cont.

Recommendations for Industry, cont.

- Toxicity testing should cover relevant toxicological endpoints, such as
  - Acute toxicity
  - Skin absorption
  - Skin irritation
  - Sensitization
  - Repeated dose toxicity
  - Mutagenicity/ genotoxicity
- Additional testing may be needed based on the results obtained from this basic test battery

Ultimate Goals of Research at FDA

- **Communication**
  - Scientific publications
  - Updates for consumers and industry on website and social media
- **Policy Development**
  - Guidance
  - Rule making/regulations
  - Educational materials
- **Enforcement Strategies and Actions**
  - Domestic facilities and products
  - Foreign facilities and imported products

Scientific Needs in Assessing Safety of Nanomaterials Used in Cosmetics

Research to Determine:

- Methods to characterize size, stability, solubility, and other properties in different solutions/vehicles
- Battery of toxicological studies comparing properties of nanoparticles vs. macroparticles or bulk phase size materials
- Information on absorption into and through skin
- Assessment of potential dermal toxicity
- Assessment of potential toxicity through other routes of administration, as needed

Nature of Research

- Percutaneous absorption studies
- Skin penetration of soft nanoparticles
  - Liposomes
  - Dendrimers
- Skin penetration of hard/metallic nanoparticles
  - coated silver nanoparticles

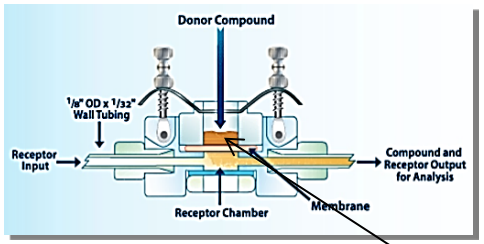
Percutaneous absorption studies were conducted on different types of nanoparticles at CFSAN

- ❑Nanosomes – soft nanoparticles
- ❑Dendrimer nanoparticles – polymeric
- ❑Silver nanoparticles – Metallic nanoparticle

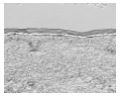
Various methods were used to characterize these nanomaterials and determine their penetration into animal and human skin

Percutaneous Absorption Studies - Methods

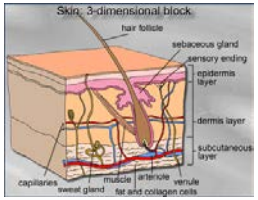
In vitro skin absorption/penetration studies in flow-through diffusion cells.



Human skin in the diffusion cell



Flow-through diffusion cell System (PermeGear In-Line ILC07)



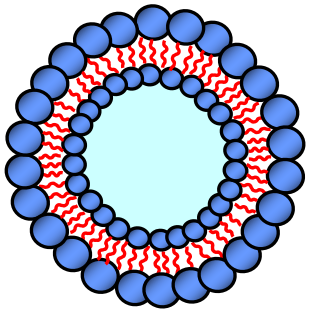
Skin Structure

- ❑ Split-thickness skin prepared with a dermatome
- ❑ Both human and animal skin models
- ❑ Skin discs mounted with epidermal side up
- ❑ Receptor fluid – physiological buffer to maintain viability - Importance of metabolism in skin
- ❑ Dosing vehicles identified (solution, oil-in-water emulsion formulation)
- ❑ Apply dosing formulation with nanoparticle test compound on skin surface
- ❑ Collect receptor fluid fractions for 24 hours
- ❑ Wash the skin surface to remove dosing formulation
- ❑ For some studies – skin surface was tape stripped to remove nanoparticles bound to surface layer of the stratum corneum

