3D bioprinted in vitro models in cancer metabolism research

ZSÓFIA GÁBRIEL[1], FATIME SZALAI[1], VIKTÓRIA VARGA[1], HENRIK WEHN OLSEN[1], NÓRA FÖRHÉCZ[1,2], BÁLINT CSIKÓS[1,2], GÁBOR PETŐVÁRI[1], ILDIKÓ KRENCZ[1], DOROTTYA MOLDVAI[1], ANNA SEBESTYÉN[1]

[1]Department of Pathology and Experimental Cancer Research, Semmelweis University, [2]Faculty of Science, Institute of Biology, Eötvös Lóránd University, Budapest

Correspondence:
Dr. Anna Sebestyén, Department of Pathology and Experimental Cancer Research, Semmelweis University, Üllői street 26., 1085, Budapest. E-mail: sebestyen.anna@semmelweis.hu, tel.: +36 1 4591500/54474

Közlésre érkezett:
2026. április 8.
Elfogadva:
2026. április 29.

Cancer metabolism is a central field in oncology, strongly serving as critical adaptation mechanism driving tumour progression, therapeutic response, and patient prognosis. The complexity of tumour metabolism remains difficult to mimic using conventional experimental conditions. Preclinical models, including in vitro 2D cultures and in vivo animal models, often fail to accurately reproduce the dynamic tissue alterations. Increasing evidence highlights the crucial role of the tumour microenvironment, nutrient availability, hypoxia, extracellular matrix components, and metabolic crosstalk between cancer, stromal, and immune cells in shaping tumour metabolic rewiring. Consequently, advanced 3D culture systems have gained significant attention, offering new perspectives for modelling cellular complexity and metabolic heterogeneity. This review summarises metabolic adaptation mechanisms influenced by microenvironment, and discusses the evolution of current modelling. Furthermore, through our own research in 3D bioprinting, we highlight the emerging role of 3D bioprinted models in tumour biology, cancer metabolism research and drug sensitivity studies. Magy Onkol 70:95-116, 2026

Keywords: *bioprinting, metabolism, cancer, in vitro, model*

A daganatok anyagcsere-változása a tumorszövet túlélését segítő alkalmazkodási mechanizmus, befolyásolja a daganatprogressziót, a betegek terápiás válaszát, így kutatása napjaink onkológiai vizsgálatainak egyik központi kérdése. A tumorokban jellemző anyagcsere-változások összetettsége és heterogenitása nehezen modellezhető hagyományos kísérleti rendszerekben. A preklinikai modellek, beleértve az *in vitro* 2D kultúrákat és az *in vivo* állatmodelleket, nem reprodukálják teljes mértékben a tumorok és mikrokörnyezetük dinamikus szöveti változásait. A tumor-, stróma- és immunsejtek közötti metabolikus kölcsönhatások, valamint a szöveti környezeti tényezők (extracelluláris mátrix komponensek vagy tápanyag- és oxigénszint) kulcsfontosságú szerepét egyre több adat támasztja alá. A gyorsan fejlődő 3D sejtkultúrás rendszerek új távlatokat nyitnak a sejtes komplexitás, az extracelluláris mátrix szerkezete, valamint az anyagcsere-folyamatok heterogenitásának modellezésében, így kiemelt jelentőségűek a kísérletes onkológiában. Összefoglaló közleményünkben áttekintjük az anyagcsere-adaptáció kulcsfontosságú mechanizmusaiban a tumor-mikrokörnyezet hatását, és bemutatjuk a jelenlegi modellfejlesztési irányokat, illetve saját modellfejlesztéseinken keresztül a 3D bionyomtatott modellek szerepét daganatbiológiai, gyógyszerfejlesztési és gyógyszerérzékenységi vizsgálatokban.

Gábriel Zs, Szalai F, Varga V, Olsen HW, Förhécz N, Csikós B, Petővári G, Krencz I, Moldvai D, Sebestyén A. 3D bionyomtatott *in vitro* modellek a daganatmetabolizmus vizsgálatában. Magy Onkol 70:95-116, 2026

Kulcsszavak: bionyomtatás, metabolizmus, tumor, *in vitro,* modell

INTRODUCTION: THE IMPORTANCE OF CANCER METABOLISM RESEARCH

After the initial „hallmarks of cancer" review (1), the past 25 years have made it clear that there is huge variability in the manifestation of cancer hallmarks throughout the tumour evolution – the continuum of tumourigenesis, from initiation through multistage carcinogenesis and progression to therapy resistance, metastasis, and ultimately causing the patient's death. The importance of these characteristics influencing the functional capabilities of tumour cells, phenotypic alterations and facilitating tumour cell growth/survival in the tumour microenvironment (TME) in temporal and spatial complexity was highlighted (1, 2). This diversity could serve as an adaptation mechanism at tissue level in progression. Histopathological heterogeneity has long been recognised by pathologists, and this complexity currently presents a major challenge for cancer research and experimental cancer modelling (3).

The recently established molecular and digital pathology methods are highly informative, though these are limited by their reliance on collected tissues. Consequently, they offer only static view of the disease at a single time point, failing to predict therapeutic outcomes. The recently evolving „liquid biopsies" provide a promising avenue to analyse disseminating tumour cells. Additionally, the other developing non-invasive imaging technologies propose solutions for monitoring, following disease features in situ (4–6).

In experimental and drug research, in vitro/in vivo models help to reproduce time-dependent tumour growth alterations, but these are not without significant limitations. In vivo, the well-organised cancer tissue and its heterogeneity support growth and survival of the tumour mass at starving conditions (altering nutrients/metabolic building blocks/oxygen/drug levels). Recently used models often fail to fully mimic e.g. structural complexity (7), the metabolic symbiosis, the characteristic biochemical and metabolic alterations, which have crucial roles in cancer progression (8). Therefore, it remains challenging to further develop and apply 3D cell cultures and more complex human cell-/tissue-derived 3D cancer models to investigate "hallmark co-targeting" strategies – simultaneous disruption of independent regulatory factors leads to more long-term adaptive responses (2, 9).

METABOLIC ADAPTATION AS AN IMPORTANT HALLMARK OF CANCERS

Alterations in cellular metabolism and energy utilisation are important features of cancer cell survival and proliferation. These metabolic abnormalities were first described by Otto Warburg and Minami Seigo in 1923, when they observed that tumour cells exhibit an unusual pattern of glucose metabolism (10). Although early research highlighted the importance of metabolic alterations in cancer, the field received less attention because of the rapid expansion of molecular genetics. In recent years, however, tumour metabolism has gained renewed interest; and metabolic pathways are now recognised as key determinants of progression and therapy response.

Cancer cells undergo metabolic reprogramming that enables them to use a wide range of nutrients to sustain uncontrolled growth and efficiently generate the energy and biosynthetic precursors required for rapid proliferation. In addition, cancer cells actively produce and secrete metabolites that influence both their own behaviour and that of surrounding stromal, immune, and endothelial cells within the TME (11). These metabolic signals contribute to the dynamic crosstalk between cancer cells and the TME, shaping tumour development and progression.

Tumour metabolism is characterised by complexity and flexibility, allowing cancer cells to survive and proliferate even under unfavourable conditions such as hypoxia, nutrient deprivation, or therapeutic stress. To meet these challenges, cancer cells rely on multiple metabolic pathways that support their demands (12, 13).

One of the most important features of cancer cells is their increased reliance on glycolysis for glucose metabolism. Glucose is rapidly converted not only to produce adenosine triphosphate (ATP), but also to generate metabolic intermediates that serve as substrates for various biosynthetic pathways. In contrast to most normal cells, which primarily utilise oxidative phosphorylation (OXPHOS), cancer cells often prefer Warburg glycolysis even in the presence of oxygen (pseudohypoxia). Warburg effect results in increased lactate production and subsequent acidification of the TME (14, 15). The accumulation of lactate contributes to several pro-tumourigenic processes, including enhanced immunosuppression, invasive capacity and the stimulation of angiogenesis (16, 17).

Beyond glucose metabolism, additional pathways contribute significantly to the metabolic adaptability of tumour cells. Glutaminolysis plays a crucial role in providing carbon and nitrogen sources for energy production, maintenance of redox balance, and the synthesis of macromolecules such as nucleotides and amino acids (18, 19). Furthermore, intermediates derived from the tricarboxylic acid (TCA) cycle serve as precursors to produce lipids, amino acids, and nucleotides, which are essential for rapidly dividing cells (20). The pentose phosphate pathway (PPP) and one-carbon metabolism provide building blocks for nucleotide synthesis and produce cofactors required for maintaining redox homeostasis. These pathways also contribute to methylation reactions that influence epigenetic regulation (21, 22). Fatty acid oxidation and acetate metabolism provide alternative sources of acetyl-coenzyme A and metabolic energy, particularly under conditions of limited nutrient availability or hypoxia (23, 24).

These metabolic alterations form a highly adaptable metabolic network that supports rapid proliferation, survival, and adaptation. While metabolic rewiring is considered universal hallmark, the relative dominance of metabolic pathways and tumor type-/environment-dependent growth signalling network result in significant intertumoural and

