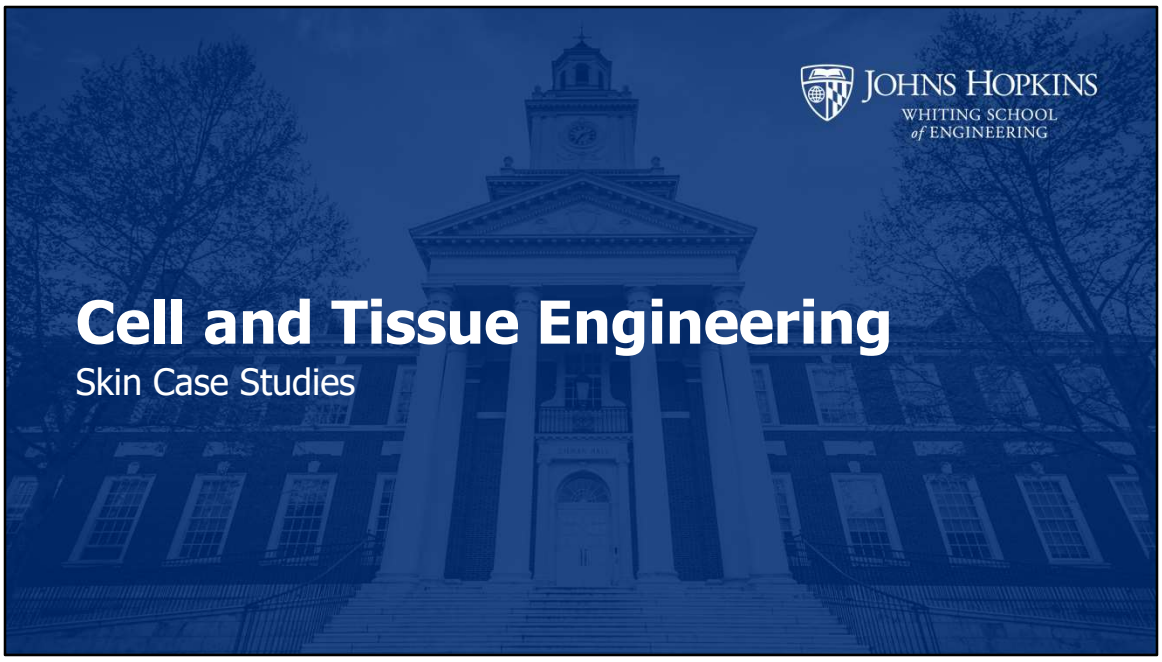
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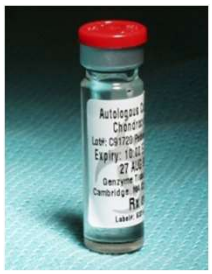
of ENGINEERING
Cell and Tissue Engineering
Skin Case Studies

Case Studies in Tissue Engineering

Carticel (autologous cultured chondrocytes)



Epicel (cultured epidermal autografts)

NEUROTUBE®

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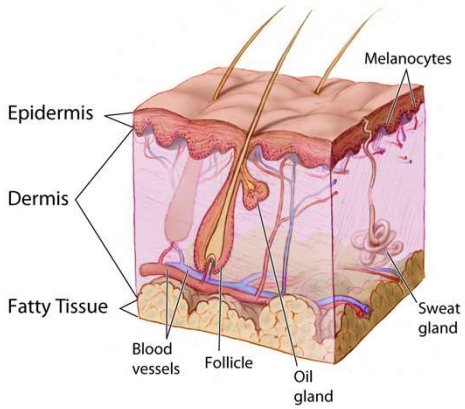
2

MACI, Smith & Nephew; GEM

Let's continue from cartilage to skin. In this video, we will look at **Epicel** – cultured autologous keratinocytes for skin regeneration

Tissue Engineered Skin – Native Skin Function

- Provides efficient **protections** against mechanical disturbances, infection and hazardous substances
- Acts as a **sensory** organ
- Helps regulate **temperature**
- Prevents excessive **fluid** loss and absorption
- Acts as an **immune** organ to detect infections, etc.
- Reduces harmful effects of **UV radiation**
- Helps in the production of **vitamin D**



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3

Wikipedia.org

Skin grafts are another area of tissue engineering where products have made it from the lab to the patient.

Skin -- which is the largest organ in your body -- protects your bodyfrom toxins, microorganisms and prevents dehydration. It is participates in **immune** system suveilance, it is highly **sensory,** and it has great **regenerative** capabilities.

A significant defect to skin -- from a burn, traumatic injury, congeital defect or disease -- can throw the human body out of **homeostasis**, leading to *death* in severe cases.

The advent of tissue engineered skin replacements **revolutionized** the treatment for wounds that could not close on their own. I cannot explain just how BIG of a deal this revolution was for burn wounds in particular.

