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Faculty of Medicine

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3D *in vitro*, *in ovo* and *in vivo* biofabricated models to study melanoma tumor dormancy and progression

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Background

Although conventional 2D cancer models remain standard in drug development, they fail to replicate the complex *in vivo* tumor microenvironment. Advanced 3D approaches, particularly bioprinting, enable more physiologically relevant models to study tumor proliferation, dormancy, and metastasis. *In vitro* systems allow controlled investigations, while vascularized *in vivo* models provide perfusable environments that better mimic native conditions. Melanoma, a highly aggressive skin cancer, progresses from primary tumors to metastasis and can enter dormant states that complicate treatment. This project aims to develop melanoma models across *in vitro*, *in ovo* (CAM), and *in vivo* platforms. By combining bioprinted bioinks with human adipose-derived stem cells (ADSCs) and using a vascularized arteriovenous loop (AVL) model, we aim to mimic tumor stages and analyze how bioink properties influence melanoma cell behavior.

Methods

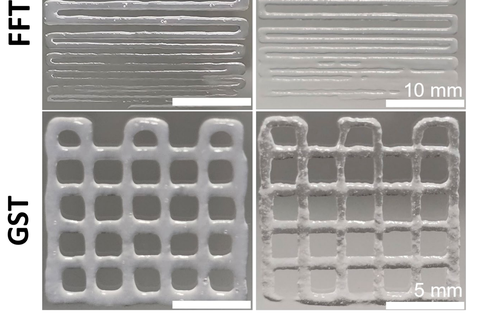
Human melanoma cell lines were cultured in two bioinks: alginate with cellulose nanofibrils (Cellink Bioink) and pre-crosslinked alginate dialdehyde-gelatin (ADA-GEL). To better mimic the tumor microenvironment, cells were co-cultured with human adipose-derived stem cells (ADSC). Cell viability and cell cycle dynamics were analyzed over 14 d using viability assays and FUCCI. Bioink properties, including mechanical behavior, pore size, and degradation, were characterized by mechanical testing and cryo-SEM. Experiments were performed in the *in ovo* CAM model to evaluate tumor growth. For *in vivo* analysis, constructs were implanted into the rat AVL model to establish a vascularized tumor environment. Tumor development, vascularization, and metastasis were assessed via immunohistology. Additionally, different geometries of 3D-printed ADA-GEL constructs were used to study the impact of scaffold design on tumor growth and tissue formation.

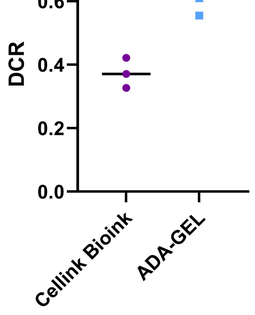
Results

Material characterization

Printability

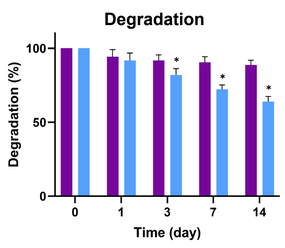
- filament fusion test (FFT) / grid structure test (GST)
- Diagonal crossing ratio (DCR): ADA-GEL 0.62 ± 0.08 / Cellink Bioink 0.37 ± 0.05
- Both inks were in category B (minimal starting fusion of strand distance 0.75 mm) based on the categorization criteria in FFT





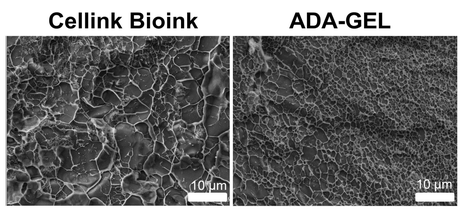
***In vitro* degradation**

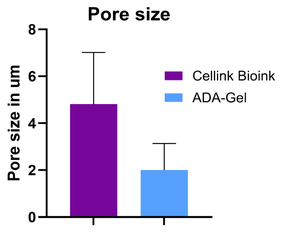
- Total weight: Higher mass loss in ADA-GEL compared to Cellink Bioink during 14 days



Morphology and pore size

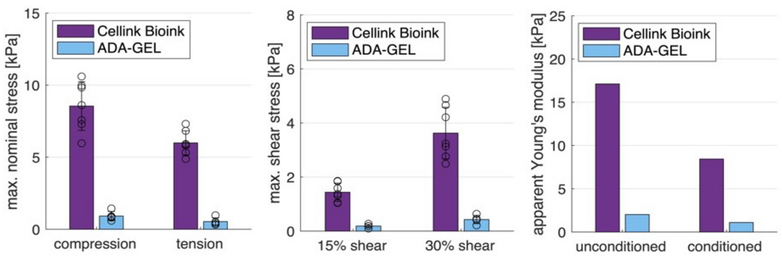
- Pore size: ADA-GEL 1.83 ± 0.48 µm Cellink Bioink 6.08 ± 2.69 µm