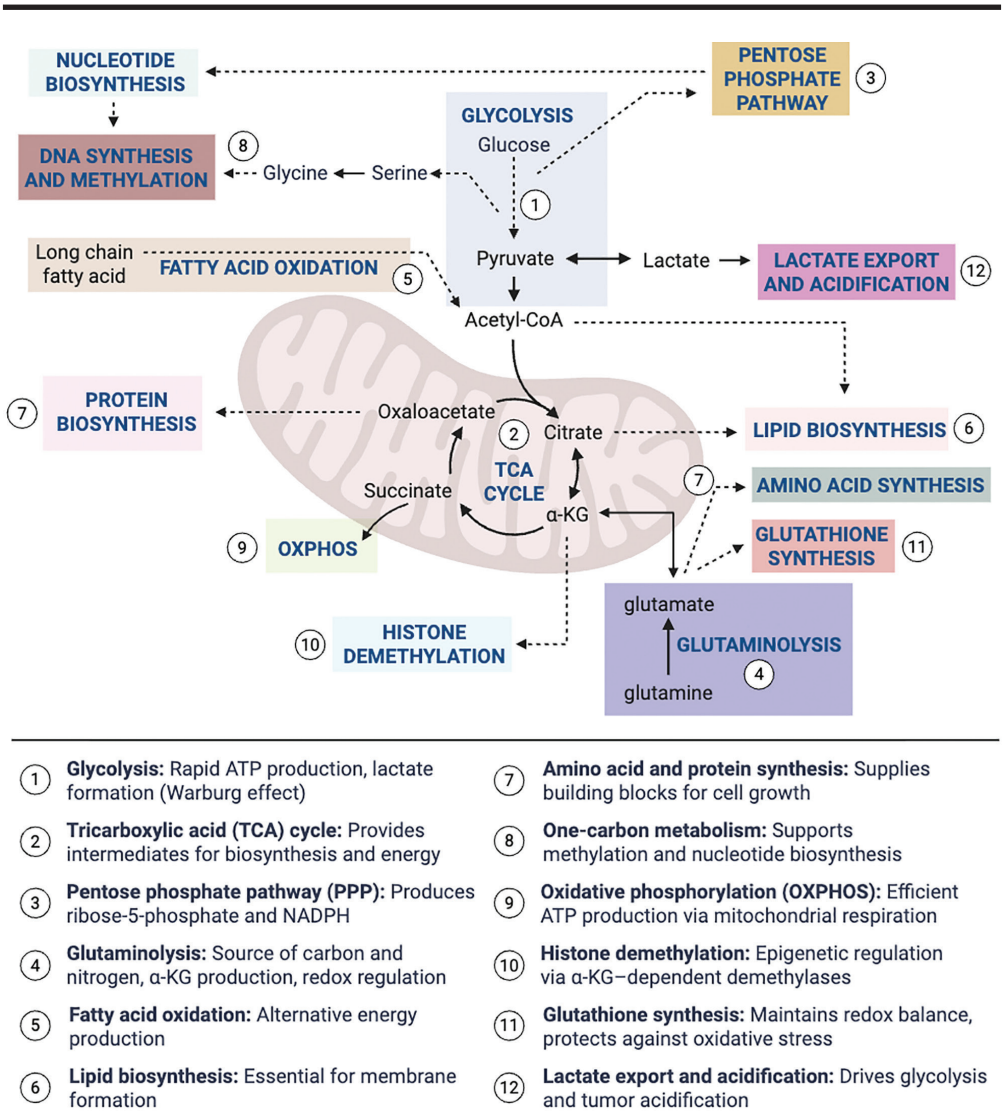

FIGURE 1. Metabolic reprogramming in cancer cells. Major metabolic pathways drive rapid proliferation, survival, and adaptation. By utilising glycolysis, glutaminolysis, and anabolic processes, tumour cells convert nutrients into energy and biomass while actively reshaping the tumour microenvironment (figure adapted from the PhD thesis of Dorottya Moldvai, 2025).

tumour tissue heterogeneity across different cancer types, influenced by their distinct genetic backgrounds. Consequently, metabolic reprogramming is now widely recognised as a defining hallmark of malignant transformation (25, 26) *(Figure 1)*.

ROLE OF THE TUMOUR MICROENVIRONMENT IN METABOLIC REWIRING

Available nutrients in the tumour microenvironment

The TME is a complex and dynamic ecosystem in which cancer cells interact with non-tumour cells, stromal cells, immune cells, and extracellular matrix (ECM) components. Rapid tumour growth creates high metabolic demands, requiring continuous access to nutrients that support energy production, biosynthesis, and cellular survival. Carbohydrates, amino acids, and lipids are critical nutrients in the TME, where various cellular components share or even compete for these resources. Cancer cells undergo rapid metabolic rewiring to efficiently utilise these substrates, enabling tumour growth. The metabolic landscape of a malignancy depends on localised oxygen/nutrient gradients and distinct oncogenic signalling. This results in a complex mosaic of metabolic phenotypes, manifesting as profound heterogeneity both between patients and within individual tumour tissues.

The metabolic reprogramming at tissue level enables metabolic symbiosis between tumour cells and surrounding stromal cells allowing for the optimisation of energy resources.

Carbohydrates

Carbohydrates, particularly glucose is a central nutrient in the TME, fuelling both the energy production and carbon intermediates required for rapid growth. While normal cells rely on mitochondrial oxidation and the TCA cycle for maximal ATP yield, cancer cells frequently undergo a metabolic shift known as the Warburg effect. This 'aerobic glycolysis' favours lactate production over OXPHOS, even when oxygen is available. Despite a lower ATP yield per glucose molecule, this reprogrammed pathway maintains a significantly higher metabolic rate, enabling tumour cells to rapidly generate energy and the precursors necessary for biosynthesis (27).

One of the benefits of shifting to Warburg glycolysis is the increased production of building blocks for cellular proliferation providing nucleic acids, amino acids, and lipids for new cells. Intermediates from aerobic glycolysis feed several biosynthetic pathways, including the pentose phosphate pathway (PPP), support nucleotide, lipid synthesis and maintain redox balance (28).

The increased glucose uptake by cancer cells is supported by several different mechanisms. Overexpression of glucose transporters GLUT1 and GLUT3 enhances glucose uptake. Several alterations in oncogenic signalling pathways, including PI3K/Akt/mTOR pathway signalling and hypoxia-inducible factor-1α (HIF-1α), stimulate glycolytic enzyme expression (such as hexokinase and phosphofructokinase), while also exhibiting increased activity, further promoting glycolytic flux (29–31).

During aerobic and/or anaerobic glycolysis, pyruvate is converted to lactate by lactate dehydrogenase A (LDHA), consequently resulting in the accumulation of lactate in the TME. Lactate, besides tumour-promoting effects (e.g. promoting angiogenesis, acidifying the TME, suppressing the immune system) may serve as an alternative energy source for surrounding cells within the oxygen-rich TME mediated by lactate dehydrogenase B (LDHB) (32) (reverse Warburg effect).

Amino acids

Amino acids are important for both metabolic and regulatory functions. In addition to serving as building blocks for protein synthesis, amino acids contribute to nucleotide synthesis, energy metabolism, redox regulation, and epigenetic modifications (33, 34).

One of the major challenges faced by rapidly dividing tumour cells is the accumulation of reactive oxygen species (ROS). The elevated metabolic activity increases ROS production, which, if left uncontrolled, can induce DNA strand breaks, subsequent mutations and/or apoptosis. To counteract this threat, cancer cells enhance their antioxidant defence mechanisms, maintaining redox balance and protecting cellular integrity. Several amino acids (glutamine, cysteine, glycine) play key roles in maintaining redox homeostasis. Glutamine is particularly important in cancer metabolism. After specific transports, mitochondrial glutaminolysis provides the carbon skeleton for non-essential amino acids and donates nitrogen for biosynthetic processes. Glutamine metabolism also supports glutathione synthesis to neutralise ROS and fuels the TCA cycle through anaplerosis, supporting both redox balance and reductive lipid biosynthesis. Several tumour types characterised by hyperactive growth signalling (e.g. KRAS mutations or mTOR hyperactivity) are highly glutamine addicted and frequently show elevated expression of glutamine transporters, glutaminase or glutamate dehydrogenase (35).

Additionally, serine and glycine drive one-carbon metabolism, providing for nucleotide biosynthesis and methylation. These pathways also generate NADPH, which contributes to antioxidant defence and reductive biosynthesis (21).

Beyond metabolism, amino acids influence gene expression and epigenetic regulation. Certain metabolic intermediates derived from amino acids serve as metabolic cofactors for epigenetic modifications. For example, S-adenosylmethionine (SAM) and acetyl-CoA serve as key donors for DNA and histone modification. Through these mechanisms, nutrient availability can directly affect chromatin structure and transcription patterns in cancer cells (36, 37).

Fatty acids and lipids

Fatty acids (FAs) and lipids function as structural membrane components, signalling molecules, and energy substrates. To sustain rapid proliferation and survive environmental stress, tumour cells often require significant alterations in lipid metabolism.

Cancer cells acquire lipids mainly in two routes. First, they enhance uptake of FAs from the ECM via specific transporters (e.g. CD36, a class B scavenger receptor). CD36 not only facilitates the entry of long-chain FAs and oxidised LDLs but also enhances the activation of several signalling pathways (such as mTOR, PPARγ, AMPK, and MAPK) (38). Second, cells activate de novo lipogenesis, converting acetyl-CoA into FAs. This endogenous synthesis reduces reliance on external sources and consumes NADPH, directly connecting lipid production to the cell's reductive capacity.

Under nutrient-deprived condition, cancer cells initiate fatty acid oxidation as a survival mechanism. By shuttling FAs into mitochondria for ß-oxidation, they generate acetyl-CoA, NADH, and FADH2, fuelling the TCA cycle and OXPHOS to maintain ATP levels.

Lipid metabolism is a double-edged sword regarding ROS. To prevent lipotoxicity and oxidative damage, excess FAs are sequestered into lipid droplets which serve as metabolic reservoirs. High ROS levels may further inhibit lipolysis to

sequester polyunsaturated FAs that are prone to peroxidation. Furthermore, tumour cells can induce mitophagy to clear ROS-generating damaged mitochondria (39). This balance is important to prevent ferroptosis (a form of regulated cell death driven by iron-dependent lipid peroxidation), by tightly regulating the saturation levels of the lipid pool and maintaining antioxidant defences, thereby promoting tumour cell survival under oxidative stress.

Hypoxia-driven metabolic adaptation in cancers

Hypoxia is both a driver and a consequence of malignant progression, creating a HIF (hypoxia-inducible factor)-dependent metabolic reprogramming that facilitates tumour proliferation, angiogenesis, immune escape, and therapy resistance. Rapid tumour expansion outpaces vascular oxygen delivery, generating a heterogeneous tissue oxygen gradient with normoxic, chronically hypoxic intermediate, and necrotic zones (40).

In normoxia, oxygen sensors (e.g. prolyl hydroxylases – PHDs) are able to hydroxylate HIF1α transcription factors, signalling the VHL protein to tag it for rapid proteasomal degradation. However, under hypoxic conditions, the inactivation of enzymes PHD1–3 stabilises HIF1α, leading to its nuclear accumulation and transcriptional activation of metabolic genes. HIF1α thereby promotes glucose uptake (increasing GLUT1-3) and anaerob glycolysis (e.g. increases the expression of hexokinases, LDHA, pyruvate-dehydrogenase-kinase 1, pyruvate kinase isoform 2), upregulates antioxidant defences to mitigate ROS-induced oxidative damage and driving a metabolic shift from oxidative phosphorylation to aerobic glycolysis (Warburg effect) (41). Cancer cells increase glutaminolysis to provide glutathione and carbon source for biosynthesis and shift the way of acquiring fats, often relying on external uptake rather than internal synthesis. In parallel, HIF1α increases the transcription of carbon anhydrase IX for pH normalisation and VEGF-A, which promotes angiogenesis. Additionally, the resulting abnormal vasculature – leaky, and poorly pericyte-stabilised – intensifies intratumoural hypoxia, a self-reinforcing cycle (40).

Importantly, the same HIF1α-mediated switch can be activated under normal oxygen levels (pseudohypoxia) through oncogenic signalling (PI3K/mTOR, RAS/RAF/MEK/Erk), or specific oncometabolites (e.g. succinate, fumarate, D-2-hydroxyglutarate, which competitively inhibit PHDs) that drives HIF1α translation independent of oxygen level (42).

