Embedded Bioprinting of Immunocompetent Tumor Models via Microporogen-Structured (μPOROS) Collagen Matrices

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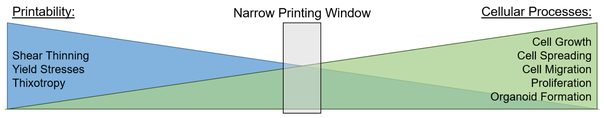
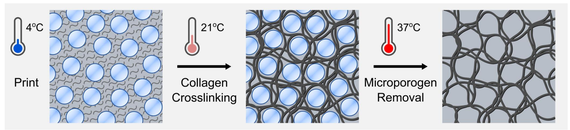
ABSTRACT

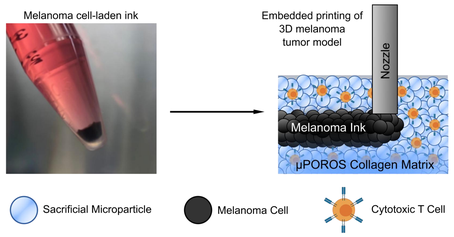
Embedded bioprinting enables the rapid design and fabrication of complex tissues that recapitulate the *in vivo* microenvironment[1-3]. However, few biological matrices provide good print fidelity while simultaneously facilitating cell function[4]. Here, we introduce a new microporogen-structured (μPOROS) matrix that addresses this unmet need[5]. We developed an embedded tumor model for immuno-oncology applications that recapitulates antigen-specific, cytotoxic T cell-mediated tumor killing in both murine and human contexts.

Bioprinting Grand Challenge: Bridging Printability & Cellular Processes



Overview of Bioprinted Tumor Models via μPOROS Matrices



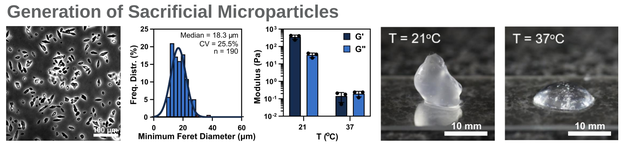


OVERVIEW OF RESULTS

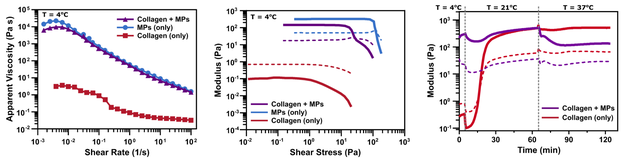
- Gelatin Type A-based sacrificial microparticles (MP) (Feret diameter ~18 μm) were generated using a modified method from previous literature[2] and compacted within prepolymer collagen type I solution to a volume fraction (φ) of 0.47.
- The sacrificial MPs first serve as rheology modifiers to improve printability at 4ºC. Following collagen crosslinking at 21ºC, the sacrificial MPs then serve as microporogens that melt at 37ºC to generate a microporous collagen network permissive to cellular processes.
- A proof-of-concept murine model recapitulated an antigen-specific anti-tumor response by embedding a murine B16-F10 cell ink at physiologically-relevant cell densities (~250 million cells mL^{-1}) within a μPOROS collagen matrix containing antigen-specific cytotoxic $CD8^+$ T cells isolated from pmel-1 C57/BL6 mice.
- In a human melanoma model, MART1 antigen-specific human cytotoxic $CD8^+$ T cells were isolated from peripheral blood of healthy donors (HLA-A*02:01) and activated by antigen presenting cell mimetic scaffolds (APC-ms)[6]. APC-ms activated cytotoxic T cells demonstrated an antigen-specific and dose-dependent anti-tumor response against human $MART1^+$ SKMEL5 (HLA-A*02:01) printed tumors.

