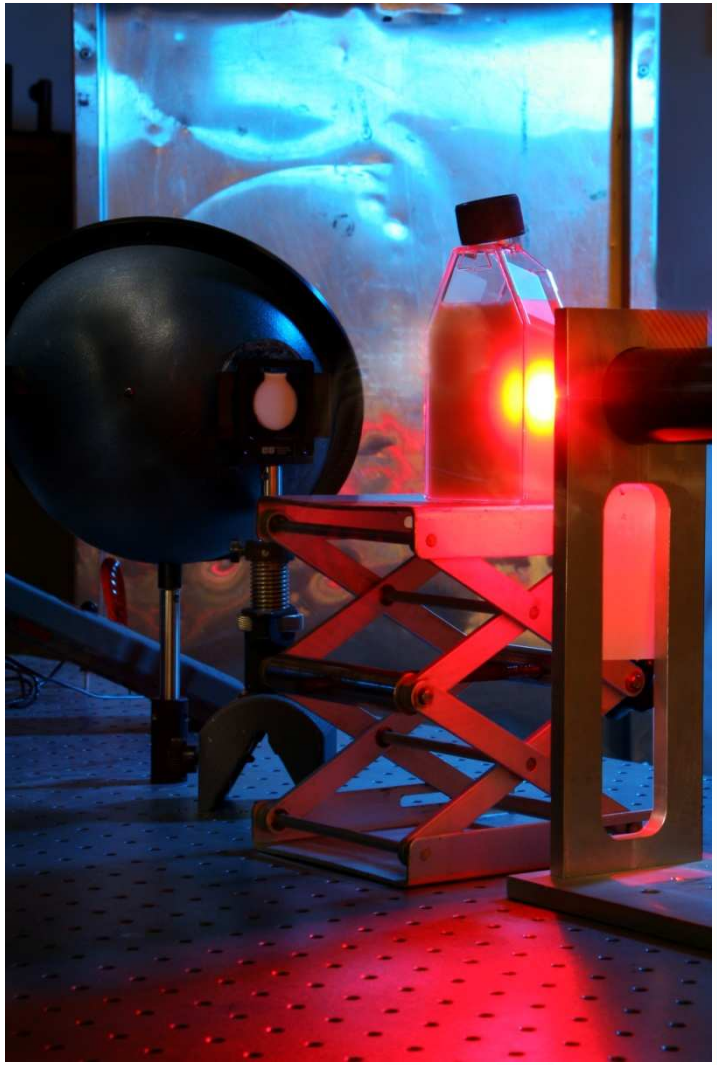
Influence of variation in melanin content on absorbance spectra of liquid skin phantoms

JE Smit, AE Karsten, AF Grobler, RW Sparrow

Presented by JE Smit

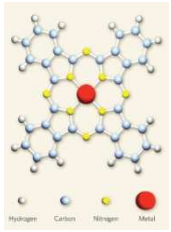
Date: 25 September 2010

Outline of presentation



- Background
  - Why study melanin?
  - Skin pigments, melanocytes, melanosomes, melanin
  - Natural vs synthetic melanin
- Results
- Conclusions

Background
Quantum Yield of PDT drugs: Why study Melanin?



PDT drug

Best solvent??

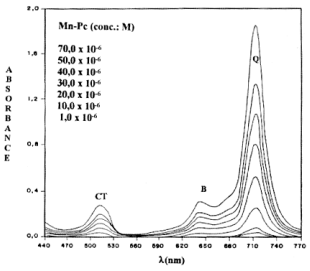
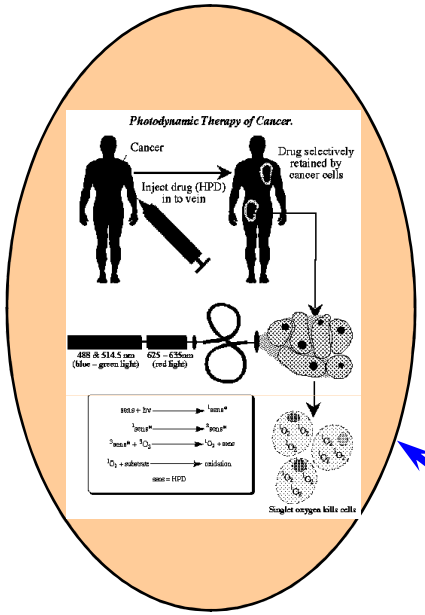
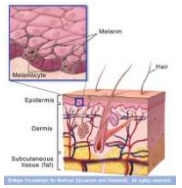


