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Understanding the passage of molecules through human skin - a tissue engineered approach

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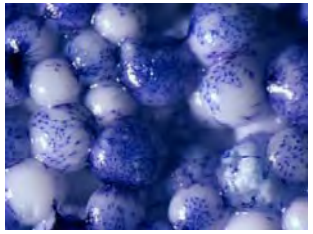
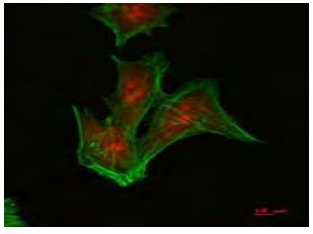
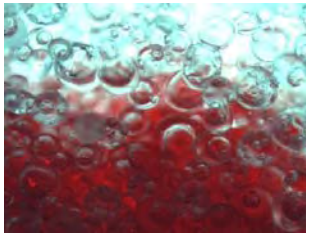
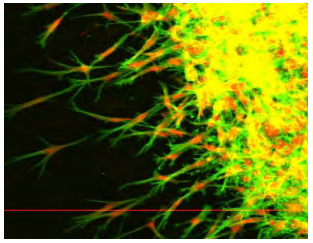





STEM
WOLFSON CENTRE FOR STEM CELLS, TISSUE ENGINEERING AND MODELLING

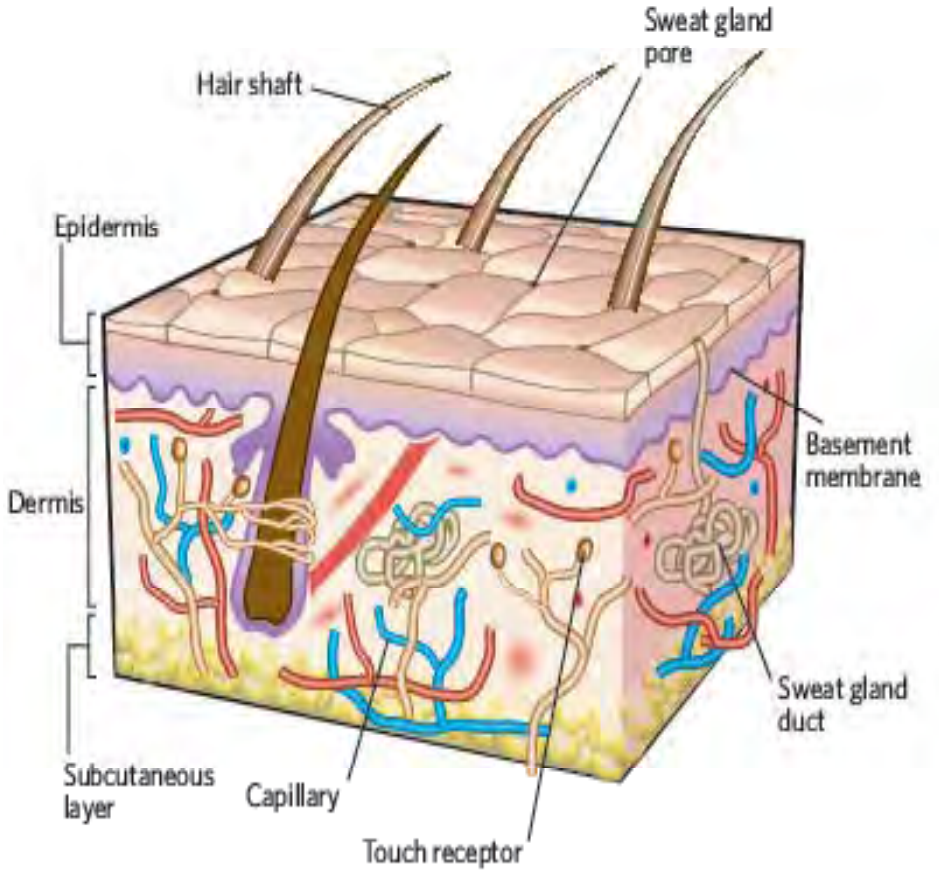
Structure of the skin

Epidermis – 0.1-0.2 mm thick

Keratinocytes migrate upwards whilst differentiating (to **corneocytes**) giving rise to keratin based **stratum corneum** providing a protective barrier against microbial and chemical assault and a highly efficient barrier for water.

Dermis – various thickness (location)

Composed primarily of **collagen type I**. Dermal inclusions, such has **hair shafts and sweat glands**, are lined with epidermal keratinocytes. Dermis is well **vascularised** and contains sensory receptors.



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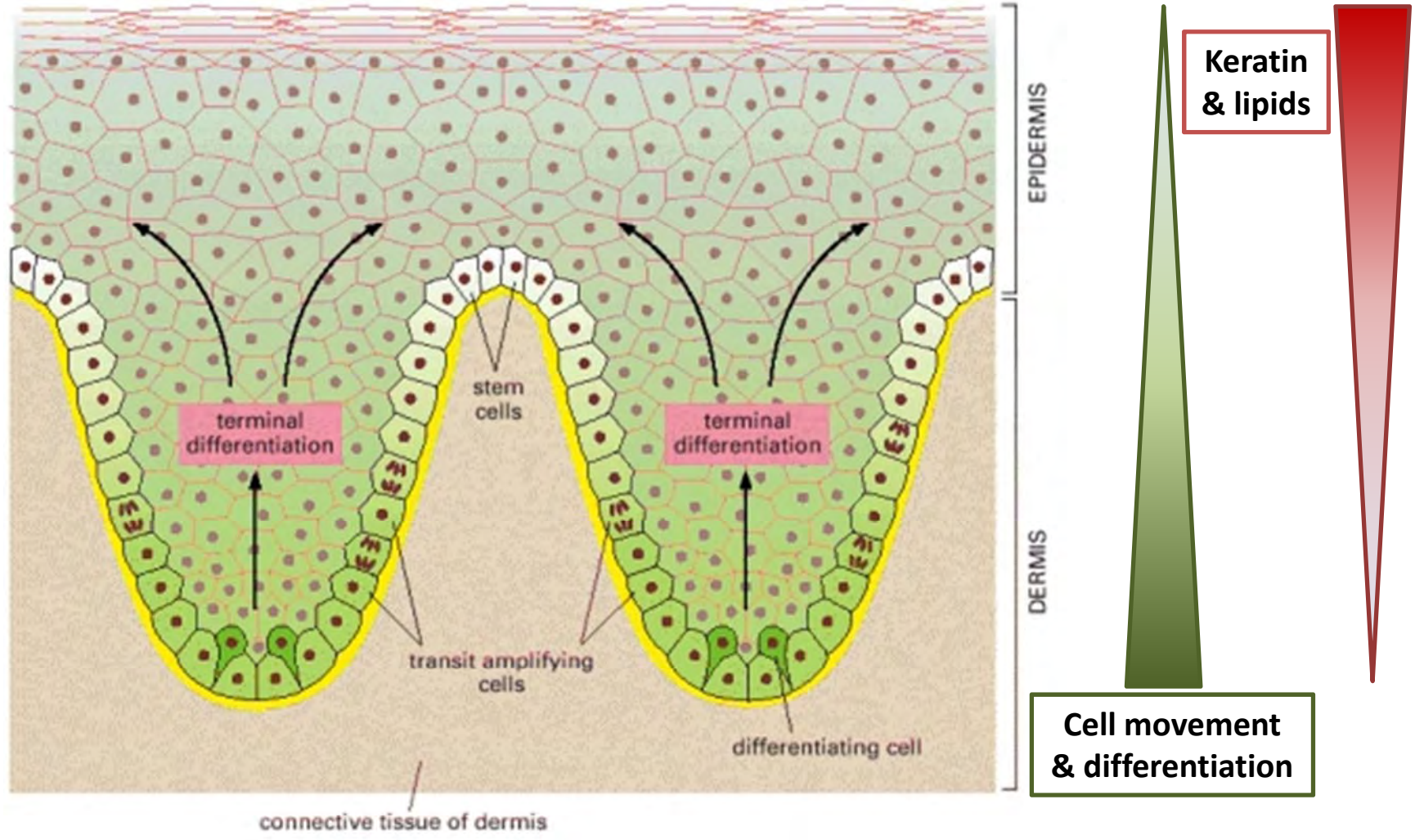
Formation of the stratum corneum



Alberts J *et al*. Molecular Biology of the Cell, 4th ed.

Routes of penetration through the skin

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Diffusional routes to penetrate normal intact human skin include:

The transepidermal route

1. Across the intact horny layer

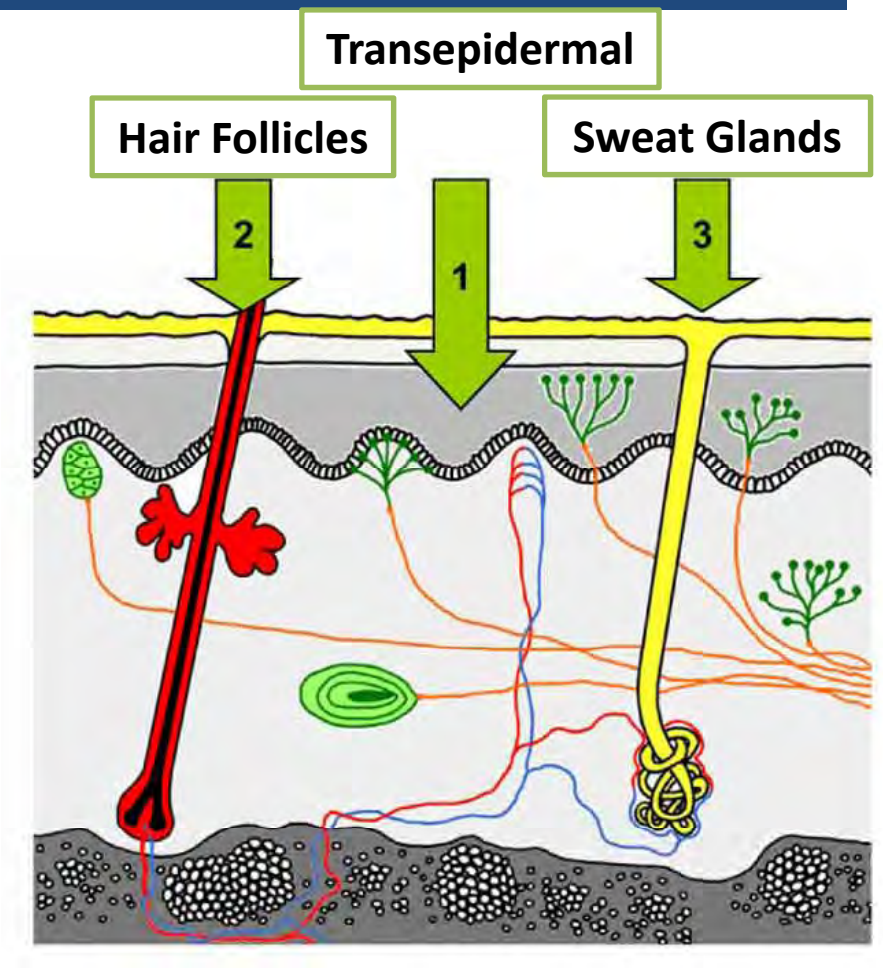
Major route for molecules and drugs either transcellular or intercellular.

