Utilization of bioprinting to fabricate tunable tissues: A polymeric perspective

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Abstract

Understanding the effects of exposure from both toxic chemicals and biological agents is a cornerstone of research at DEVCOM Chemical Biological center, with current pursuits encompassing the complexity of all human physiological components such as age, ethnicity, and gender. Historically, 2D cell culture has been implemented to understand the effects of such agents on the warfighter, but limitations with this approach have been well documented such as the challenge to translate experimental results to real-life scenarios. Consequently, new techniques have been explored to allow for more translatable data such as animal testing, organ on a chip, and most recently, 3D printing of tissue mimics. There are still limitations with platforms such as animal testing and organ on a chip but bioprinting has emerged to address some of these issues. As 3D printing has taken off into manufacturing and innovation, bioprinting emerged to fabricate 3D scaffolds of cells within a polymer matrix that can be specifically tuned to mimic a wide range of human physiology. This allows for a better model than traditional 2D cell culture as the 3D matrix provides a more realistic model of living tissue. Bioprinting allows for this flexibility as properties such as elasticity and thickness can be changed in the material formulation and printing parameters. To better understand these material formulations and to benefit downstream analysis such as permeation through the 3D construct, polymer properties must be understood. This collaborative effort at DEVCOM CBC focuses on implementing bioprinting as a realistic approach to better understand possible effects on the warfighter. Using an ocular model as the guide, a tissue often not investigated, the formulation and printing parameters highlighted above are explored using various polymers and crosslinkers to better understand gel properties through the printing process and final 3D construct. The resulting printed ocular models are then characterized to determine the similarity to the physiological tissue.

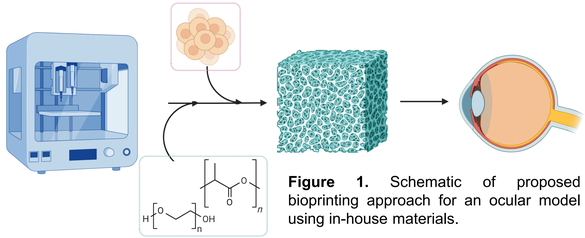
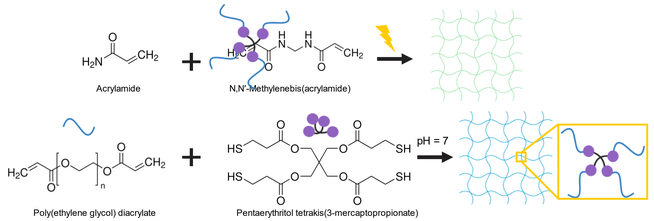


Figure 1. Schematic of proposed bioprinting approach for an ocular model using in-house materials.

Hydrogel Properties

Figure 2. Schematic of proposed hydrogel system: UV polymerization of polyacrylamide (PAM) crosslinked with N'N'-methylenebisacrylmide and thiol-Michael addition of polyethylene glycol diacrylate (PEGDA) with pentaerythritol tetrakis (3-mercaptopropionate). PAM is UV polymerized at 365 nm using Irgacure 2959. PEGDA undergoes polymerization at pH 7 and serves as a viscosity modifier during the printing. The polymerization rate temperature dependent



