Development of a 3D Vascularized Skin-on-Chip (VascSOC) Platform

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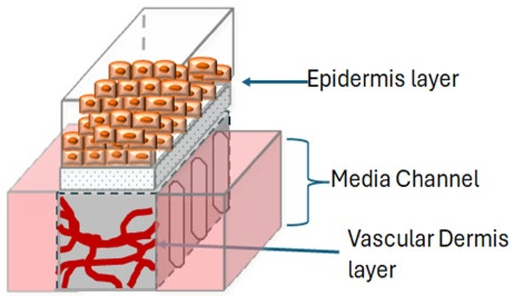
Introduction

Burn injuries result in lifelong physical and psychological scarring, causing pain and influencing mental health, quality of life, ability to return to work and subsequent mortality. A full-thickness (third-degree) burn extends through the full dermis and is not typically painful owing to damage to the nerve endings, limiting warfighter motility. Key to increased burn preparedness is research of various treatments that currently utilize animal models. Despite significant differences in skin histology and pathology between humans and animals. Microphysiological systems (MPS) have vastly improved the quality of lead compound discovery due to improved human modeling. Unlike traditional well plate assays, MPS utilize microfluidics to create a more natural setting for the cells. This improvement in environment provides a setting that more closely mimics human physiological conditions. Triton is developing a 3D vascular Skin-on-Chip (VascSOC) platform and burn assay which will improve upon therapeutic discovery and wound model over traditional 2D *in vitro* assays and burdensome animal models. This will be achieved by 1) developing a vascularized skin model, 2) developing thermal and directed energy burn wound assays, and 3) fabricating a unique microfluidic device for our skin model.

Overview & Features

Major features of VascSOC platform:

1) Triton has developed protocols to form a 3D vascular network through vasculogenesis, and a stratified epidermis using primary microvascular endothelial, dermal keratinocyte, and dermal/lung fibroblast cell types. This improves upon traditional 2D *in vitro* assay and advances 2D MPS skin platforms.
2) A microfluidic MPS device. This contains and air-liquid interface for epidermal differentiation, a center chamber for a vascular network embed in a gel matrix, and specialized pillars for vascular lumen formation. Designed to be compatible with common lab equipment



Materials & Methods

3D Vascular Skin Culturing

The center (vascular dermis) section of our microfluidic device is seeded with microvascular endothelial and fibroblasts embedded in fibrin gel. Cells undergo vasculogenesis and form a vascular network connecting both media channels. Upon completion of vasculogenesis, epithelial keratinocytes are seeded upon the vascular network and differentiated in a stratified, multilayer epidermis. This forms a permeable epidermis layer with a perfusable vascular dermis layer for the transfer of biomarkers and therapeutics.

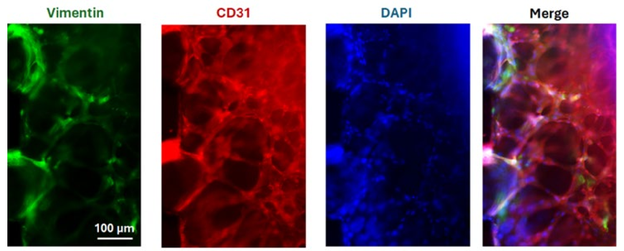
Microfluidic Device Fabrication

Skin Microfluidic device is constructed using a combination of acrylic molds, biocompatible adhesives, and high-resolution SU-8 pillar formation.

Thermal Wound Induction

Burn wounds were induced to completed skin models via 2 methods, heat metal rods (thermal) and laser (directed energy, DE). Thermal wounds were induced by heating a stainless-steel rod to >85 °C and applied to epidermis for 7 and 20 seconds. DE wounds were induced using a high powered 980 nm laser (1 Amp, 19 V) and applied for 6 seconds with equates roughly to 20 J. These methods represent 3rd degree burns. Both methods were assessed for cell viability using MTT colorimetric dye.

Results



Immunofluorescent imaging of fully formed microvascular network

Microvascular network was stained with vimentin (cytoskeleton), CD31 (endothelial marker), and counter stained with DAPI (nuclear stain). This confirms the 3D lumen formation with the correct presenting biomarkers.

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(a) This material is based upon work supported by the US Office of Naval Research under Contract No. **N00014-25-C-2408**
(b) Any opinions, findings and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the US ONR.
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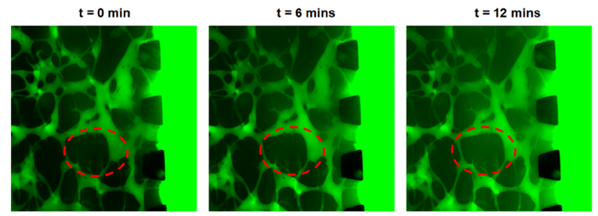
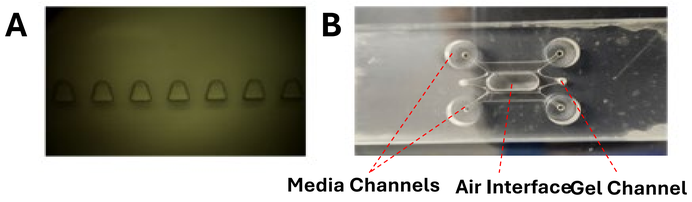
Results Cont.

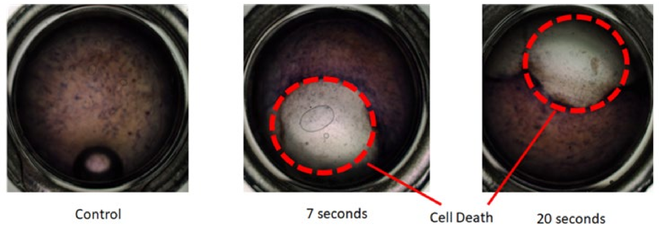


10K Dextran-GFP perfusion through microvascular vessels into extracellular gel matrix over time.

10K Dextran-GFP (0.1 mg/mL) was applied to media and allowed to perfuse over 12 minutes. Red circle highlights perfusion over time of dextran.



A) SU-8 pillars (0.25 mm H x 0.15 mm W x 0.15 mm L)
B) Complete microfluidic device highlighting media channels, gel channels (vascular network), and air interface (stratified epidermis)



Thermal wound induced by heated rod
Cell viability determined by MTT

Representative image of thermal wound assay. Red circles indicate cell death and purple represent viable cells.

Conclusions & Future Efforts

- Triton has presented evidence of a 3D vascular Skin-on-Chip that can be applied to modeling burn wounds generated via thermal and DE insult and has developed a MPS microfluidic device that allows for 3D vascular networks with a stratified epidermis.
- Future efforts include further validation of VascSOC through flow cytometry and RT-qPCR analysis. Additionally, a burn wound fungal assay is being developed to help screening for emerging antifungal therapeutics.