Fish Skin Grafts Promote Superior Cell Ingrowth Compared to Amnion Allografts, Human Cadaver Skin and Mammalian Extracellular Matrix (ECM)

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Why do we look at cell ingrowth?

- The most obvious feature of chronic wounds is the failure to re-epithelialize
- Failure is most often due to keratinocyte migration problems rather than failure to proliferate
- Failure of migration may occur due to
  - Lack of functional Extracellular Matrix for adherence
  - Excessive inflammation and protease activity
  - Altered cytokine expression and distribution

Cell Migration as marker of functional Extracellular Matrix

- Achieving cell ingrowth into a skin substitute demonstrates functional ability to
  - Protect ECM components, growth factors and receptors from degradation
  - Modulate inflammatory response
  - Provide scaffold to direct cells into the injury as well as stimulating them to proliferate, differentiate and synthesize new ECM

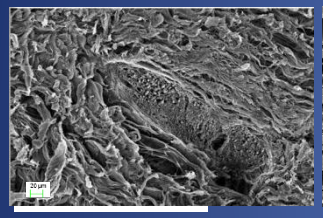
Study goals

1. Compare the structure of human skin to common skin substitutes from:
   - Fish skin
   - Human amnion/chorion
   - Bovine Pericardium
   - Porcine small intestinal submucosa
   - Porcine urinary bladder
2. Quantify the different rates of cellular ingrowth between two skin substitutes
   - Fish skin
   - Human amnion/chorion

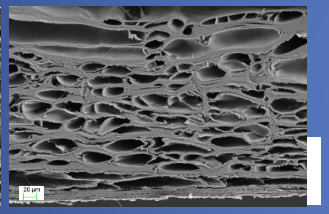
Structure comparison

- Structure comparison was done by Scanning Electron Microscopy imaging
- Porosity was measured by number of pores and pore sizes.

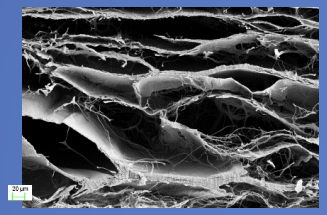
Structural Comparison with Other Tissue Based Products



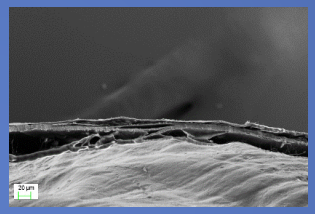
Human **Skin**



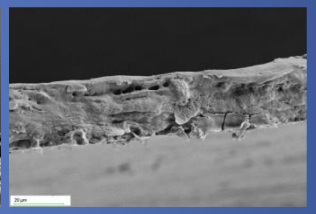
Acellular Fish **Skin** Graft



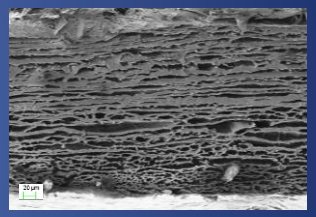
Porcine **Urinary Bladder** Matrix



Porcine Small **Intestinal Submucosa**



Human **Amnion/ Chorion Membrane** allograft



Bovine **Pericardium**

- No disease transmission risk

→ Gentle processing

→ Preserves lipids

→ Preserves structure

- Disease transmisson risk

→ Harsh Processing with Detergents

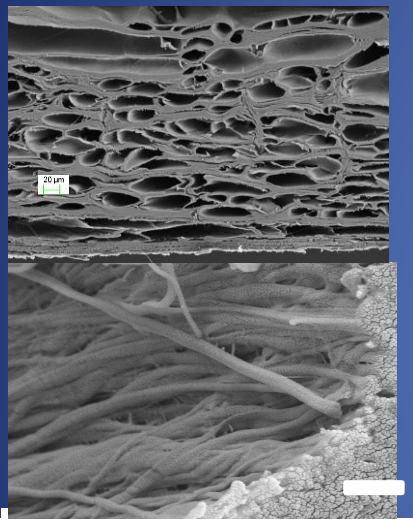
→ Lipid removal

→ Structure Denaturation

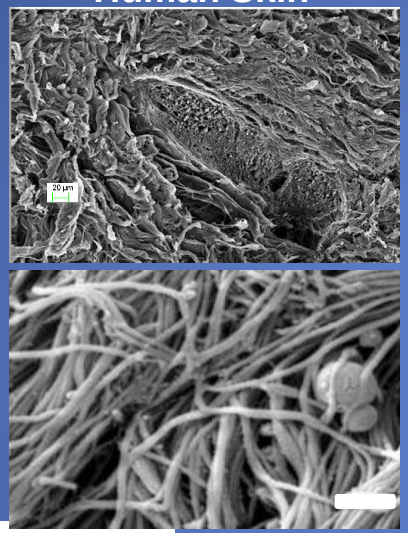
Crapo, P. M., Gilbert, T. W. & Badylak, S. F. An overview of tissue and whole organ decellularization processes. Biomaterials 32, 3233–3243 (2011).

