# **MelanoMEMS:** Advancing Melanoma Cancer Research Through Skin-On-A-Chip

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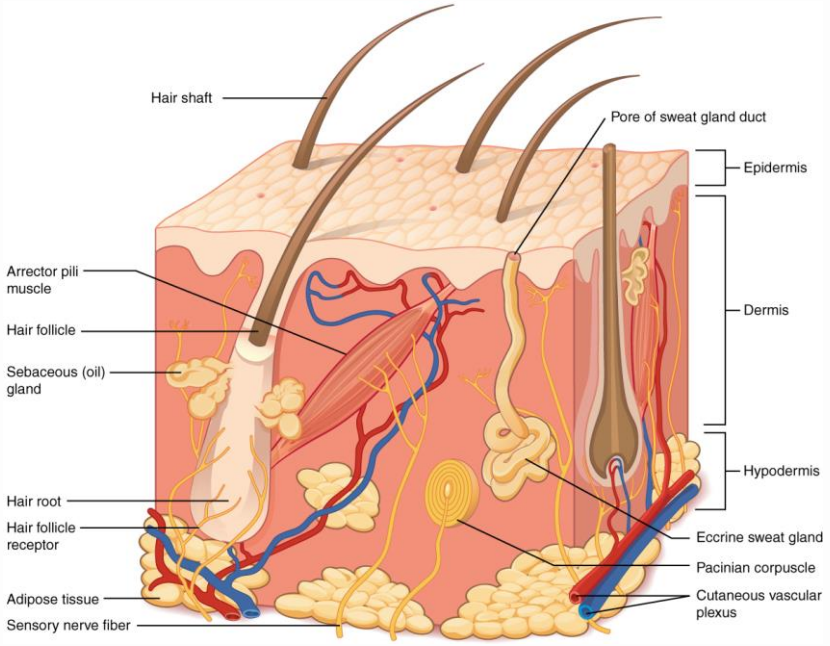
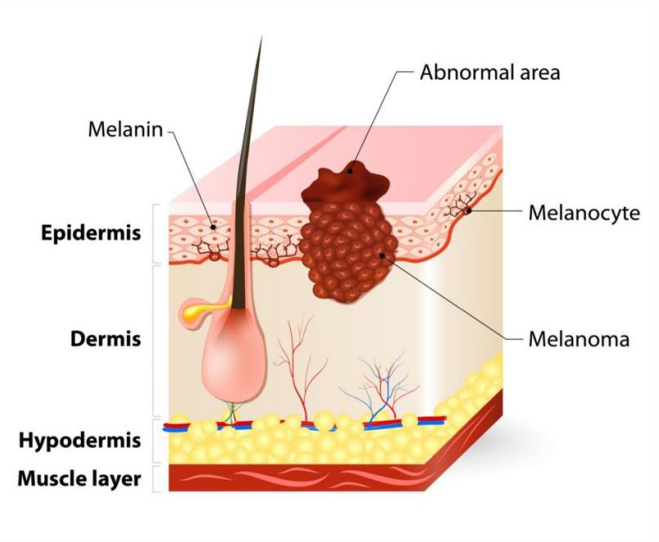
# Skin Physiology

- Largest organ in the human body, made up of three layers
  - Epidermis - external environment protection
  - Dermis - assists in thermoregulation and sensation
  - Hypodermis - energy storage and connects skin to muscles and bones
- Provides protection against mechanical, thermal, and chemical injuries
- First line of defence against pathogens