Mechanical properties

- Cellink Bioink constructs exhibited higher stresses in comparison to ADA-GEL
- Cellink Bioink displayed a higher Young's modulus compared to ADA-GEL in both unconditioned and conditioned states

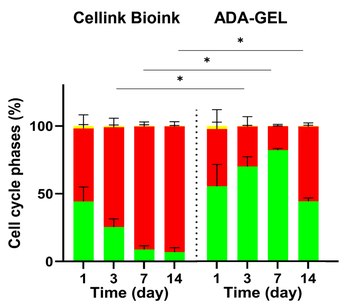


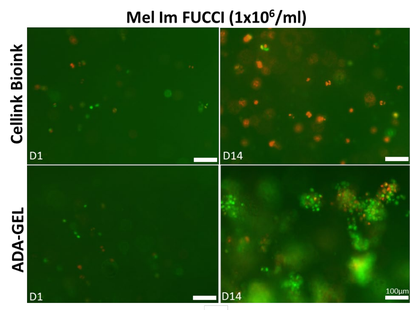
Biofabricated 3D *in vitro* constructs

Melanoma labeled-FUCCI

- Cellink Bioink: G1 phase (dormant)
- ADA-GEL: G2-S-M phase (progression)

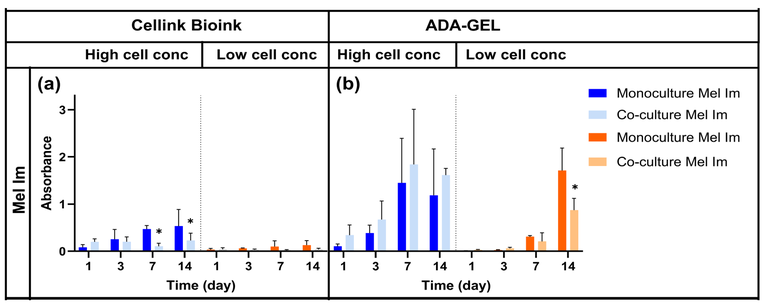
FUCCI color scale of cell cycle phases
Cdt1 indicates G1 state while geminin indicates S/G2/M state, during the transition from G1 to S phase, both proteins are present which lead to a yellow signal.



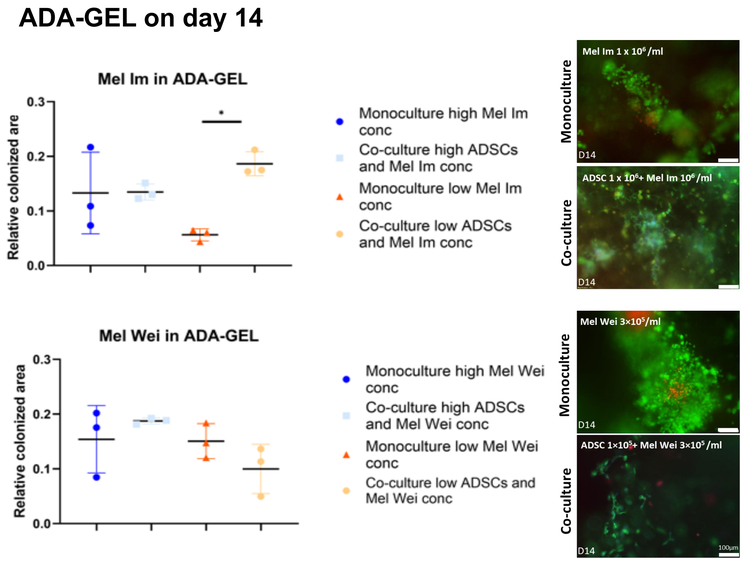


WST-8 assay

- Higher cell viability in ADA-GEL compared to Cellink Bioink
- Higher cell viability in monoculture compare to co-culture