The appendageal route

2. Through the hair follicles

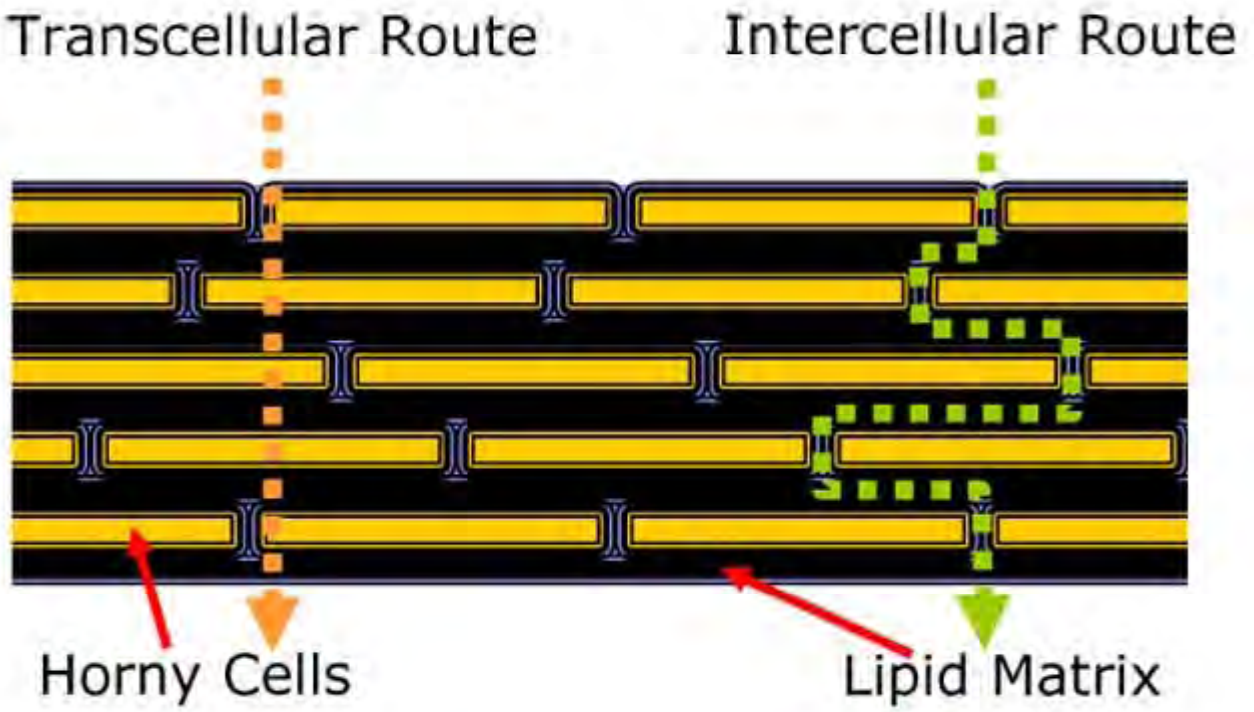
3. Through the sweat glands

Minor route (minor importance due to small area (0.1% of the total skin area) – offers **high permeability** for **ions and large polar molecules**.



Routes of Penetration through the Skin
http://www.skin-care-forum.basf.com

Routes of penetration through the skin



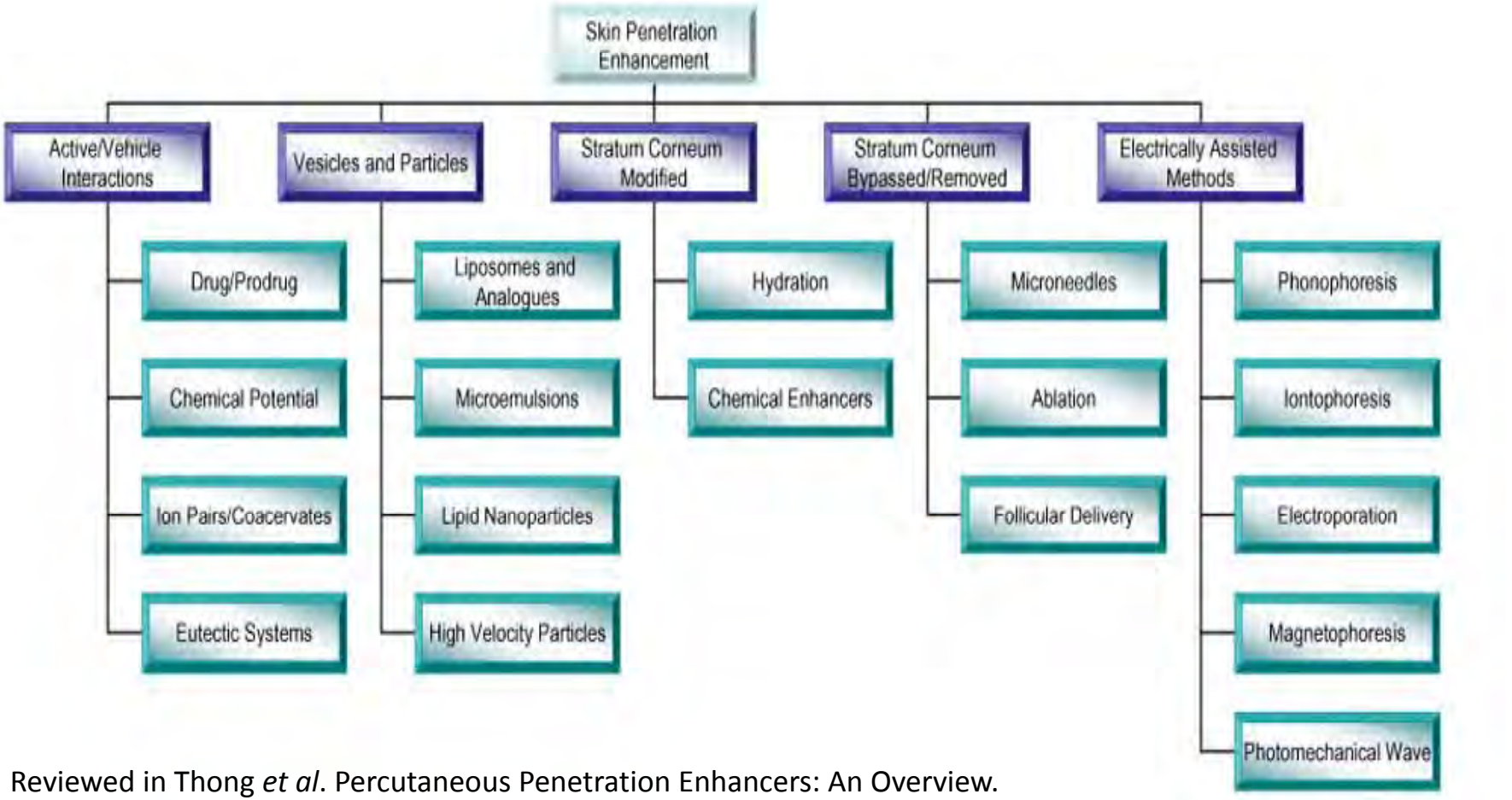
Hydrophilic molecules

Lipophilic molecules

Routes of Penetration through the Skin
http://www.skin-care-forum.basf.com

- The principal pathway taken by a penetrant is decided mainly by the partition coefficient (log K).
- Most molecules pass the stratum corneum by both routes.
- Intercellular pathway is the principal route and major barrier to the permeation of most drugs.

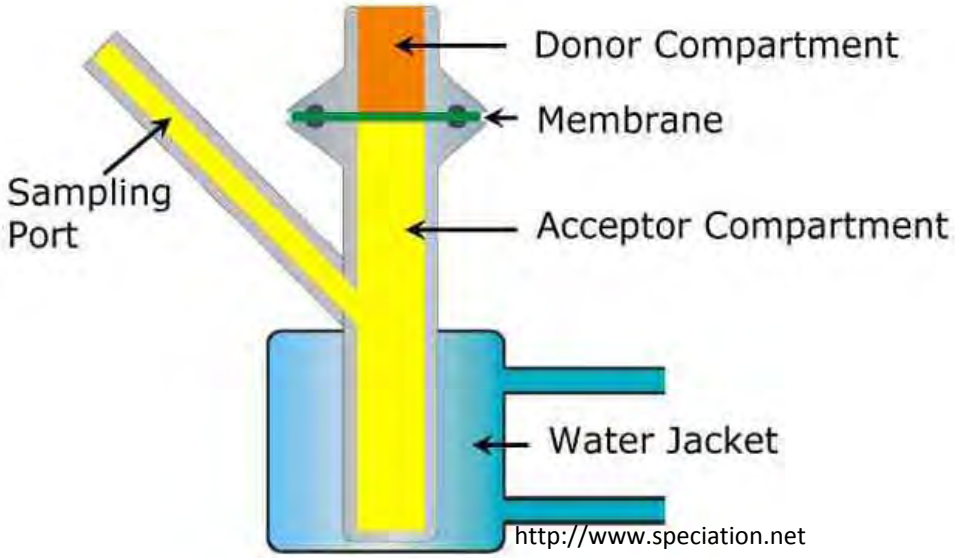
Skin Penetration Enhancement



Reviewed in Thong *et al*. Percutaneous Penetration Enhancers: An Overview. Skin Pharmacol Physiol 2007; 20:272-282.