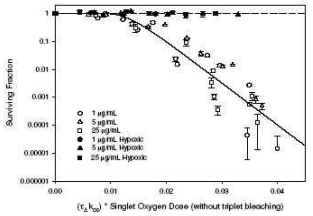
FIGURE 1 - Absorption spectra of Mn-Pc in ethanol at 298.15 K

Drug concentration?





Different skin colours:
Melanin concentrations

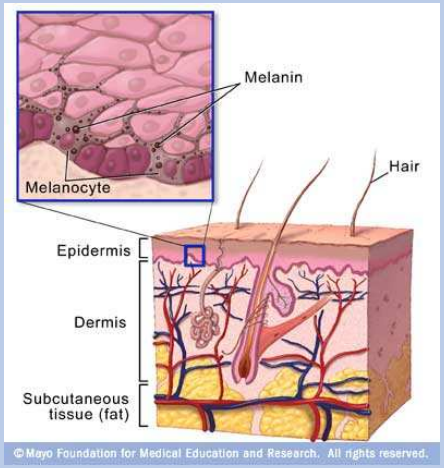


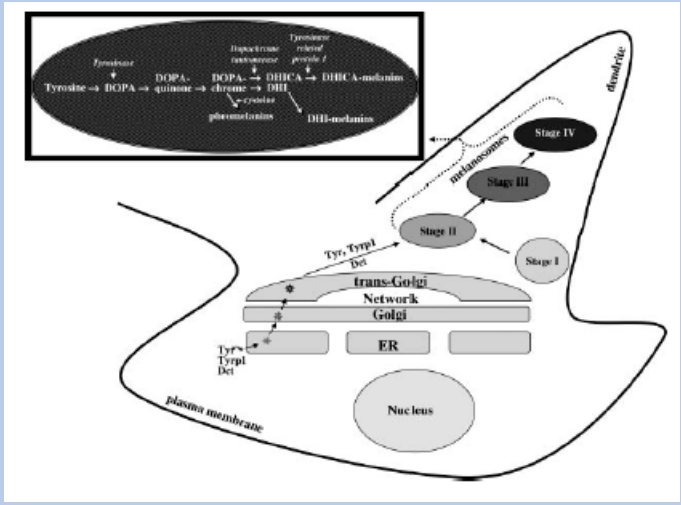
Singlet oxygen lifetimes
and concentrations?