Melanoma colonization in mono- and co-culture in ADA-GEL on day 14

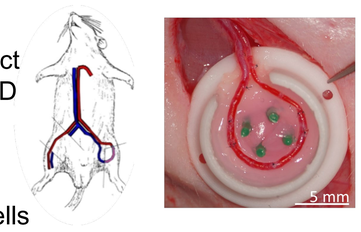


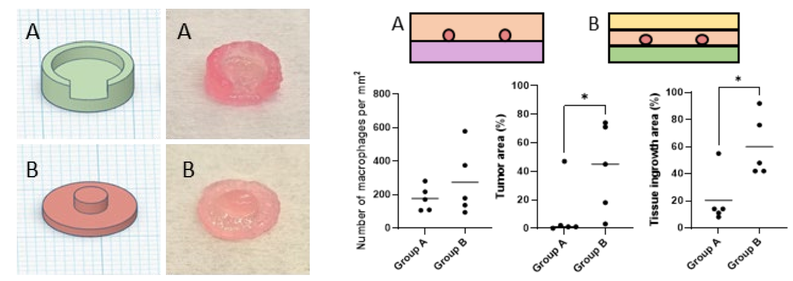
- In high cell conc: lower colonization area in co-culture
- In low Mel Im cell conc: higher colonization area in co-culture
- In low Mel Wei cell conc: lower colonization area in co-culture

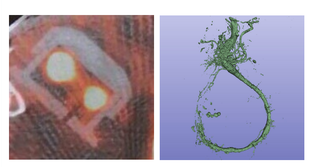
Biofabricated and vascularized 3D *in ovo* and *in vivo* constructs

Development of a 3D printed bioink system for the *in vivo* Arteriovenous loop (AVL) model

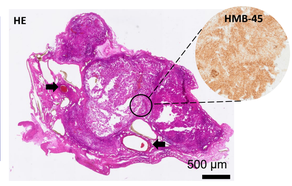
- Establishment of 3D printed construct
- Implanting melanoma cell-loaded, 3D printed, pre-cross-linked ADA-GEL
- Analysis of tumor growth and neovascularization
- Metastatic behavior of melanoma cells



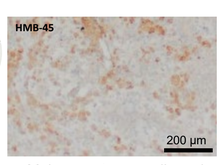




PET-CT (*in vivo*) and µCT (*ex vivo*) images of AVL chambers



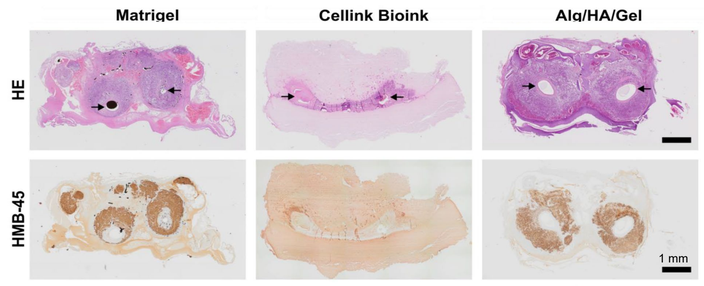
Histological cross sections of AVL explants, highlighting melanoma tumor growth



Melanoma tumor cells and cell clusters in rat *Lymphonodus subiliacus*

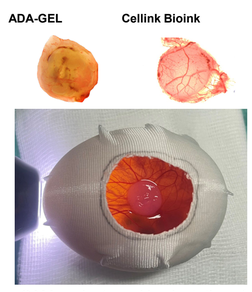
Utilization of established bioink models in the AV loop *in vivo* cast model

- It was possible to mimic *in vivo* progression (Matrigel and Alg/HA/Gel) as well as dormancy (Cellink Bioink) states of tumor development

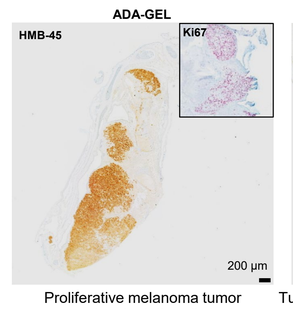


Pre-screening of established bioink models in the Chicken chorioallantoic membrane (CAM) model (3R concept)

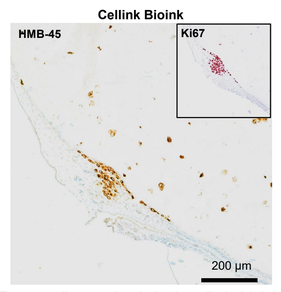
- It was feasible to replicate *in ovo* the different tumor development stages and to observe tumor cell migration



CAM explants and implantation of Cellink Bioink into the CAM model



Proliferative melanoma tumor areas and metastatic cell clusters



Tumor cells remained single cells in bioink, however, cell migration was observed

Conclusion and outlook

- ADA-GEL promotes melanoma cell growth, while Cellink Bioink replicates the dormant stage of melanoma
- Co-culture with human adipose-derived stem cells (ADSC) enhances physiological relevance and reveals interactions with melanoma cells
- Both the CAM model and rat AVL model overcome limitations of non-vascularized *in vitro* cell culture techniques
- Well suited to study various cancer development stages and metastatic tumor behavior
- Provide a valuable platform to investigate mechanisms of drug resistance development

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