Metabolic symbiosis within tumours

The TME is a heterogeneous ecosystem in which cancer cells, non-cancer cells, immune cells, cancer-associated fibroblasts (CAFs), adipocytes (CAAs), and endothelial cells take part in continuous metabolic crosstalk through nutrient competition, metabolite exchange, and reprogramming, consequently determining tumour development, progression, and immune evasion *(Figure 2).*

Proliferating tumour cells monopolise limited metabolic substrates and modify the milieu through metabolites such as lactate, adenosine, ROS, kynurenines, and lipid peroxides. In the TME, proliferating cancer cells outcompete the immune cells for nutrients, drive metabolic reprogramming that favours glycolysis and acidification; in addition, cancer cells can easily consume alternative substrates, including glutamine and fatty acid oxidation. Anti-tumoural, effector immune cells ($CD8^+$ T cells, NK cells, tumour-associated macrophages [TAM], M1 subset) depend on glycolysis and mitochondrial fitness with limited metabolic flexibility. In the tumour tissue, $CD8^+$ T cells are often glucose-starving and fail to maintain the glycolytic rate necessary for cytotoxicity and cytokine production, finally driving T cell exhaustion (43).

In contrast, pro-tumour immune cell populations, such as regulatory T cells (Treg), myeloid-derived suppressor cells (MDSCs), and TAM M2 phenotype, exhibit high metabolic flexibility; preferentially utilising fatty acid oxidation (FAO) and oxidative phosphorylation (OXPHOS), enabling them to thrive under hypoxia, acidosis, and nutrient scarcity, thereby reinforcing immunosuppression. The immune-metabolic rewiring leads to metabolic "zoning" within the TME: nutrient- and oxygen-deprived "cold" niches favour exhausted or suppressive immune phenotypes, whereas vascularised tertiary lymphoid structures with sufficient glucose support effector cell function, creating "immune hot" zones (43).

Accumulated toxic metabolites such as lactate, a major product of Warburg-type and pseudohypoxic glycolysis, act as active metabolic and signalling intermediates. Beyond acidifying the TME, lactate specifically supports immunosuppressive milieu (suppressing cytotoxic $CD8^+$ and NK cells, and promoting Treg expansion and M2 macrophage polarisation) (44). Additionally, cancer cells force autophagy of CAFs to serve building blocks/nutrients for themselves. Furthermore, cancer tissue-driven lactate fuels CAF metabolism (by reverse Warburg) and mediates histone lactylation, establishing a metabolic epigenetic feedback loop that stabilises tumour growth and survival by oncogenic, angiogenic, and glycolytic programs (HIF- and pseudohypoxia-driven) (45).

Moreover, oxidised lipids and lipid peroxides in lipid-rich TME induce ferroptotic susceptibility and mitochondrial dysfunction in effector T cells through CD36 receptor expression; concurrently promoting M2 macrophage and Treg phenotypes (46).

Finally, CAFs and CAAs, contributing to metabolic symbiosis, strongly remodel tumour tissue metabolism through coordinated lactate and fatty acid handling and promote immune suppression. Oxidative tumour cells and cancer-associated fibroblasts import lactate via MCT1, recycling the glycolytic output of hypoxic or pseudohypoxic cells and maximising overall tumour energetics (47). In parallel, CAAs release free fatty acids utilised by tumour cells for membrane synthesis, ß-oxidation, and stress adaptation, while driving lipid overload and exhaustion in $CD8^+$ T cells (46).

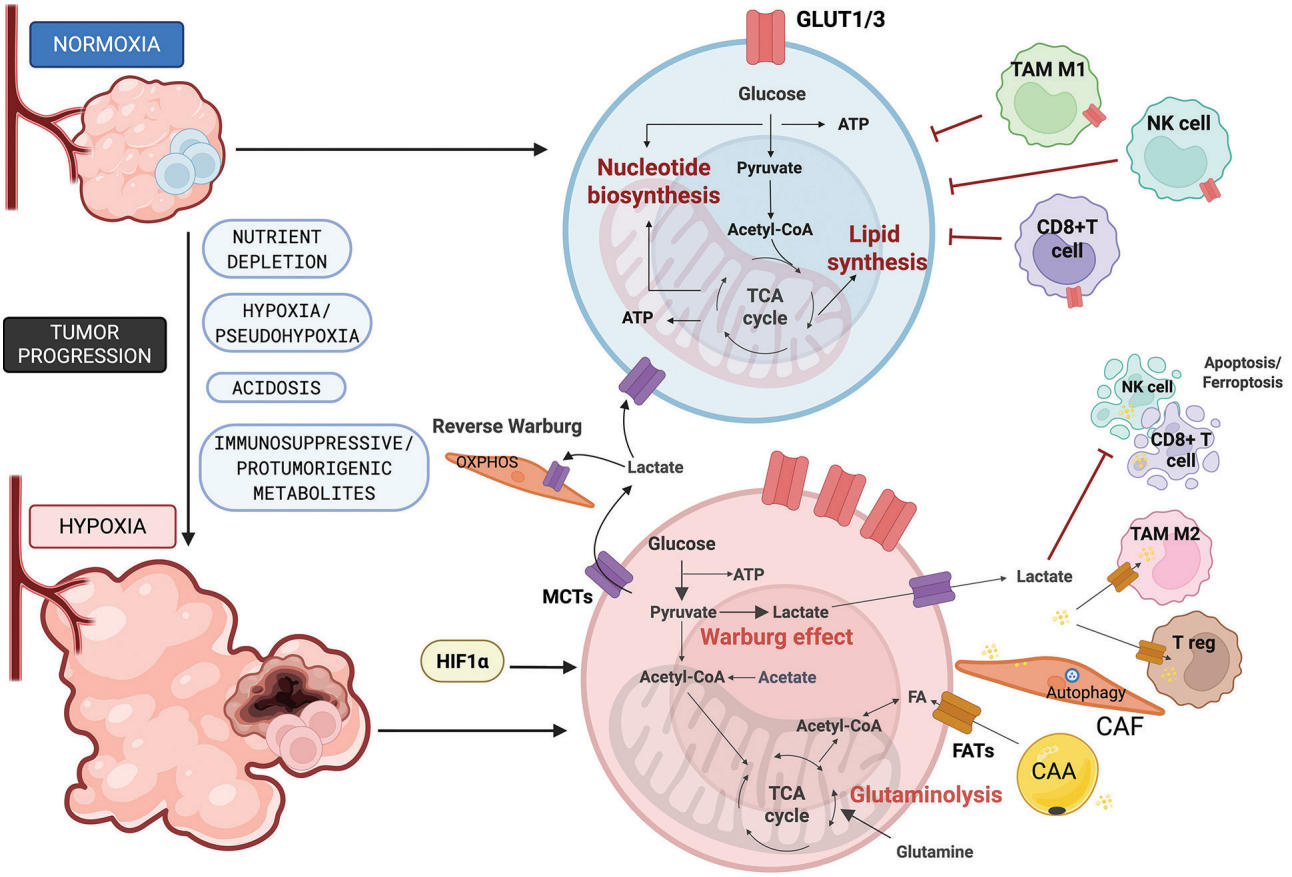

FIGURE 2. Metabolic alterations and symbiosis rewired cancer tissues. The figure simplifies the alterations from the normoxic, glucose-rich environment to nutrient-deprived, hypoxic, acidic, immunosuppressive regions of a tumour. The increasing distance from blood vessels and the decline in available nutrients force metabolic shifts, orchestrated by tumour cells and their adaptation mechanisms. Overexpressing glucose transporters (GLUT1/3) enable tumour cells to monopolise glucose utilisation, using OXPHOS or Warburg effect depending on hypoxia signalling activation. Under normoxic conditions ("immune hot zones"), activated immune cells – in addition to tumour cells – are also able to consume available glucose and attack/inhibit the growth of tumour mass. Simultaneously, decreasing oxygen and nutrient levels, combined with increasing lactate production (acidification) force additional adaptation mechanisms across all cells in the TME. To orchestrate nutrient utilisation, glucose uptake dominates in tumour cells, however, other nutrients also fill the energy demand of the rapidly growing/cycling tumour mass, e.g. cancer-associated adipocytes (CAA) and fibroblasts (CAFs, forced into autophagy) provide fatty acids (FAs) and/or glutamine. These supply metabolites or building blocks for the TCA cycle, macromolecule synthesis and/or redox balance maintenance. Tumour cells begin to overexpress fatty acid transporters (FATs) for the uptake of FAs, increasing external lipid usage for new biosynthetic mechanisms and/or fatty acid oxidation (FAO). CAFs can act as metabolic donors or "spare" glucose for tumour cells by FAO or lactate utilisation. In oxygen-rich areas, CAFs can uptake lactate, supporting metabolic symbiosis through reverse Warburg mechanism. In nutrient-deprived "fasting" conditions, regulatory immune cells (Tregs, M2 macrophages and MDSCs) adapt by increasing the expression of the fatty acid transporter CD36 as a survival mechanism to maintain immunosuppressive environment. In the competition for glucose, in glucose-poor areas $CD8^+$ T cells and NK cells attempt to adapt by FA uptake, thus lipid deposition from the TME. This lipid overload leads to metabolic exhaustion, functional silencing, and ultimately ferroptosis or apoptosis, neutralising anti-tumour immune defence. (The normoxic, hypoxic tumour cells are indicated with blue and red colour, respectively; GLUT1/3: glucose transporter 1-3, TAM M1 and M2: tumour-associated macrophages M1 and M2, Treg: regulatory T cells, NK cells: natural killer cells, FA: fatty acids, FATs: fatty acid transporters, MCTs: monocarboxylate transporters, CAF: cancer-associated fibroblasts, CAA: cancer-associated adipocytes, HIF1α: hypoxia induced transcription factor 1α.)

LIMITATIONS OF CURRENT PRECLINICAL MODEL SYSTEMS

The pharmaceutical industry is facing significant challenges nowadays, especially in the field of oncology drug development *(Figure 3)*. According to a 2022 Deloitte report, the average cost of developing a new therapeutic has risen to over 2.2 billion USD when accounting for failed candidates. In parallel, the duration of clinical trials has also increased, with oncology studies extending from approximately 9.4 years in 2018 to 11.6 years in 2022 (48). Similarly, based on 38 FDA-approved drugs in 2019, median and mean R&D costs were estimated at 708 million USD and 1.31 billion USD, respectively, after adjusting for capital costs and discontinued projects (49). An analysis of 631 clinical projects conducted in Japan between 2012 and 2022 reported phase-specific costs of 7.8 million

USD (Phase I), 15.95 million USD (Phase II), and 38.38 million USD (Phase III), with corresponding median durations of 20, 29, and 31 months, respectively. It is important to note that projects targeting approval outside Japan were much more expensive (median Phase III cost was 74.16 million USD in these cases) (50).