http://www.skin-care-forum.basf.com

Skin Permeability Studies – *in vitro*

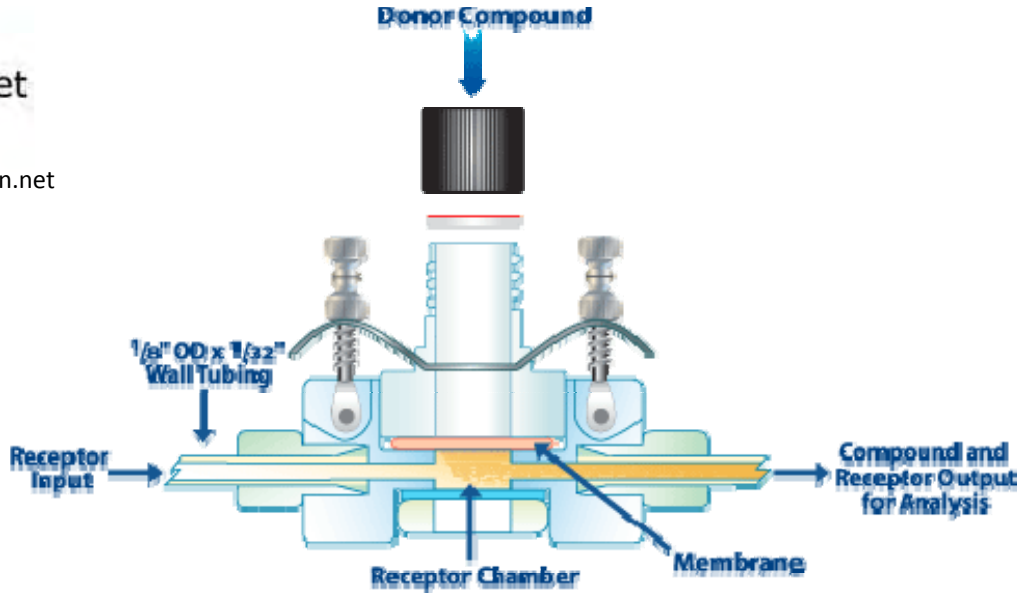


http://www.speciation.net

Franz diffusion cell

Diffusion & Flow Perfusion Cells

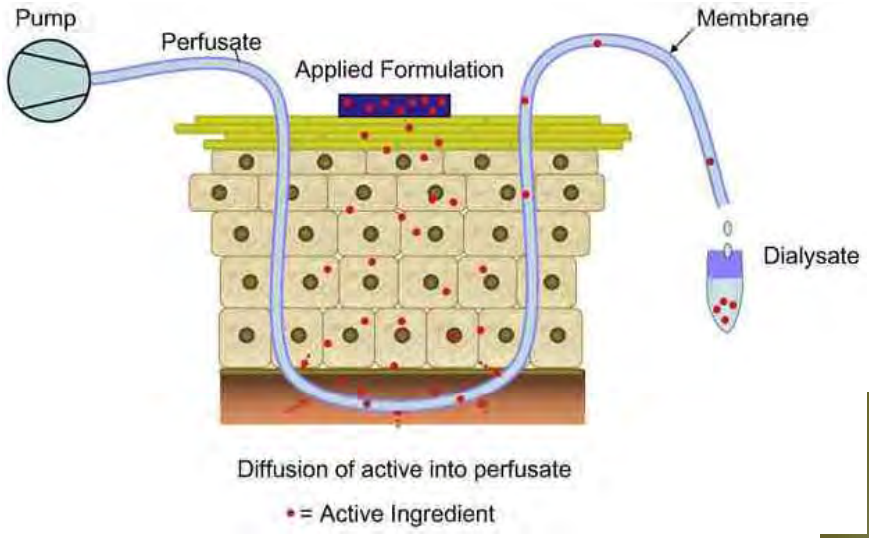
Membrane can be substituted for cell cultures or *ex vivo* skin.



http://www.permegear.com/inline.htm

Bronaugh flow cell

Skin Permeability Studies – *in vivo*



Microdialysis

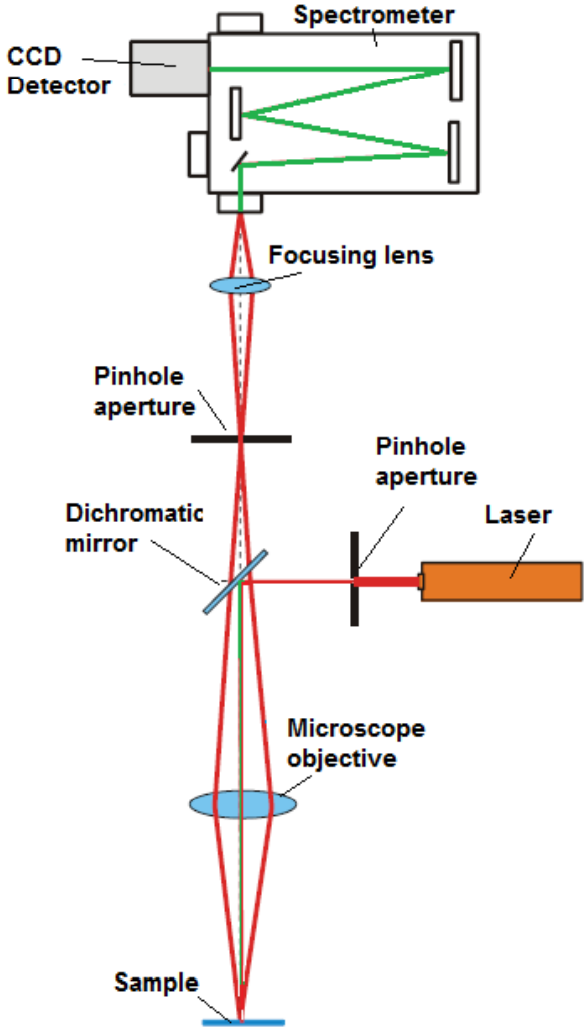
http://www.skin-care-forum.basf.com

Microdialysis & Tape Stripping

Tape Stripping

http://www.skinandallergynews.com

Skin Permeability Studies – confocal Raman



- **Raman spectroscopy** relies on Raman scattering of monochromatic light.
- The spectrum of the **energy difference** between the absorbed and emitted photon is termed the **Raman spectrum** providing a chemical fingerprint for individual chemicals.
- **Confocal Raman** spectroscopy allows **spatial Raman spectra** gathering (z-stack).
- **Non-invasive, non-destructive and label free** allowing timepoint analysis of **multiple chemicals** (delivery vehicle & active) on the **same tissue sample**.
- Detailed information on **chemical ingress**, differentiates between **delivery rates**, **solvent monitoring** in one assay.
- Can be **used *in vivo*** allowing ***in vitro/in vivo* correlations** to be established.

Skin Permeability Studies – experimental setup

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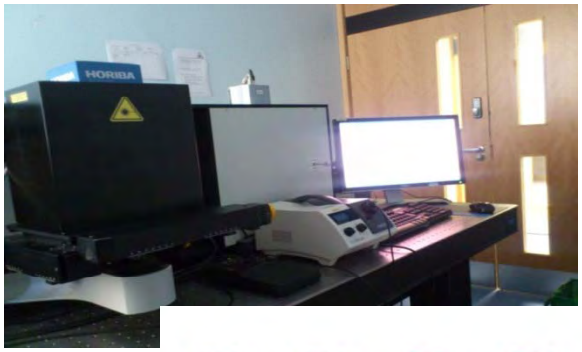
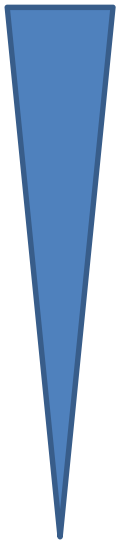
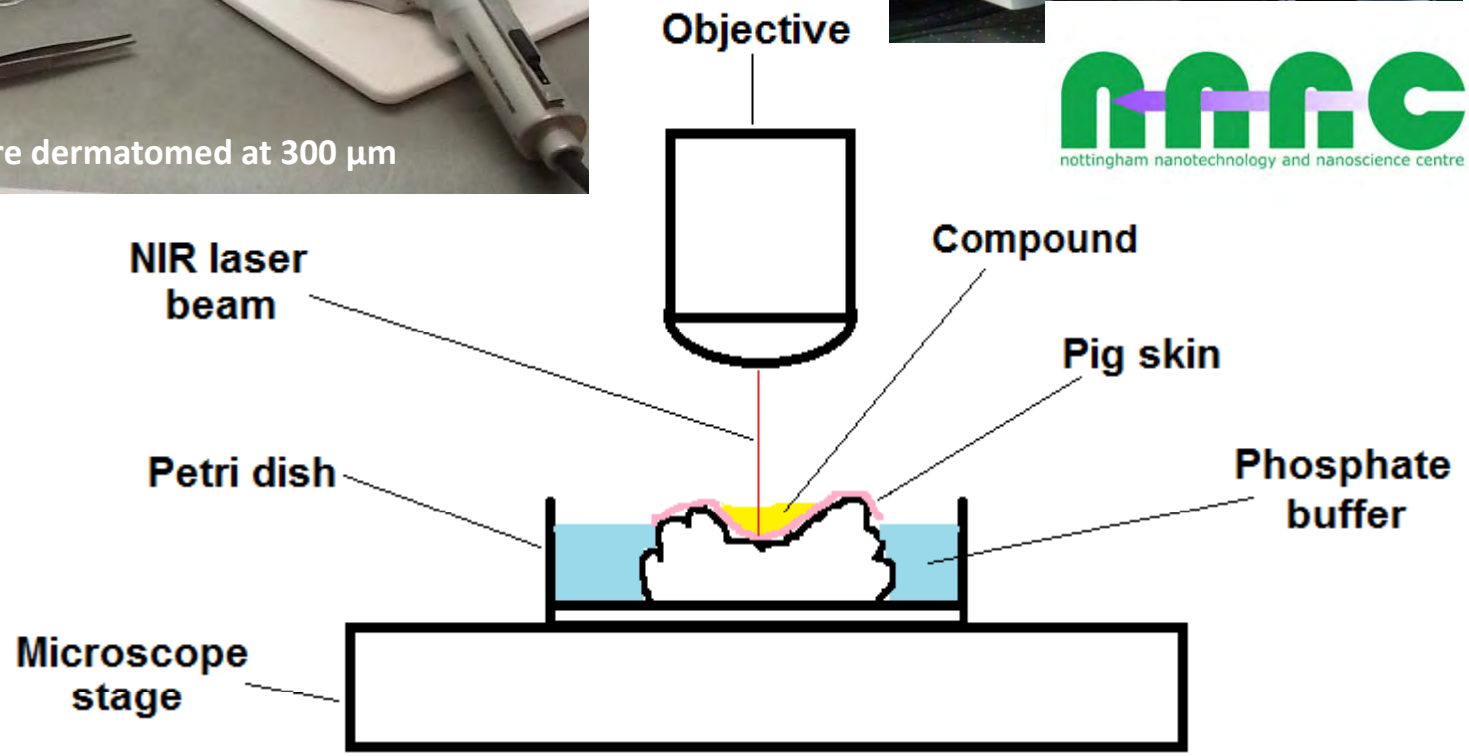


nottingham nanotechnology and nanoscience centre

Chemicals applied to the skin and penetration monitored over 24 hour period using confocal Raman spectra collected from the underside of the sample.