Intracellular drug uptake

our future through science

Background
Skin pigments, Melanocytes, Melanosomes, Melanin





Schematic of a melanocyte: the engine for melanin production

Costin and Hearing *FASEB J.* **21**(4) 2007

Skin pigments

- Melanin (epidermis), Haemoglobin (dermis), Bilirubin, Carotene, Keratin

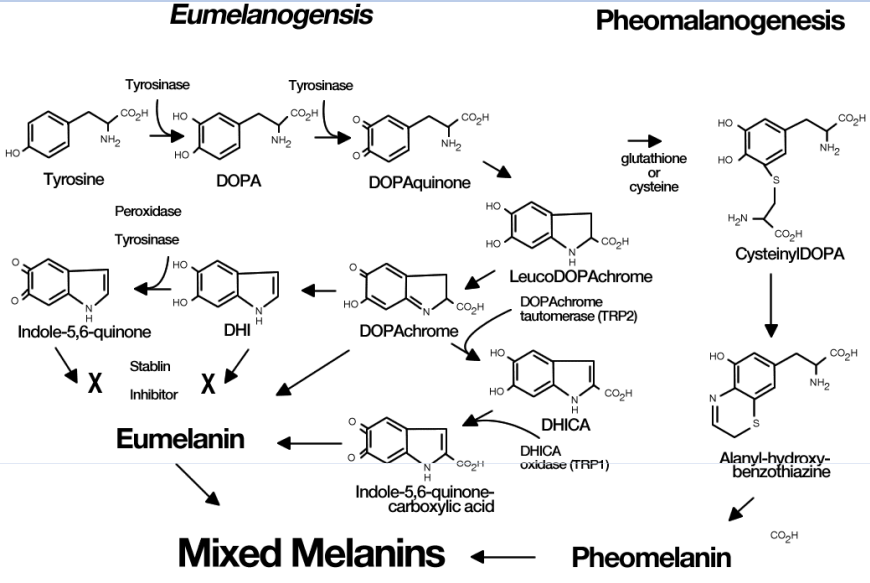
Melanocytes

- Located at epidermal-dermal junction
- Melanosomes
- Melanin biosynthesis
- In contact with ~ 40 keratinocytes through dendrites
- Transfer melanosomes to keratinocytes

Melanosomes

- Highly organised membrane bound organelles
- Four stages of maturation
- Melanin synthesis takes place inside

Background
Skin pigments, Melanocytes, Melanosomes, Melanin



Melanogenesis
http://photoprotection.clinuvel.com/custom/uploads/20080116_03_01_02_02_v1%281%29.gif

Melanin

- Polymorphous multifunctional biopolymers
- Eumelanin, pheomelanin, mixed melanins, neuromelanin
- Eumelanin: black-brown
- Pheomelanin: yellow-reddish
- Protective role: bind to cations, anions, drugs, chemicals etc.

Inside keratinocytes

- Melanin granules accumulate above nuclei
- Absorb harmful UV-R
- Changes in environment can cause increase in melanin production

Background
Natural melanin vs Synthetic melanin

Natural melanin

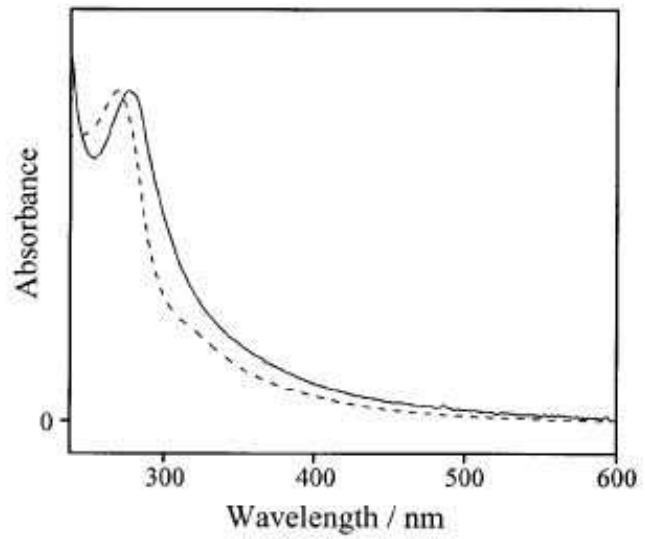


Figure 2. The optical spectra for (—) MW < 1000 hair eumelanin is compared to that for (- - -) MW < 1000 *Sepia* eumelanin showing similar electronic features. The spectra are normalized at 276 nm for display purposes only.

