Bioprinting a skin model to assess the toxicity of sulfur mustard

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Background

Bioprinting technology has emerged as innovative platform to develop customizable organ models. By combining traditional additive manufacturing techniques and biological components, such as cells, growth additives, and other biomaterials, bioprinters can create customizable organ models on-demand. With advanced multi-extruder bioprinters, layers of different cells and materials can be printed. This is especially relevant for the skin, as it is structured in various layers of cells (Figure 1). Complex skin models are necessary to assess vesicants of interest, such as sulfur mustard (HD), in the chemical and biological defense community.

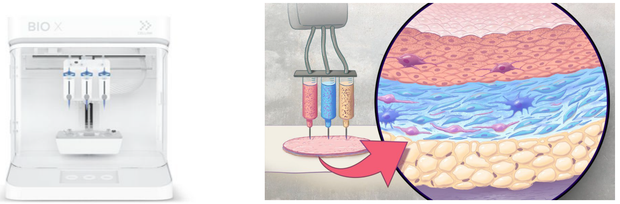
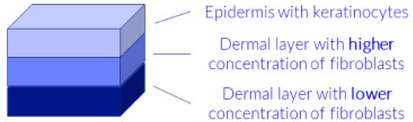


Figure 1. CELLINK BIO X Bioprinter (left) and graphic of bioprinting different skin layers (right) [1].

CELLINK Skin Methodology

The CELLINK Skin kit (CELLINK, IKC207000305) and protocols were used to build this multi-layered skin construct. The printed model consists of three layers of normal human dermal fibroblasts (NHDF) and normal human epithelial keratinocytes (NHEK) (Lonza; CC-2511, #00192907) for physiological relevance (Figure 2).



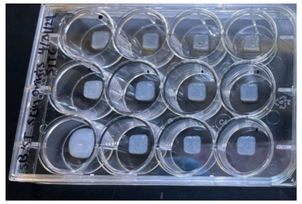


Figure 2. Illustration of the CELLINK Skin model (top) [2] and printed CELLINK Skin Models (bottom).

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Bioprinted Skin Constructs

After bioprinting, the skin models were chemically crosslinked overnight, according to the CELLINK Skin protocol [2]. The bioprinted skin constructs were transferred to air-liquid interface (ALI) with Transwell® inserts after 3 days in culture (Figure 3). Basal media was compromised of a 75:25 ratio of keratinocyte and fibroblast growth media. Media was changed every 2-3 days on the samples. On Day 14 of culture, the bioprinted skin samples were exposed.

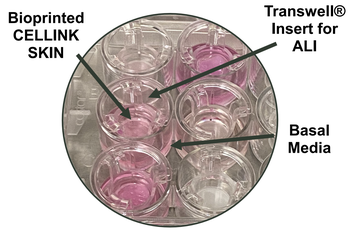

Figure 3. Bioprinted CELLINK Skin placed under ALI.

Sulfur Mustard Exposure

Due to acute hazards with HD, all experiments were performed by qualified personnel in certified chemical fume hoods equipped with an advanced filtration system that protects the user and the environment at DEVCOM CBC according to all Federal, State, and International guidelines. Neat HD was diluted with corn oil into a 10 mL working stock at a concentration of 15.97 mM and then diluted further. Workflow for exposures conducted are shown below (Figure 4).

- *Concentrations: 15.97, 7.99, 4.00, 2.00 mM*
- *Media, Vehicle, Positive Controls*
- *Remove treatment conditions*
- *Replace with culture media*
- *alamarBlue Analysis (Quantitative)*
- *Histology/Staining (Qualitative)*

Figure 4. Workflow of HD Exposures on Bioprinted Skin

HD exposures were also conducted on monolayer cultures for NHDF and NHEK to serve as a benchmark. However, due to corn oil vehicle treatment, cellular viability was extremely low. Instead, the bioprinted skin viability was compared to MATTEK EpiDerm FT [3]. The observed LC_{50} in the MATTEK EpiDerm FT was between 1.57 and 3.14 mM (Figure 5) and the observed in the bioprinted skin samples was greater than 8 mM (Figure 5). Additionally, a full dose response was not observed with the bioprinted skin samples. Histology was also conducted on the bioprinted samples after exposure (Figure 6), however, there are no observed differences between treatment groups.

Results

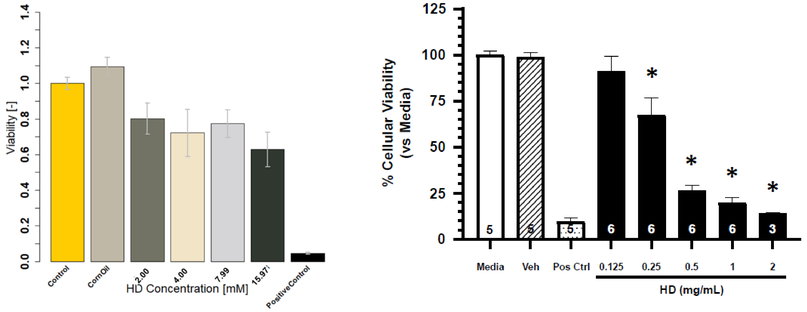

Figure 5. Cellular viability on bioprinted skin with Normal Human Dermal Fibroblasts and Normal Human Epithelial Keratinocytes (left) compared to cellular viability on MATTEK EpiDerm FT [3] (right) 24 hours after one hour of HD exposure in a corn oil carrier.

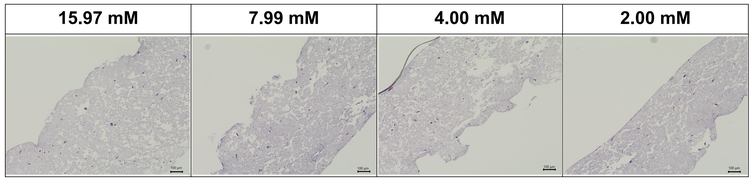

Figure 6. Hematoxylin and eosin staining on the bioprinted constructs after treatment. Brightfield images (10X) taken on Keyence BZ-800 microscope. Scale bars are 100 µm.

Conclusions and Future Work

This project provided proof-of-concept foundational work to explore bioprinting technology for developing in-house skin models for exposure against vesicants. CELLINK Skin methodology was utilized and produced viable skin prints with two primary cell types found in the epidermis and dermis layers. More research and development is required to finetune and streamline these models for validity and repeatability. Additionally, further work needs to be conducted for optimizing HD dosing for *in vitro* models.

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The views expressed in this abstract are those of the authors and do not necessarily reflect the official policy or position of the Department of Defense or the U.S. Government.)

References: 1. Image adapted from Donny Bliss/NIH. 2. CELLINK. https://www.cellink.com/product/cellink-skin/ 3. Angelini DJ, Jenkins CC, Rizzo GM, Clay AE, Horsmon JR, Renner JA, Dhummakupt ES. Multiomic analysis of Lewisite-exposed human dermal equivalents. Society of Toxicology Annual Meeting; Salt Lake City, UT; March 10-14, 2024.