Skin Permeability Studies – confocal Raman

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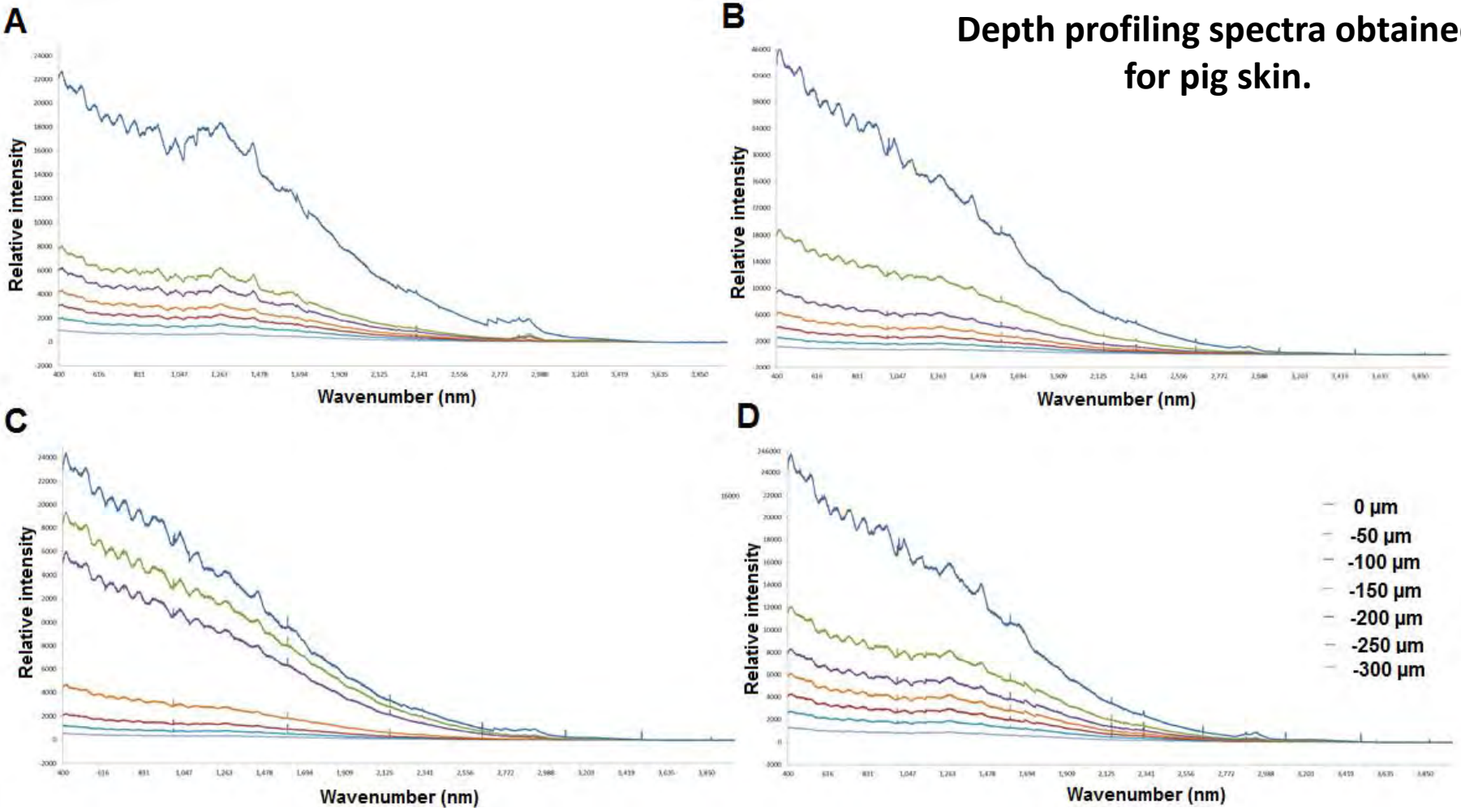
Depth profiling spectra obtained for pig skin.



Skin Permeability Studies – Methyl Benzoate

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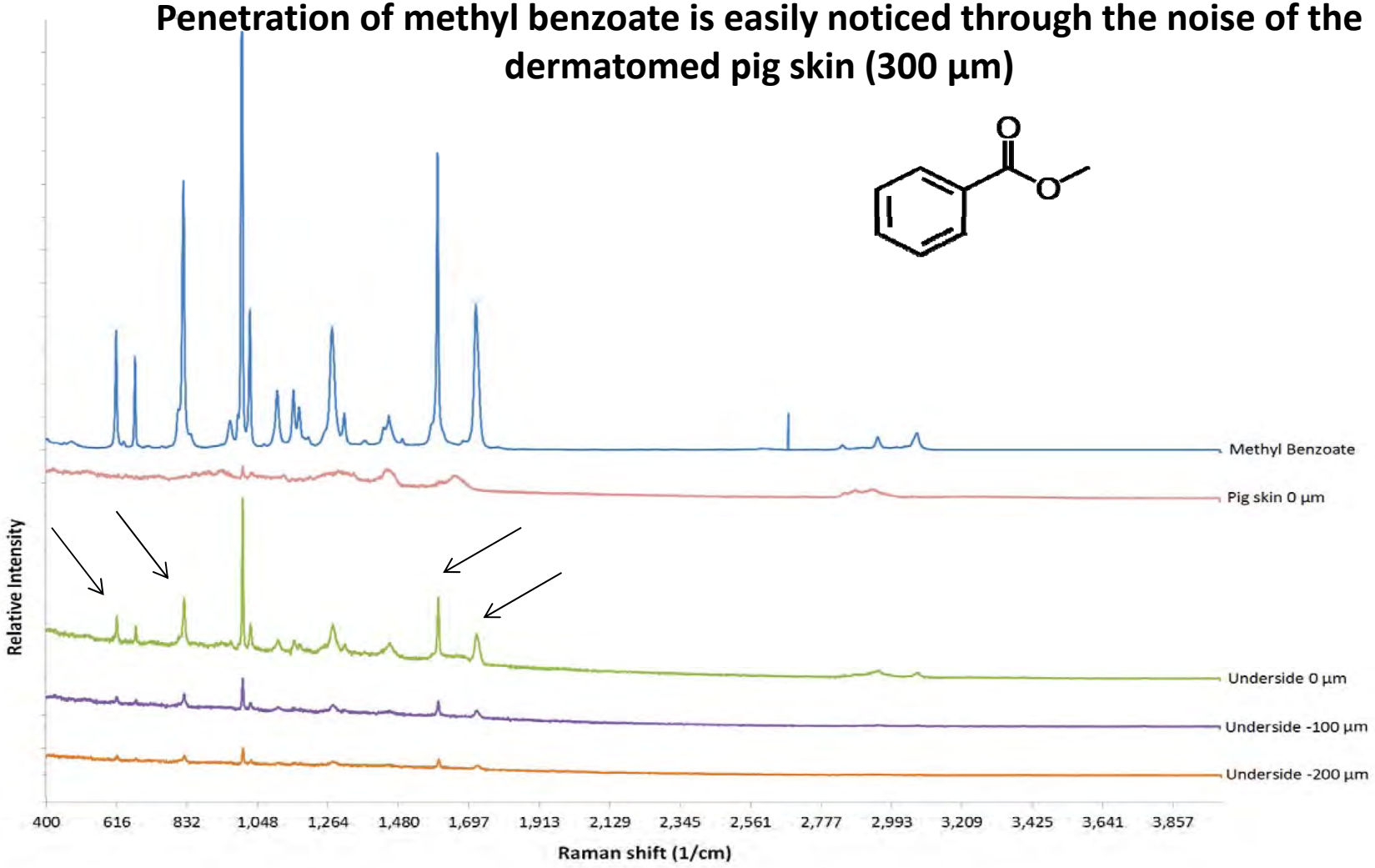
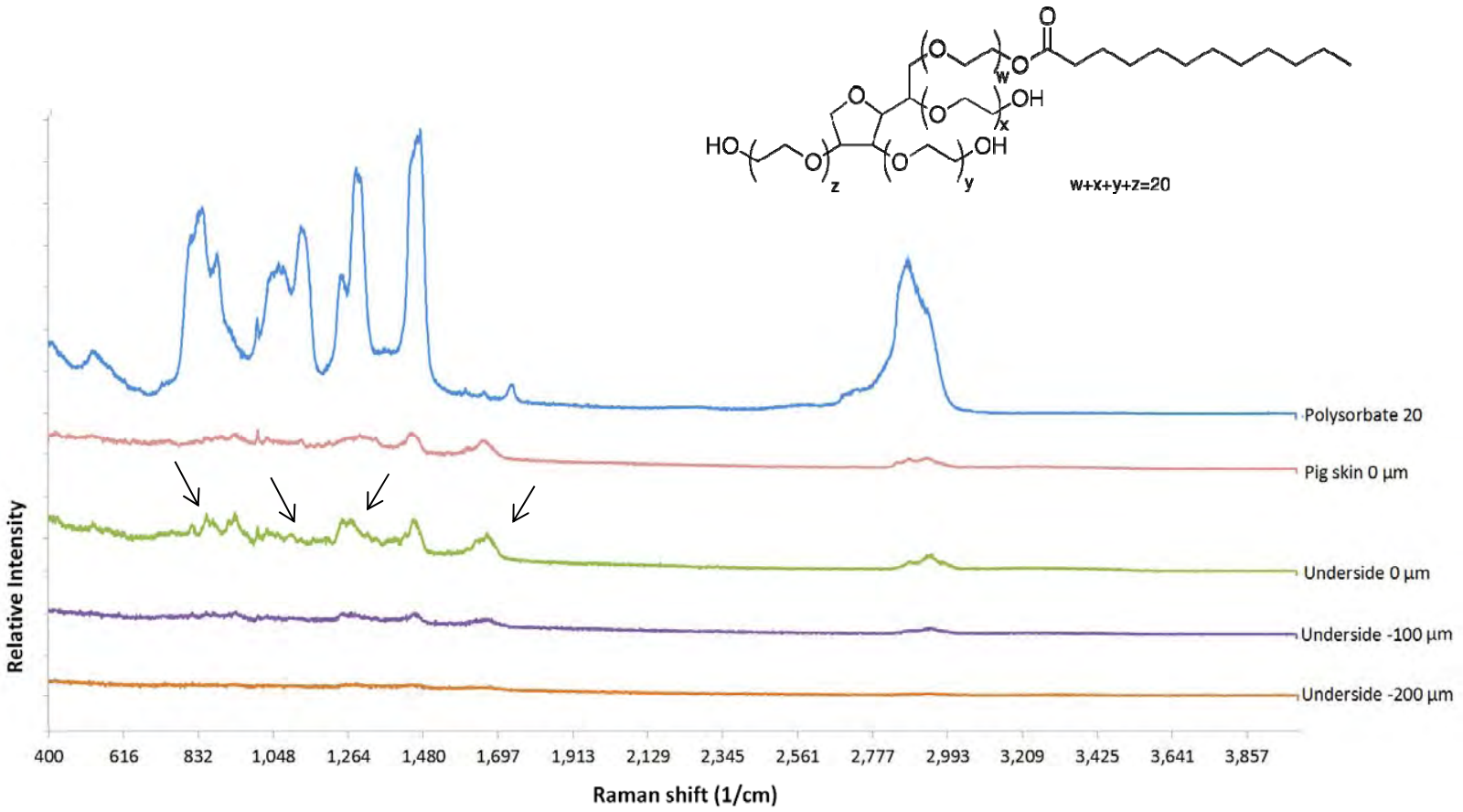
Penetration of methyl benzoate is easily noticed through the noise of the dermatomed pig skin (300 µm)