Where we were once reliant only on **autografts --** which were limited in size, risk of pain scarring and infection -- we now have *multiple* commercial products that use small cell biopsies, or in some cases no cells at all, to cover large wound sites – All now without the need to scrape the skin off of any remaining part of your body or use cadaveric skin grafts (a treatment which has an interesting ethical backstory).

Tissue Engineered Skin – Design Criteria

Normal Skin Function

- Able to resist infection
- Able to prevent water loss
- Able to withstand shear forces

Business Criteria

- Cost-Effective
- Widely available
- Long shelf life and easy to store

Wound Treatment Criteria (Use)

- Lack of antigenicity
- Flexible Thickness
- Durable with long-term wound stability
- Conformable to irregular wound surfaces
- Easily secured and applied

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Here is a list of the ideal **features** – of a skin substitute – this type of list should look familiar to you – it's a list of design criteria.

As you can see it meets the natural functions of skin -- withstanding **mechanical** forces, preventing **dehydration**, and resisting **infection**.

Additionally, for business reasons, a tissue engineered skin graft should be **cost effective**, widely available, have a long shelf life.

For clinical reasons, the product should also **conform to irregular wound** surfaces and be easiled applied.

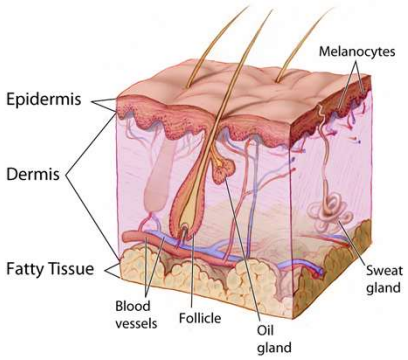
There is one more criteria that didn't make this list which we've discussed several times this semester… do you have any ideas of what that might be?

Boyce, Steven T., and Andrea L. Lalley. "Tissue engineering of skin and regenerative medicine for wound care." *Burns & trauma* 6 (2018).
- https://burnstrauma.biomedcentral.com/articles/10.1186/s41038-017-0103-y

- Engineering of biologic skin substitutes has progressed over time from individual applications of skin cells, or biopolymer scaffolds, to combinations of cells and scaffolds for treatment, healing, and closure of acute and chronic skin wounds. Skin substitutes may be categorized into three groups: acellular scaffolds, temporary substitutes containing allogeneic skin cells, and permanent substitutes containing autologous skin cells. Combined use of acellular dermal substitutes with permanent skin substitutes containing autologous cells has been shown to provide definitive wound closure in burns involving greater than 90% of the total body surface area. These advances have contributed to reduced morbidity and mortality from both acute and chronic wounds but, to date, have failed to replace all of the structures and functions of the skin. Among the remaining deficiencies in cellular or biologic skin substitutes are hypopigmentation, absence of stable vascular and lymphatic networks, absence of hair follicles, sebaceous and sweat glands, and incomplete innervation. Correction of these deficiencies depends on regulation of biologic pathways of embryonic and fetal development to restore the full anatomy and physiology of uninjured skin.

Tissue Engineered Skin – Vascular Structure

- **Connects epidermis to deep layers**
- **Damaged in burns and diabetic ulcers**



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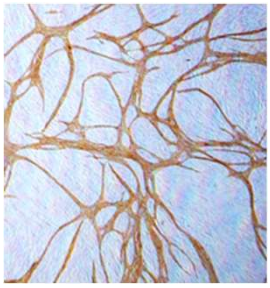
Let's go back to this schematic of the skin for a moment. One of the key features that skin must possess is a **working vasculature** – one that is connected from the epidermis down to the tissue below the skin.

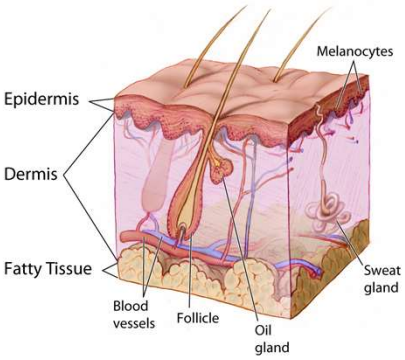
As we've discussed earlier this semester connecting the artificial tissue with the host vasculature is essential to the **survival** of the that artificial tissue. One of the main complications with different types of skin products is **sloughing** due to the absence of proper vascularization.

This has been a major roadblock in the development of tissue engineered skin – in part because the host vasculature under the wound site is often *damaged*. This is the case of both **burns** and **diabetic ulcers** – the two *most common* indications for skin substitutes.

Stimulating Vascular Growth

HUVECS
human umbilical vein endothelial cells
+
Collagen I
Fibronectin
+
Bcl-2
(survival gene)





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Angiogenesis.nl

Research on enhancing vacularization in grafts has included cell, matrix, and factor components.