Nofsinger et al. *Biopolymers* **67**(4-5) 2002

Synthetic melanin

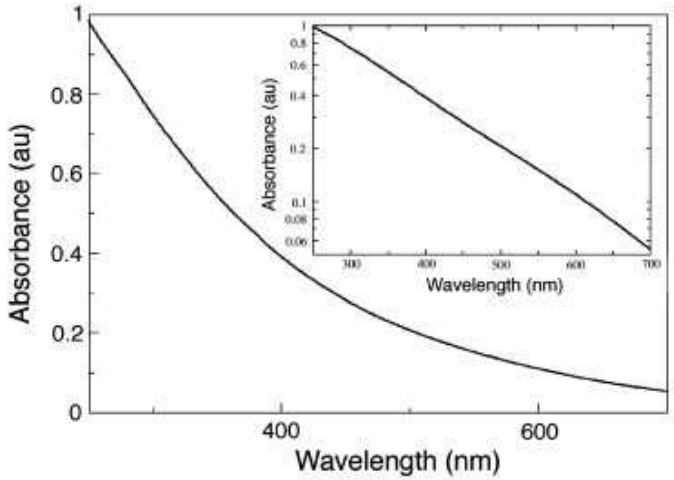
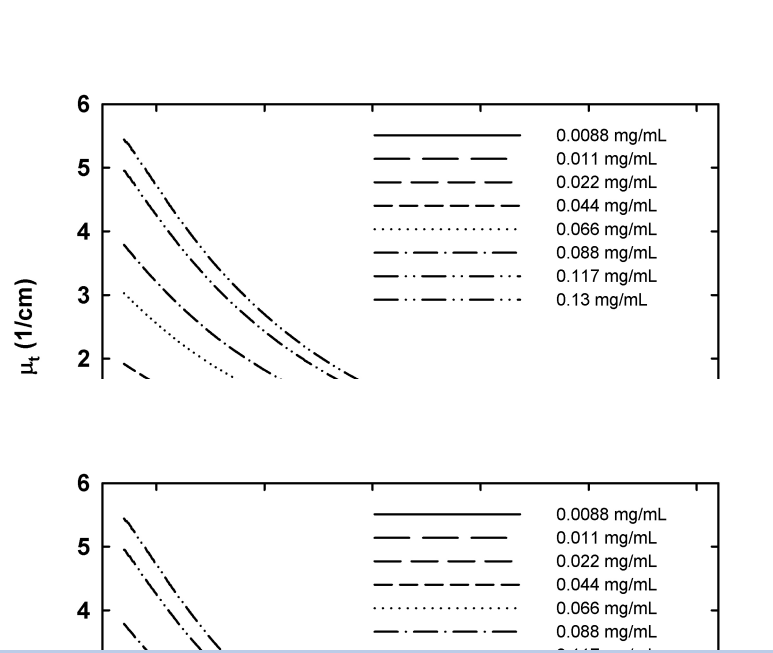
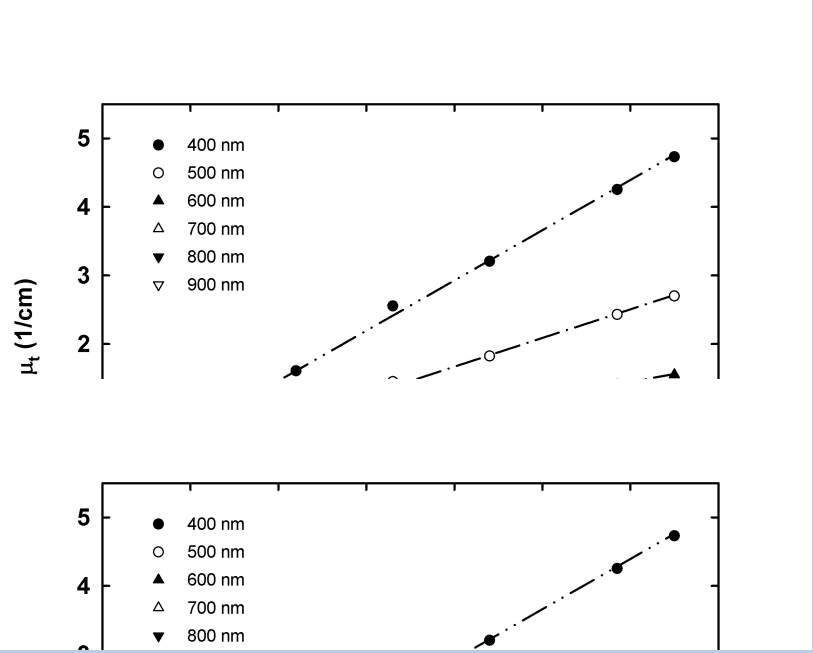


Figure 3. The well-known and much reported monotonic, broad band UV-visible absorption spectrum of eumelanin (synthetic material derived from the non-enzymatic oxidation of dl-DOPA). The spectrum fits closely a single exponential (insert) and no distinct chromophoric peaks are visible. Figure adapted from (Meredith et al., 2006).

