# Mimicking breast cancer tissue using 3D bioprinted patient-derived models

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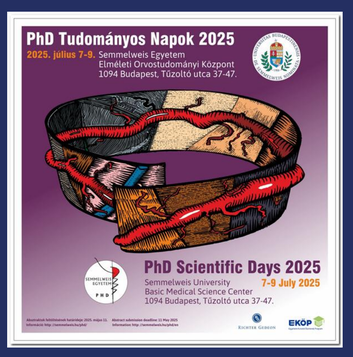



## INTRODUCTION

Conventional two-dimensional (2D) in vitro models often fail to replicate the drug sensitivities of cancer patients, limiting their value in personalized oncology. Three-dimensional (3D) cell culture models are being developed to better mimic the physiological conditions of in vivo tumors and to assess therapeutic responses more accurately – especially patient-derived models.

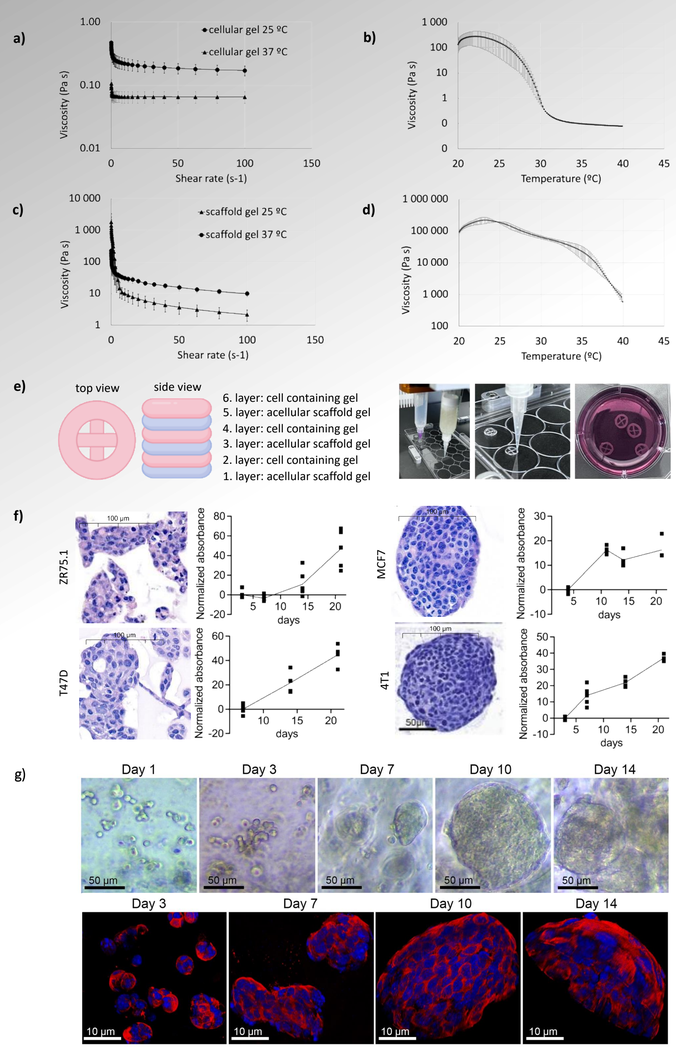
## AIMS AND METHODS

This study aimed to establish and validate an in vitro culturing method using a 3D bioprinted breast cancer tumor model (4T1 cell line) and to compare its biological and pharmacological characteristics with traditional 2D, spheroid, and in vivo (xenograft and allograft) models. Additionally, patient-derived equivalents of the same models were established by isolating tumor cells from 4T1 tumors grown in BALB/c mice, characterized by flow cytometry (panel: CD3, CD4, CD8, CD11b and CD45R/B220). Comparative analyses were performed to assess tissue heterogeneity, growth potential, and therapeutic responses.

