Notes on Skin
For the skin papers, don’t worry about the details so much as the systems they are using and the important considerations in using them.

SKIN! (modified from wikipedia)
Skin is the largest organ in the body, accounting for ~15% of your body weight. Its purpose is to protect you from the rest of the world (barrier function). It also insulates you, produces vitamins B and D, senses touches, and excretes sweat, is involved in heat regulation as well as fat and water storage. There are a lot of immune cells associated with skin since it’s a prime site of pathogen infiltration.

The outermost layer of skin, the epidermis, is made up of stratified (layered) squamous epithelium with an underlying basement membrane (basal lamina → extracellular matrix).

It contains no blood vessels, and is nourished by diffusion from the dermis. The main type of cells in the epidermis are keratinocytes. Melanocytes (pigment) and Langerhans cells are (immune cells) also present.

Cells are formed through mitosis (division) at the innermost layers (basal layer). They move up the strata, changing shape and composition as they differentiate and become filled with keratin. Keratin is a tough insoluble protein that makes the skin tough and almost completely waterproof (up to the horny layer). They eventually reach the top and become sloughed off. This process is called keratinization and takes place within about 30 days. This layer of skin is responsible for keeping water in the body and keeping other harmful chemicals and pathogens out.

The epidermis is comprised largely of cells with direct contact to each other, there is little extracellular matrix in this region (except at the bottom).

The dermis lies below the epidermis and contains a number of structures including blood vessels, nerves, hair follicles, smooth muscle, glands and lymphatic tissue. It is made up of dense connective tissue - collagen, elastin and reticular fibres are present.

Erector muscles, attached between the hair papilla and epidermis, can contract, resulting in the hair fibre pulled upright and consequentially goose bumps. The main cell types are fibroblasts, adipocytes (fat storage) and macrophages (immune cells).

p.s. the wikipedia entry on skin is particularly entertaining all round.
Schematic of tissue engineered epidermis
For these culture systems, keratinocytes are first cultured in monolayer on a permeable membrane, submerged in culture media.

Under the appropriate conditions, the cells begin to pile up (stratify) and differentiate as they do in skin. Early culture conditions involved leaving the cell layers submerged.

More recently, culture conditions in which the top layer of cells is exposed to air (air/liquid interface cultures) have been developed. For reasons I don’t think are well understood, it causes further differentiation of the keratinocyte layers. In particular, it leads to a layer resembling the outer layer of the epidermis that acts as a hydrophobic barrier in vivo – making it much more skin like and appropriate for application of topical agents.

One thing to note about this type of tissue engineered skin is that there is no scaffold since it’s mostly cells normally anyway.

Also, in the papers they talk about co-cultures with different cell types as well as full-thickness cultures (this means both epidermis and dermis). Presence of other cell types could clearly change how the tissue acts and how cells within it interact with each other.

(Pay attention to how they describe the creation of the dermis… It might look familiar…)

**More Vocab**

Transdermal means across the skin.

Drug metabolism and targeting are concerns when trying to deliver drugs. Often you have to deliver a drug systemically (to the whole body) so that it gets diluted and also taken up, and possibly metabolized (lost), in non-intended places. Drugs are often also taken orally, meaning they get into a person systemically that way. This loss and dilution means that you have to give more than you would otherwise want to which may lead to toxicity. Drug targeting and other methods of more efficient drug delivery are big topics. (Therapeutic window in class.)

Percutaneous means adsorbing or moving across the unbroken skin.

Gene microarrays are very hot right now because they can provide vast amounts of information about gene expression. They consist of an array of gene segments. Samples with a mix of fluorescently labeled DNA are added and “hybridize” (bind) to the gene segments. The plate is washed and the fluorescence is measured. This technique provides information about expression levels of many genes at one time.
Information on *in vivo* testing From “Recommended Performance Standards for In Vitro Test Methods for Skin Corrosion” (iccvam.niehs.nih.gov/methods/ps/ps044510.pdf)

Skin corrosion refers to the visible destruction or irreversible alteration of skin following exposure of the skin to a chemical substance. Skin corrosivity has traditionally been assessed by applying the test substance to the skin of living animals and evaluating the extent of tissue damage after a fixed period of time (OECD 2002b; EPA 1998). Some U.S. regulatory authorities require determination of corrosivity using three categories of responses, as provided in **Table 1-1** (EPA 1998; DOT 2003a, 2003b).

**Table 1-1  Skin Corrosive Category and Subcategories**

<table>
<thead>
<tr>
<th>Corrosive Category (category 1) (applies to authorities not using subcategories)</th>
<th>Potential Corrosive Subclasses(^1) (UN Packing Group Classification(^2))</th>
<th>Corrosive in at least 1 of 3 animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrosive</td>
<td>Corrosive subcategory 1A (I)</td>
<td>≤3 minutes</td>
</tr>
<tr>
<td></td>
<td>Corrosive subcategory 1B (II)</td>
<td>&gt;3 minutes / ≤1 hour</td>
</tr>
<tr>
<td></td>
<td>Corrosive subcategory 1C (III)</td>
<td>&gt;1 hour / ≤4 hours</td>
</tr>
</tbody>
</table>

\(^1\) Classifications designated by the United Nations (UN) Globally Harmonised System for the Classification and Labelling of Chemical Substances and Mixtures (GHS) (UN 2003a).

\(^2\) Corresponding UN packing group classifications to be used for the transport of dangerous goods (UN 2003b).

Ethical discussion:

Try to step back from what you instinctively believe in about use of animals in research/testing while you read the ethics papers. Try to evaluate all of the positions from a neutral place.

Ethics language is very different from the other papers we’ve been reading. Spend time shifting gears to read/think in a different way.

Web sites with alternate to animal testing info:
 http://iccvam.niehs.nih.gov/
 http://altweb.jhsph.edu/index.htm