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Recent advances in 3D bioprinted tumor models for personalized medicine

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ABSTRACT

Cancerous tumors are among the most fatal diseases worldwide, claiming nearly 10 million lives in 2020. Due to their complex and dynamic nature, modeling tumors accurately is a challenging task. Current models suffer from inadequate translation between *in vitro* and *in vivo* results, primarily due to the isotropic nature of tumors and their microenvironment's relationship. To address these limitations, hydrogel-based 3D bioprinting is emerging as a promising approach to mimic cancer development and behavior. It provides precise control over individual elements' size and distribution within the cancer microenvironment and enables the use of patient-derived tumor cells, rather than commercial lines. Consequently, hydrogel bioprinting is expected to become a state-of-the-art technique for cancer research. This manuscript presents an overview of cancer statistics, current modeling methods, and their limitations. Additionally, we highlight the significance of bioprinting, its applications in cancer modeling, and the importance of hydrogel selection. We further explore the current state of creating models for the five deadliest cancers using 3D bioprinting. Finally, we discuss current trends and future perspectives on the clinical use of cancer modeling using hydrogel bioprinting.

Introduction

Despite the rapid progress in modern medicine, cancer remains one of the deadliest diseases worldwide. According to the World Health Organization's (WHO) 2020 data, there were nearly 20 million new cases of various types of cancer and almost 10 million cancer-related deaths worldwide, accounting for nearly one in six deaths overall [1]. The National Cancer Institute defines cancer as a disease in which some cells grow uncontrollably and spread to other parts of the body, making the disease highly diverse as tumors can grow in almost any part of the body [2]. Fig. 1 shows the most common types of diagnosed cancer cases and the leading causes of cancer-related deaths in the USA in the years 2010–2020. The data are limited to the USA because of being the most available and reliable in this decade. While the data may vary based on gender and location, the values in the USA are mostly proportional to those globally collected for the world cases. In 2020 breast cancer was the most common type of cancer reaching over 227 thousand cases in the USA and over 2.2 million cases in the world. However, the deadliest cancer in 2020 was lung cancer, accounting for 138 thousand deaths in the USA and 1.8 million deaths in the world [3,4]. Over the past recent years, the total incidence for cancers has been increasing, especially breast and prostate cancers, while early diagnosis techniques have also been developing quite fast. Advancement of new surgical techniques, and targeted therapies have resulted in significant drop of death count, which is especially the case for lung cancer. What is worth mentioning is that the mortality of cancers steadily decreased each year, as the total case count of presented cancer types has increased by 11%. Overall, the death count has reduced by around 4% over the last decade. It's essential to note that these figures only reflect diagnosed cancer cases, and the actual number of affected individuals may be much higher, particularly in regions with limited access to healthcare.

Despite the millions of cases, billions of dollars spent on cancer research, and the joint effort of the greatest scientists in the world, tumors are considered as one of the main health threats in the modern world [5]. There is no single treatment method applicable for all kinds of cancer. One of the most common therapies is the usage of chemotherapeutic agents, but is far from ideal, mostly because of the high burden on the organism, variable success rate, and the long recovery time [6].

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Other promising methods for treating tumors are targeted approaches such as the usage of nanoparticles or therapeutic peptides, immunotherapies and notch targeted strategies [7]. However, none of them is close to becoming the gold standard of tumor treatment, mainly due to the high costs of the therapy and usefulness only for selected cancer types. It is worth mentioning that most of our knowledge about cancer development is based on *in vivo* observations of patients and histopathological studies. There is still very little knowledge of why tumor cells start to appear, how they develop into cancers and why some of them are malignant. The graphical interpretation of cancer development is presented in Fig. 2b. In order to find better therapies, those questions have to be answered first. In the last years, it was observed that long-used 2D *in vitro* cancer models are too simplified to properly assess the viability of new therapies [8]. The main aspects which have to be included in new models are the gradient growth of tumor cells in the extracellular matrix (ECM), thus isotropic influence on cell viability, cell-cell adhesion and signaling [9]. Early studies show that tumor cells can change their 3D microenvironment to generate local hypoxia, which can lead to excessive cell proliferation [10]. Even much more expensive animal studies are also not satisfactory due to their limited ability to mimic human diseases [11]. Few new approaches were developed to overcome those downsides, mainly: organotypic slices of cancer tissues, multicellular tumor spheroid models, multilayered cell cultures, and cell-seeded scaffolds. While some advancements in knowledge have been achieved through these methods, they suffer from issues with the reproducibility of results and necessitate years of experience in the field to implement successfully [12].