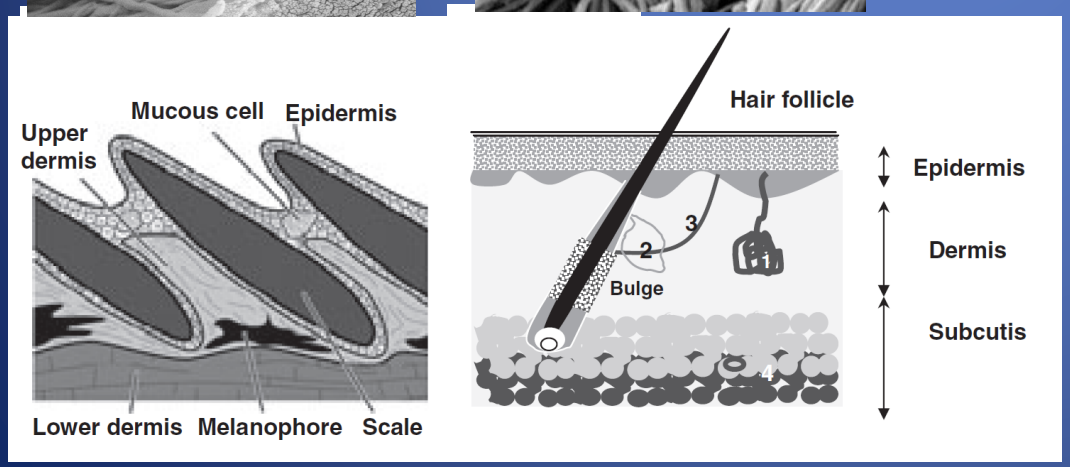
Human- and Fish Skin Structural Comparison

Acellular Fish Skin



Human Skin





- Fundamental structure is similar
- **The key difference is:**
  - Scales (all removed) instead of hairs
- **Omega3**
  - **Fish skin: >30%**
  - **Human skin: <1%**
  - **Amniotic Membrane: <1%**

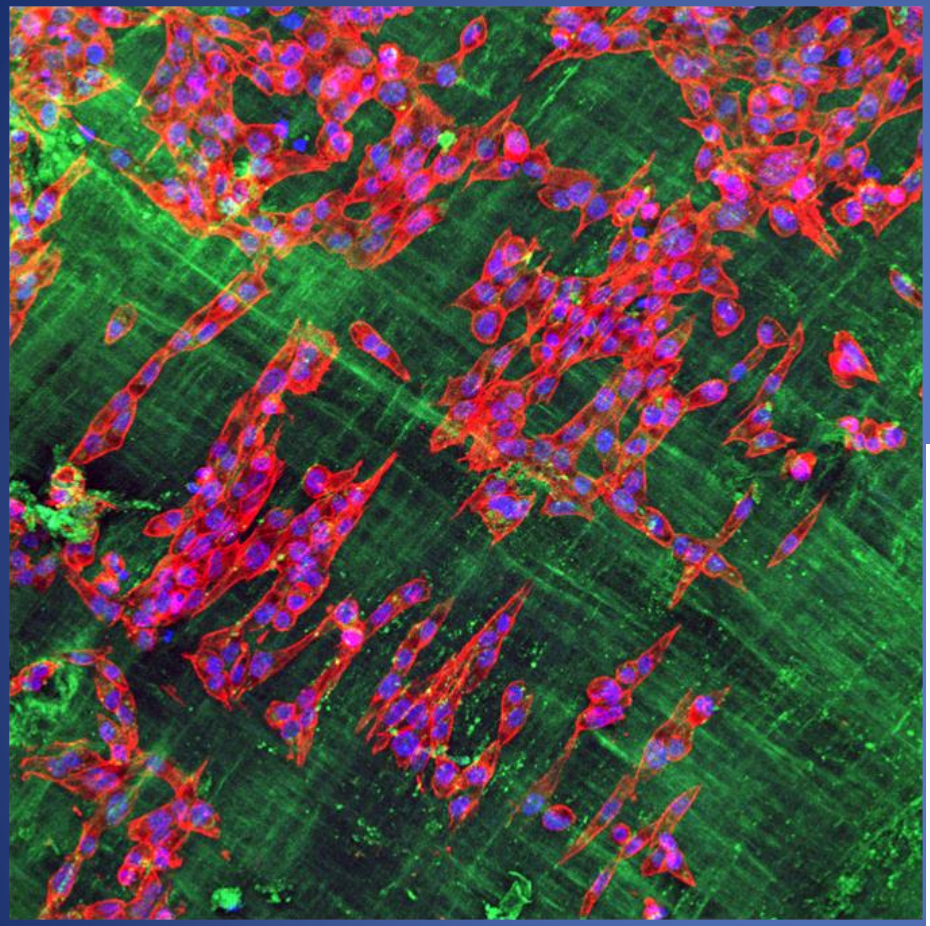
Rakers S. Et al. 'Fish matters': the relevance of fish skin biology to investigative dermatology. Experimental Dermatology 2010; 19: 313–324.