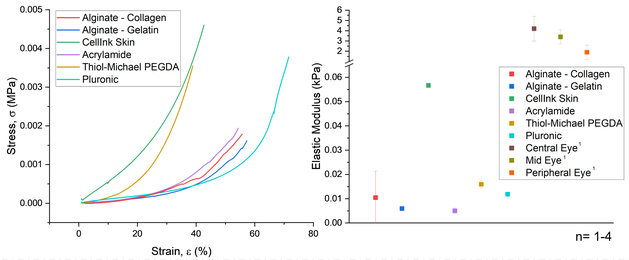
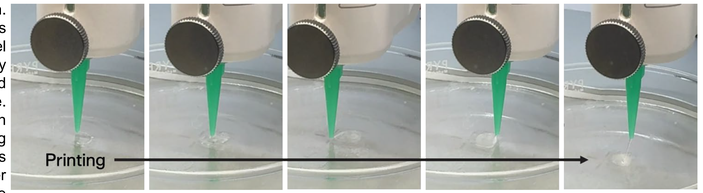
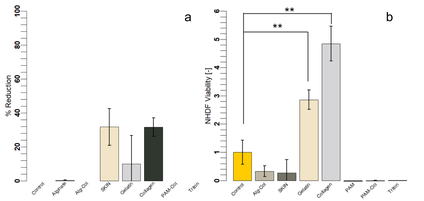


Figure 3. Representative Stress-Strain curve and elastic modulus of samples compared to literature values. Compression testing shows that all gels exhibit elastic moduli 2 orders of magnitude lower than ocular values[1], which can be adjusted by increasing the crosslinking density. The toughness of the gels highlights their brittleness, with Thiol-Michael PEGDA performing comparably to the commercial product, CellInk Skin.

Figure 4. Bio-X printing of thiol-Michael PEGDA formulation. The printing process moves from left to right, with the gel maintaining its shape initially coming out of the nozzle and spreading out over time. Increasing the polymerization rate with heat and modifying collagen by adding thiol groups will enhance control over polymerization and improve cellular compatibility



Cell Viability



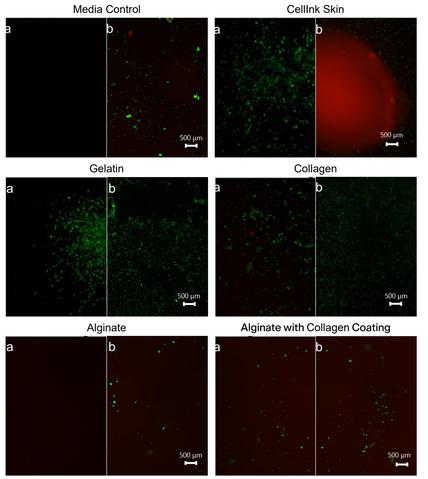


Figure 5. Cell viability of a) corneal epithelial cells (CEC) (P4) and of b) fibroblasts (NHDF) (P7) assessed using almar Blue. Images on the left show CECs while the right displays fibroblasts. Fibroblasts exhibited the highest attachment to gelatin and collagen, with no attachment to the commercial substrate, Cellink Skin. CEC's exhibited poor survival across most substrates, and optimization of CEC's are ongoing.

The bar chart on left represents CEC data, and the right shows fibroblast data. Data is normalized to a) 100% reduced alamar Blue reagent and to b) a control sample cultured on tissue culture plastic. N=3. Error bars are one standard deviation.

Conclusions

Bioprinting is an emerging technology that enables the investigation of cellular responses in environments that better mimic native tissue. It allows for printing with multiple materials and cell lines to create synthetic models, such as ocular and dermal tissue. However, commercial products often consist of inconsistent components, making their roles unclear. This study aims to evaluate and tailor each component to create more biologically relevant environments. Biopolymers like gelatin and collagen improve cell viability, and collagen will be incorporated into the system for multiple functions. The polymerization process is being optimized for both biocompatibility and tunability to improve printing. The goal is to ensure proper mixing of cells and polymers without damage and to control polymerization speed during printing. The thiol-Michael addition offers better control of reaction speed via temperature, rather than relying solely on UV, which can vary based on the printer's UV power. Thiol-functionalize collagen will regulate crosslinking density and mesh size, while UV crosslinking will lock the structure in place with acrylamide.

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1. Mikula, E. R., Jester, J. V., & Juhasz, T. (2016). Measurement of an Elasticity Map in the Human Cornea. Investigative ophthalmology & visual science, 57(7), 3282–3286. https://doi.org/10.1167/iovs.15-18248

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