A response to the need to deepen understanding of the genesis and characteristics of cancer is bioprinted models, which are supposed to mimic cancerous *in vivo* environments very precisely. In contrast to previously developed methods, 3D bioprinting can be used for manufacturing reproducible models, which are much cheaper to produce and can involve the usage of different cell types and complex structures. The first research papers devoted to obtaining three-dimensional cancer models started to appear at the turn of 2013 and 2014. Since that time, the field of "cancer 3D bioprinting" has been developing rather rapidly – according to Scopus data 3 articles appeared in 2013 and grew up to 125 published articles in 2022 with the "cancer 3D bioprinting" phrase in title, abstract, or keywords. Most of the works focus on breast, liver and lung cancers (Fig. 2a). 3D bioprinting is a special variant of three-dimensional additive manufacturing that, instead of traditional filament or granulate, uses bioink. The most common type of bioink is scaffold-based bioink, where cells are loaded in polymer hydrogels of natural or synthetic origin and fabricated into 3D printouts [13]. Hydrogels are used for bioprinting applications due to their unique properties and the possibility of closely imitating human tissues, but they have to meet several requirements to be bioprintable and to finally be used as a scaffold for cancerous research models. They have to have very specific rheological properties, excellent biocompatibility to create a beneficial environment for cells' development, suitable mechanical strength, processability, etc. [14,15]. 3D models obtained this way may be a major breakthrough in cancer research and may help to further understand the mechanism and genesis of this complex disease. In the following review, the comprehensive description of materials, methods and different types of cells used in cancer bioprinting will be presented along with some new perspectives that appeared in this field more recently and may hopefully bring improvements in cancerous tumors treatment and patient care.