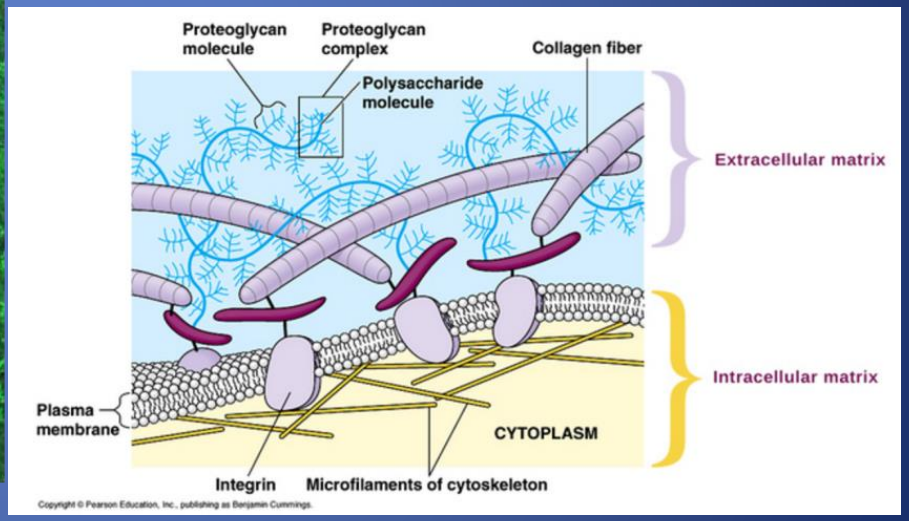
Cell ingrowth assay

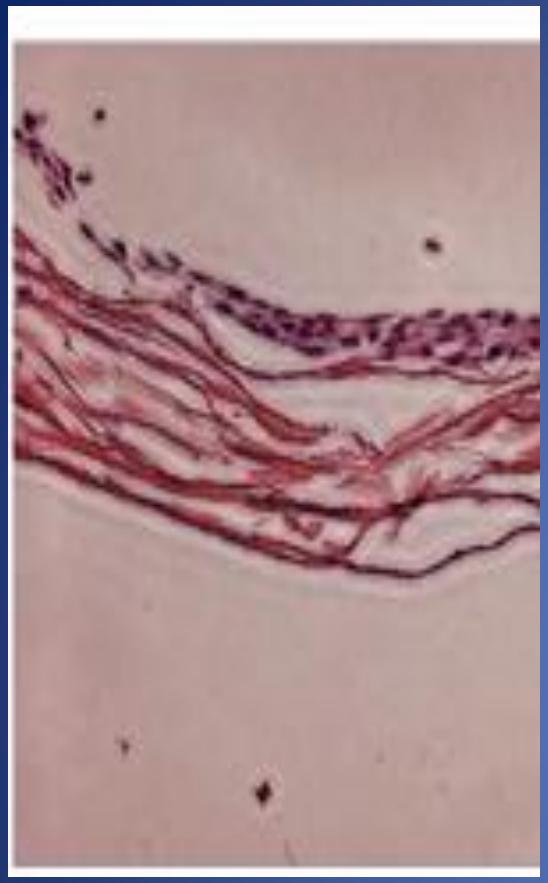
- Biologic materials were seeded with NIH 3T3 mouse embryo fibroblast cell line.
- Each material received 16,000 cells
- Plates were incubated for 12 days