# Melanoma Cancer Background

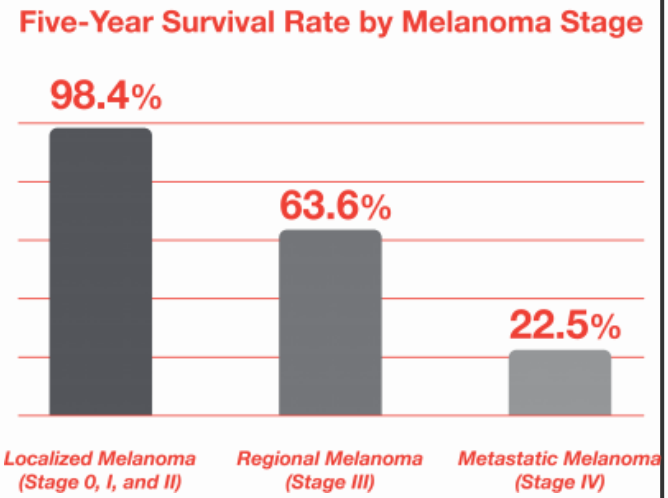
- Malignant melanoma is a type of skin cancer caused by the malignant transformation of melanocytes
  - Like other skin cancers, melanoma risk exponentially increases with prolonged exposure to UV radiation, especially from sunburns
  - Melanoma risk is increased in those who
    - Have prolonged and unprotected UV radiation exposure
    - Have a history of childhood tanning



# Melanoma Background (contd.)

Compared to other skin cancers, such as squamous cell carcinoma and basal cell carcinoma:

- Melanoma is much less common, but much more likely to spread to the lymph nodes
- Melanoma is the most dangerous form of skin cancer
    - 2024 estimations, via American Cancer Society
        - 100,640 Americans diagnosed with Melanoma
        - 8,290 Americans will die from Melanoma



# Current Needs

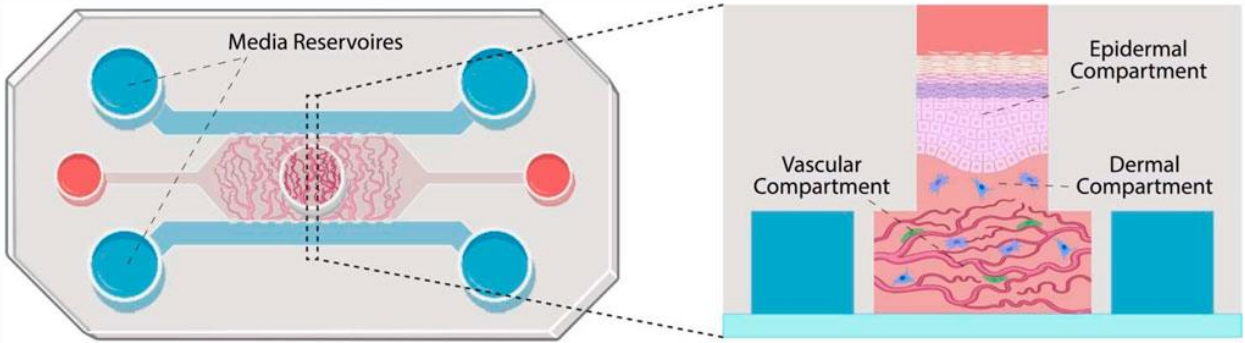
- Malignant Melanoma is a heterogeneous disease, it has a complicated aetiology
    - Molecular analyses reveal consistent genetic patterns among melanoma subtypes, but reveals little on disease spread
- Animal models are used to study melanoma development, and to assess efficiency and safety of drugs preclinically
    - Limited approach to research treatments, as animal models:
        - May not be able to accurately predict human responses to treatment options due to differences in skin physiology and immunology
        - Time and money consuming, and also raises ethical concerns

# Current Challenges

- Tumor Cell Cultures
  - Reductionist approach
    - No microenvironment
    - Altered proliferation rates
    - Aberrant responses to drug therapies
- Animal Models
  - Different than human physiology, potentially different responses to drugs
  - Ethical concerns
- In-Vitro Computer Simulations
  - Demands many inputs and conditions, and needs experimental validation

# Requirements for SoC

- Multilayered structure to replicate main layers of skin
- Mimics vascularization
    - Allow for perfusion & nutrients
- Cell culture of melanoma tumors & immune cells (macrophages, T-cells)
- Recreate shear stress & mechanical stress on cells
- Method to track melanoma tumor migration & cell proliferation