Meredith et al. *Soft Matter* **2**(1) 2006

Results
Melanin only samples





Attenuation coefficient as a function of (a) wavelength and (b) melanin concentration for synthetic eumelanin samples at pH ~7.01.

Results
Skin-like phantoms: Comparison to *in vivo* human skin

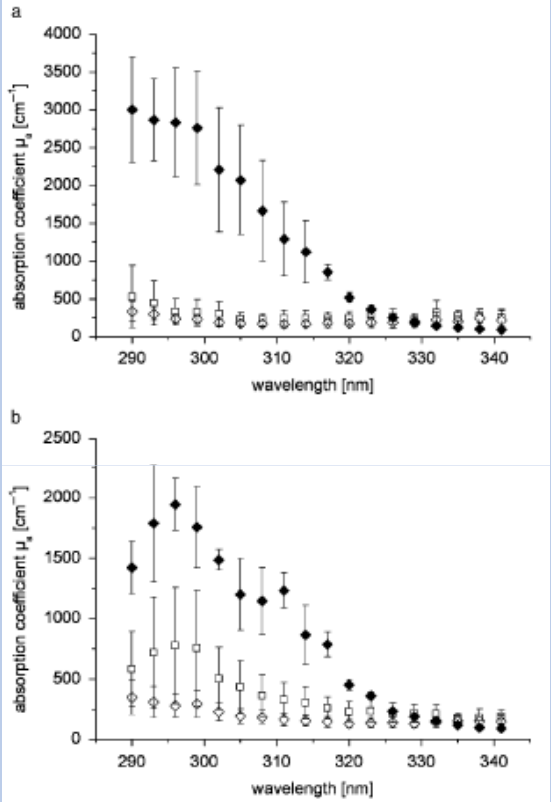
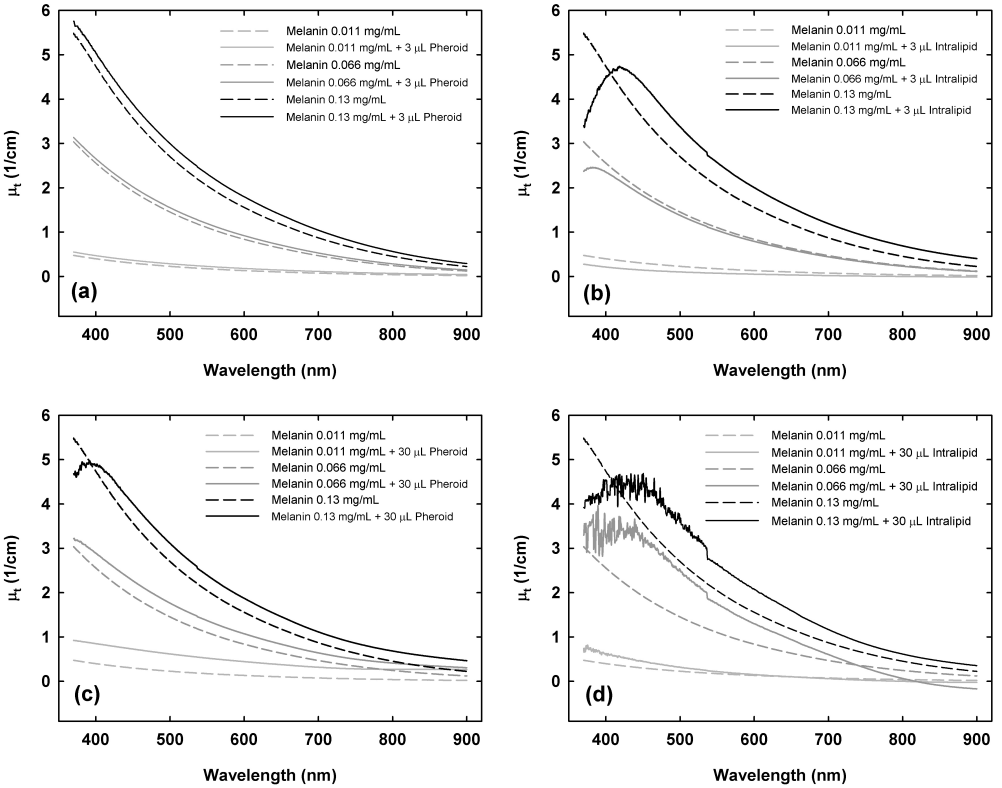