Based on the IQVIA Global Oncology Trends 2025 report, oncology research activity seems to expand in recent years, with clinical trial numbers increasing compared to 2019 and a growing focus on novel technologies such as cell and gene therapies, antibody-drug conjugates (ADCs), and multi-specific antibodies, which now represent a substantial proportion (35%) of ongoing studies. Although clinical development productivity has improved since 2019, it remains lower than in other therapeutic areas, despite a steady rise in the number of newly approved oncology drugs (novel active substances in oncology between 2015 and 2019 had an average of 16; in 2020–2024 had an average of 25). At the same time, patient access to innovative treatments is increasing and global spending on oncology medicines is rapidly growing, further driven by the adoption of novel and costly therapies in earlier lines of treatment across many high-income countries. Global spending on cancer medicines, measured at list prices, reached 252 billion USD in 2024 and is projected to increase to approximately 441 billion USD by 2029 (51).

The limitations of current preclinical model systems highly affect the success rate of drug development; these preclinical model systems were summarised and compared in *Table 1* (52–58). While in silico approaches (performed entirely on computer or via a simulation software) enable rapid and cost-efficient screening, their predictive value remains dependent on experimental validation. Traditional in vitro models, particularly two-dimensional (2D) cell cultures fail to recapitulate the complexity of the TME. These systems lack key components such as stromal and immune cells, vascularisation, and physiologically relevant gradients, resulting in discrepancies between in vitro findings and clinical outcomes (52).

In vivo models attempt to address some of these limitations by incorporating living organisms. However, they are limited to model species-specific differences, next to high costs, ethical concerns, and limited applicability in certain therapeutic areas (e.g. immunotherapy). Notably, a substantial proportion of drug candidates that demonstrate efficacy in animal models ultimately fail in clinical trials (53).

In response, there has been growing interest in advanced three-dimensional (3D) in vitro models, including organoids,

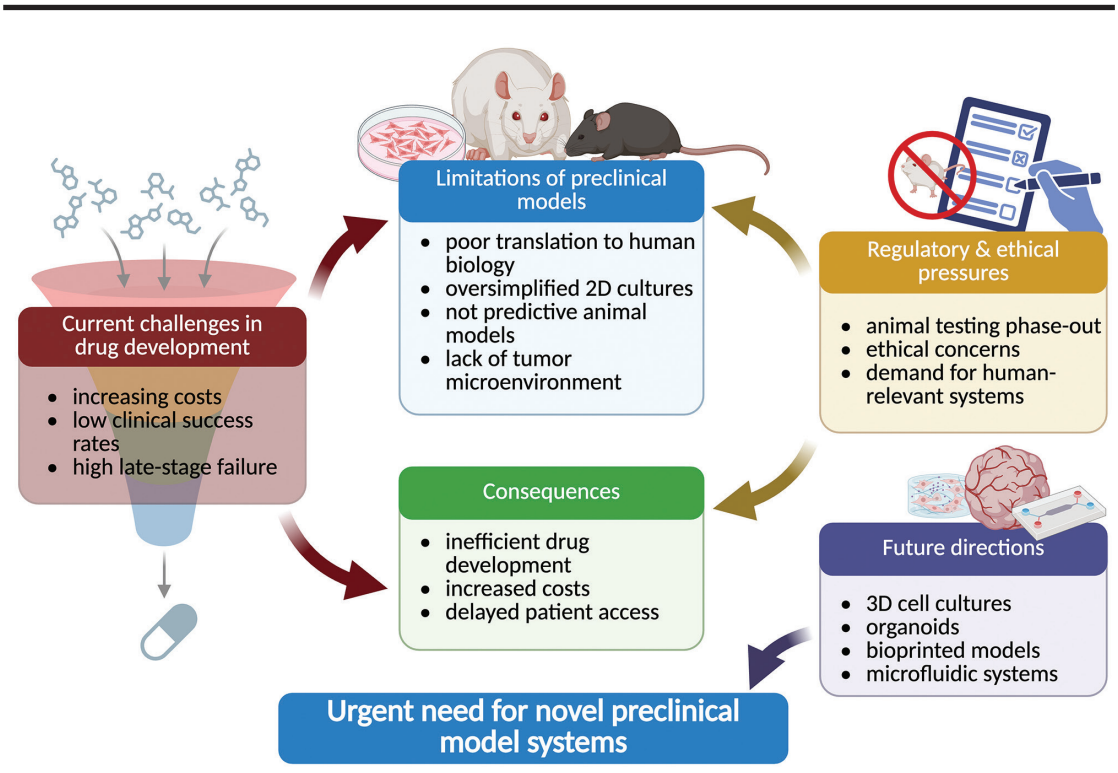

FIGURE 3. Key challenges and solutions in drug development. Schematic overview of the key challenges in current drug development, highlighting the limitations of preclinical models, regulatory and ethical pressures, and their consequences. These factors collectively drive the urgent need for more predictive, human-relevant systems.

TABLE 1. Comparative overview of different preclinical model systems (in silico, 2D cell culture, spheroids, organoids, 3D bioprinting, and in vivo)

Feature / Model	In silico	2D culture	Spheroids	Organoids	3D bioprinted culture	In vivo
oxygen gradient	–	–	++	++	+++	+++
nutrient diffusion	–	–	++	++	+++	+++
cell-cell interactions	–	+	++	+++	+++	+++
metabolic heterogeneity	–	–	++	+++	+++	+++
cell-ECM interactions	–	–	+	+++	+++	+++
tissue architecture	–	–	+	+++	+++	+++
tumour microenvironment (TME)	–	–	+	++	+++	+++
vascularisation modelling	–	–	–	+	++	+++
immune system components	–	–	–	+	++	+++
drug penetration modelling	–	+	++	++	+++	+++
reproducibility	+++	+++	++	++	++	–
cost/time efficiency	+++	+++	++	++	–	–
scalability (high-throughput)	+++	+++	++	+	+	–
human relevance	+	+	++	+++	+++	++
genetic manipulability	+++	+++	++	++	++	+
ethical constraints	+++	+++	+++	++	++	–

The values in the table show the degree of representation for each characteristic: - indicates the absence, while +++ signifies strong availability/representation.

spheroids, and 3D bioprinted tissue mimetic structures. These systems better mimic the structural and functional characteristics of human tumours, including cellular heterogeneity and microenvironmental interactions. Among these, 3D bioprinting represents a promising approach, allowing precise spatial organisation of multiple cell types and ECM components. Furthermore, integration with microfluidic technologies, such as organ-on-a-chip systems, enables dynamic modelling of physiological conditions, including fluid flow and nutrient gradients. Collectively, these innovations hold significant potential to improve the predictive accuracy of preclinical testing and enhance the efficiency of drug development (52, 54).

3D BIOPRINTED TUMOUR MODELS AND CURRENT METABOLISM RESEARCH

3D bioprinting has emerged as a new and rapidly developing human tissue modelling technology, allowing for the precise deposition of living cells in specified biomaterials (bioinks), and added further defined materials (e.g. defined nutrients, growth factors) to recreate the heterogeneous tissue and (in cancer research) the tumour microenvironment (TME) (55).

To apply 3D bioprinting in tissue modelling is generally a multi-stage workflow (56). Their steps depend on the modelled tissues and the applied technologies (e.g. extrusion-based, droplet-based, digital light processing and laser-assisted printing). The workflow includes many preprocessing steps: 1. selecting/designing the optimal structures using CAD (computer-aided design) software which allows to build different compartments from e.g. tumour core to periphery, mimicking oxygen and nutrient gradients and cellular heterogeneity; 2. selecting/preparing (producing/isolating) different cell types (cell lines) and the matching bioinks (e.g. GelMA, alginate-based inks, decellularised ECMs); 3. establishing the applicable printing (speed, temperature, pressure, etc.) and post-printing processes (e.g. bioink polymerisation, maintaining tissue formation and growth); 4. selecting and establishing the protocols for the finally used growth, sensitivity or further molecular biology tests (e.g. proliferation/viability tests, cell recovery and further tissue handling technologies could be established before performing the experiments). For 3D bioprinting to be successful, the bioinks and cells must meet several strict criteria which need to be considered: 1. inks must be biocompatible and non-toxic, with appropriate permeability for the oxygen and nutrient diffusion; 2. the bioink must have the right viscoelasticity and stiffness compared to the real tissue; 3. the printing process must not