# Proposed BioMEMS Solution

A skin-on-a-chip system that mimics microenvironment of melanoma tumors for studies of tumor progression & metastasis

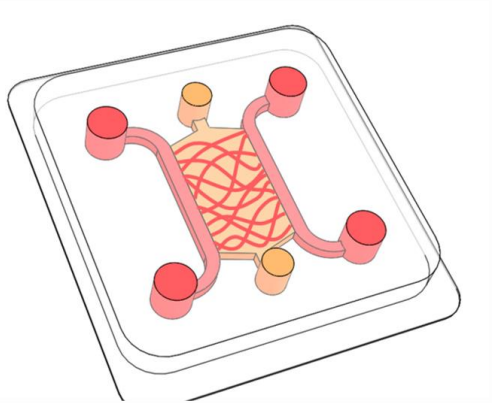
- **Design of skin-on-a-chip system**
    - BioInk to mimic ECM, layers of skin, & vasculature
    - Microfluidic channels to simulate perfusion & shear stress on cells
    - PDMS as structural framework & substrate
- **Melanoma Microenvironment**
    - Choose a melanoma cell line w/ known migration & proliferation characteristics
    - Co-culture immune cells & fibroblasts to mimic microenvironment
- **Melanoma Migration & Proliferation Tracking**
    - Electrochemical biosensors to detect melanoma biomarkers
    - Optical biosensor to monitor cellular processes

# Device Fabrication

# Fabrication Materials

- **PDMS**
  - Good biocompatibility & oxygen permeability
  - Transparent - real-time visualization of tumor behavior
  - Mechanical flexibility to resemble deformation & stretching of skin
  - Beneficial for microfluidic integration & vascularization
- **PET Porous Membranes**
- **BioInk using 3D bioprinting**
  - Closely mimic ECM composition
  - Composition of bioink can be customized to replicate different skin regions
- **Human-derived melanoma cells** (SK-MEL-28)
- **Human-derived keratinocytes (HaCaT), fibroblasts (Fb), endothelial cells (HUVEC)**

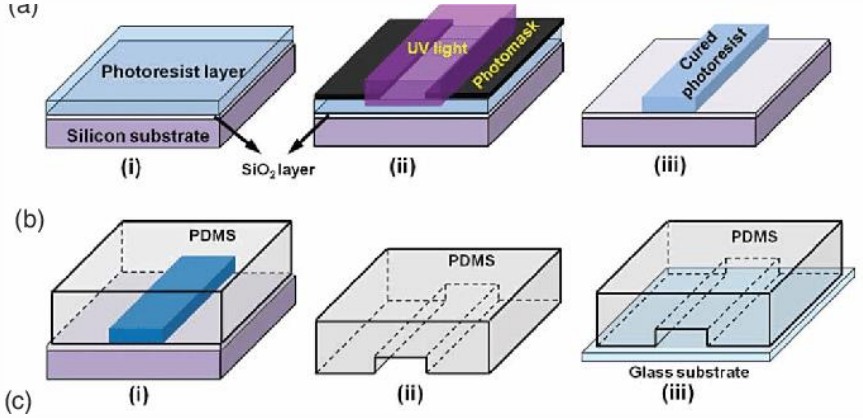
# Microfluidic Channel Design



- Design created using CAD, used to mimic vasculature
- Diameter 30~300 μm and depth of 200~800 μm
- **Channel network design**
  - Branching structure
  - Incorporate branching & bifurcated channels to mimic arterial-venous networks & capillaries
- **Channel geometry**
  - Channels of various curvatures, angles, & lengths
- **Fluid flow characteristics**
  - Constrictions & expansions to regulate shear stress & flow rate
  - Incorporate valves/flow regulators to mimic dynamic regulation of flow