- Samples examined under
  - Light microscope for Hematoxylin and Eosin staining
  - Confocal microscope for Fluorescent stains

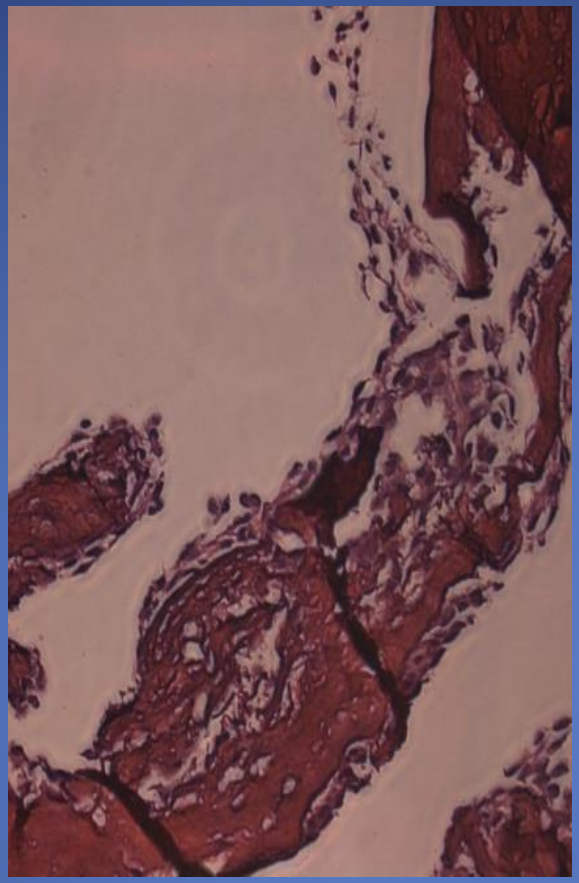
Fibroblasts Attachment and Migration on Fish Skin



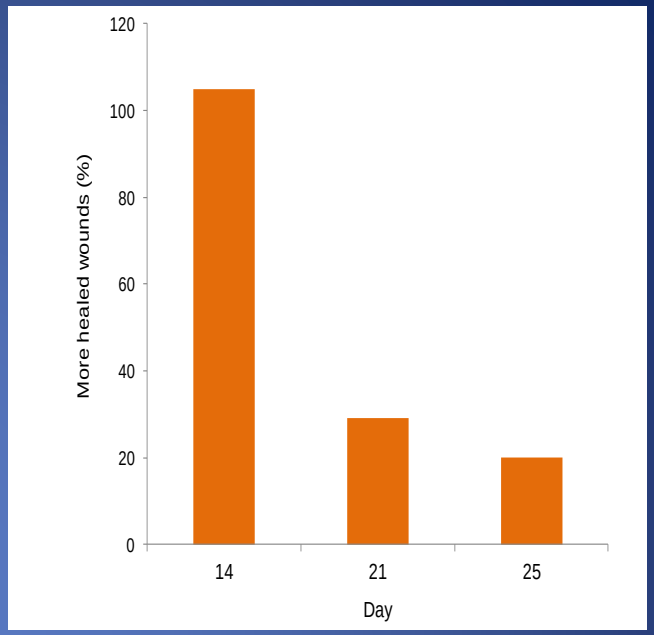




Porcine small intestinal submucosa



Fish skin graft



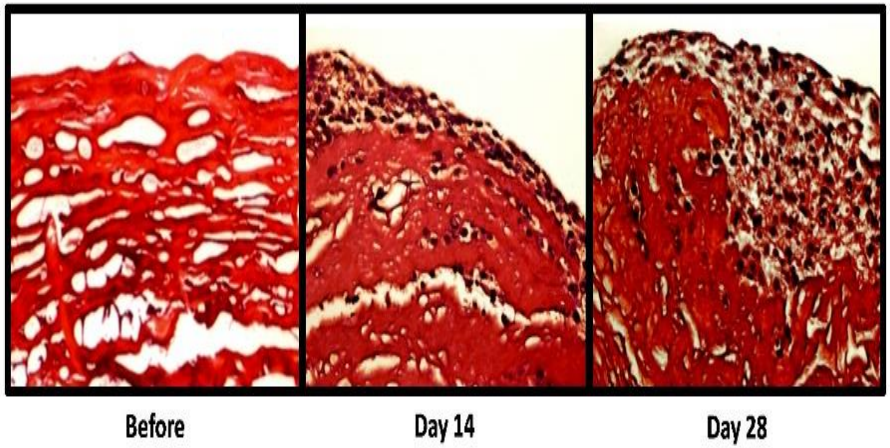
Parallel group, double-blind, non-inferiority, randomized controlled, punch graft clinical trial on 81 healthy individuals.