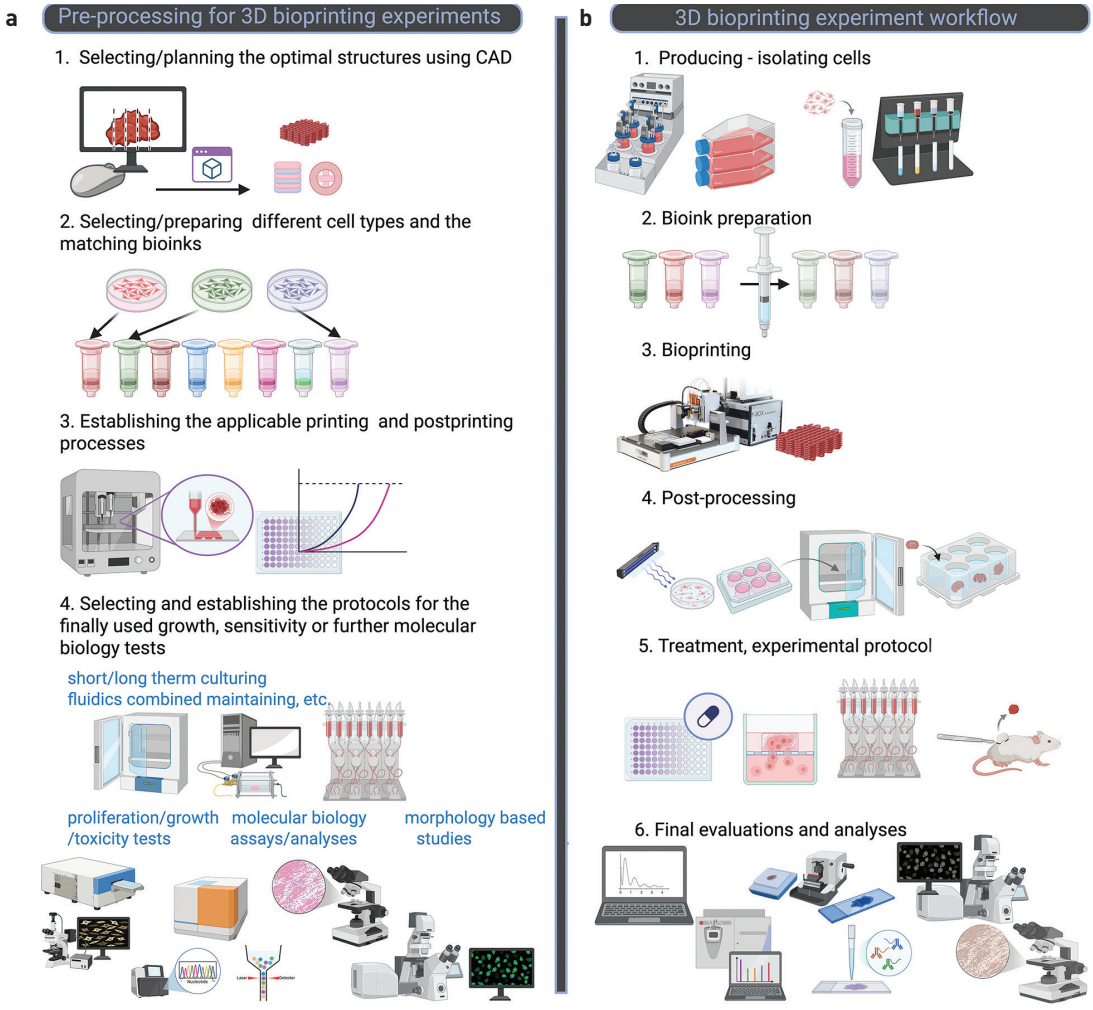

FIGURE 4. Experimental design and optimisation/pre-processing steps – workflow using 3D bioprinted cancer models. a) The main steps and processes necessary before performing 3D bioprinting with cancer and/or primary isolated cells from human cancer tissues. Prior to the initiation of 3D bioprinting several critical parameters must be standardised beyond designing the printing architecture. The primary focus involves the optimisation of cellular components, whether utilising established cell lines or isolating primary cells from tissue dissociation. This is followed by the selection of biocompatible bioinks tailored to the specific mechanical and biological requirements of the target tissue. The establishment of the bioprinting procedure itself requires assessment of cell viability and the structural integrity of the newly formed tissue, the selection of the experimental timeline (the initiation and duration of treatments). Furthermore, the crucial preparatory steps involve the optimisation and validation of analytical assays specifically for 3D constructs (establishing and optimisation of molecular biology techniques, proliferation assays, and metabolic tests) to ensure they provide accurate data from the printed "tissues". These optimisation steps are mandatory prerequisites for ensuring the reproducibility and validity of the subsequent experimental series. b) The experimental workflow necessitates the expansion or isolation of the required cells (cell populations), the preparation of specialised bioinks and scaffold materials, and the execution of the bioprinting process. This is followed by post-printing maturation, the application of experimental treatments, and the final analysis of the resulting data.

damage the living cells and their tissue forming and/or growing capacity (pressure, temperature, shear stress, cross-linking toxicity, etc.); 4. the ink and finally, the built matrix must have predictable biodegradation rates to maintain the printed structures during the experiment (57); additionally, 5. the timeline for printing, tissue maturation, treatments and sample preparation and handling must be optimised for the final goal of analysing e.g. drug sensitivity or molecular/pathomorphological changes *(Figure 4).*

In the last decade, despite the high interest in 3D bioprinting research and 3D bioprinted research model applications, the literature remains heavily weighted toward review publications (more than 40% of all 3D bioprinting publications in cancer research), which highlights that more

robust inflow of primary research data is required for future innovation. In terms of cancer types studied, the literature is dominated by high-prevalence and highly malignant cases, such as breast, lung, gastrointestinal and skin malignancies or brain, pancreas tumours (58).

A tumour is a dynamic ecosystem that evolves over time, especially when under the stress of chemotherapy or immunotherapy, and this evolution is in correlation with their spatial and temporal heterogeneity. The recently published experimental results and their current advancements can revolutionise cancer research and cancer model building by developing the creation of highly complex, tissue-specific TME. As evidenced by recent studies, the field has progressed from the addition of tumour cells to simple 3D printed matrix scaffold and printed spheroid cultures to sophisticated multicellular models, including tumour-stroma, tumour-adipocytes, and vascularised systems, such as the BLISH technology – blood-lymphatic integrated system with heterogeneous melanoma spheroids (59) created by 3D bioprinting and different types of bioinks (60). 3D bioprinted models are nicely developing across various cancer types (glioblastomas, pancreatic, breast, gastric cancers and melanomas) *(Table 2)*. These models mainly use extrusion-based bioprinting (or droplet-based printers) with different (cell type-dependent) bioinks/biomaterials (alginate, cellulose, GelMA, collagens, matrigel or decellularised ECM, etc.). Biomaterial development is crucial in future cancer models, especially in patient-derived tumour cell culture applications. The modelling of complex tumour development (growth, pressure changes, and tissue hardening/fibrosis) will be performed in the future by hybrid systems (as 4D bioprinting) that utilise "smart" materials capable of responding to environmental changes, such as pH, ion, or cytokine concentrations. This allows for the real-time modification and monitoring of matrix stiffness. Regarding these, the current developments are advancing in four primary directions: 1. 4D bioprinting (creation of structures responsive to external or internal stimuli that track the dynamics of tumour changes in both space and time); 2. integrating biosensors (built-in detectors for the continuous monitoring of hypoxia, lactate levels, and pH fluctuations); 3. representing structural complexity (representing the processes of vascularisation and metastasis through tumour-on-a-chip and microfluidics-integrated models); 4. application of patient-derived models (in vitro testing of personalised therapies and drug combinations using cells obtained directly from the patient's tumour). The ultimate goal is the creation of unified integrated platforms that, beyond the 3D structure, incorporate a fourth dimension (encompassing temporal changes, bio-sensing, complex organisational processes, and individual patient specificity), thereby enabling the long-term, live following of tumours under controlled in vitro conditions (61).

Metabolic monitoring in these new models is beginning to incorporate optical sensors to detect metabolic changes (the alterations of specific metabolite concentrations) in real-time, which could be essential for understanding how tumours reprogram their energy consumption to survive and develop drug/treatment resistance mechanisms. Furthermore, the integration of metabolic monitoring, e.g. the use of optical sensors in a pancreas cancer model (62), is a critical milestone. This metabolic insight could be a key to developing more effective targeted therapies and accelerating the transition of these models from the model research laboratories to basic, pharmaceutic research, and clinical applications.

Our previous research studies show that while 2D cell cultures and 3D spheroids differ significantly from xenografts, 3D bioprinted tissue-like structures closely recapitulate in vivo characteristics (63, 64). Using the Shannon diversity index, we found nearly identical metabolic enzyme expression patterns and – in the in vitro studies – similar drug sensitivities between 3D bioprinted models and xenografts. Immunohistochemistry confirmed that 3D bioprinting successfully mimics the spatial and tissue heterogeneity of metabolic enzymes *(Figure 5),* ECM and adhesion proteins, the complexities that are absent in 2D cell cultures. These results validate 3D bioprinted models as superior models for replicating better in vivo morphological characteristics and metabolic profiles (58, 63, 64).

The main advantage of 3D bioprinting in metabolism research is the ability to recreate the pathological gradients of the TME. The controlled thickness of the printed materials and the density of the cells can influence and induce normoxic shell and hypoxic core within a single printed spheroid model, as well as in a more complex multicellular structure.

CURRENT CHALLENGES AND FUTURE PERSPECTIVES – TRANSLATIONAL FRONTIERS IN 3D BIOPRINTING: FROM TUMOUR MICROENVIRONMENT TO PERSONALISED ONCOLOGY

Advances in 3D bioprinting facilitate studies of tumour-TME interactions and tumour progression (65). By incorporating non-tumour cells to increase complexity, these models provide insights into drug sensitivity and immune evasion. Moreover, the integration of fluidics and long-term maintenance of complex 3D bioprinted tissue cultures allows for the study of tumour evolution and metastatic progression.

Current challenges include the standardisation and integration of 3D bioprinting with other technologies, particularly for drug development and toxicity studies. Extrusion-based (EbP) and droplet-based bioprinting (DbP) facilitate high cell viability, the use of diverse biomaterials, reduced costs, and increased automation (66). DbP enables higher resolution and high-throughput testing (67). Additionally, the integration of cell-spheroid printing with organoid technology increases tissue mimicking complexity (68, 69). Consequently, it has been suggested that 3D bioprinting be categorised into basic cell bioprinting

TABLE 2. Current applications of 3D bioprinting across tumour types

Tumour		Research focus	Cell line	Bioprinting type	Bioink	Model complexity	Year	Author
Brain cancer	Glioma	tumour-stroma interactions	GSC23 (GBM) hMSC	extrusion bioprinting, core-shell method	sodium alginate, gelatin, fibrinogen	multicellular (tumour cells + stroma MSCs)	2017	Dai X et al.
		high throughput drug screening	patient-derived cells	droplet bioprinting (384-well plate)	alginate	monoculture	2020	Golebiewska A et al.
		high throughput drug screening	patient-derived cells	droplet bioprinting (96-well plate)	methacrylated ColI, HA	monoculture	2020	Maloney E et al.
		modelling tumour heterogeneity	TS576 (GBM) CW468 (GBM stem cells) HUVEC	DLP-based bioprinting	GMHA, GelMA	multicellular (tumour region + endothelial region + stiff ECM + soft ECM)	2021	Tang M et al.
		CAR-T therapy evaluation	patient-derived cells SNC109 (CAR-T cells) mock-T cells	DLP-based droplet bioprinting (96-well plate)	GelMA, HAMA	multicellular (tumour cells + CAR-T cells)	2026	Li X et al.
		drug response (CDK4/6 inhibitors) and TME connections	patient-derived cells	extrusion bioprinting	sodium alginate, gelatin, GelMA	monoculture or multicellular (tumour cells + astrocytes)	2026	Kaps P et al.
		chemokine microenvironment	patient-derived MSCs U251 (GBM) DK-MG (GBM)	extrusion bioprinting	CELLINK Laminink521, CELLINK Bioink	multicellular (tumour cells + stromal MSCs)	2024	Zielniok K et al.
		TME influencing progression	patient-derived cells U87 (GBM)	extrusion bioprinting	GelMa, HA	monoculture	2025	Schroyer KAR et al.
		anticancer cell therapy system	NHF1 (fibroblast) LN229 (GBM) U87 (GBM) GBM8 (GBM stem cells)	CLIP bioprinting	GelMa	multicellular (bioprinted fibroblast constructs + tumour cell suspension)	2026	Kass L et al.
		high throughput drug screening assay	JHH520 (GBM) cAP–0002GFP (primary brain endothelial cells) cAP–0031 (astrocytes) cAP–0030 (pericytes)	extrusion bioprinting	fibrinogen	multicellular (endothelial cells + astrocytes + pericytes in ring pattern, tumour cells in the centre)	2024	Tung YT et al.
		response to current therapy (TMZ + radiation)	patient-derived cells hMSC	extrusion bioprinting	CELLINK Bioink	multicellular (tumour cells + hMSC)	2024	Oliver L et al.
		heat-inducible CAR-T cell therapy	CW468 (GBM stem cells) U87 (GBM) primary T-cells	SLA	GelMa, GMHA	multicellular (tumour cells + T cells)	2024	Tang M et al.

