Experimental techniques: The Skin; Biocompatibility

Dr. Gábor Erős
Structure of the skin

- Stratum corneum
- Stratum granulosum
- Stratum spinosum
- Stratum basale

- Sweat duct
- Sebaceous gland
- Arrector pili muscle
- Sweat gland
- Hair follicle

- Blood vessels
- Fat lobules

EPIDERMIS

DERMIS

SUBCUTANEOUS TISSUE
Possibilities

- Human trials

- Animal experiments

- *In vitro* examinations
Cosmetic products and their ingredients must not be tested in animal experiments!!!
Questions and problems

- Barrier function and transdermal drug delivery
- Contact dermatitis: allergic or irritant
- Rosacea
- Sun damage, UV-radiation
- Melanoma and other skin tumors
- Wound healing
Methods

- Inspection, palpation
- Photography (photo analysis)
- Non-invasive dermatophysiological methods
- Ultrasound
- Confocal laser scanning microscopy
- Tape stripping + spectroscopy
- Intravital videomicroscopy
- Histology, immunhistochemistry
Animals used for dermatologic research

- Large animals (pig)
- Guinea pig, hamster, rabbit
- Mouse

There is a difference between HAIRLESS mouse and NUDE mouse

NUDE: immun-compromitted
HAIRLESS: mutant, but NOT immun-compromitted

(SKH-1 and SKH-2)
Dermatophysiology: Transepidermal water loss (TEWL)

- Diffusion principle in a opened chamber

- Two pairs of sensors (temperature and relative humidity) inside the cavity

- \( \text{g} / \text{m}^2 \times \text{h} \)

- dermatology, cosmetology, etc.
TEWL measurement in animal
Dermatophysiology: Melanin & Erythema (Mexameter)

- 3 specific wavelengths
- Reflection is measured, absorption is calculated
- Special wavelength corresponding to the absorption peak of hemoglobin
Dermatophytophysiology: Skin Surface Hydratation (Corneometer)

- Capacitance measurement of a dielectric medium
- Temperature stability
Dermatophysiology: other parameters

- pH
- Sebum on the skin surface
- Elasticity
- Skin topography (UV-light video camera)
Ultrasound

- Tumor
- Burn injuries
- Skin aging
- Edema?
- High frequency (15-70 MHz)
Confocal laser scanning microscopy

- Depicts skin structures **horizontally** in cellular resolution

![Images of skin structures]

- Stratum corneum
- Stratum granulosum
- Papillary dermis
Tape stripping + spectroscopy

- Adhesive tape to collect corneocytes

- Analysis by means of Fourier transform infrared spectroscopy (FTIR)

- Human trials, animal experiments (Csizmazia et al., *Pharm Dev Technol* 2010; Csizmazia et al., *J Drug Del Sci Tech* 2011)
Animal model of different skin disorders: Irritant contact dermatitis

- Non-specific and non-immunological

- External chemical or physical impacts

- Inducible in rodents with different agents

- Sodium lauryl sulphate (SLS), SDS

- SLS: anionic surfactant
Other agents inducing skin irritation

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Maximal response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl salicylate</td>
<td>20 min</td>
</tr>
<tr>
<td>Phenol</td>
<td>1 hr</td>
</tr>
<tr>
<td>Croton oil</td>
<td>6 hr</td>
</tr>
<tr>
<td>Benzalkonium chloride</td>
<td>6 hr</td>
</tr>
<tr>
<td>Ethyl phenylpropiolate</td>
<td>8 hr</td>
</tr>
</tbody>
</table>

(Patrick et al., *Toxicol Appl Pharmacol* 1985)
Irritant contact dermatitis: confocal microscopy images and routine H&E staining

Disruption of stratum corneum

Parakeratosis

Microvesicle containing inflammatory cells

Hicks et al., J Am Acad Dermatol; 2003
Animal model of different skin disorders: Allergic contact dermatitis

- Mouse model: repeated application of 2,4,6-trinitro-1-chlorobenzene (TNCB) induces chronic allergic contact dermatitis (skin swelling + scratching behaviour)

(Yamaura et al., J Toxicol Sci 2011)
Animal model of different skin disorders: Wound healing

(Sorg et al., *J Anat* 2007)
Animal model of different skin disorders: Transdermal drug delivery

- Forming of a skinfold

- „Sandwiching” of the skinfold with titanium frames

- Forming of a circular wound

- Fixation of the buffer-containing cylinder

(Erős et al., Scientific World Journal (accepted for publication))
Animal model of different skin disorders: Melanoma

- Large animal model: swine, horse (no sunlight etiology)
- Goat (similar to that of caused by chronic sunlight exposure)
- Guinea pig, hamster (dimethylbenzanthracene – DMBA, metastases) In guinea pig: similarities to human
- Mouse: VERY DIFFICULT!
  - DMBA + UV
  - Transgenic mice: HGF/SF (neonatally)
Biocompatibility 1.

Biosafety
- Appropriate host response (local and systemic)
- Absence of cytotoxicity, mutagenesis, carcinogenesis

Biofunctionality
- Ability to perform the special task
Biocompatibility 2.

Biomaterials and components relevant to \textit{in vivo} assessment of tissue compatibility:

- Additives, process contaminants, residues
- Leachable substances
- Degradation products
- Other components in the final product
- Properties and characteristics of the final product
Biocompatibility 3.

Body contact and contact duration of implanted materials:

- Surface devices
- Skin
- Mucosal membranes
- Breached or compromised surfaces
- External communicating devices
- Blood (direct or indirect)
- Tissue / bone / dentin

Contact duration:
- Limited (≤24 h)
- Prolonged (>24 h and <30 days)
- Permanent (>30 days)

(FDA-categories)
Biocompatibility 4.

Biological response tests:
(prior to clinical testing)

- Cytotoxicity
- Sensitization
- Irritation
- Intracutaneous reactivity
- Systemic toxicity (acute)
- Subchronic toxicity
- Genotoxicity
- Implantation
- Hemocompatibility

- Chronic toxicity
- Carcinogenicity
- Reproductive and developmental toxicity
- Biodegradation

INITIAL EVALUATION STEPS

SUPPLEMENTARY EVALUATION STEPS