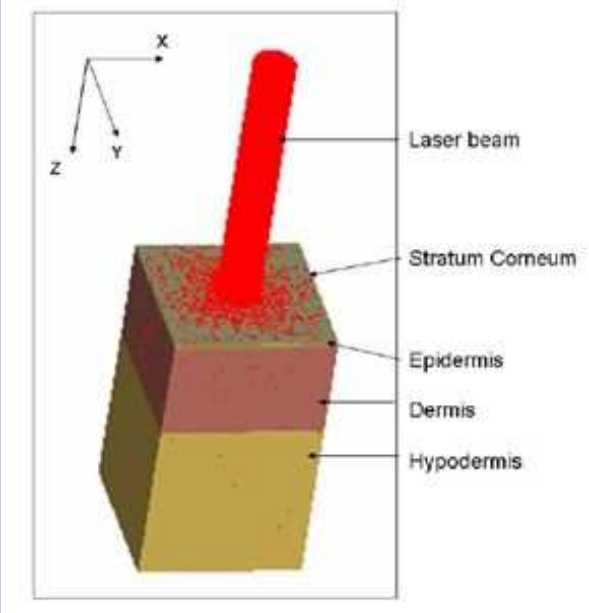
Fig. 5. Typical absorption spectra for subjects with low natural ultraviolet (UV) exposure (a) and high natural UV exposure (b). Each diagram shows a dataset from one example subject of the respective group. Measurements were taken at the volar (◇) and dorsal (□) sides of the forearm as well as on the ball of the thumb (◆) (thenar) where the horny layer is particularly thick. Error bars show standard deviation from mean for three measurements at closely adjacent areas.

Meinhardt et al. *Photoderm, Photoimm & Photomed.* ***24***(2) 2008



Attenuation coefficient as a function of wavelength for skin-like phantoms containing (a) 3 µL Pheroid™, (b) 3 µL Intralipid, (c) 30 µL Pheroid™ and (d) 30 µL Intralipid. Phantoms are compared to the melanin only samples of same concentration from previous slide.

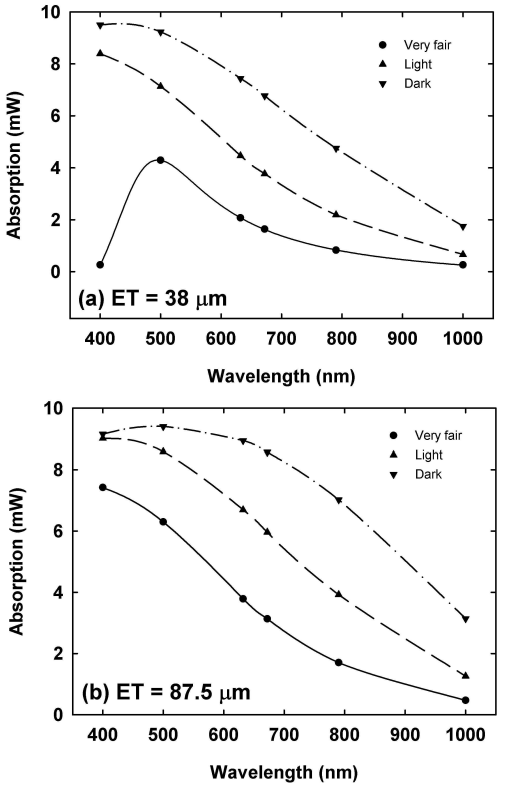
Results
Realistic skin model (ASAP from Breault Research Organisation Inc)



The Realistic Skin Model showing the different layers of the skin as well as the laser beam (Gaussian profile and beam diameter of 0.4 mm). The red spots on the model boundaries are the photons leaving the model.