Skin Permeability Studies – Tween 20

Penetration of Tween 20 is difficult to detect through the noise of the dermatomed pig skin (300 µm)



Skin Permeability Studies using Confocal Raman

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Pharm Res (2011) 28:858–872
DOI 10.1007/s11095-010-0342-0

RESEARCH PAPER

Ingredients Tracking of Cosmetic Formulations in the Skin: A Confocal Raman Microscopy Investigation

Matthias Förster • Marie-Alexandrine Bolzinger • Delphine Ach • Gilles Montagnac • Stephanie Briançon

Bonnist *et al*. Measuring the Penetration of a Skin Sensitizer and Its Delivery Vehicles Simultaneously with Confocal Raman Spectroscopy. Skin Pharmacology and Physiology 2011; 24:274-283.

JDDG
618 Original Article DOI: 10.1111/j.1610-0387.2011.07657.x

In vivo Monitoring of epidermal absorption of hazardous substances by confocal Raman micro-spectroscopy

Horst Christoph Broding[1], André van der Pol[2], Johanna de Sterke[2], Christian Monsé[1], Manigé Fartasch[1], Thomas Brüning[1]
(1) Institute for Prevention and Occupational Medicine of the German Social Accident Insurance-Institute of the Ruhr-University Bochum (IPA), Bochum, Germany
(2) River Diagnostics B.V., Europoint IV, Rotterdam, The Netherlands

Tissue Engineering

Tissue Engineering Approaches

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WEDNESDAY, OCTOBER 25, 1995 NATION

Scientists Grow Human Ear on Mouse

Tissue engineering research is 'promising'

By Katharine Webster
Associated Press

Boston

It sounds like something from a carnival sideshow: "The Mouse With a Human Ear on Its Back." But it is real, and it is alive.

This mouse, and others of its kind, are at the leading edge of a science known as tissue engineering, which someday may allow laboratories to grow skin and cartilage for transplant in humans.

The mouse in question is in the laboratory of Dr. Charles Vacanti, a University of Massachusetts anesthesiologist.

Linda Griffith-Cima, an assistant professor of chemical engineering at Massachusetts Institute of Technology who helped Vacanti grow the first ears on mice, said she did it at the request of a plastic surgeon from Children's Hospital, Dr. Joe Upton.

"He said, 'I see these kids who are born without ears. And I have boys who come in whose ears have been chewed off in playground fights, and I can't sew them back on because they're so chewed up,'" Griffith-Cima said.

So she created an earlike scaffolding of porous, biodegradable polyester fabric. Then she and Vacanti distributed human cartilage cells throughout the form and implanted the prototype ear on the back of a hairless mouse.

The mouse, specially bred without an immune system that might reject the human tissue, nourished the ear as the cartilage cells grew to replace the fiber.

"You end up with a piece of cartilage in the shape of an ear," Griffith-Cima said.

The mouse remains healthy and alive after the ear is removed, the researchers said.

Other researchers, including Vacanti's older brother, Dr. Joseph Vacanti, a surgeon at Children's Hospital, and his close friend Dr. Robert Langer, a professor of chemical engineering at MIT, are also at work on tissue engineering. They have grown livers, skin, cartilage, bone, ...ters, heart valves, tendons, intestines, blood vessels and breast tissue on polymer scaffolding.

Although no tissue products have yet become available to the public, skin products are in the advanced stages of clinical

Tissue Engineering Strategy

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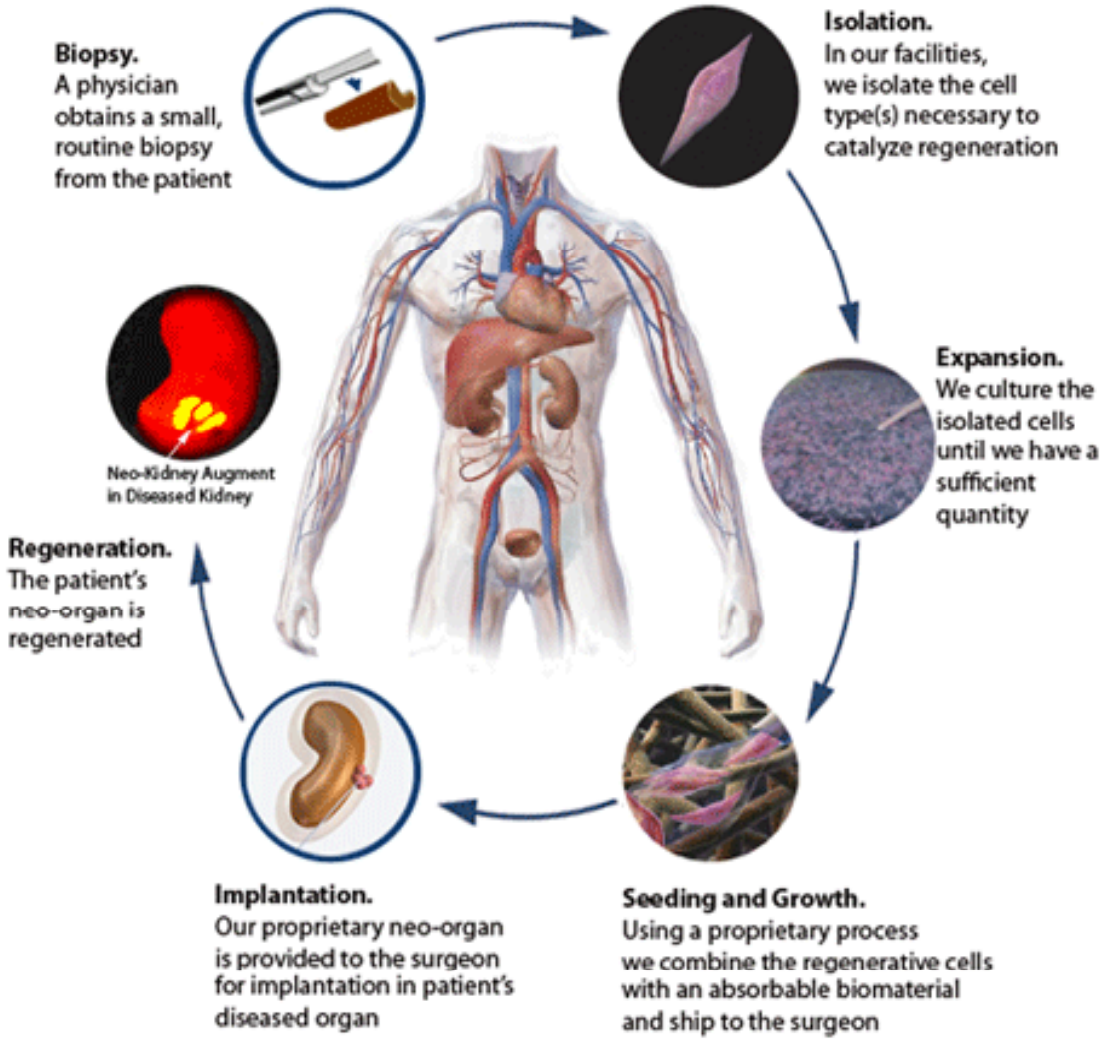
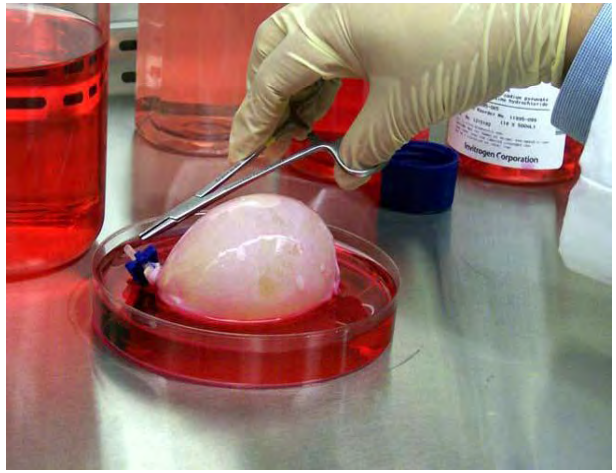


http://www.tengion.com/technology/platform.cfm

Tissue Engineering Approaches

http://news.bbc.co.uk/1/hi/7735696.stm



BLADDER (TENGION)

Myskin™

- Medical grade **silicone** sheet
- Surface deposition of plasma polymerised **acrylic acid**.
- Acrylic acid allows the growth of a layer of proliferative, sub-confluent, **autologous keratinocytes** prior to transplantation onto wound bed.

SKIN

NATURE|Vol 445|22 February 2007|doi:10.1038/nature05664

Tissue Engineered Skin

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Box 1 | Examples of currently available skin-replacement materials

Epithelial cover

Involves the delivery of autologous keratinocytes as one of the following:

- An integrated sheet such as Epicel (Genzyme Tissue Repair). This is developed from the methodology originally pioneered in 1981 (ref. 12). A biopsy of the patient's cells is grown into an integrated sheet and enzymatically detached for delivery to the patient[43].
- Subconfluent cells on a carrier such as Myskin (CellTran)[47]. Cells are delivered to the patient before they reach confluence on a chemically defined carrier dressing.
- Small sheets cultured from a patient's hair follicles such as Epidex (Modex Therapeutics)[48].
- A spray such as CellSpray (Clinical Cell Culture). Subconfluent cells are expanded in the laboratory and made into a suspension in which they are transported. They are then delivered to the patient as a spray[45].