MATERIALS CHARACTERIZATION:

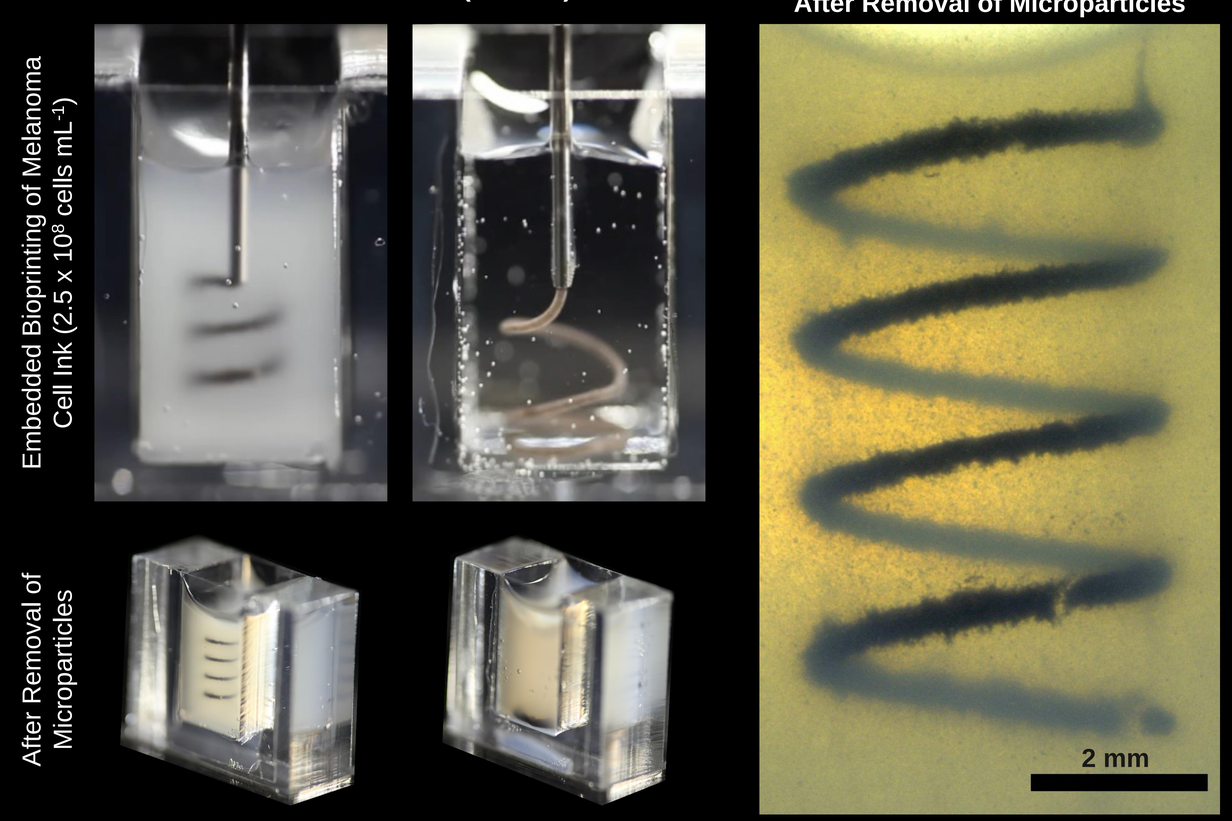
Generation of Sacrificial Microparticles

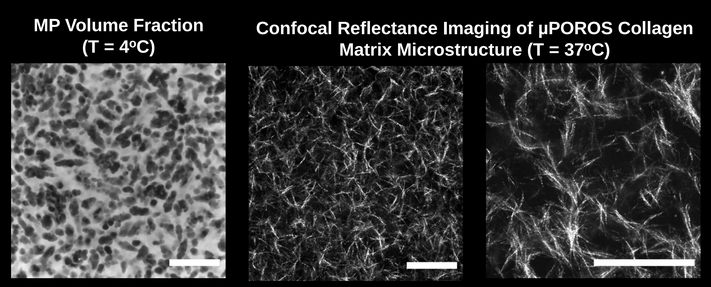


Rheological Bulk Characterization of μPOROS Matrices



Printable μPOROS Collagen Matrices Enable Fabrication of Complex Tumor Models for Immuno-Oncology