## RESULTS

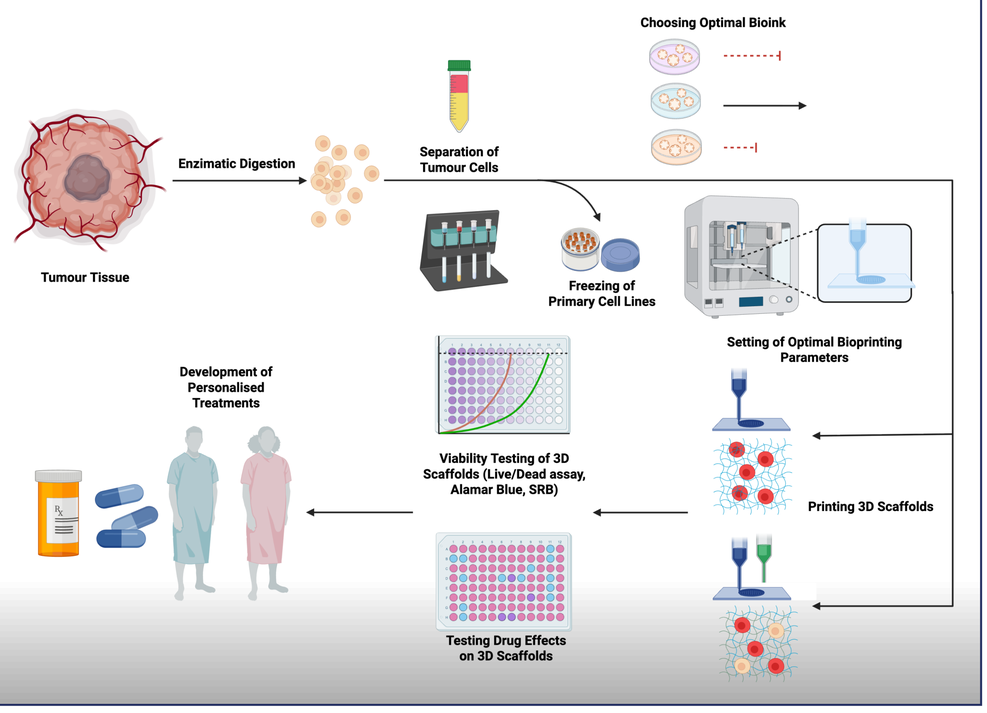
The 3D bioprinted tumor models exhibited architectural and cellular complexity closely resembling in vivo tumors. Morphological and functional evaluations revealed that these models maintained tumor-like structure, proliferation rates, and drug response patterns that were more consistent with in vivo conditions than those seen in 2D cultures or spheroids. Notably, drug sensitivity in 3D bioprinted models paralleled that of syngeneic tumors regrown in BALB/c mice (allograft tumor), demonstrating improved prediction of therapeutic efficacy.

**Figure 1: Creation of 3D bioprinted tissue-mimetic structures (TMS) from various breast carcinoma cell lines**




**a, c)** Viscosity curves of the cell-laden (3% alginate + 1% gelatin) and scaffold (6% alginate + 11% methylcellulose) hydrogels at different temperatures.
**b, d)** Temperature-dependent changes in the viscosity of the cell-laden (b) and scaffold (d) hydrogels.
**e)** Schematic representation of the 3D bioprinted tissue-mimetic structures composed of six alternating layers of "cell-laden" hydrogels containing cells and "scaffold" hydrogels providing structural integrity.
**f)** Hematoxylin-eosin staining of formalin-fixed, paraffin-embedded (FFPE) sections of 3D bioprinted tissue-mimetic structures created from different breast carcinoma cell lines. Growth of the 3D bioprinted structures was monitored over three weeks using the sulforhodamine B (SRB) assay. Scale bars are indicated. The cell lines used were: **ZR75.1** – luminal B breast carcinoma; **T47D, MCF-7** – luminal A breast carcinoma; **4T1** – Balb/c metastatic mouse breast carcinoma.
After one week of culture post-bioprinting, tissue formation was observed in all cell lines. The scale bar represents 100 µm.
**g)** Cell growth of 4T1 cells observed in 3D bioprinted TMSs observed by inverted microscope (Olympus CK-2). Tumour formation in 4T1 TMSs detected by confocal microscopy (Leica Sp8 Lightning – LAS X software) (actin -phalloidin-red, nuclei – Hoechst-blue). Scale bars are indicated.