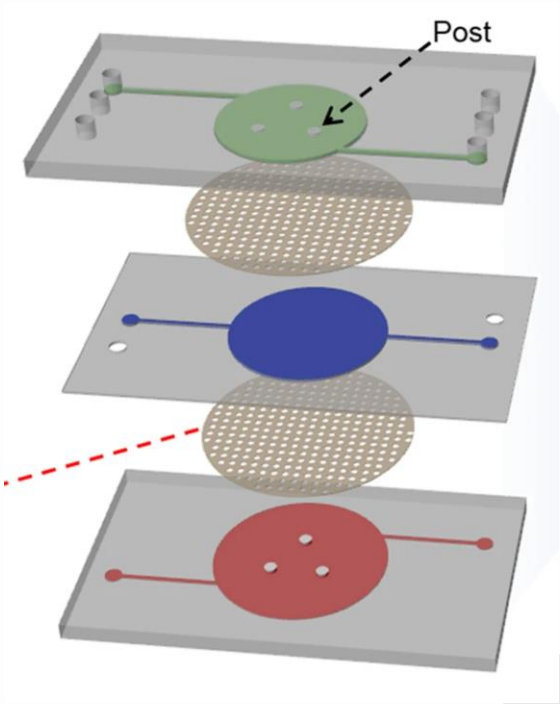
# Contact Photolithography

- **Used to pattern microfluidic channel designs onto PDMS** surface → Good for precise & complex channel designs
- Transfer channel pattern onto the PDMS surface using a photomask and UV exposure.
    - Exposed PDMS become crosslinked, forming desired channel structures.
- Plasma oxidation bonding to glass for sealing



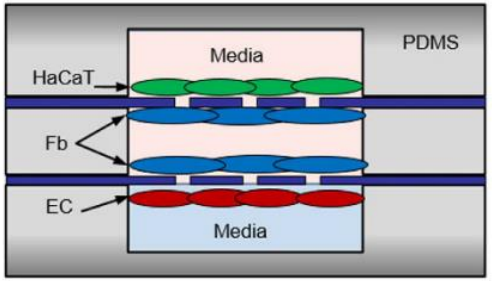
# Fabrication Integration

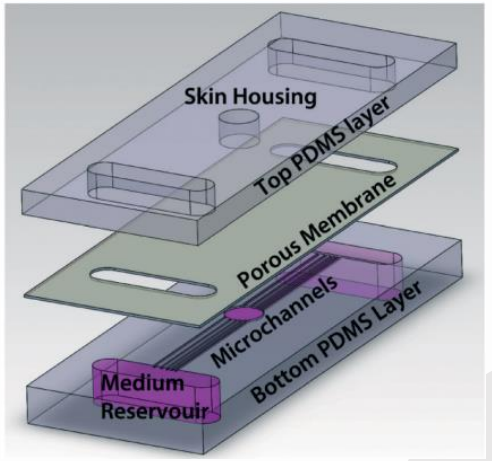
1. **Contact lithography** for patterning on PDMS that to create microfluidic channels & platform for bioprinting
2. **3D Bioprinting**: Use BioInk to deposit biomaterials onto PDMS surface to create tissue architecture and mimic ECM & skin composition
3. **APTES bonding:** Surface treatment using APTES to form covalent bonds between PDMS and PET porous membranes
4. **Layering:** Separate epidermal, dermal, & vasculature PDMS layers with PET membranes
5. **Cell Seeding:** Melanoma, HaCaT, Fbs, and HUVEC onto membranes