Dermal replacement materials

- Donor skin[34]: skin from screened skin donors can be used to provide either a temporary wound cover or a permanent source of allodermis.
- Integra[21] (Integra LifeSciences): an alternative to donor skin that provides a vascularized dermis for a subsequent split-thickness skin graft.
- Alloderm (Lifecell): freeze-dried human donor dermis[35].
- Dermagraft (Advanced Biohealing): a synthetic material conditioned with donor fibroblasts[28].
- Transcyte (Advanced Biohealing): similar to Dermagraft but with a silicone membrane to act as a temporary epidermal barrier[29].
- Permacol (Tissue Science Laboratories): porcine skin that provides a temporary wound dressing[36].

Epidermal/dermal replacement materials

- Apligraf (Organogenesis): this combines allogeneic keratinocytes and fibroblasts with bovine collagen to provide a temporary skin-replacement material suitable for use in chronic wounds but not major burns[30].
- Orcel (Ortec International): combines allogeneic keratinocytes and fibroblasts with bovine collagen to provide a temporary skin-replacement material suitable for use in chronic wounds[31].
- Cincinnati skin substitute, or Permaderm (Cambrex): comprises autologous keratinocytes and fibroblasts crafted into reconstructed skin with bovine collagen. Can provide a permanent skin substitute for burns patients[37].

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Alternatives to Animal Testing

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- EU directives (REACH and European Cosmetic) to find alternatives to animal testing of chemical additives using in human skin products.

- Provide a 'living' skin model in contrast to dying excised skin but barrier function and cell co-culture is vital.

- Two reconstructed skin models **EpiDerm™** and **EpiSkin™** have been successfully validated for skin irritancy (and corrosive) testing (ECVAM)

"The EpiSkin™ test method was validated as a potential stand-alone method, capable of reliably distinguishing non-irritant from irritant chemicals. The EpiSkin™ test method can thus replace the Draize skin irritation test, a classic test introduced into safety tests for drugs and chemicals 60 years ago.

The EpiDerm™ test method did not qualify as a stand-alone replacement but was recommended for the identification of irritant chemicals, and thus to be used as a constituent of a testing strategy. A modification of the Epiderm™'s Standard Operating Procedure (SOP) and/or Prediction Model was recommended by ESAC. In 2008, the modified EpiDerm™ test method was validated."

EpiSkin™

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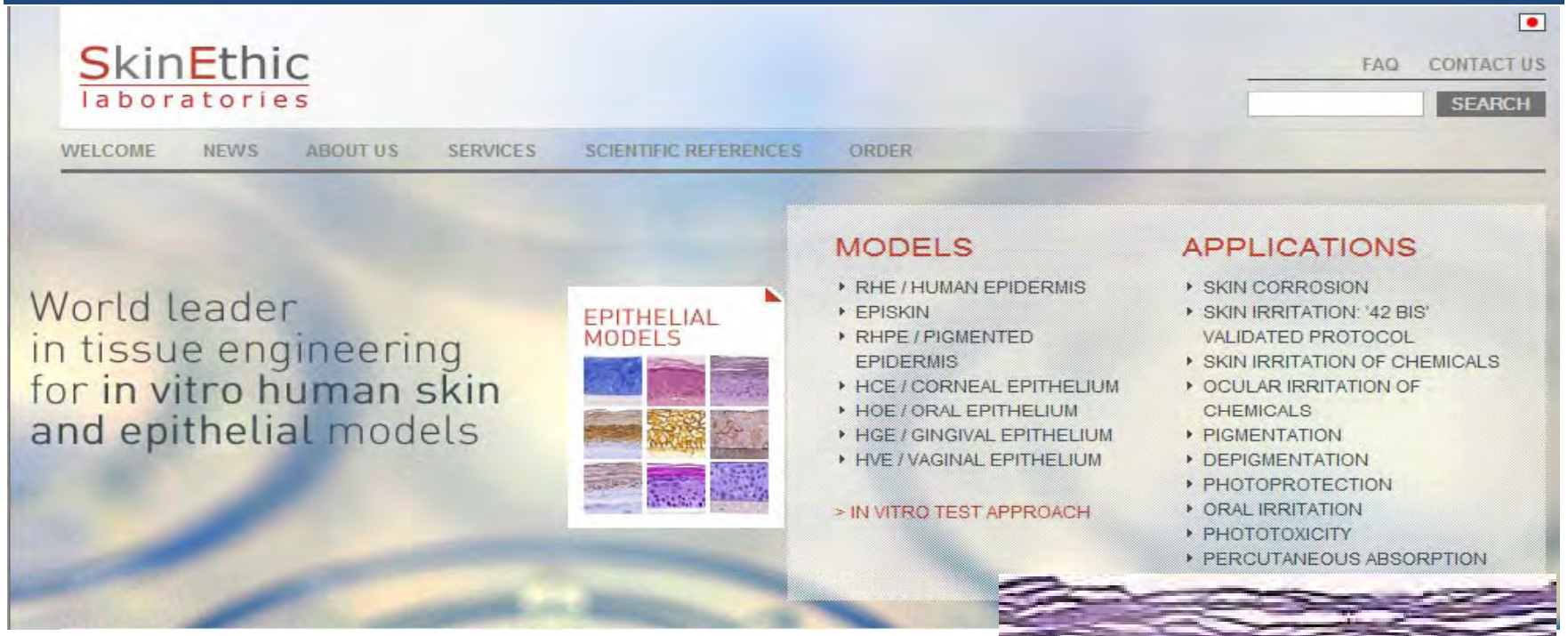
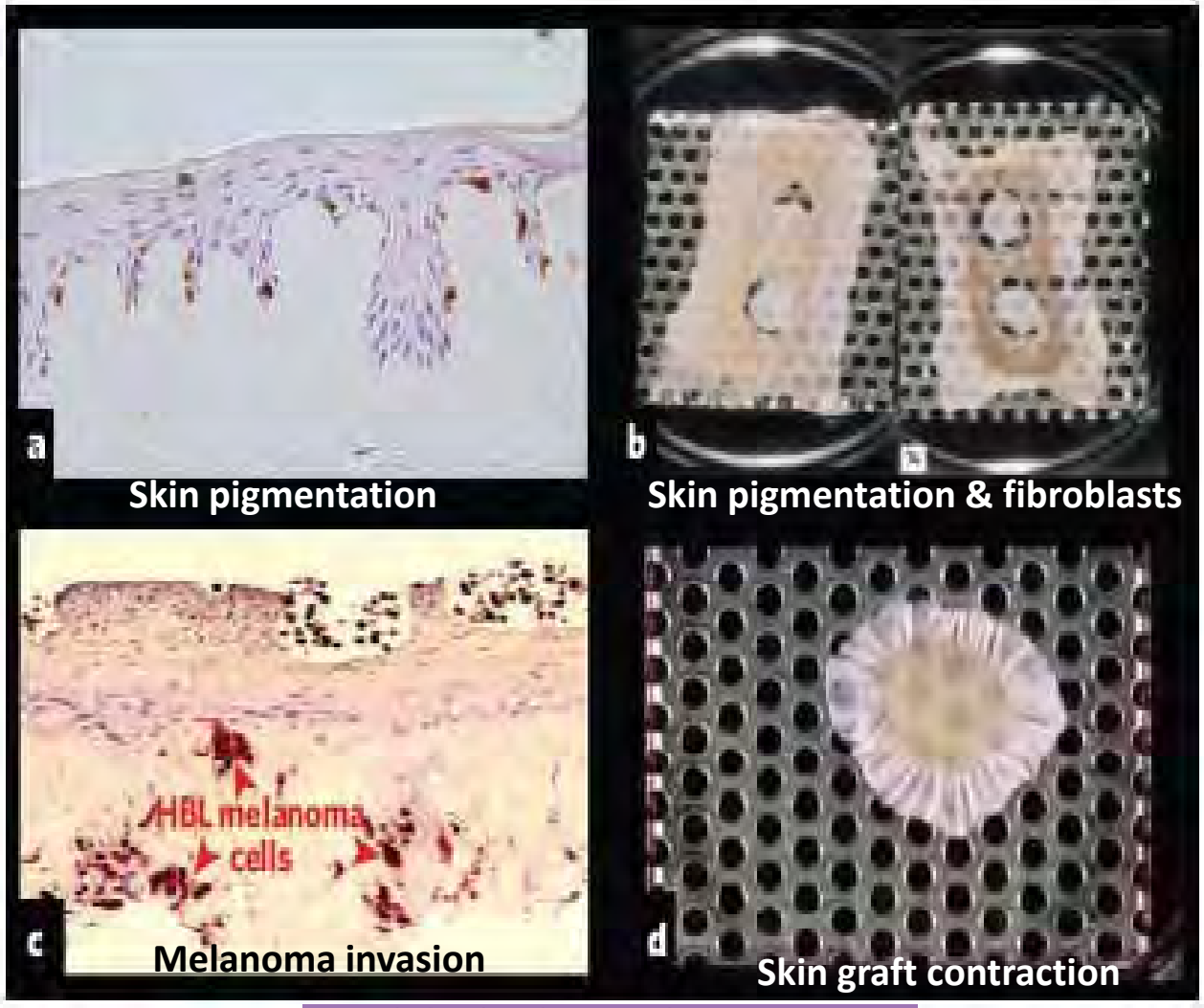


The **EpiSkin™** - adult human keratinocytes cultured on a collagen substrate. Submerged culture followed by culture at the air-liquid interface results in the reconstruction of an epidermis with a functional horny layer.