TABLE 2. ***(cont.)*** Current applications of 3D bioprinting across tumour types

Tumour		Research focus	Cell line	Bioprinting type	Bioink	Model complexity	Year	Author
Brain cancer	Glioma	high throughput drug screening assay, which mimics intratumoral heterogeneity	U87MG (GBM) T98G (fibroblast)	extrusion droplet bioprinting	alginate	monoculture or multicellular	2024	Lee G et al.
		vascularisation of GBM in different TME	GSC23 (GBM) HUVEC	extrusion bioprinting	alginate, gelatin	multicellular (tumour-laden scaffolds in interior dish + endothelial cells in exterior dish)	2023	Wang X et al.
Gastrointestinal	Colorectal cancer	prediction of chemotherapy effectivity	patient-derived cells	extrusion bioprinting	alginate, GelMa	monoculture	2023	Sun H et al.
		colorectal cancer model with glycosylated hydrogels	HT29 (CRC) patient-derived cells	extrusion bioprinting	HA, functionalised gelatin	monoculture	2023	Cadamuro F et al.
		printing with colorectal cancer stem cells	SW480 (CRC) patient-derived cells	extrusion bioprinting	GelMa-nanoclay	monoculture	2022	Zhang Y et al.
		screening oncolytic viruses	patient-derived cells	extrusion bioprinting	CELLINK Bioink	monoculture + viral infection	2023	McGuckin C et al.
		demonstration of oncolytic viral chemotherapeutic delivery	JVE-103 (CRC) JVE-253 (CRC)	extrusion bioprinting	CELLINK Bioink	monoculture + viral infection	2025	McGuckin C et al.
		modelling tumour invasion and predicting treatment responses	patient-derived cells	acoustic bioprinting	GelMa	multicellular (healthy organoid + tumour)	2022	Chen H et al.
		multicellular model for drug screening	SW480 (CRC) THP-1 (AML) HUVEC	extrusion bioprinting	gelatin, sodium alginate	multicellular (tumour cells + stromal cells)	2023	Wang P et al.
		ECM affects oncolytic virus efficacy	HT29 (CRC) CAF	extrusion bioprinting	gelatin, alginate, fibrinogen	multicellular (tumour cells + CAF)	2024	Marquette CA et al.
		modelling selective efficacy of drugs	HT29 (CRC) HCT116 (CRC)	extrusion bioprinting	dynamic bioink composition	monoculture	2025	De Nobrega DD et al.
		predicting patient-specific drug response	patient-derived cells	extrusion bioprinting	PCL, alginate	multicellular (tumour cells + immune cells)	2025	Han J et al.
	GBC	modelling TME and intratumoural heterogeneity	GBC-SD (GBC) CCC-ESF-1 (fibroblast) THP-1 (AML) HUVEC	extrusion bioprinting	gelatin, alginate	tetra-culture (tumour cells + endothelial cells + fibroblasts + immune cells)	2024	Jin Y et al.

TABLE 2. ***(cont.)*** Current applications of 3D bioprinting across tumour types

Tumour		Research focus	Cell line	Bioprinting type	Bioink	Model complexity	Year	Author
Gastrointestinal	Pancreatic cancer	mimicking TME for drug screening	BxPC-3 (PC)	extrusion bioprinting	Coll, GelMa, alginate	monoculture	2025	Gato-Diaz U et al.
		patient-derived 3D models drug sensitivity correlates with clinical outcome	patient-derived cells	extrusion bioprinting	alginate, gelatin	monoculture	2025	Sun H et al.
		tumour-stroma interactions in drug screening	BxPC-3 (PC) dermal fibroblasts	extrusion droplet bioprinting	GelMa	multicellular (tumour cells + fibroblasts)	2023	Huang B et al.
		desmoplastic spheroids better mimic the native tumour	PANC-1 (PC) fibroblasts	extrusion bioprinting	GelMa, gelatin, collagen I	mono- or multicellular (tumour cells + fibroblasts)	2024	Wei X et al.
		cancer-stroma model for drug screening	PANC-1 (PC) fibroblasts	extrusion bioprinting	GelMa, HAMA	multicellular (tumour cells + fibroblasts)	2024	Monteiro MV et al.
	Gastric cancer	constructing a physiologically relevant model	patient-derived cells HGC27 (GC)	extrusion bioprinting	GelMa	monoculture	2025	Ju M et al.
		drug response and gene profile	patient-derived cells fibroblasts	extrusion bioprinting	dECM (gastric)	multicellular (tumour cells + fibroblasts)	2025	Choi Y et al.
		high-throughput drug screening and decision-making model	patient-derived cells	light-assisted 3D bioprinter	GelMa, HAMA	monoculture	2025	Du L et al.
	Liver cancer	simulating cancer initiation from primary hepatocytes	mouse-derived primary hepatocytes	extrusion bioprinting	gelatin, sodium alginate	monoculture	2025	Zhao L et al.
		TME model for progression and drug response tests	SMMC-7721 (HCC) HDF (fibroblasts) HUVEC	extrusion bioprinting	GelMa, gelatin	multicellular (tumour cells + fibroblasts + endothelial cells)	2024	Wang X et al.
		dynamic culture for drug screening	Hep3B (HCC)	extrusion droplet bioprinting	alginate	monoculture	2025	Joshi P et al.
Genitourinary cancers	Prostate cancer	multicellular metastasis model	C4-2B (PrC) PC-3 (PrC) M-22 (fibroblasts)	extrusion bioprinting	GelMa, CSMA, HAMA	multicellular (tumour cells + fibroblasts)	2023	Xu K et al.
		modelling bone metastasis of PrC	mBMSCs MC3T3 (pre-osteoblasts) C4-2B (prostate cancer)	extrusion bioprinting	dECM (porcine), alginate	multicellular (tumour cells + MSCs + osteoblasts)	2025	Chen X et al.

TABLE 2. ***(cont.)*** Current applications of 3D bioprinting across tumour types

Tumour		Research focus	Cell line	Bioprinting type	Bioink	Model complexity	Year	Author
Genitourinary cancers	Bladder cancer	tissue-on-a-chip model to simulate immunotherapy	T24 (bladder carcinoma) MRC-5 (fibroblast) HUVEC (endothelial cell) THP-1 (human monocytic leukaemia cell line)	multilayered tissue-on-chip, extrusion bioprinting	GelMa	multicellular (bladder carcinoma, fibroblasts, endothelial cells and macrophages)	2022	Lee S et al.
		tissue-on-a-chip model to mimic the TME	T24 (bladder carcinoma) 5367 (bladder carcinoma) MRC-5 (lung fibroblast) HUVEC THP-1 (AML)	tissue-on-chip, extrusion bioprinting	GelMa	multicellular (tumour cells + fibroblasts + endothelial cells + macrophages)	2021	Kim JH et al.
		high throughput tissue-on-a-chip	T24 (bladder carcinoma) 253J (bladder carcinoma) MRC-5 (lung fibroblast) MBT2-luc (mouse bladder cancer) HUVEC THP-1 (AML)	tissue-on-chip, extrusion bioprinting	GelMa	multicellular (tumour cells + fibroblasts + endothelial cells + macrophages)	2023	Choi J et al.
		modelling drug screening	5637 (bladder carcinoma) T24 (bladder carcinoma)	extrusion bioprinting	GelMa	monoculture	2019	Kim MJ et al.
		cytotoxic and chemosensitising effects	RT4 (bladder carcinoma)	extrusion bioprinting	alginate, gelatin	monoculture	2021	Miranda MA et al.
		quantifying intratumoral heterogeneity	patient-derived cells	inkjet-based single-cell bioprinting	Matrigel	monoculture	2020	Yoon WH et al.
	RCC	intercellular transfer of mitochondria	786-O (RCC)	extrusion bioprinting	gelatin, alginate, Coll	monoculture	2021	Herrada-Manchón H et al.
		organoids for personalised therapy	patient-derived cells	extrusion bioprinting	gelatin, alginate, Matrigel, GelMa	monoculture	2025	Mao S et al.
Breast cancer	Luminal A	tumour-adipose tissue model	MCF-7 (BC) ADSC	extrusion bioprinting	alginate, gelatin	multicellular (tumour cells + stromal cells)	2020	Chaji S et al.
		tumour-adipose tissue model	MCF-7 (BC) ADSC	extrusion bioprinting	dECM (porcine), GelMa, alginate, Coll	multicellular (tumour cells + stromal cells)	2022	Blanco-Fernandez B et al.

TABLE 2. ***(cont.)*** Current applications of 3D bioprinting across tumour types

Tumour		Research focus	Cell line	Bioprinting type	Bioink	Model complexity	Year	Author
Breast cancer	Luminal A	stroma-mediated modulation of ECM and radiosensitivity	MCF-7 (BC) HC-6071 (CAF) H-6071 (fibroblast) HUVEC	extrusion bioprinting	ColMA, HAMA, IKVAV peptide gel	multicellular (stromal periphery + tumour centre)	2024	Desigaux T et al.
		tumour-stroma model for drug testing	HBF MDA-MB-231 (BC) MCF-7 (BC) TeloHAEC (endothelial cell)	extrusion bioprinting	dECM, HAMA	multicellular (tumour region + stroma region)	2023	González-Callejo P et al.
		metastasis to bone	MDA-MB-231 (BC) MCF-7 (BC) osteoblast HUVEC	SLA	GelMA, PEGDA, hydroxyapatite	multicellular (tumour cells + bone + vessel)	2020	Cui H et al.
		paracrine interaction analysis	MCF-7 (BC MDA-MB-231 (BC)	extrusion bioprinting	Coll, alginate, HA, gelatin	multicellular (adipose tissue + tumour spheroids)	2024	Jeong W et al.
		stem cell and EMT markers	MCF-7 (BC) T47D (BC) MDA-MB-231 (BC)	extrusion bioprinting	CELLINK Bioink	monoculture	2025	Szostakowska-Rodzoś M et al.
		metastasis-on-a-chip	MCF-7 (BC) MDA-MB-231 (BC) SVEC4-10 (endothelial cell)	extrusion bioprinting	ColMA, HAMA, GelMa	multicellular (tumour cells + endothelial cells)	2025	Chang TW et al.
		gravitational forces modulate the invasiveness	MCF-7 (BC) MDA-MB-231 (BC)	SLA	GelMa, PEGDA	monoculture	2025	Breideband L et al.
		cancer-stroma microenvironment model	ADSC MCF-7 (BC) MDA-MB-231 (BC)	extrusion bioprinting	GelMa	multicellular (tumour cells + ADSC)	2025	Romanazzo S et al.
		drug screening	MCF-7 (BC) MDA-MB-231 (BC)	DLP-based bioprinting	GelMa	monoculture	2025	Mei X et al.
	Luminal B .	metabolic characterisation	ZR751 (BC)	extrusion bioprinting	gelatin, alginate, methylcellulose	monoculture	2022	Dankó T et al.