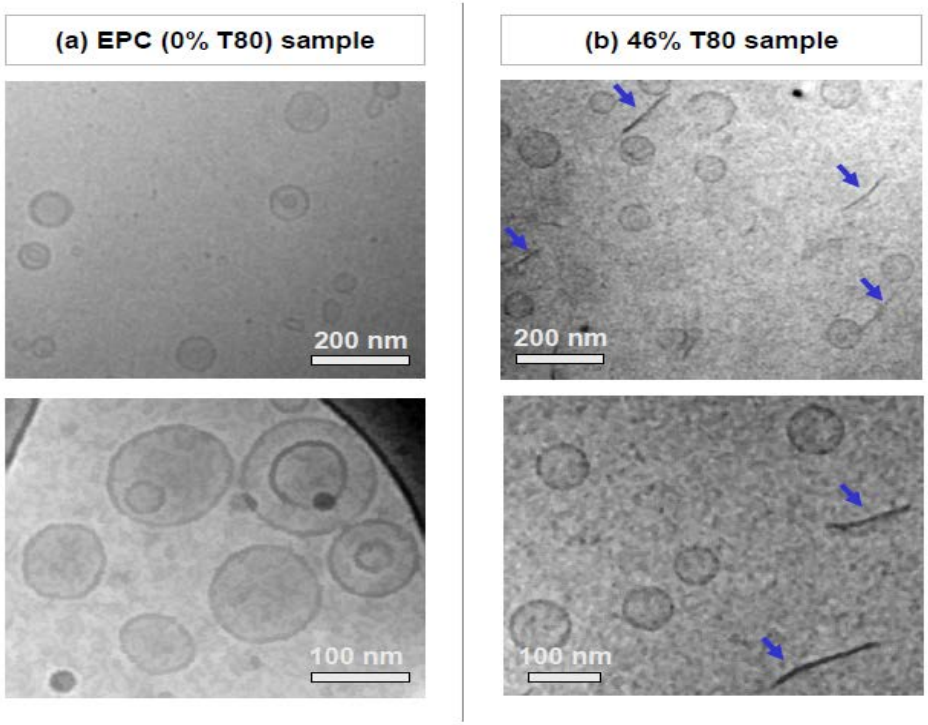
Skin Penetration of soft nanoparticles – “Flexible” liposomes

Liposomes

•Liposomes are self assembled capsules formed in water by lipids. “Flexible” liposomes are believed to have deformable bilayers that enable the liposomes to squeeze through smaller openings in the skin structure



•Studies were done to look at phase behavior, structure and extent of dermal penetration of these flexible liposome formulations to understand the mechanism of penetration. Studies done in hairless guinea pig and human skin

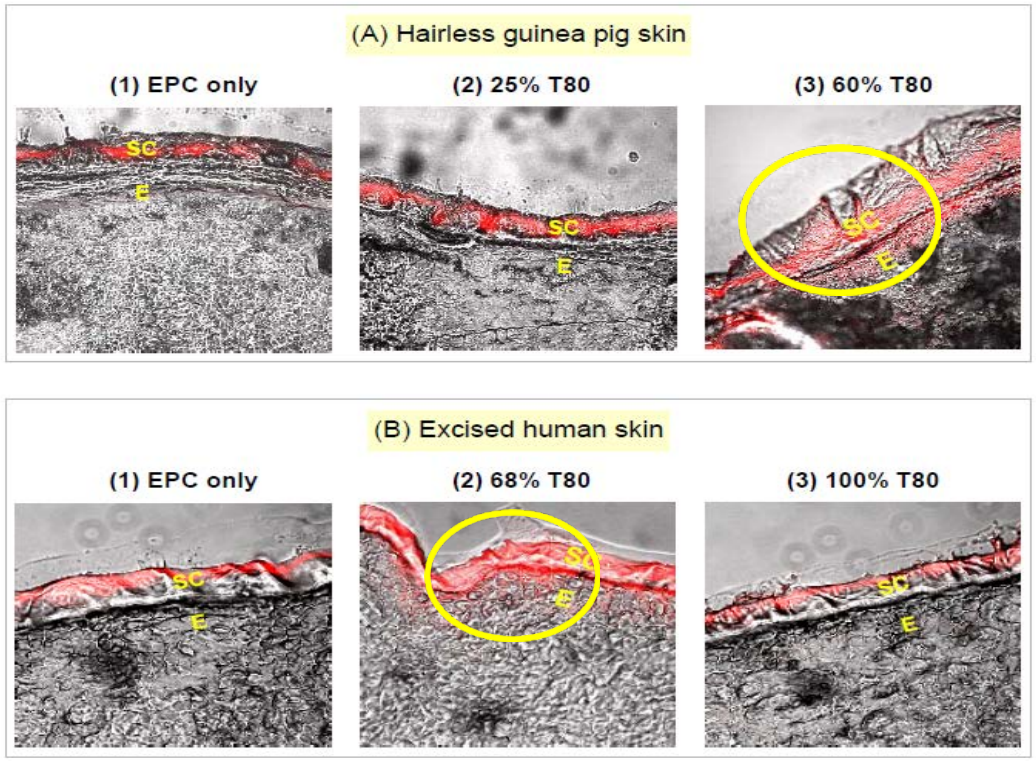


Cryo-TEM images of formulations corresponding to “rigid” (a) and “flexible” (b) liposomes.