Tissue Engineered Skin - future uses



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- Co-culture of keratinocytes with fibroblasts shown to be essential for keratinocyte expansion and toxicity tolerances.
- Inclusion of melanocytes for skin pigmentation and the influence of fibroblasts.
- Studies of melanoma invasion to tissue engineered skin.
- As a model of skin graft contraction (keratinocytes gather dermis)

Tissue Engineered Skin – a synthetic approach

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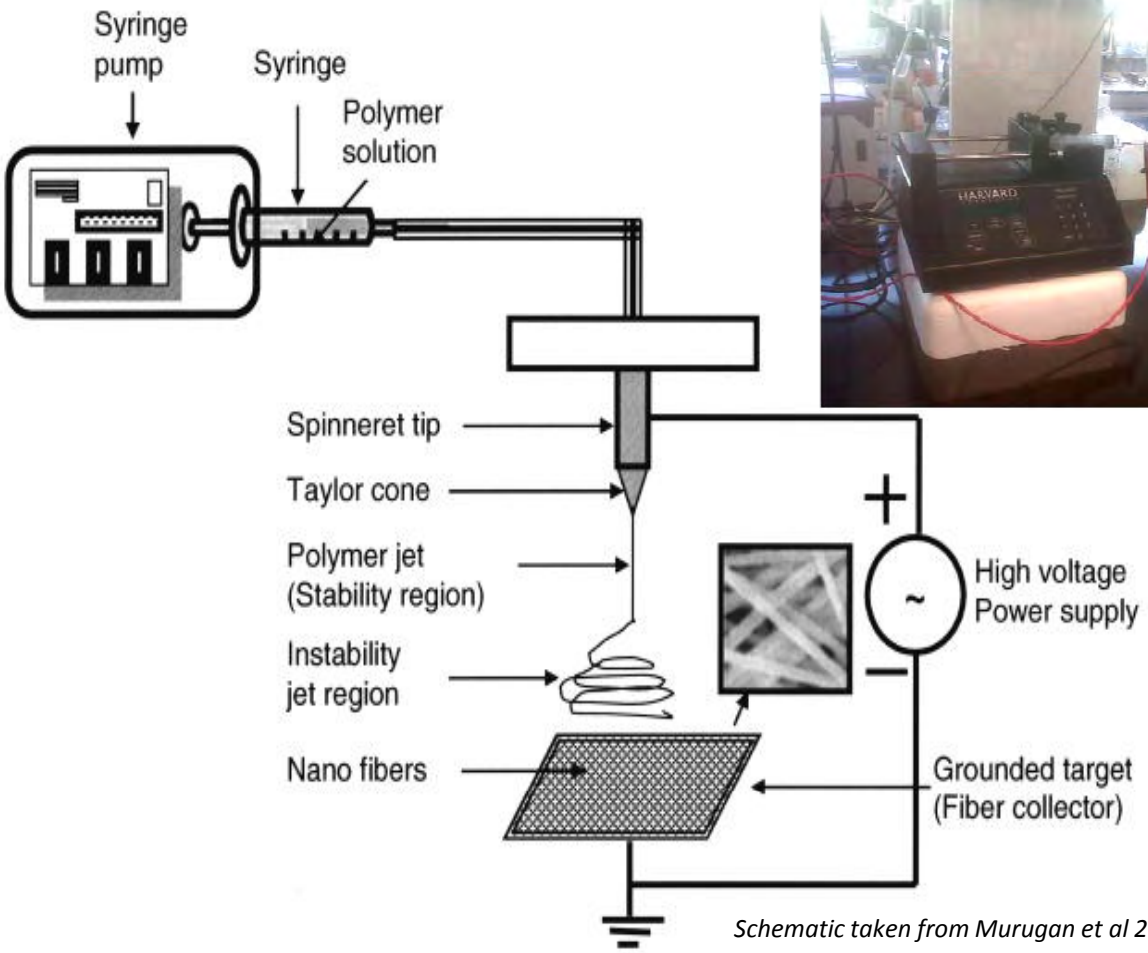
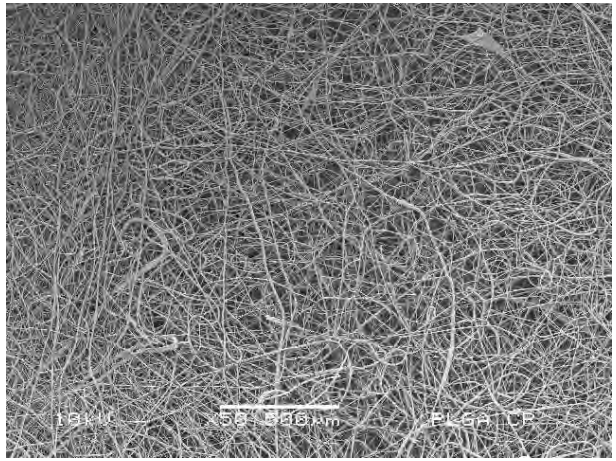
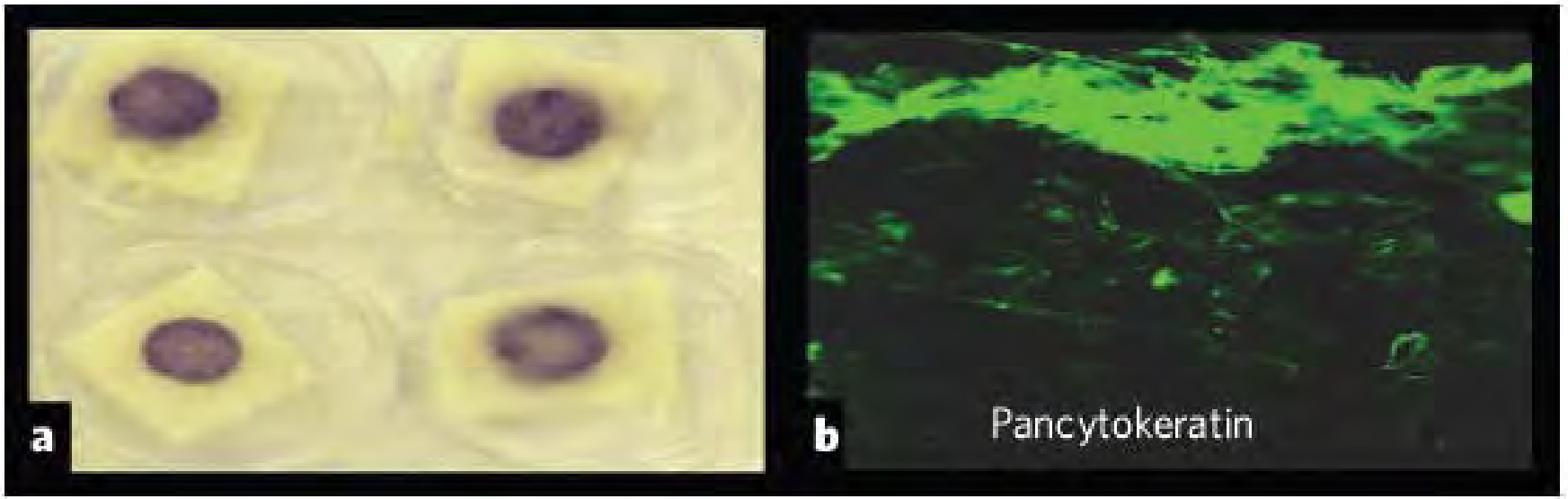


Electrospinning



Schematic taken from Murugan et al 2006

Tissue Engineered Skin – a synthetic approach



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- **Electrospun polystyrene** scaffold (10 um diameter fibres).
- **Keratinocytes, Fibroblasts and Endothelial cells** (10 day culture including ALI).
- Expression of **keratin** representing differentiated keratinocytes (stratum corneum).

Electrospinning patterned scaffolds

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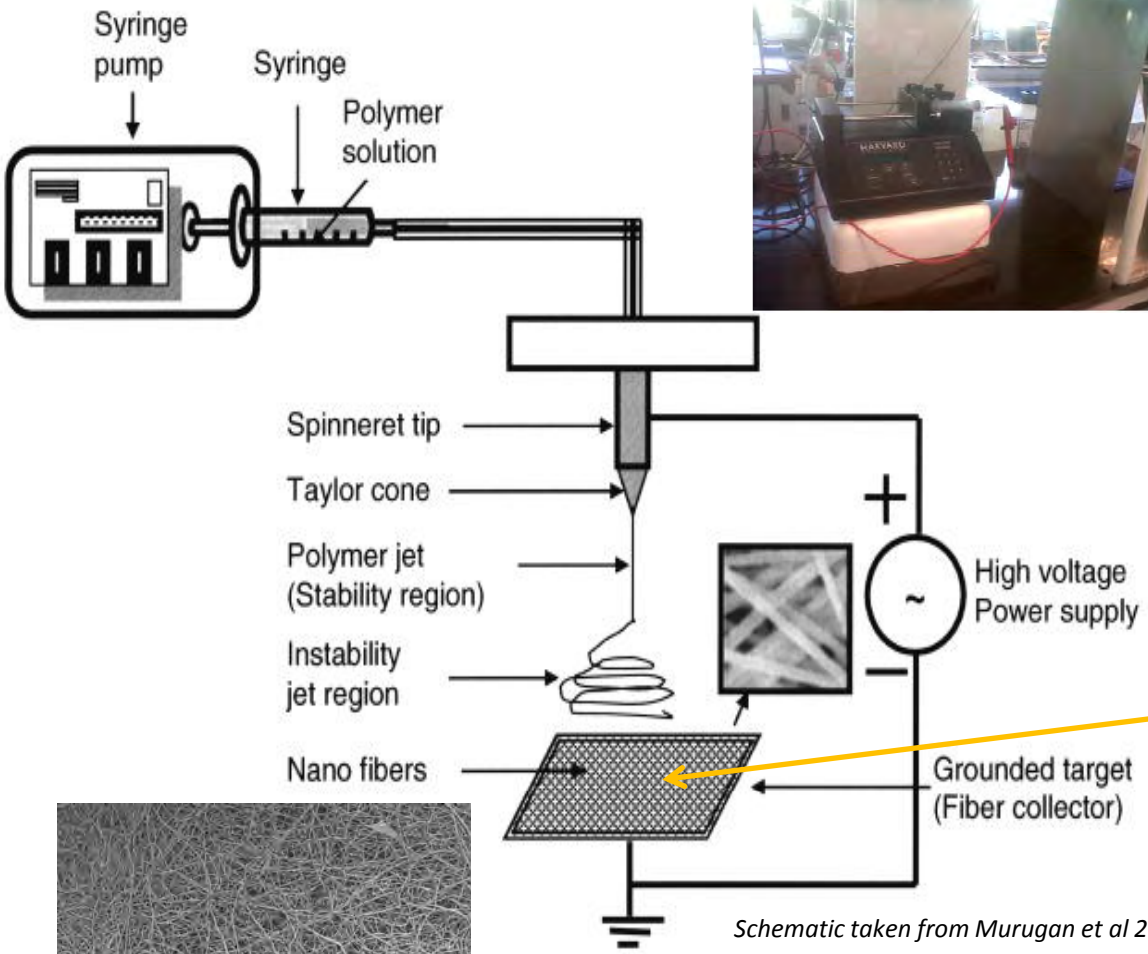
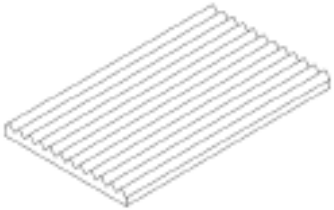
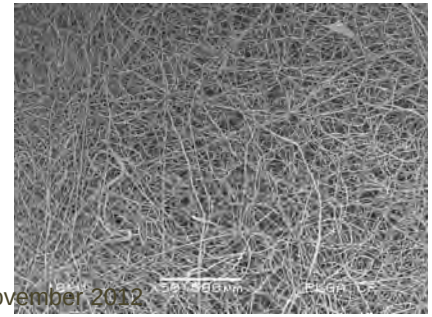