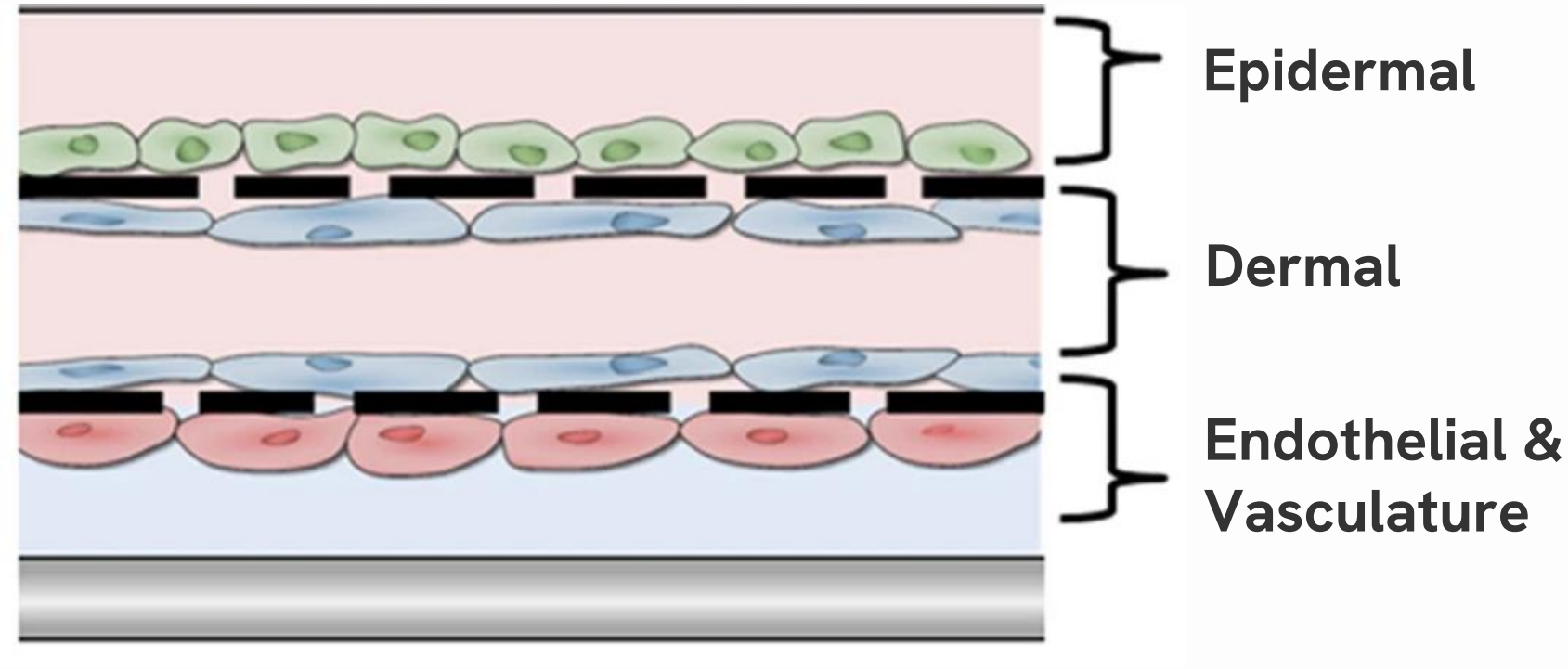


# Cell Seeding and Vascularization

- Skin layers:
  - **Epidermis** → HaCaT cells + melanocytes (Me)
  - **Dermis** → Fb cells
  - **Endothelium** → HUVEC
- Seed HaCaTs+Mes and Fbs first, then flip chip over and seed with HUVECs and Fbs
- **Vascularization**:
  - Microchannels beneath endothelium layer for medium to transport nutrients, oxygen, and waste products
  - Separated by porous PET membrane



