POLBIONICA

Vascularized inkjet-bioprinted liver cancer organoids as a platform for anticancer drug screening

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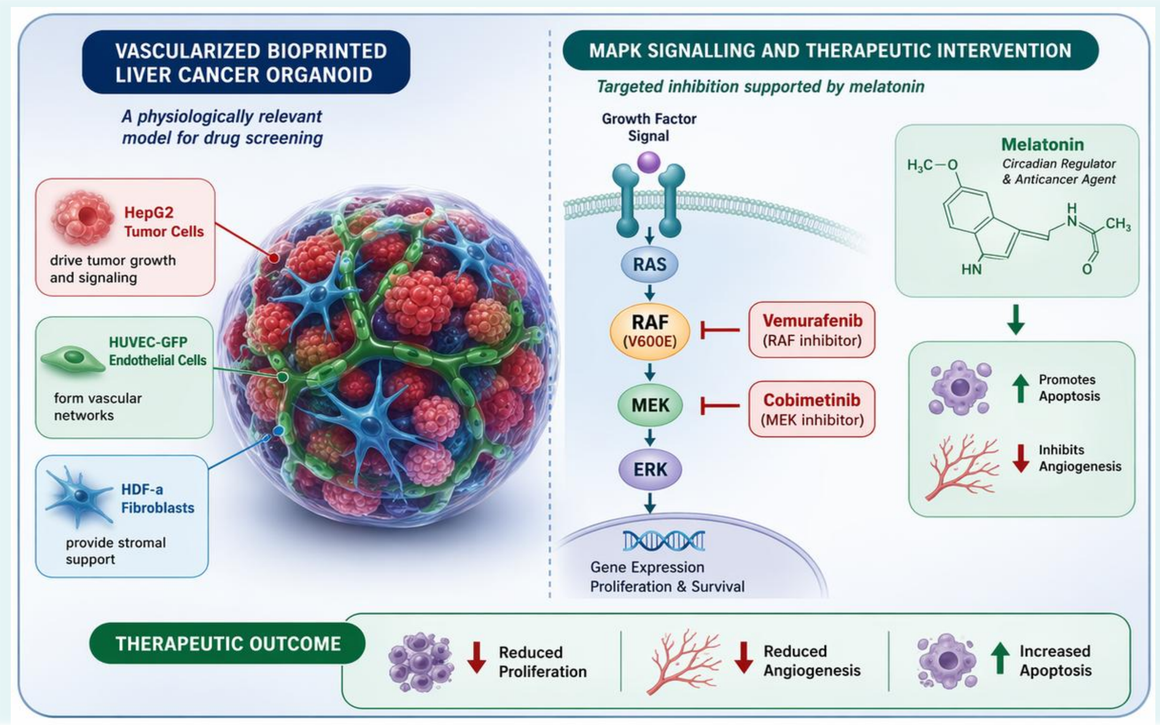
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INTRODUCTION

Conventional 2D models fail to recapitulate key features of tumor biology, including three-dimensional architecture, cellular heterogeneity, and cell–cell and cell–matrix interactions, as well as vascularization which limits their predictive value in anticancer drug development and contributes to poor clinical translation.

Organoid-based systems have emerged as advanced in vitro models that better mimic tissue organization and microenvironmental signaling. When combined with inkjet bioprinting, they enable precise spatial control over tumor composition, allowing integration of cancer, stromal, and endothelial cells within a reproducible architecture and supporting the formation of vascular-like networks.

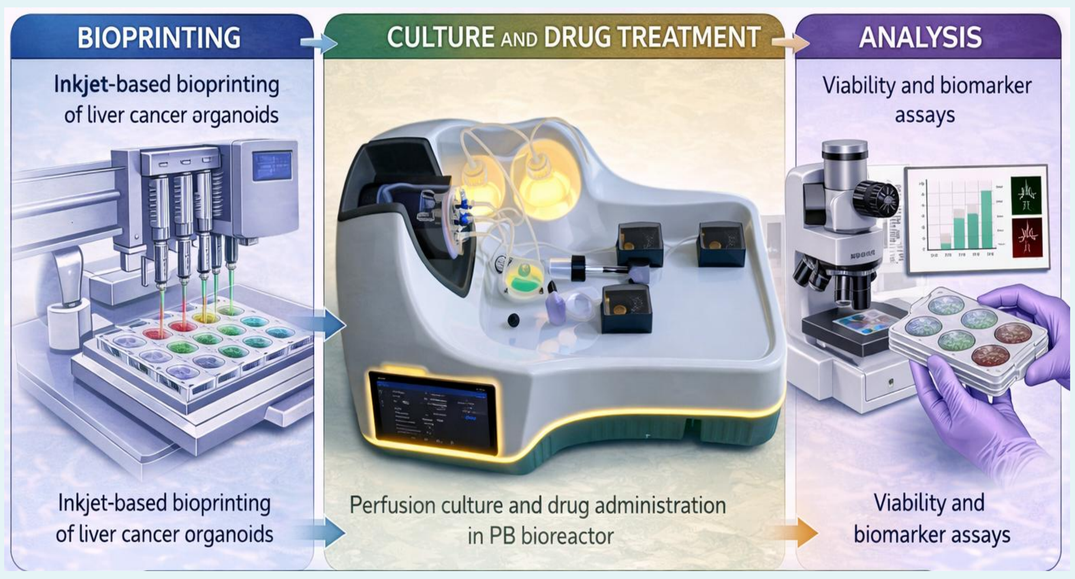
Here, we present a pilot study demonstrating the feasibility of a vascularized, inkjet-bioprinted 3D liver cancer organoid platform for the evaluation of targeted therapies and combination treatments, including melatonin and the mitogen-activated protein kinase (MAPK) pathway inhibitors, in a scalable and physiologically relevant system.