HUVECs -- or human umbicial vien endothelial cells -- have been repeatedly shown to **spontaneously** form tube structures *similar* to blood vessels in culture, in matrices rich in collagen I and fibronectin.

When implanted under the skin in a mouse, they will connect (anastomose) with the mouse vaculature and become **perfused**. Additional studies report that forced expression of the **bcl-2 gene** – this is a *survival* gene that keeps cells from **apoptosis --** can increase the vascular density in the construct prior to implantation and fosters remodeling behavior in the new networks, leading to the formation of arteriolar and venular vascular types.

Vascularization of Tissue Engineered skin grafts remains a challenge for indications where the underlying vascular bed is heavily damaged – deep burns and trauma that exposes underlying structures.

BCL2 = B-cell lymphoma 2. Discovered in it's role in pathology of cancer, but has a normative role in vasculature as well.

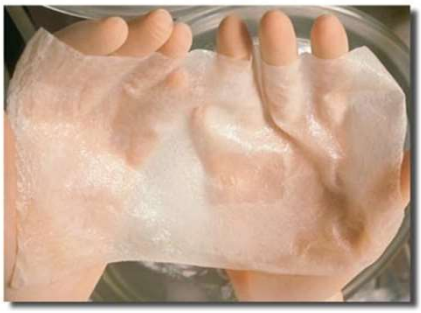
Revascularization occurs via a graduated process: The graft is initially nourished by oxygen diffusion from the wound bed in the first 24 hours, with revascularization occurring in the subsequent 24 to 48 hours. Thus any clean wound with a well-vascularized bed can accept a skin graft. Wounds with denuded bone, tendon, or exposed implants will not accept a graft.

Although there have been considerable improvements in tissue-engineered skin grafts, none of them could reproduce the normal architecture of natural skin, including hair follicles, Langerhans cells, sebaceous glands, and sweat glands.

Shahin, Hady, et al. "Vascularization is the next challenge for skin tissue engineering as a solution for burn management." *Burns & trauma* 8 (2020).

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7396265/
- Combination of the right cells with scaffolds of proper physio-chemical properties, vascularization can be markedly enhanced. The material effect, pore size and adsorption of certain proteins, as well as the application of appropriate growth factors, such as vascular endothelial growth factors, can have an **additive** effect.

Tissue Engineered Skin - Integra



TRADE NAME		COMPOSITION	APPROXIMATE COST per square cm
Biobrane ™ (Dow Hickam/Bertek Pharmaceuticals, Sugar Land, TX)		1.Silicone 2.Nylon Mesh 3.Collagen	$0.82
Transcyte® (Advanced Tissue Sciences Inc., La Jolla, CA)		1.Silicone 2.Nylon Mesh 3.Collagen seeded with neonatal fibroblasts	$9.16
Apligraf® (Organogenesis Inc., Canton, MA and Novartis Pharmaceuticals, East Hanover, NJ)		1.Neonatal keratinocytes 2.Collagen seeded with neonatal fibroblasts	$16.52
Dermagraft® (Advanced Tissue Sciences Inc., La Jolla, CA)		1.Polyglycolic acid or polyglactin-910 seeded with neonatal fibroblasts	$8.31
Integra® (Integra Life Sciences Corporation, Plainsboro, NJ)		1.Silicone 2.Collagen and glycosaminoglycan	$3.86
Alloderm® (Life Cell, Woodlands, TX)		1.Acellular de-epithelialised cadaver dermis	$6.86
Epicel™ (Genzyme, Cambridge, MA)		1.Cultured autologous keratinocytes	$16.28
Laserskin™ (Fidia Advanced Biopolymers, Italy; Vivoderm™ by ER Squibb & Sons Ltd., United Kingdom)		1.Cultured autologous keratinocytes 2.Hyaluronic acid with laser perforations	n/a
Cadaveric allograft (from non-profit skin banks)		1.Cryopreserved in order to retain viability 2.Lyophilized 3.Glycerolized	$0.70

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Tissue Engineering, Saltzman

There are a host of products available to patients right now. This the table from your text indicating different products, their structures, compositions, and cost per square centimeter.

The first solutions were completely **acellular** – using silicone with collagen and other matrix components -- to induce **local angiogenesis**. These designs became the clinical product **Integral** – shown on the left.

You can see that comapred to the other products, Integra is relatively **simply** in structure, but at the higher end **pricewise**. This is the most *widely used* skin substitute for burn patients where there is no **time** for growing cells for the graft. After a period of healing the **silicone layer** is removed and can be replaced with the **grown epidermal graft**.