The comparison material was a porcine derived ECM. Every participant had two 4mm punch graft biopsy wounds made on the upper forearm and then a sheet of either fish skin or porcine ECM was placed in the wounds. Every participant came for a follow up at day 14, 21, 25 and on day 28, the end points of the study. On day 28 all wounds had essentially healed as expected

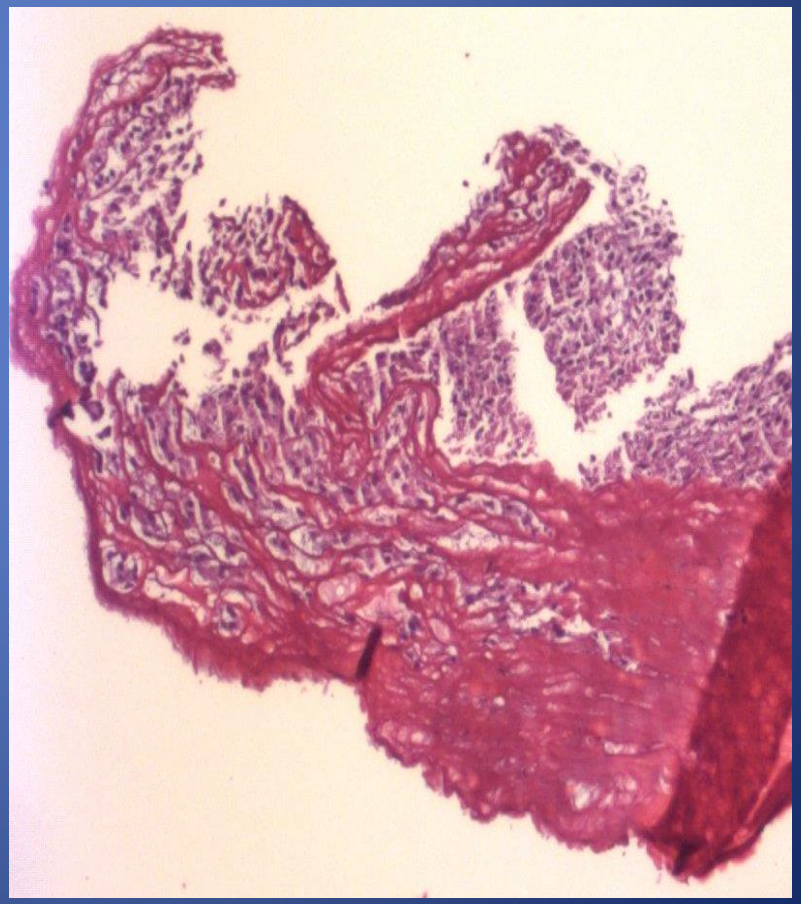
Overall the fish skin showed significantly faster healing ($p=0.041$).