AIMS

The aim of this study was to develop and validate a vascularized inkjet-bioprinted 3D liver cancer organoid model and to evaluate its potential as a platform for screening targeted therapies and combination treatments, including melatonin and clinically relevant kinase inhibitors.

MATERIALS AND METHODS



Biofabrication of organoids:

Vascularized liver cancer organoids generated using inkjet bioprinting:

- composite bioink: GelMA, HAMA, and dECM,
- multicellular model including: HepG2, HDF-α, HUVEC-GFP cells,
- reconstruction of tumor microenvironment with stromal and vascular components,
- enabling reconstruction of a multicellular tumor microenvironment.

Culture conditions:

- dynamic perfusion culture in *Bioreactor ResearchLine (Polbionica)* for 14 days,
- medium collected at days: 1, 3, 7, 10, and 14,
- analysis of secreted biomarkers over time.

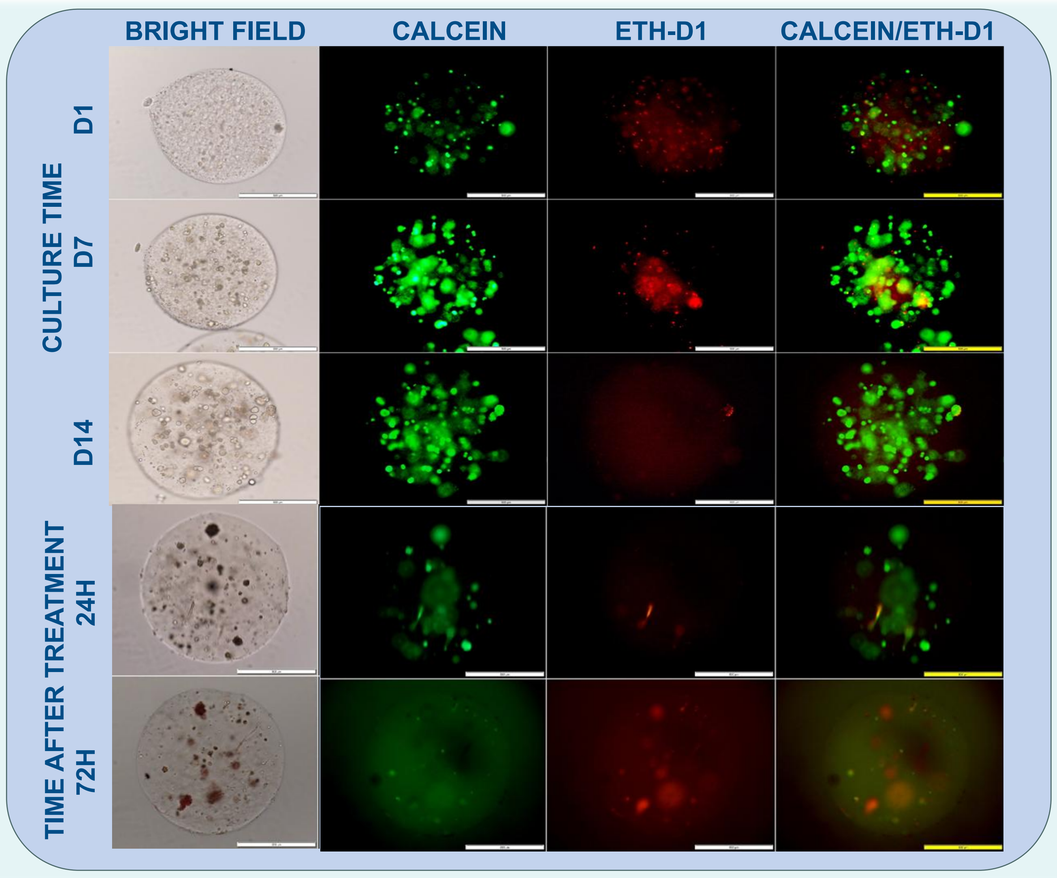
Drug treatment:

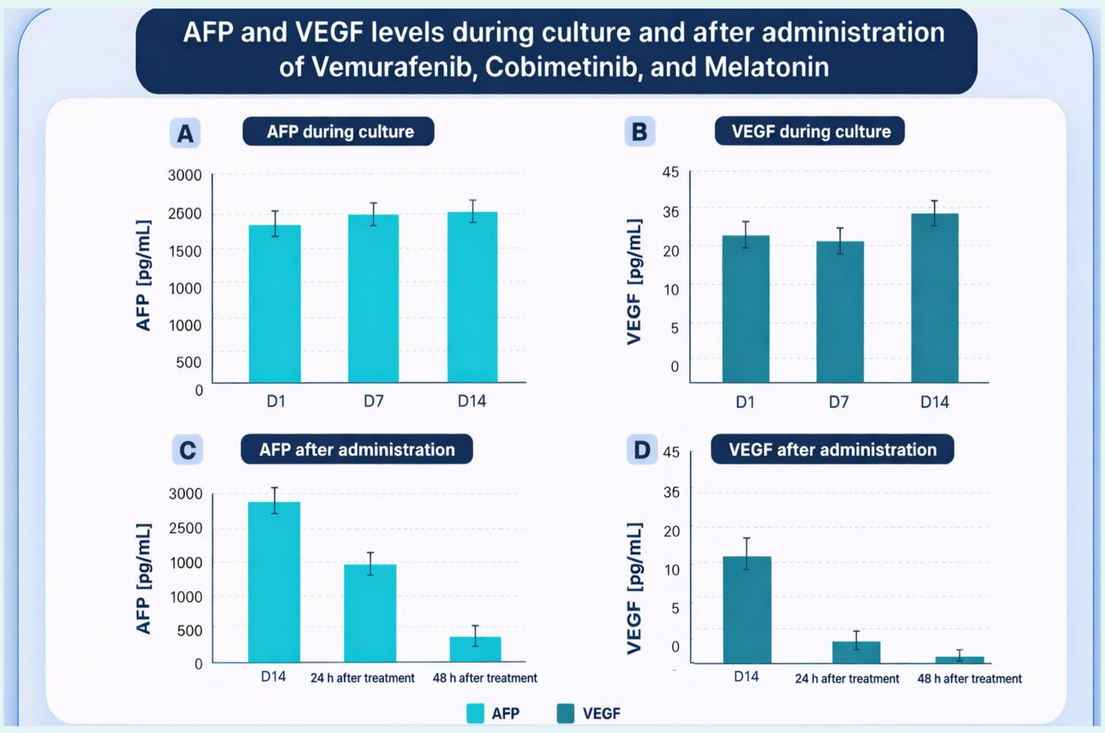
- initiated on day 14,
- combination therapy: Vemurafenib, Cobimetinib, and Melatonin under perfusion conditions,
- samples collection after 24, and 48 hours.

Analysis:

- cell viability: calcein/ETH-D1 staining,
- functional biomarkers: AFP, VEGF,
- morphology & vascularization: fluorescence microscopy and immunohistochemical staining.

RESULTS





Organoid formation and baseline characterization (Day 1–14):

- Inkjet-bioprinted liver cancer organoids maintained high viability (>80%) and formed stable, compact 3D structures throughout the 14-day perfusion culture.
- AFP and VEGF levels progressively increased, confirming sustained tumor activity and pro-angiogenic signaling.
- By day 10, endothelial cells self-organized into microvascular-like networks integrated within the tumor microenvironment.

Response to treatment (24–72 h):

- Treatment with Vemurafenib, Vobimetinib, and Velatonin induced a strong therapeutic response, reflected by an approximately 80% reduction in AFP secretion and a marked decrease in VEGF levels.
- Viability analysis demonstrated an approximately 82% reduction in viable tumor cells, accompanied by an increase in dead (ETH-D1-positive) cell populations.
- Morphological analysis revealed a clear disruption of organoid structure and integrity following treatment.

CONCLUSION

- The vascularized inkjet-bioprinted liver cancer organoid model represents a physiologically relevant platform for reconstructing the tumor microenvironment, integrating cancer, stromal, and endothelial components under dynamic perfusion conditions.
- The system showed high stability and viability, with increasing AFP and VEGF levels, confirming functional tumor and pro-angiogenic activity.
- The model responded effectively to combination therapy, showing reduced AFP and VEGF levels along with decreased cell viability.
- These findings support the use of vascularized 3D bioprinted organoids as scalable platforms for preclinical drug screening and precision oncology.

Conflicts of Interest: Michał Wszola and Marta Klak are the co-founders of Polbionica Ltd