The structure of the “flexible” liposome formulations are actually mixtures of liposomes and micelles.

“Rigid” (a) liposomes appear unilamellar or bilamellar.

“Flexible” (b) – liposomes reveal a combination of spherical unilamellar liposomes and a number of disklike micelles (marked by arrows). The disks are seen from the side, which is why they show up as dark lines or curves in the images.



Confocal microscopy images showing indirect evidence for the penetration of EPC/T80 ("flexible") liposomes into the epidermis of (A) hairless guinea pig skin; and (B) excised human skin. The red fluorescence (lipid dye) penetrates significantly through the stratum corneum (labeled SC) and into the epidermis (labeled E) , as seen in Figures A3 and B2.

Percutaneous Penetration of Dendrimer Nanoparticles

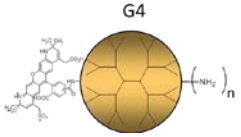
- Dendrimers are highly branched stable polymeric nanoparticles with terminal functional groups capable of binding other molecules which could be used to increase delivery of chemicals into skin

- Dendrimers can be synthesized to specific nano-sizes, compositions, and surface chemistries. PAMAM dendrimers consist of terminal poly(amidoamine) end groups, with each branching set is referred to as a “generation” (G) containing terminal amine groups

- There is concern about nanoparticles potentially increasing the skin absorption of ingredients that are currently considered safe in cosmetic products. The surface charge of the dendrimers may affect skin absorption of bound cosmetic ingredients

- Pig skin is often used as a model for human skin. Are they comparable for skin absorption studies of nanoparticles?

Methods:



•Different size PAMAM dendrimers, G3, G4, G5 and G6 were synthesized (at Texas Christian University)

•A photo stable fluorescent dye - Alexa Fluor 568 was conjugated to terminal amines (positive charge) on PAMAM dendrimers generations 3-6 via amide bonds.

•To determine surface charge effects in skin penetration – compounds were further modified to provide negatively charged and neutral dendrimers.

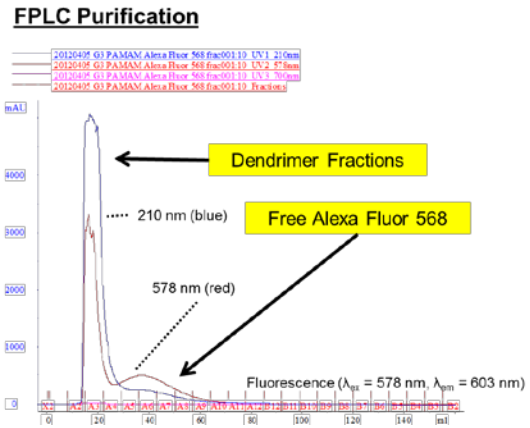
•Dendrimers were purified to remove free dye and characterized by the Nanotechnology Characterization Laboratory.

•Dendrimers dosed on skin using solution and an oil-in-water emulsion representative of a cosmetic lotion at low and high doses on both excised pig and human skin for 24 hours.

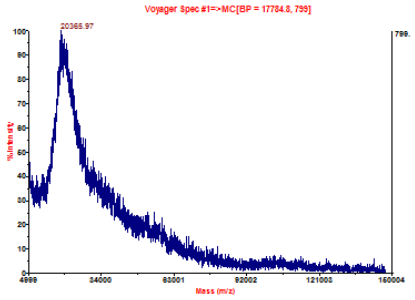
•Skin penetration was determined by localization of fluorescence in the skin layers using confocal microscopy.