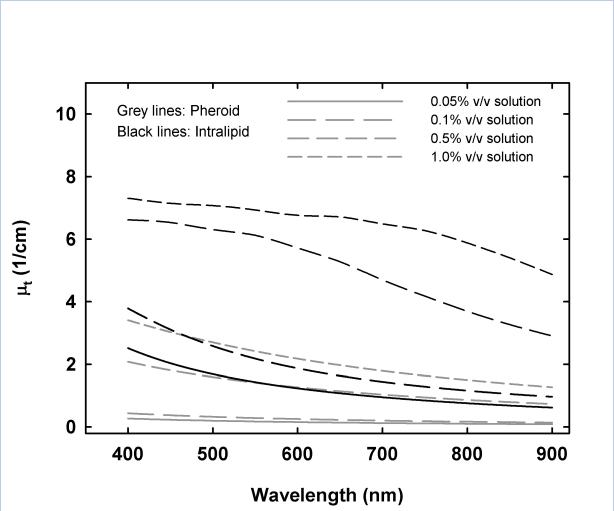
Volume fraction of melanin used for the different skin tones	
Skin tone	**Volume fraction of melanin in the epidermis**
Very fair	0.0255
Fair	0.0505
Light	0.0865
Medium	0.135
Dark	0.305

Zonios et al. *J Invest Dermatol.* ***117***(6) 2001

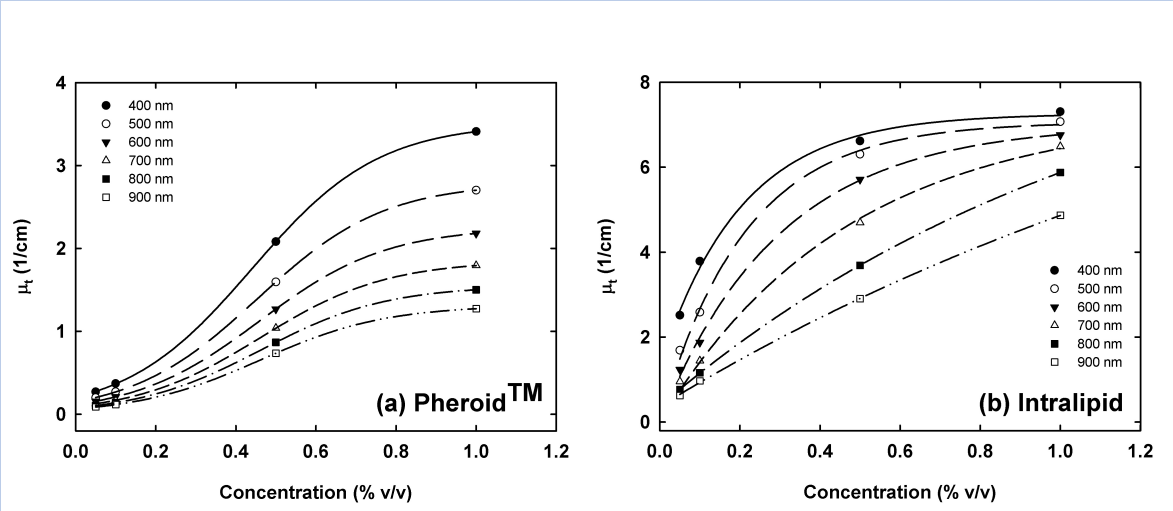


Comparison of the cumulative absorbed power (mW) as a function of wavelength for epidermal thicknesses (ET) of (a) 38 μm and (b) 87.5 μm up to a skin depth of 0.12 mm.