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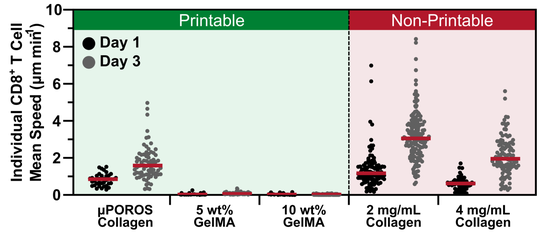
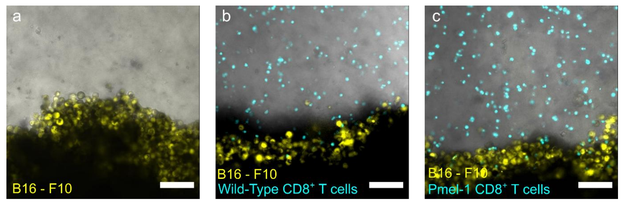
2. Lee A, Hudson A, et. al. *Science* (2019).
3. Bhattacharjee T, et. al. *Science Advances* (2015).
4. Chaudhuri O, et. al. *Nature* (2020).
5. Reynolds D, et. al. *Advanced Materials* (2023).
6. Cheung A, et. al. *Nature Biotechnology* (2018).

BIOPRINTED MURINE MODEL:

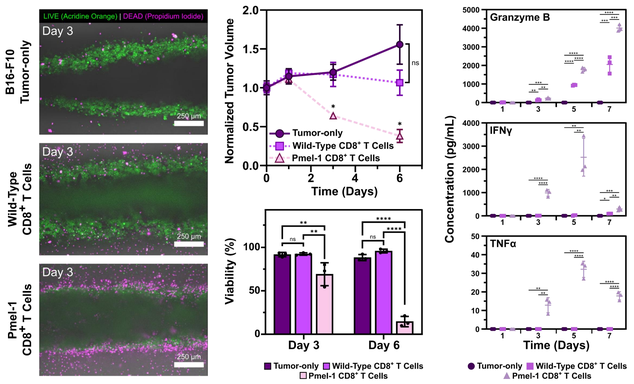
Murine $CD8^+$ T Cell Migration Assay



Patterning of Murine Tumor Cells and $CD8^+$ T Cells

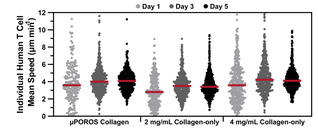


Murine Antigen-Specific Anti-Tumor Response

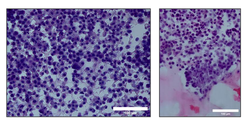


BIOPRINTED HUMAN MODEL:

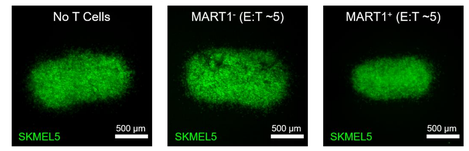
Human $CD8^+$ T Cell Migration Assay



H&E Bioprinted SKMEL5 Tumor



Human Antigen-Specific Anti-Tumor Response



SUMMARY & FUTURE DIRECTIONS:

We have created a new class of printable materials, microporogen-structured (μPOROS) matrices, for embedded bioprinting that use sacrificial microparticles as both rheology modifiers and microporogens. We generated 3D tumor models (murine & human) for immuno-oncology applications with high cellular density and immune cell-tumor interactions akin to their *in vivo* counterparts. We showed that cytotoxic T cells migrate and exhibit tumor antigen-specific killing. Looking forward, we anticipate that our μPOROS matrices coupled with embedded bioprinting may be adopted for creating human tissues for disease modeling and therapeutic applications.

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