TABLE 2. ***(cont.)*** Current applications of 3D bioprinting across tumour types

Tumour		Research focus	Cell line	Bioprinting type	Bioink	Model complexity	Year	Author
Breast cancer	TNBC	modelling tumour-stroma microenvironment	MDA-MB-231 (BC) ADSC	extrusion bioprinting	thiol-modified HA	multicellular (tumour cells + stromal cells)	2021	Horder H et al.
		investigating metastasis to bone	MDA-MB-231 (BC) osteoblast HBMMSC	SLA	GelMA, nHA	multicellular (tumour cells + osteoblasts + MSCs)	2016	Zhou X et al.
		bone metastasis model and effects of cold atmospheric plasma treatment	MDA-MB-231 (BC) HBMMSC	extrusion bioprinting	alginate, gelatin	multicellular (tumour cells + stromal cells)	2025	Bouret LM et al.
		effect of stiffness on cell behaviour and drug resistance	MDA-MB-231 (BC) patient-derived cells	extrusion bioprinting	HA, alginate, gelatin, Matrigel	monoculture	2026	Jeong W et al.
		cancer stemness, invasion, and drug efficacy	MDA-MB-231 (BC) HASCS	extrusion bioprinting	dECM (porcine), GelMa, alginate, Coll	multicellular (tumour cells + HASCs)	2025	Blanco-Fernandez B et al.
		drug dynamics in a tissue-like environment	MDA-MB-231 (BC) fibroblasts	extrusion bioprinting	TALG, MCMC	multicellular (tumour cells + fibroblasts)	2025	Troncoso-Afonso L et al.
Gynaecologic cancer	CervC	measurement of drug diffusion	HeLa (CervC)	extrusion bioprinting	alginate, mannitol	monoculture	2023	Becconi M et al.
		bioprinted CervC model	HeLa	extrusion bioprinting	HEC, alginate	monoculture	2021	Gospodinova A et al.
		comparison with 2D cultures	HeLa	extrusion bioprinting	gelatin, alginate, fibrinogen	monoculture	2014	Zhao Y et al.
	Ovarian cancer	role of GLUT1 transporter in CAFs in OC characteristics	SKOV-3 (OC) CAF	extrusion bioprinting	gelatin, alginate	multicellular (tumour cells + CAF)	2025	Zhang D et al.
		patient-specific therapy response	patient-derived cells	extrusion bioprinting	GelMa	monoculture	2024	Zhang J et al.
		drug screening platform	SKOV-3 (OC)	extrusion bioprinting	aCNC, aHA, gelatin nanocomposite	monoculture	2024	Wang Y et al.
		immunomodulatory properties of cowpea mosaic virus on OC	ID8/Defb29/VEGF-A-Luc (murine OC) macrophage	DLP-based bioprinting	GelMa	multicellular (tumour cells + macrophages)	2024	Xiang Y et al.
		high throughput drug screening	SKOV-3 (OC) MeWo (CAF)	extrusion bioprinting	alginate, gelatin	multicellular (tumour cells + CAF)	2022	Baka Z et al.

TABLE 2. ***(cont.)*** Current applications of 3D bioprinting across tumour types

Tumour		Research focus	Cell line	Bioprinting type	Bioink	Model complexity	Year	Author
Skin cancer	Melanoma	TME affecting drug screening	451Lu, WM164 (melanoma) Hs27, HDF (fibroblast)	extrusion bioprinting	dECM (porcine)	multicellular (tumour cells + fibroblasts)	2024	Vázquez-Aristizabal P et al.
		drug-induced apoptosis	A375 (melanoma)	extrusion bioprinting	GelMa	monoculture	2024	de Villiers M et al.
		testing drug-loaded microneedle therapy on skin cancer model	B16-F10 (mouse melanoma) fibroblasts keratinocytes HUVEC	extrusion bioprinting	GelMa, alginate, gelatin	multicellular (tumour cells + fibroblasts + keratinocytes + endothelial cells)	2024	Barros NR et al.
		CSC-based malignant melanoma model for personalised cancer treatment	A375, Mel-1 (melanoma) HUVEC MSCs fibroblasts keratinocytes	extrusion bioprinting	agarose, Coll	multicellular (three layered melanoma skin texture)	2023	López de Andrés J et al.
		blood-lymphatic integrated system	Malme-3M, SK-MEL-2, SK-MEL-28, A375-P (melanoma) HDF HDMEC HDLEC melanocytes	extrusion bioprinting	dECM (skin, vascular tissue), alginate	multicellular (melanoma + blood vessel + lymphatic vessel)	2022	Cho WW et al.
		construction of cancer-vascular model	SK-MEL-28 (melanoma) HUVEC	extrusion bioprinting	dECM (skin, vascular tissue), alginate	multicellular (tumour cells + endothelial cells)	2021	Kim BS et al.
Lung cancer	Lung adenocarcinoma	vascularised lung cancer model	A549 (NSCLC) MRC-5 (fibroblast) HUVEC	extrusion bioprinting, core-shell method	GelMA/Matrigel, HAMA/fibrin	multicellular (tumour cells + fibroblasts + endothelial cells)	2026	Gao Q et al.
		tumour progression and cell-ECM interactions	A549 (NSCLC)	two-photon mediated laser-assisted bioprinting	PEGDA/gelatin	monoculture	2025	Dong Y et al.
		radiotherapy research	A549 (NSCLC) primary fibroblast	extrusion bioprinting	alginate, gelatin	multicellular (tumour cells + fibroblasts)	2024	Mei Y et al.
		perfusable lung cancer model	H358 (NSCLC)	DLP-based bioprinting	GelMa	monoculture	2023	Mei Y et al.
		anticancer drug evaluation	A549 (NSCLC)	inkjet-based droplet bioprinting	alginate, HA, peptides	monoculture	2023	Dong Q et al.

TABLE 2. ***(cont.)*** Current applications of 3D bioprinting across tumour types

Tumour		Research focus	Cell line	Bioprinting type	Bioink	Model complexity	Year	Author
Lung cancer	Lung adenocarcinoma	cancer-on-a-chip model to study metastasis and therapy response	A549 (NSCLC) fibroblast	extrusion bioprinting	PDMS, GelMA	multicellular (tumour cells + fibroblasts)	2022	Das P et al.
		3D culture environment regulating actin cytoskeleton	LL/2 (mouse Lewis lung carcinoma) A549, NCI-H1975 (NSCLC)	DLP-based bioprinting	polyethene oxide-GelMA	monoculture	2021	Hu Q et al.
Musculoskeletal tumours	Osteosarcoma	drug screening platform	MG-63 (osteosarcoma)	extrusion bioprinting	dECM (osteosarcoma), alginate, methylcellulose, GelMA	monoculture	2026	Domigues MF et al.
		osteosarcoma-on-a chip model for drug testing	MG-63 (osteosarcoma) MSCs HUVEC	extrusion bioprinting	GelMa, hydroxyapatite	multicellular (tumour cells + MSCs + endothelial cells)	2025	Jaiwal C et al.
		identification of therapeutic targets	HOS, 143B, U2-OS (osteosarcoma)	extrusion bioprinting	GelMa, HAMA	monoculture	2022	Lin Y et al.
	Rhabdomyosarcoma	macromolecular crowding tuned extracellular matrix deposition	RH30, RD (rhabdomyosarcoma)	extrusion bioprinting	agarose, Matrigel	monoculture	2022	D'Agostino S et al.

Abbreviations: aCNC: aldehyde-modified cellulose nanocrystals, ADSC: adipose-derived stromal cells, aHA: aldehyde-modified hyaluronic acid, AML: acute myeloid leukaemia, BC: breast cancer, CAF: cancer associated fibroblasts, CervC: cervical cancer, CLIP: continuous liquid interface production, ColI: collagen I, ColMA: methacrylated collagen, CRC: colorectal cancer, CSMA: methacrylate-modified chitosan, dECM: decellularised extracellular matrix, DLP: digital light process, ECM: extracellular matrix, GBC: gallbladder carcinoma, GBM: glioblastoma, GC: gastric cancer, GelMa: gelatin methacrylate, GMHA: glycidyl methacrylate hyaluronic acid, HA: hyaluronic acid, HAMA: methacrylated hyaluronic acid, HASCS: human adipose derived stem cells, HBF: human breast fibroblast, HBMEC: human brain microvascular endothelial cells, HBMMSC: human bone marrow-derived mesenchymal stromal cells, HCC: hepatocellular carcinoma, HDF: human dermal fibroblast, HDLEC: human dermal lymphatic endothelial cells, HDMEC: human dermal microvascular endothelial cells, HEC: hydroxyethyl cellulose, hMSC: human mesenchymal stem cells, HUVEC: human umbilical vein endothelial cells, mBMSCs: primary mouse bone marrow mesenchymal stem cells, MCMC: methacrylated carboxymethyl cellulose, MSCs: mesenchymal stem cells, nHA: nanocrystalline hydroxyapatite, OC: ovarian cancer, PEGDA: polyethylene glycol diacrylate, PC: pancreatic cancer, PDMS: polydimethylsiloxane, PCL: polycaprolactone, PrC: prostate cancer, RCC: renal cell carcinoma, SLA: stereolithography, TALG: thiolated alginate, TME: tumour microenvironment