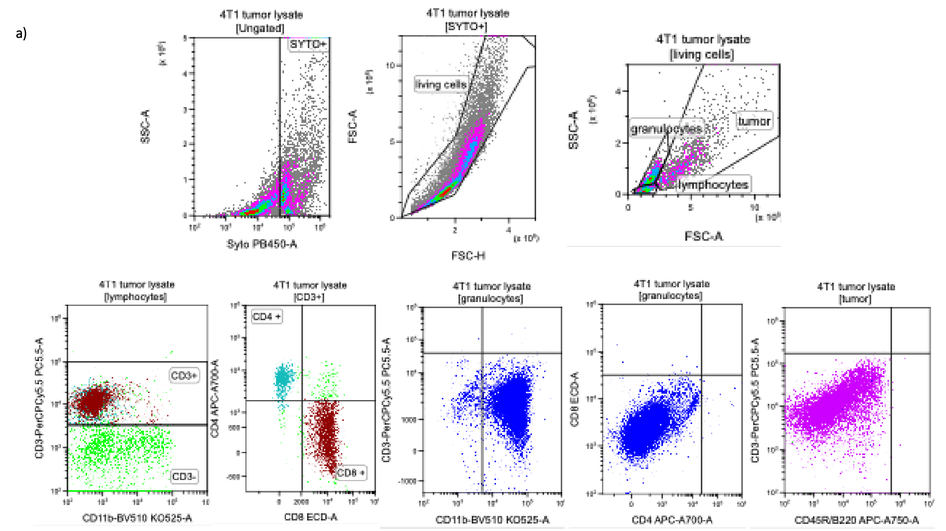
**Figure 2: Schematic illustration of creating patient-derived 3D bioprinted tissue-mimetic structure (TMS) to support therapeutic decision-making.**




## CONCLUSIONS

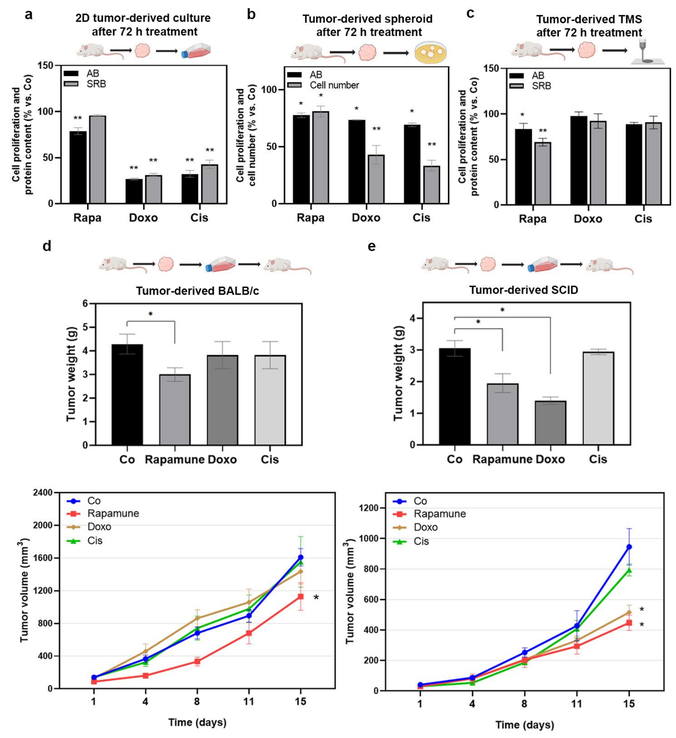
Our findings indicate that 3D bioprinted breast cancer models provide a more physiologically relevant and reproducible platform for evaluating drug responses compared to conventional in vitro systems and PDX models. This approach holds strong potential for advancing personalized oncology by bridging the gap between in vitro testing and in vivo therapeutic outcomes.