Results
Skin-like phantoms: Pheroid™ vs Intralipid

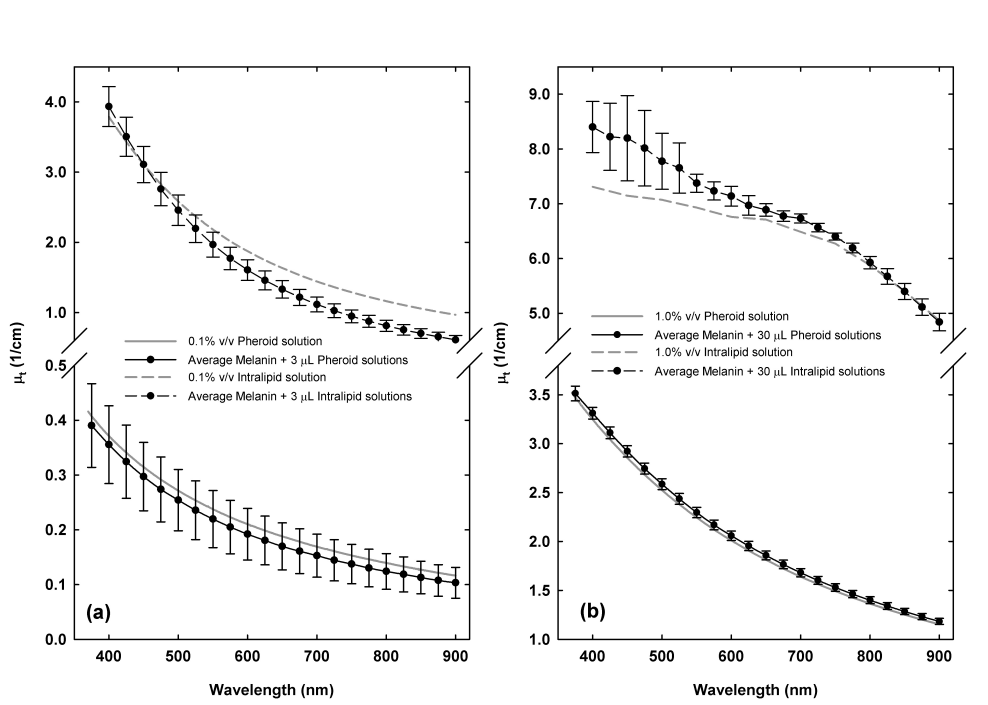


Comparison absorbance spectra for increasing concentrations of Pheroid™ (grey lines) and Intralipid (black lines).



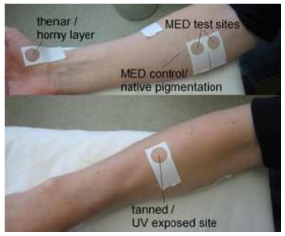
Attenuation coefficient as a function of (a) Pheroid™ and (b) Intralipid concentration over the wavelength range 400 to 900 nm.

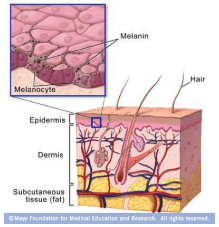
Results
Skin-like phantoms: Melanin interaction with lipid particles

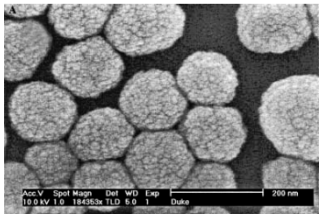


Average of skin-like phantoms made up of 0.0088 to 0.044 mg/mL melanin concentrations with added (a) 3 µL and (b) 30 µL Pheroid™ and Intralipid respectively. Corresponding melanin only concentrations were used as references / blanks.

Conclusions







Conclusions

- Synthetic eumelanin may be more similar to epidermal eumelanin than previously thought
- Phantoms may be able to represent the optical characteristics of real skin
- Difference in interaction between high and low concentration melanin samples with lipid particles
- Difference in melanin interaction with Pheroid^{TM} and Intralipid

What next?

- Meinhardt et al. (2008): non-monotonic absorbance UV behaviour due to keratin. More realistic phantoms – adding keratin
- Comparison between the Pheroid^{TM} phantoms and melanosomes from different skin phototypes could not be made. To verify the true amount to which these phantoms represent real skin, such a comparison needs to be made.
- Addition of gelatine to make solid phantoms

Thank You

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