In Vitro and In Vivo Histology

H&E images of cell infiltration into fish skin from vitro experiments,

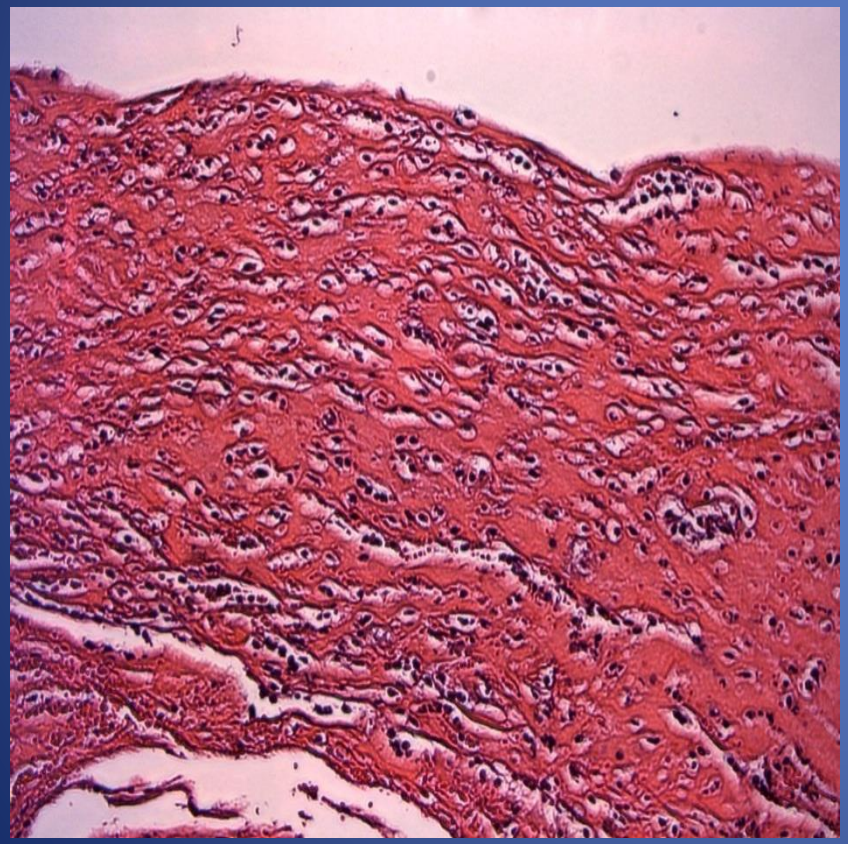


From human implants – showing cell infiltration

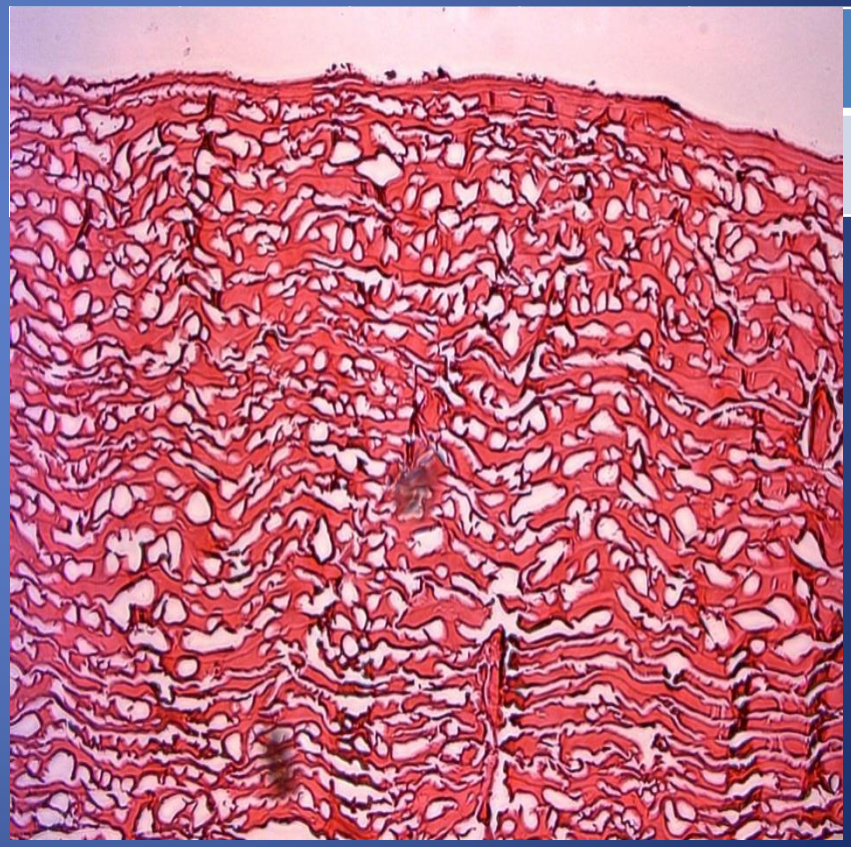


Ideal Pore size for early cellular population

Cellular ingrowth



Acellular fish skin



Cell Ingrowth

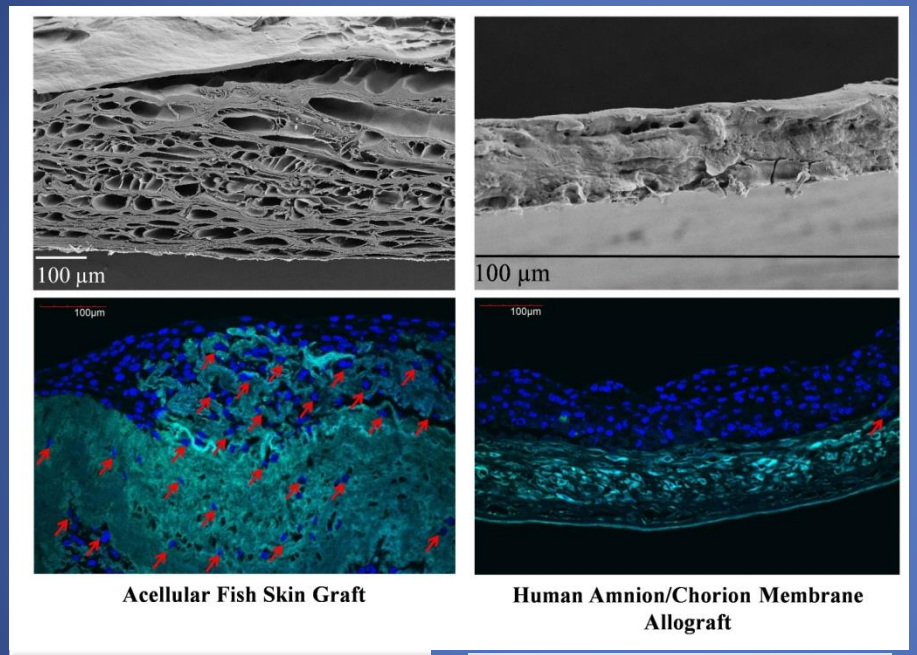
Fish Skin

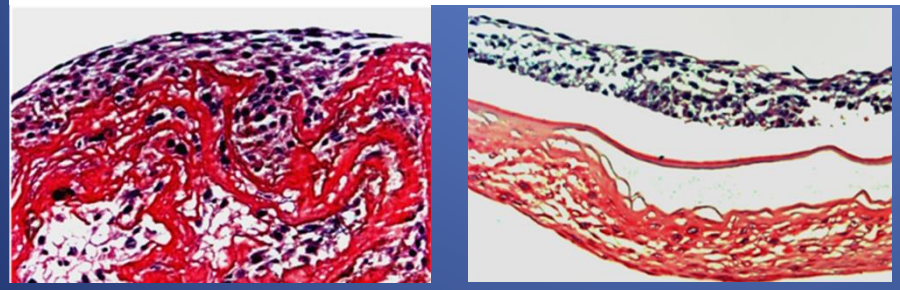
Human Amnion/Chorion Membrane Allograft

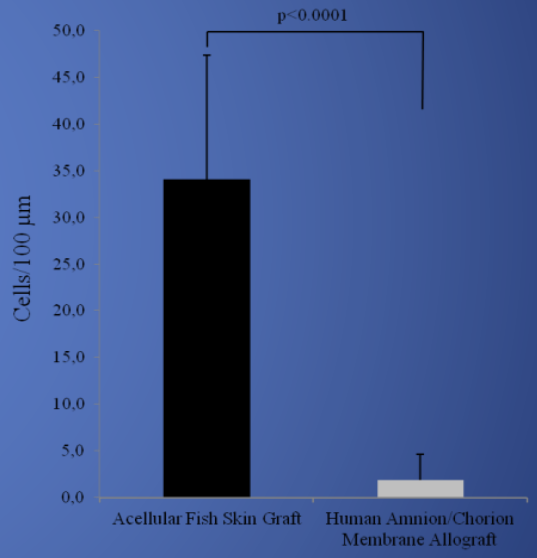
SEM

Fluorescent Labeling

H&E Staining



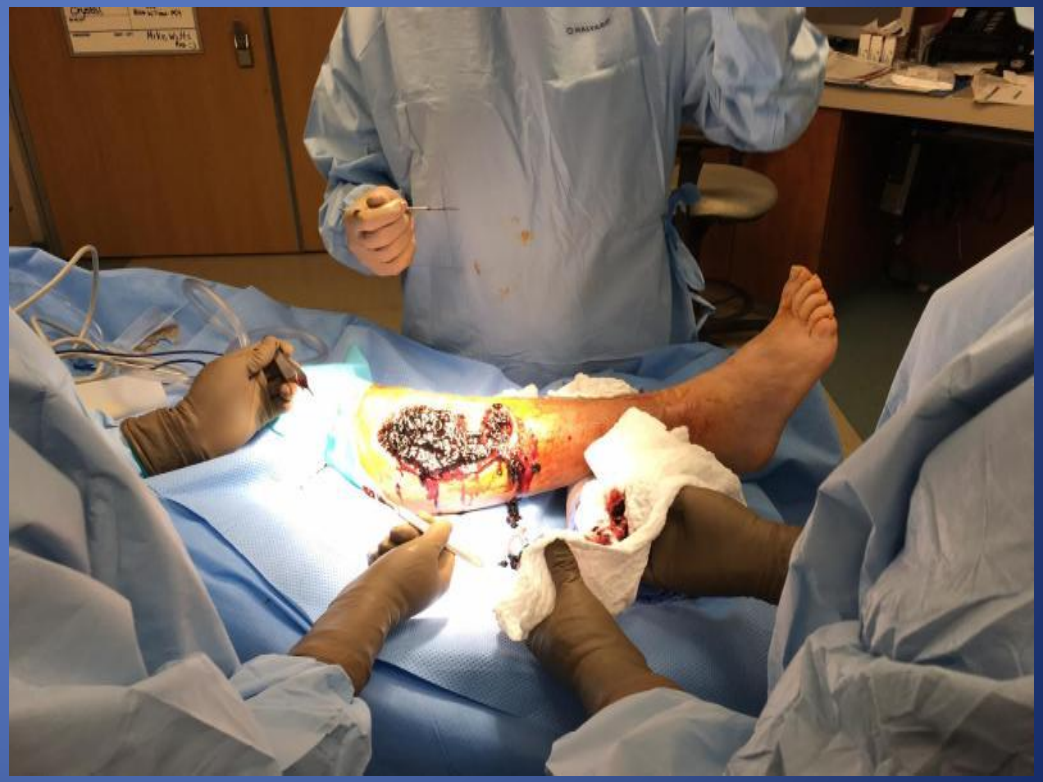




Case Study-real world use of fish skin

- 73 y/o diabetic male with a leg ulcer sustained while camping and hitting leg on the metal grate around the fire approximately 2 weeks prior to initial presentation
- Presented to primary care physician who immediately referred patient to our wound center