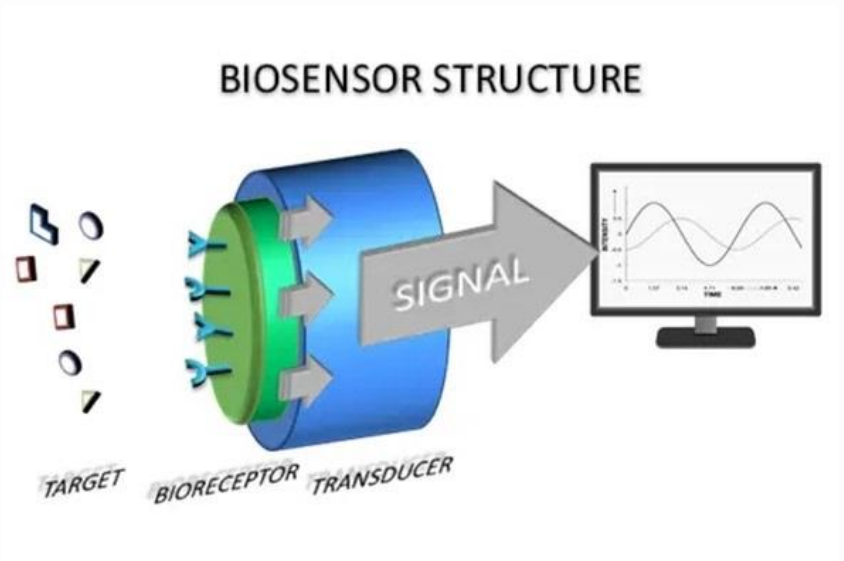






Cross-sectional diagram of PDMS layers with seeded cells and PET membranes

# Biosensors

- **Impedance-based biosensors:** Sense changes in impedance as tumor migrates across membrane
- **Microfluidic biosensors**: Captures antigens within microchannels and detect melanoma cells flowing through channels
- Biosensors functionalized w/ melanoma-specific antigens and receptors (CD63, MART-1)

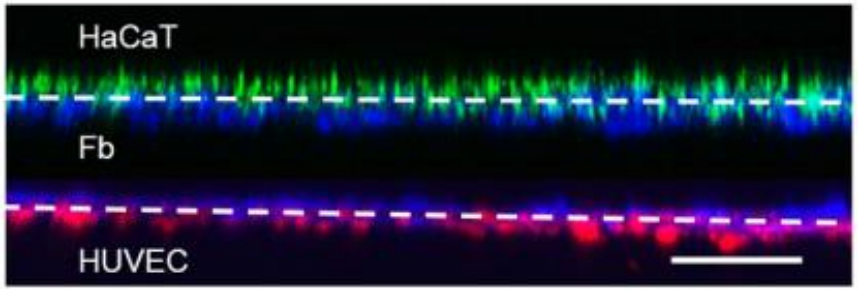


# Testing & Validation

# Testing

- Proper development of cell culture
  - Cell staining and 3D fluoroscopy
  - Demonstrate uniform proliferation in all layers

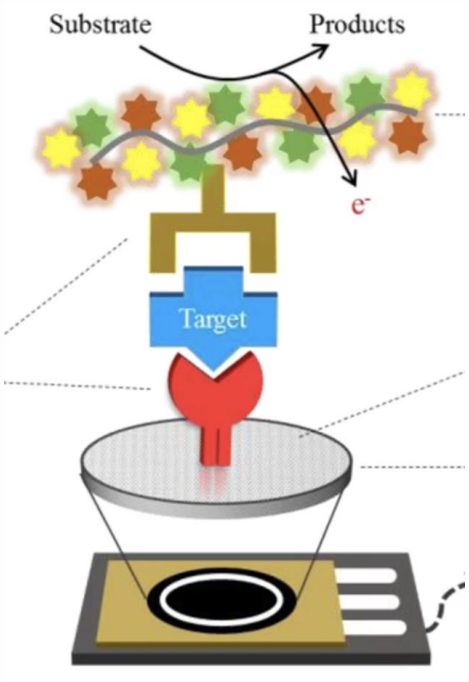
| Cell Type | Fluorescent Marker |
|---|---|
| HaCaT | Green CMFDA |
| Fb | Blue CMF2HC |
| HUVEC | Red CMTPX |



# Testing

Tracking melanoma migration and proliferation

- Biosensors at outlet allow for analysis of biomarkers with clinically significant characteristics
  - Melanoma Inhibitory activity protein (MIA)
  - Tyrosinase
  - S100B
- Amperometric electrochemical sensor using enzyme bioreceptors




https://biomaterialsres.biomedcentral.com/articles/10.1186/s40824-019-0181-y/figures/1