Schematic taken from Murugan et al 2006

Rapid prototyped resin formers





Patterning Electrospun Scaffolds

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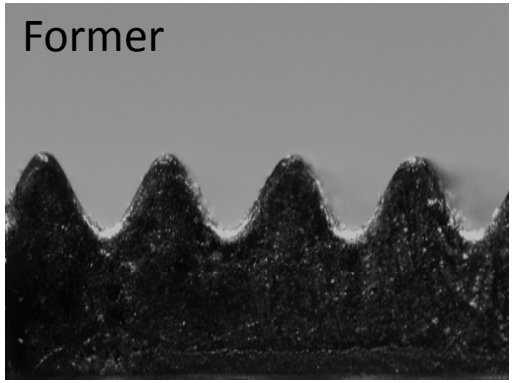
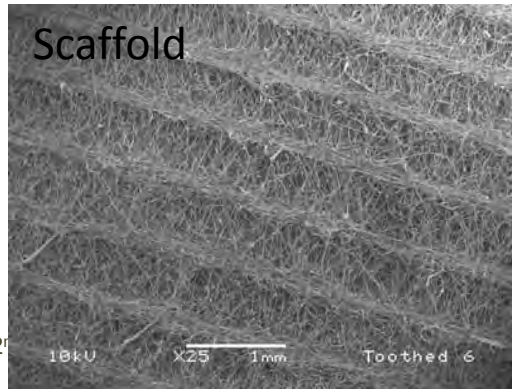
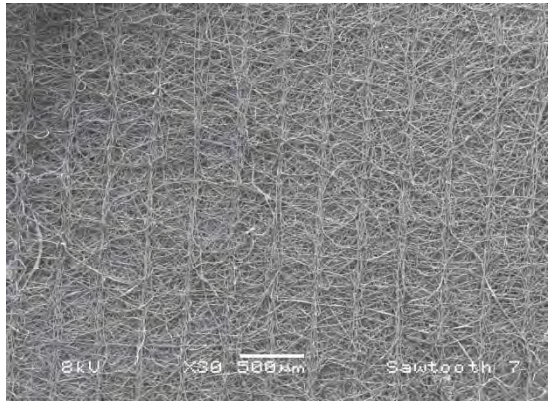
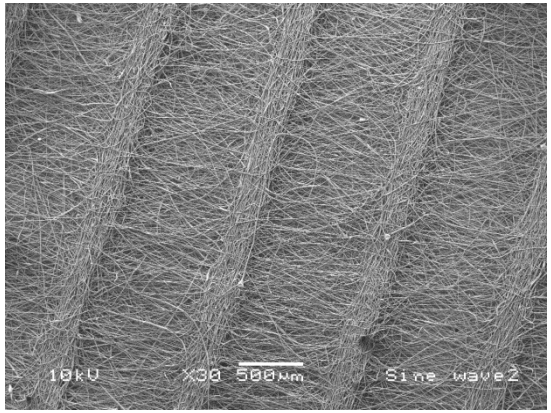
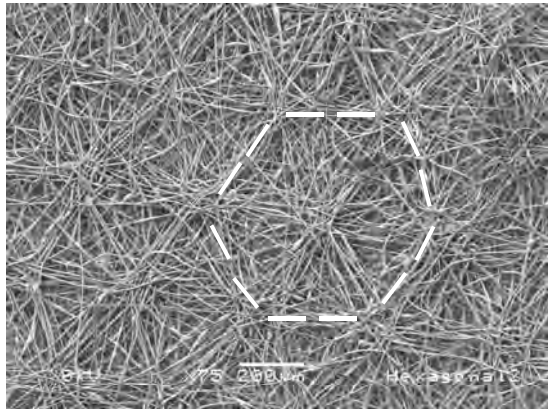


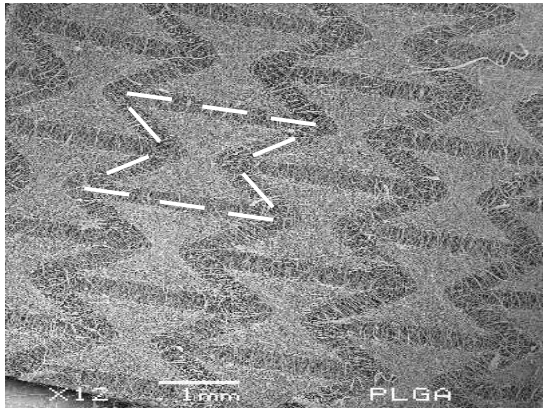


- Patterning technique is versatile and not exclusive to polymer type.









Introducing the macroarchitecture of the epidermal basement membrane

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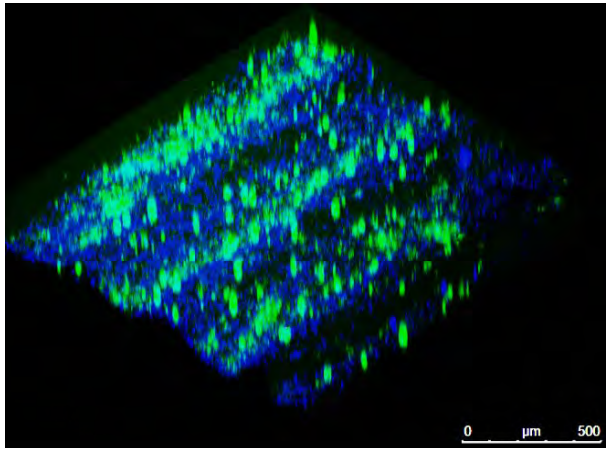
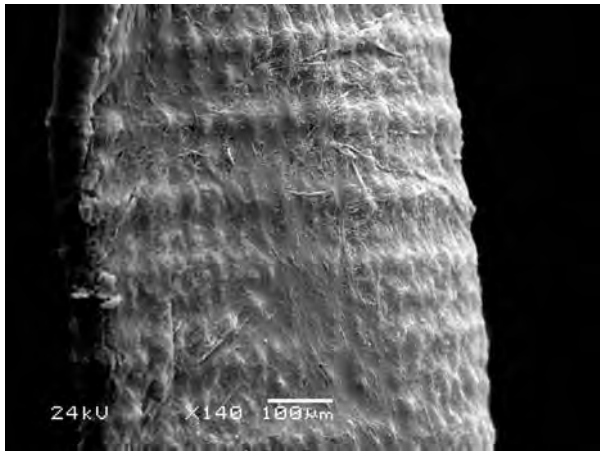
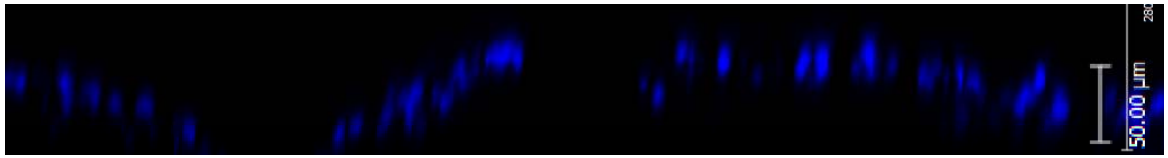
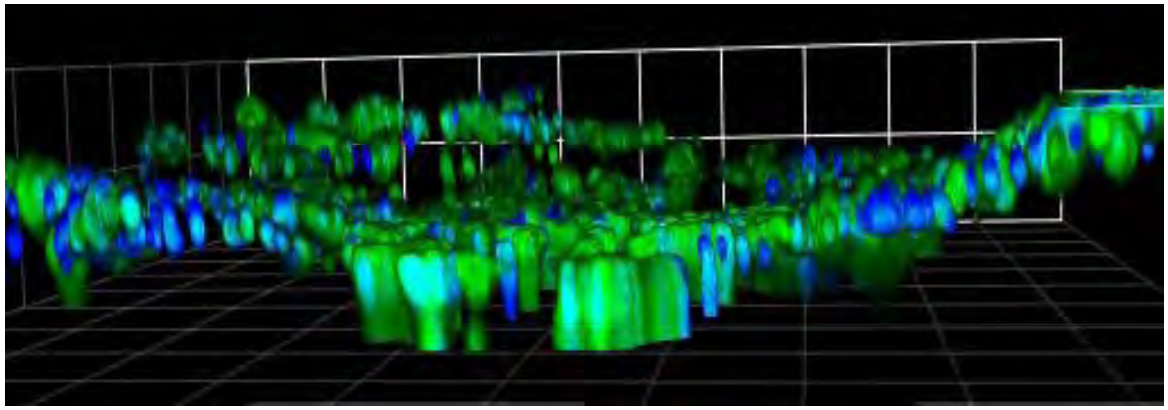


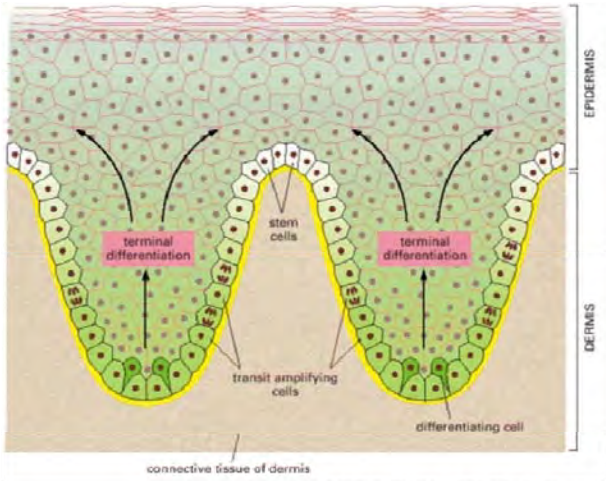


- Patterned scaffolds guide cell adhesion.
- Cells are able to proliferate on the scaffolds and the pattern remains.





Collaboration with Prof Fiona Watt



Alberts *et al.* Molecular Biology of the Cell, 4th ed.

Gavin Morris & Catherine Rogers

Summary

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- **Skin is an effective barrier against the penetration of chemicals.**

- **Numerous methods employed to overcome this barrier for drug and chemical delivery.**

- **A number of different methods used to monitor penetration of chemicals through the skin.**

- **Confocal Raman spectroscopy is a non-invasive, non destructive technique for monitoring the passage of chemicals through the skin both *in vitro* and *in vivo*.**

- **Tissue engineering strategies have provided skin tissues for *in vitro* assays.**

- **Important that the tissue mimic truly replicates the *in vivo* situation including 3D co-culture.**

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