- Ulcer was too large and possibly too deep to attempt debridement in the wound center
- Taken to the operating room for initial debridement

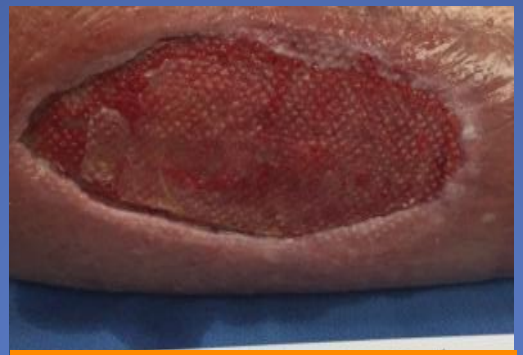
Case Study -Initial presentation



Case Study

Case Study - Rapid and steady healing progress throughout the weeks

Week 1
105cm^2

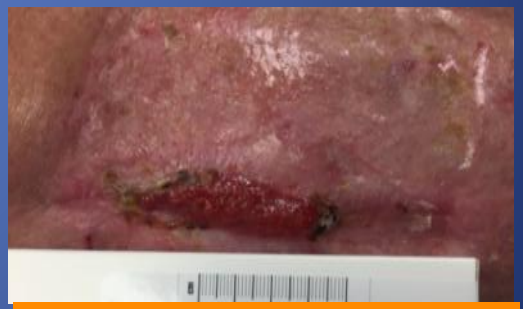


Week 2
72cm^2

Week 4
50cm^2

Week 6
36cm^2

Week 10
16cm^2



Week 14
5cm^2

Healed-16 weeks with 6 applications of fish skin

Key difference of Fish Skin

- No viral transmission from N. Atlantic Cod to humans
- Allows for gentle processing retaining
  - Three Dimensional Structure
  - Fatty Acids – Omega3
  - Extracellular Matrix Component
- No Cultural or religious concerns
- Wild caught through responsible fisheries

Discussion on Fish Skin

- Promising skin substitute for tissue regeneration
- Has shown faster healing in RCT (n=162) compared to mammalian sourced skin substitutes
- Rich in Omega3 fatty acids that
  - Promotes cell migration
  - Natural bacterial barrier without cytotoxicity
  - Inflammation modulation

Conclusion

1. Fish skin with highest resemblance to human skin
   - Dermal origin of the Fish Skin maintains structural similarity to human skin superior to skin substitutes of other tissue origins

2. Fish skin shows superior cell ingrowth
   - The native bioactive structure of acellular fish skin supports superior three-dimensional ingrowth of cells compared to human amnion/chorion membrane
   - Structural preservation in biologic tissue products is important for their ability to support cellular ingrowth
   - Faster healing time compared to porcine tissue
   - Further ongoing studies are underway with other mammalian tissues and human cadaver tissue suggest more rapid cellular ingrowth leading to faster healing times

Thank you