# Material Biocompatibility

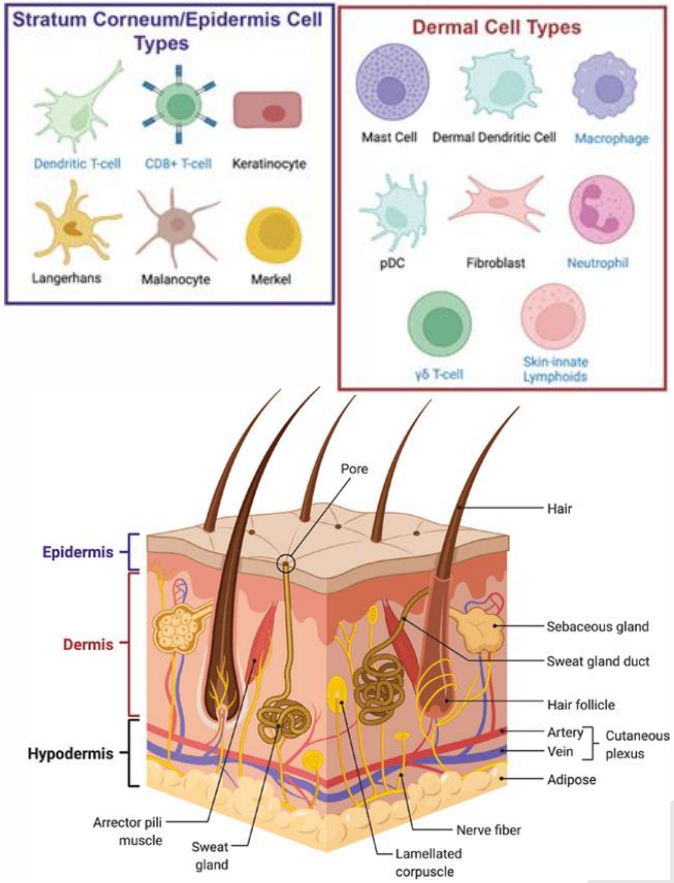
Even in *in vitro* modeling, material biocompatibility must be considered for modeling purposes

- PDMS - Polymer
  - Highly compatible with most organic systems
- PET Porous Membranes - Polymer
  - Treated PET surfaces offer extraordinarily high bacterial resistance, further promoting biocompatibility
- BioInk using 3D bioprinting
  - Properties can be manipulated to meet current needs, very high biocompatibility

# Limitations

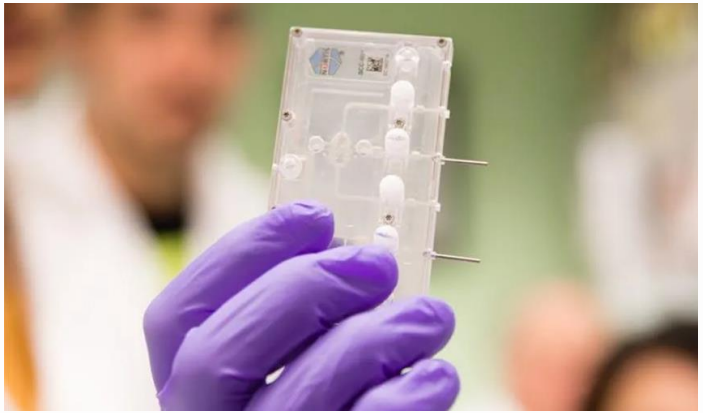
- Validity of miniaturized skin model
  - Renewal cycle of skin (stem cells continuously differentiate in basal layer, outer layers of epidermis shed)
  - Monolayers vs complex 3D structure

- Longevity of SoC model
  - Melanomas can take weeks/months to grow
  - Current skin models have only shown stability up to ~1 month



# Future Directions

- Melanoma drug testing
  - Most common treatment option for melanoma is surgery
  - SoC technology would allow for the testing of alternative, non-invasive treatments
- Sunscreen/UV exposure testing
  - Test preventative effects of sunscreens, effect of UV exposure levels without the need for human subjects



# Questions?

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