Tissue Engineered Skin - Biobrane

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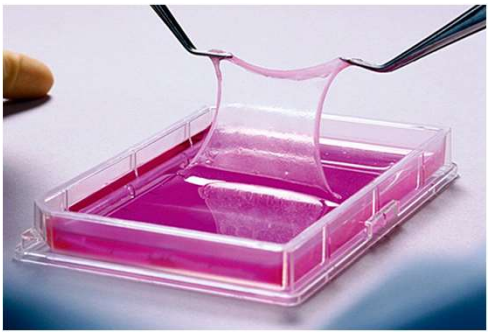
We introduced an example of an acellular graft at the start of this talk – **Biobrane** is a silcone, nylon/collagen composite, again with the silicone on top.

The price point for this product is just a **fraction** of the Integra product. Instead of replacing the silicone with epidermis – the top of this graft **spontaneously delaminates** upon re-epithelializatin by the body.

Although these acellular products have the great benefits of being available on demand – they are shipped and stored **dry**, then **rehydrated** prior to application -- they also have their **faults**. They are not prefered for **facial** grafts because of their mechanical properties – **puckering** and **creasing** around small curves.

Tissue Engineered Skin - Epicel



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There are also tissue engineered solutions which contain **cells** – **Epicel** from Genzyme (now Vericel, the same company that makes MACI) is an example.

This is a graft made from **cultured autologous keratinocytes**. The company claims that from 2 biopsies, each the size of a postage stamp, they can grow enough replacement skin to cover the entire human body. With this potential, it is no surprise that this solution is indicated for patients that have **severe full-thickness wounds which** cover 30% or more of their body.

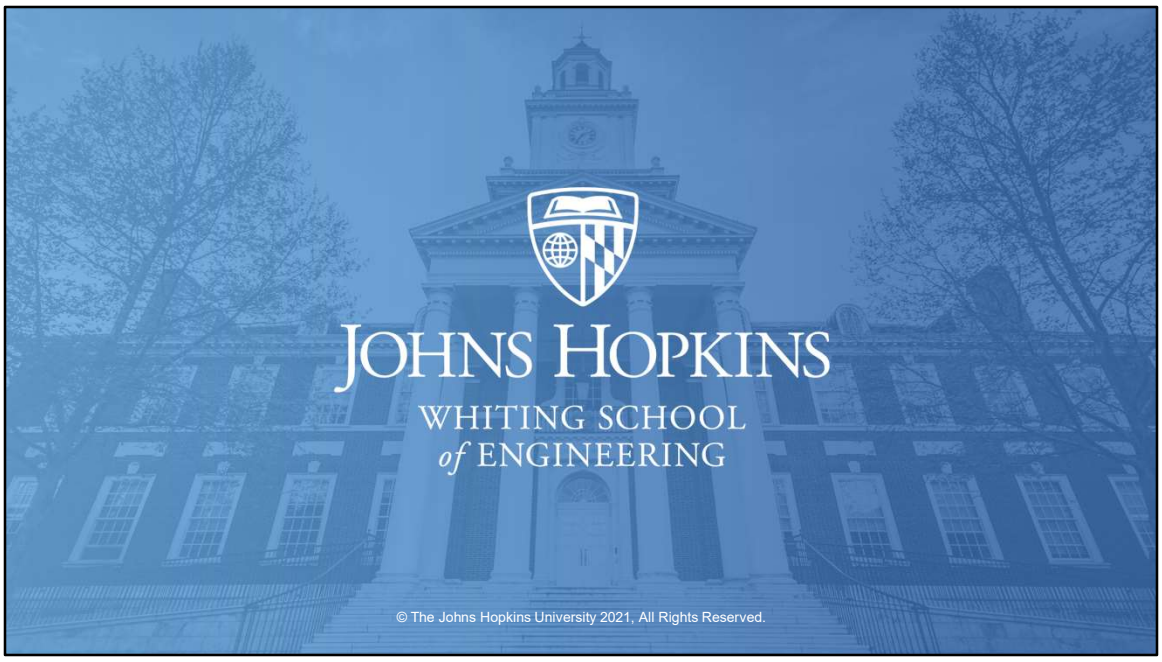
After the biopsies are shipped to Genzyme (in Massachusetts) they culture the keratinocytes in **50cm2 sheets,** like the one shown on the slide, that range in thickness from **2-8 cells** thick. This takes 3-4 weeks.

During culture the cells are exposed to **murine cells** which results in **trace amounts** of these cells in the final graft. For this reason the FDA considers this a **xenograft** product, **precluding** reciptients from donating blood or other tissues in the future.

It is currently an FDA approved as a HDE – humanitarian exemption. It does not have 510k approval, it's limited to treating a certain number of patients per year - We'll

talk more about this in our next module on how to translate products through the regulatory framework to reach patients.

Update 2017: Humanitarian Device Exemption for burn patients up to 360, 400 per year. Now lives with CBER as an approved blood product
https://www.fda.gov/media/103147/download
Carcinoma risk https://www.fda.gov/media/102746/download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