**Figure 4: Flow-cytometric characterization of cells isolated from *in vivo*-grown 4T1 tumors**



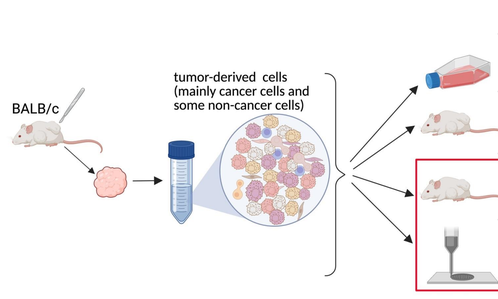

**a)** Digested tumor sample was tested on FACS Navios (Beckman Coulter) using Navios and Kaluza softwares. Used antibodies: CD45R/B220-APC-Cy7, CD3-PerCP-Cy5.5, CD4-Alexa 700, CD8-PE-Texas Red (ECD), CD11b-BUV510.
**b)** The cellular composition of the isolated suspension, distinguishing tumor cells, lymphocytes, and granulocytes; based on FSC and SSC characteristics and surface-marker expressions.

**Figure 5: Patient-derived tumor model highlighted that 3D bioprinted tissue-mimetic structures (TMSs) could mimic the drug response of patients**




Drug sensitivity differences in breast cancer models using tumor-derived cells from *in vivo* growing tumors. Tumor tissues were removed from BALB/c mice, and subsequently cells were isolated and cultured in various conditions:
a) two-dimensional (2D) tumor-derived culture,
b) tumor-derived spheroid,
c) three-dimensional (3D) bioprinted tumor-mimicking structures (TMSs) (tumor-derived TMS),
d) syngeneic tumors in BALB/c (tumor-derived BALB/c), and
e) xenografts in severe combined immunodeficiency (SCID) mice (tumor-derived SCID).
AB - Alamar Blue; Cis - cisplatin (*in vitro*, 10 µM or *in vivo*, 1 mg/kg); Co - control; Doxo - doxorubicin (*in vitro*, 50 ng/mL or *in vivo*, 2 mg/kg); Rapa, rapamycin (in vitro, 50 ng/mL) or Rapamune (*in vivo*, 3 mg/kg); SRB - sulforhodamine B. *p < .05; **p < .01. Three parallel measurements (six replicates in each) for in vitro and two parallel experiments (five replicates in each group) for in vivo experiments were conducted. Error bars indicate standard deviation (SD).

**Figure 6: Comparison of tissue-derived tumor models tested. The patient-derived cultures established and maintained in an inappropriate environment may show different therapeutic responses**




| | Rapa | Doxo | Cis |
|---|---|---|---|
| Tumor-derived - *in vitro* 2D | Resistant | Sensitive | Sensitive |
| Tumor-derived - xenograft in SCID mice | Sensitive | Sensitive | Resistant |
| Tumor-derived - syngeneic tumor in BALB/c mice | Sensitive | Resistant | Resistant |
| *In vitro* 3D bioprinted Tumor-derived-TMS | Sensitive | Resistant | Resistant |

Three-dimensional (3D) bioprinted tumor-mimicking structures (TMSs) more accurately represent in vivo drug responses—compared drug sensitivity across the tested tumor-derived models. Tumor-derived cultures under different conditions exhibit altered therapeutic responses, as observed. Based on our presented experimental drug tests, rapamycin/Rapamune (Rapa), doxorubicin (Doxo), and cisplatin (Cis) sensitivity/resistance detected in the appropriate models were indicated in the right table. Our study demonstrated that in vitro 3D bioprinted TMSs most closely mimic the in vivo therapeutic response of syngeneic tumors to the tested treatments (highlighted in red).

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