Bioprinting contribution to modeling complex cancer tumors

Bioprinting methods in cancer modeling

3D bioprinting is the direct evolution of the conventional 3D printing

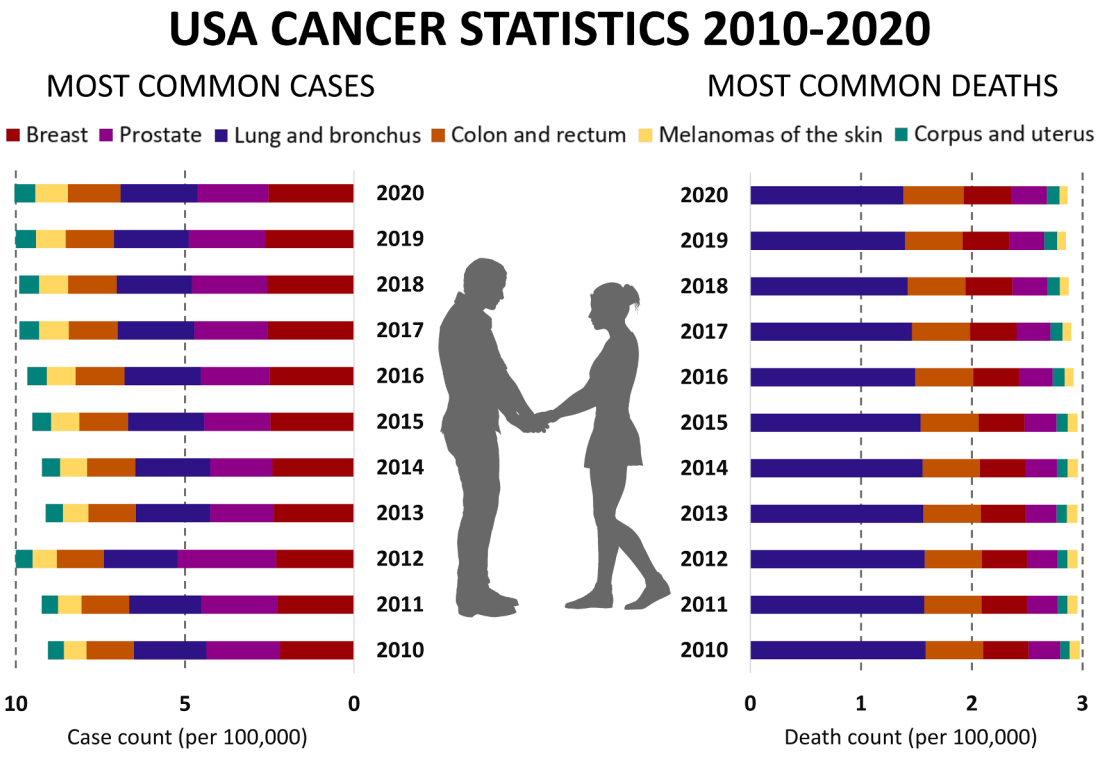

Fig. 1. Comparison of the number of most common cases of cancer and most common deaths caused by cancer in the USA in the years 2010–2020. Only in 2020 six most common types of cancer summed up to over 1 million new cases with breast cancer being the most frequently appearing reaching over 227 thousand new cases. On the other hand, the deadliest type of cancer in 2020 occurred to be lung cancer with over 138 thousand deaths, while all six types totaled over 286 thousand deaths [3]. Two trends can be seen: constant growth of new cancer cases each year, most likely due to improvement in cancer diagnosis, and a slight decrease in deaths, most probably connected to the development of medical knowledge in cancer treatments.