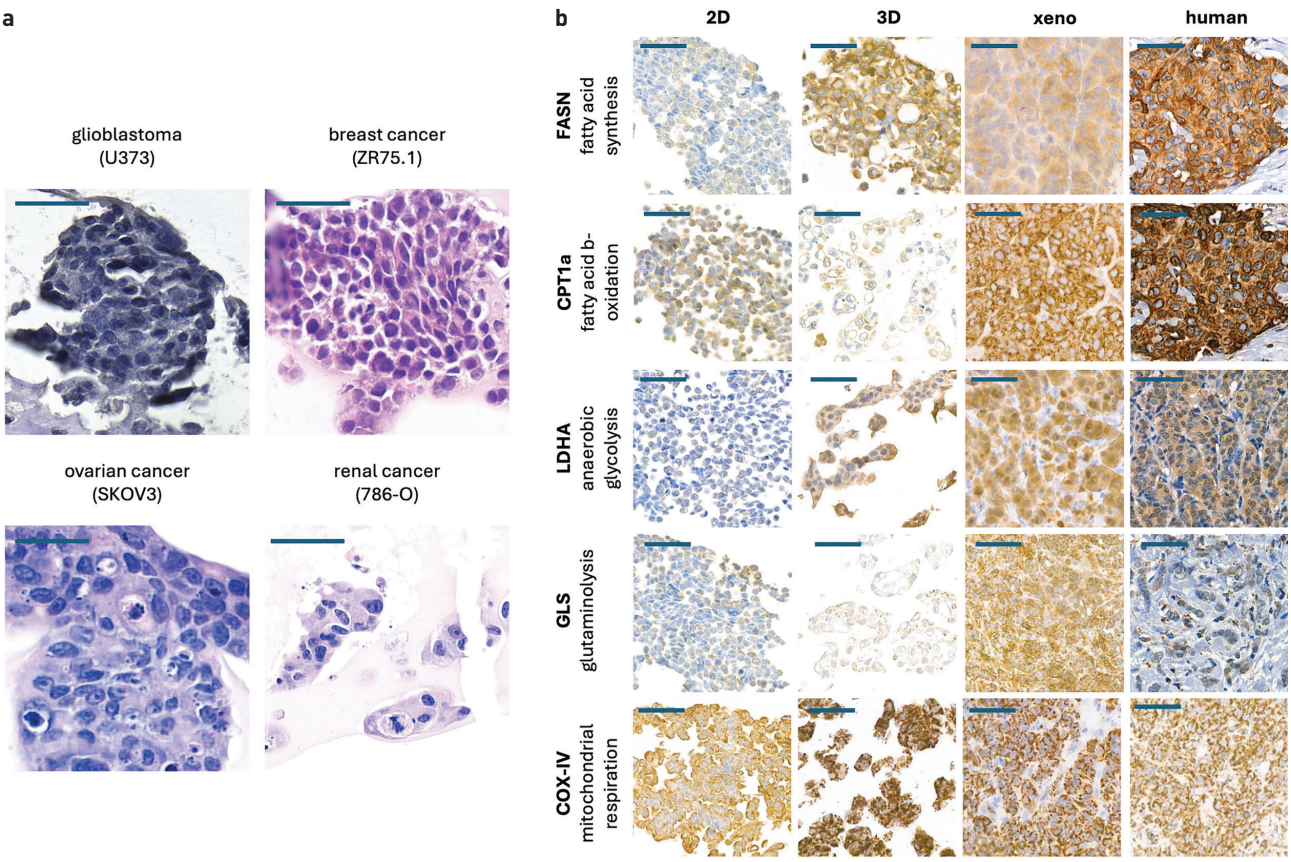

FIGURE 5. a) Haematoxylin-eosin staining of 3D bioprinted tumour models from different tumour types (glioblastoma – U373; breast cancer – ZR75.1; ovarian cancer – SKOV3; renal cancer – 786-O). Scale bar = 50 µm. **b)** Comparison of different metabolic pathways in 2D monoculture, 3D bioprinted tissue-mimetic structures, SCID xenograft model and human breast cancer tissue. (FASN: fatty acid synthase – fatty acid synthesis – Cell Signaling, #3180, 1:50; CPT1a: carnitine palmitoyltransferase I – fatty acid oxidation – abcam, ab128568, 1:1000; LDHA: lactate dehydrogenase A – anaerob glycolysis – Cell Signaling, #3582, 1:400; GLS: glutaminase – glutaminolysis – abcam, ab156876, 1:100; COX-IV: cytochrome C oxidase (complex IV) – mitochondrial respiration – Cell Signaling, #4850, 1:1000). Cell line: ZR75.1. Scale bar = 50 µm. DAB (3.3'-diaminobenzidine) chromogen, with haematoxylin counterstaining.

(suitable for standardised drug testing) and advanced aggregate bioprinting, which allows for the construction of more complex tumour models (70).

Further challenges involve combining 3D bioprinted models with immune cells to illustrate the crosstalk within the TME. These models are continuously evolving through the incorporation of macrophages, CAR T cells or various types of CAFs and immune cells (71–73).

Increased stiffness in correlation with tissue-like 3D structures and ECM accumulation is highly associated with invasiveness and metastatic capacity. Therefore, 3D in vitro models serve as powerful tools in metastasis studies, bridging the gap between traditional 2D metastatic property assays and in vivo metastasis models. In these advanced models, researchers utilise microcapsules (74) or precisely deposited bioinks to mimic the vessel structures and metastatic niches (for instance, the combined use of hyaluronic acid and GelMA in scaffolds for tumour-vessel-bone cocultured 3D bioprinted breast cancer cells) (75, 76). These models combined with fluidic systems will enhance time- and site-dependent mimicking potential in the future (77).

The final goal is to apply this technology in pre-clinical testing to replace animal experiments with human cell-based disease models. Furthermore, patient-derived (PD) cells can be used with 3D bioprinting for personalised drug selection. While the first patient tumour tissue-derived bioprinting attempts began at the end of the last decade, comparative predictive analyses are now ongoing across many cancer types. The highest numbers of PD models are currently in gastrointestinal tumours (gastric, colorectal, pancreatic cancers, cholangiocarcinomas and liver cancers), lung cancers, breast cancers, and glioblastomas with emerging reports in bladder cancer and melanoma (78–83).

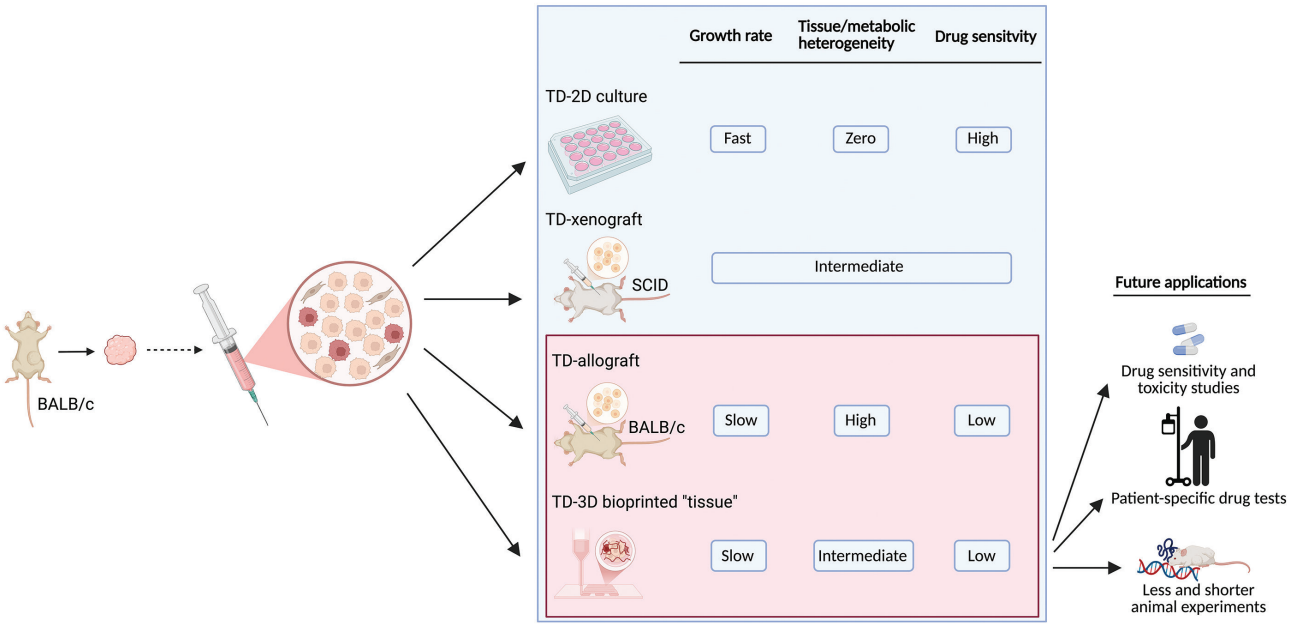

FIGURE 6. 3D bioprinted models accurately reflect the in vivo recurrence of the original tumours. Based on our comparative study using tissue-derived cells in 2D cell, 3D bioprinted in vitro cultures, as well as in vivo xenograft (SCID mice) and allograft (BALB/c) models, we found that the 3D bioprinted tumour-like tissue structures effectively mirrored the slower growing, lower drug sensitivity and reasonable tissue heterogeneity of in vivo tumours. This work demonstrates that 3D bioprinting tumour models more closely mimic in vivo therapeutic responses and tissue characteristics than traditional in vitro and patient-derived methods. Therefore, these evolving models could enhance the efficacy of drug sensitivity/toxicity tests including personalised drug tests, additionally, could help replacement in using animal models [figure adapted from Petővári et al., 2025 (64)].

Our own research significantly contributes to the development of patient-derived 3D bioprinting. Based on our recently published results using primary isolated cells from mouse mammary tumours, we compared drug sensitivity results from 3D bioprinted tissue models with 2D, xeno- and allograft models derived from the same cell source. Notably, the 3D bioprinted models reflected the results obtained in the recurrence-modelling allograft cases *(Figure 6).* Our experiments were performed using a mouse breast cancer cell line ("tumourous mice as patients"). Our recent works focus on applying patient-derived cells and comparing the clinical relevance of the use of our 3D bioprinted tissues for personalised drug screening applications (64).

These findings clearly indicate the accelerating development of this technology, not only in basic research and modelling but also in PD models and personalised treatment. However, it is crucial to recognise that the development of complex PD bioprinted models requires a multidisciplinary approach. Close collaboration is essential among biologists, biomedical scientists, biophysicists and bioengineers, as well as oncologists, pathologists, radiologists and surgeons. Through the synergy of these fields, increasingly realistic in vivo-like models and long-awaited PD drug pre-selection tests will emerge. Achieving this will require not only standardisation but also extensive clinical validation where patient outcomes are directly compared with the results of these bioprinted assays (60, 70, 77).

Funding: This research and the associated infrastructure were supported by Semmelweis University and the National Research, Development and Innovation Office of Hungary (TKP2021-EGA-24 [A.S.], NKFI-K-142799 and 152525 [A.S.], and NKFI-PD-146373 [I.K.]); 2025.2.1.1-EKÖP-2025-00014 [D.M.]; EFOP-3.6.3-VE-KOP-16-2017-00009 [F.S.]; the National Bionics Program (ED_17-1-2017-0009); and the University Research Scholarship Programmes funded by the Ministry for Culture and Innovation.

Acknowledgments: The authors would like to express their sincere gratitude to everyone who contributed to their 3D bioprinting research. We offer special thanks to Andrea Kárpáti and Csilla Kurucz for their invaluable assistance with histological sectioning, and to András Sztodola for his dedicated work conducting the animal experiments. We also thank Regina Raffay, Titanilla Dankó, Hunor Sántha, Risa Miyaura and Dániel Sztankovics for their contributions during the initial stages of our 3D bioprinting projects.

All figures were generated via BioRender.

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