Characterization:

- Free unconjugated fluorophore was removed by ultrafiltration followed by gel filtration, and characterized by 1H Nuclear Magnetic Resonance (NMR) Spectroscopy
- UV-Vis Spectroscopy
- Fluorescence Spectroscopy
- **Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) Mass Spectroscopy – determine molecular weight**.
- High Pressure Liquid Chromatography (HPLC)
- **Fast Pressure Liquid Chromatography (FPLC)** – used for purification – separation of the free dye and dendrimer fractions



MALDI-MS G4 PAMAM Dendrimer-Alexa Fluor 568



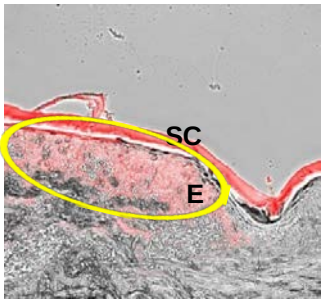
RESULTS:

•Most fluorescence seen in the skin remained in the stratum corneum and hair follicles of both pig and human skin.

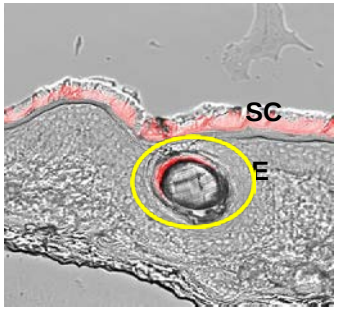
•Pig skin appears to be more permeable to the larger dendrimers compared to human skin. Human skin penetration of dendrimers was not as extensive as pig skin.

•Dendrimers penetrated most when applied in solution compared with emulsion applications. Dendrimers applied in emulsion mostly did not penetrate beyond the stratum corneum (barrier layer) of human skin.

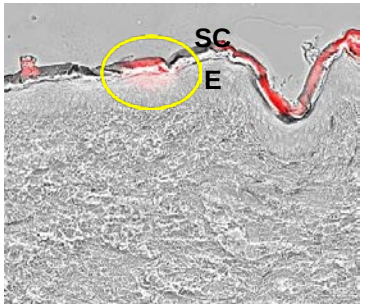
Pig Skin – Aqueous solutions high dose



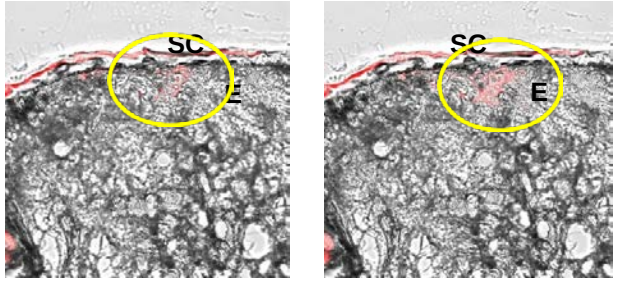
Pig Skin – Aqueous solutions low dose



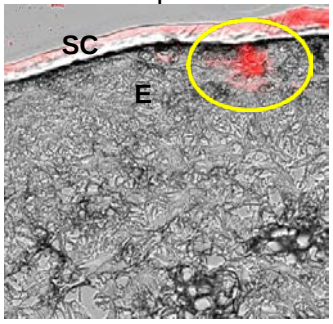
Pig Skin – Emulsion



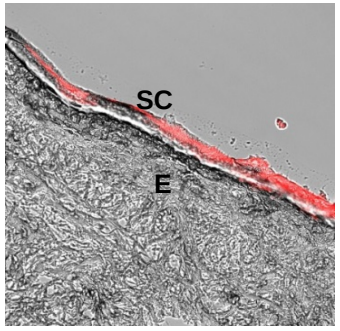
Human Skin – Aqueous solutions high dose



Human Skin – Aqueous solutions low dose



Human Skin – Emulsion



SC= Stratum corneum; E=Epidermis

In Vitro Penetration of Coated Silver Nanoparticles into Human and Pig Skin

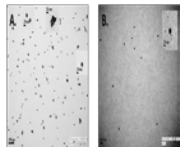
Objective:

To investigate the extent of penetration of 20nm silver nanoparticles into human and pig skin using an *in vitro* system with the following coatings:

- Polyethylene glycol (*PEG; neutral*)
- Citrate (*CIT; negative*)
- AgNPs were dosed on skin in water or O/W emulsion formulation - prepared at low (0.001%) and high (0.01%) dose concentrations reflecting nanosilver content in commercially available cosmetics: 10ppm to 100ppm

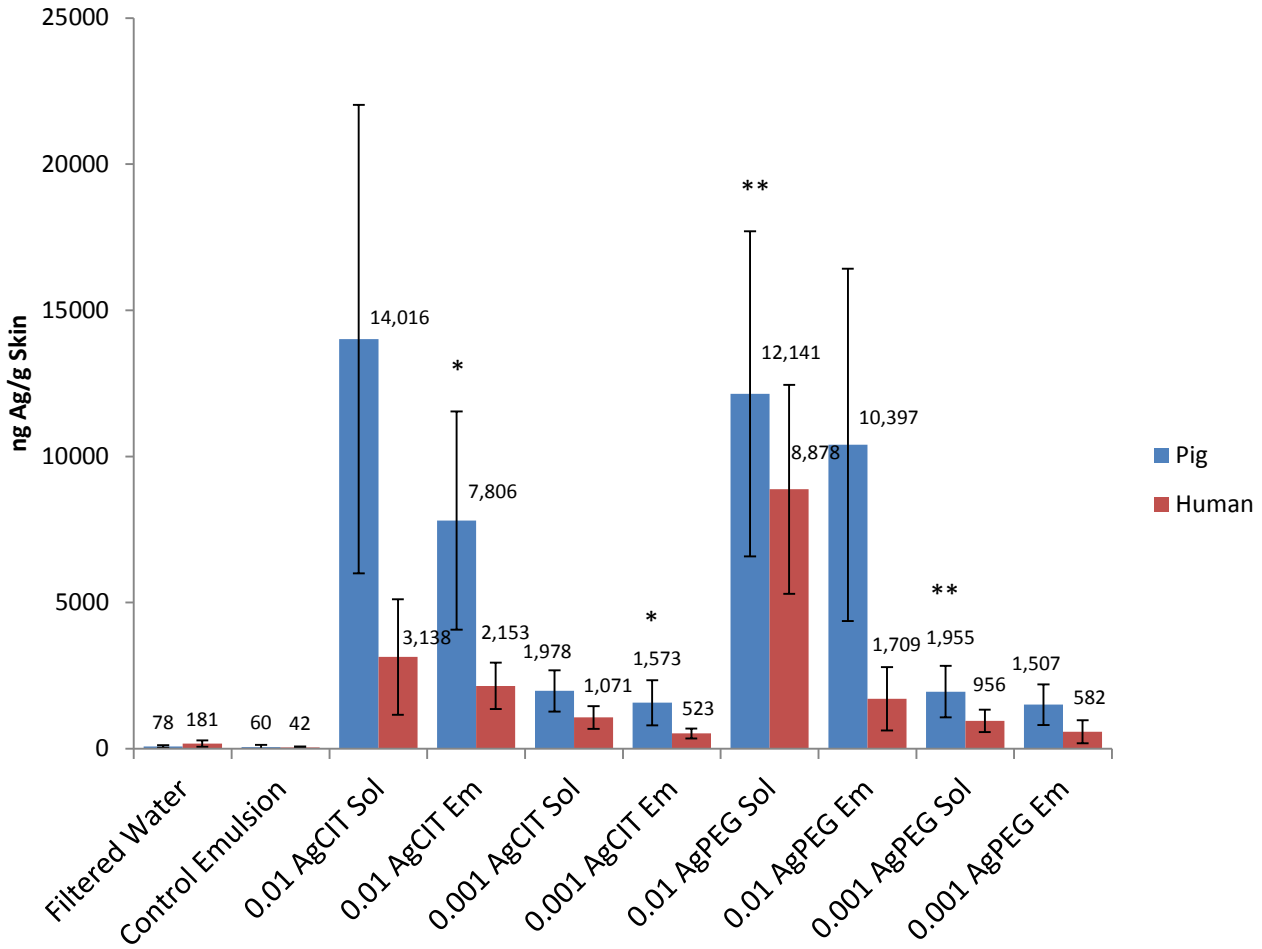
Characterization and Preparation

- 20nm Biopure™ Silver nanoparticles from nanoComposix were characterized to confirm size, shape, and agglomeration state
- **Extent of skin penetration was determined by ICP-MS and TEM analyses**



TEM; 20 nm Citrate (A) and PEG (B) coated AgNPs

ICP-MS: Total Silver Content of Pig vs Human Skin



Results:

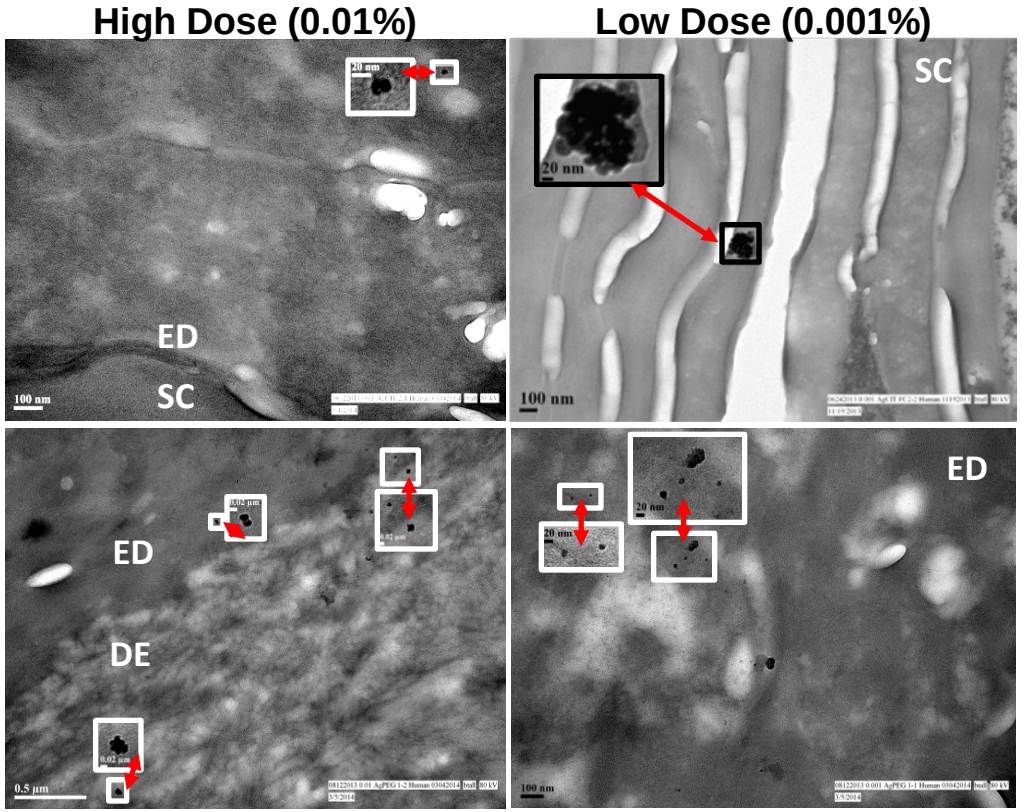
Silver uptake was greater using the solution vehicle than the cosmetic formulation (emulsion).

Silver uptake was greater in pig skin than human skin.

Silver uptake was dose-dependent:
Mostly, the higher doses of the AgNPs penetrated the skin greater than the low doses.

Mean ± SD
* And ** = $p < 0.05$

Human Skin TEM: Ag-CIT & Ag-PEG Solutions



SC = Stratum corneum ED = Epidermis DE = Dermis

- 20 nm silver nanoparticle singlets, doublets and agglomerates were found throughout layers of skin.
- PEG-coated silver nanoparticles penetrate deeper (DE) than CIT-coated silver nanoparticles (SC & ED).
- Surface-coating and charge may determine extent of silver nanoparticle penetration into the skin.

Summary

- In vitro test models can be used to evaluate personal care product safety
- Skin absorption studies were successful in measuring exposure to nanoparticles
- Physicochemical properties could be used to predict skin penetration of new untested nanoparticles
- Characterization is nanoparticle specific

Limitations of Characterization

- Confocal microscopy – auto-fluorescence of the skin will interfere with the fluorescence of the dye – correct for skin auto-fluorescence
- Cosmetic matrices may make it challenging to characterize nanoparticles realistically in the finished product

Conclusion

- The safety of a cosmetic product using nanomaterials should be evaluated by analyzing
  - physico-chemical properties
  - relevant toxicological endpoints of each nanoparticle
  - expected exposure levels
  - intended use of the finished product
- The overall safety substantiation of the product should be done under the intended conditions of use
- Manufacturers are encouraged to contact FDA early in the product development process

For updates and further information on the guidance document
http://www.fda.gov/Cosmetics/GuidanceRegulation/GuidanceDocuments/ucm300886.htm.

Thank You For Your Interest!

Questions?