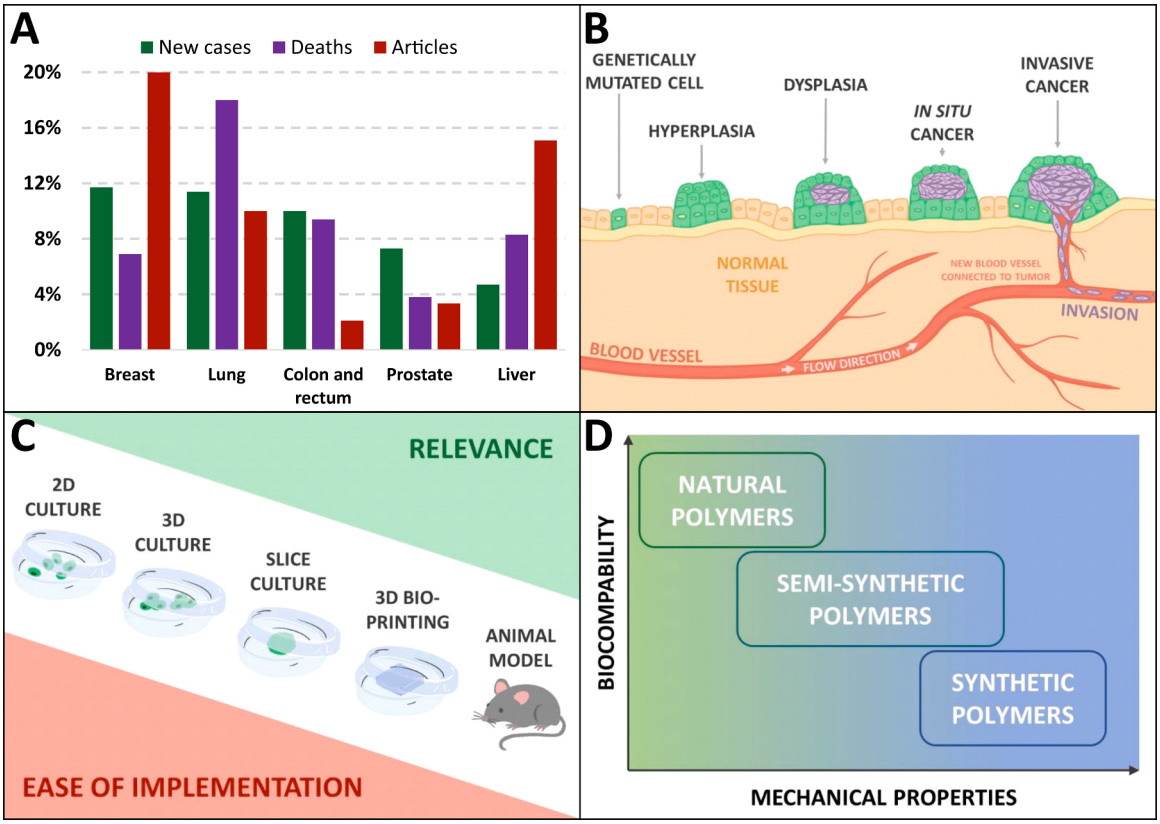

Fig. 2. A. A comparison of new cases and deaths caused by cancer in 2020 with the percentage of articles focused on specific types of cancerous models. Articles researching the five most common cancer types globally take up almost 50% of all cancerous models' articles and what is more, almost 20% of all articles focus on models for breast cancer (Scopus database, search within title and keywords: (cancer OR tumor) AND (bioprinting OR bioprinted) AND (*type of cancer*)) [4]. B. Development of cancer. Five stages of cancer growth are shown, starting with mutation of a single cell, through hyperplasia and dysplasia to the creation of *in situ* cancer and its invasive form with its own newly developed blood vessels. Own elaboration based on [46]. C. A visual presentation of the dependence between ease of implementation and relevance of different types of models used for cancer treatment. The more complex the model, the more relevant it gets in terms of tissue-mimicking and the harder it gets to implement such a model. The 3D bioprinted model is the closest relevance to the living organism model while still being ethically acceptable. Own elaboration based on [47]. D. The relation between biocompatibility and mechanical properties for natural, semi-synthetic, and synthetic polymers used in 3D bioprinting. Natural-based hydrogels are characterized by good and excellent biocompatibility but have poor mechanical properties in contrast to synthetic polymers that have better mechanical properties, but usually poor biocompatibility. The compromise between these properties is semi-synthetic polymers with higher mechanical values than natural polymers while maintaining biocompatibility on satisfying levels.

method. Bioprinters use bioinks, which are materials containing cells suitable for automated processing. Those materials include *e.g.*, alginate, collagen, fibrin, gelatin, gellan gum, silk, polycaprolactone (PCL) or poly (ethylene glycol) (PEG) [16]. Before the actual bioprinting process, the crucial pre-bioprinting stage takes place. The 3D anatomical visualizations are prepared using different high-resolution imaging techniques, such as magnetic resonance imaging (MRI) or computerized tomography (CT). Then obtained medical images are processed and 3D models are designed using computer-aided design (CAD) and manufactured with layer-by-layer deposition. Besides the modeling process, pre-bioprinting also contains an essential step of isolation and differentiation of cells, that are going to be seeded on the printed scaffolds [17]. After the pre-bioprinting stage is completed, the scaffolds are finally printed out. There are many techniques of 3D bioprinting, but there are three most common methods, that are also successfully used in bioprinting of 3D cancerous models: extrusion, stereolithography (SLA), and inkjet bioprinting. Extrusion bioprinting is the most popular technique. In this technique, the material is controllably ejected using pneumatical or mechanical force through a small diameter nozzle. The primary advantage of this process lies in its simplicity, which facilitates easy customization and enables the use of diverse materials, including those with high viscosity values. Additionally, the process is cost-effective, making it an attractive option. The disadvantage of extrusion bioprinting is most of all a shear stress that has a huge negative impact on cell viability [18,19]. The second of the most commonly used techniques, which is inkjet 3D printing can be divided into two main methods: continuous and drop-on-demand (DOD) inkjet printing. The continuous method uses liquid-forming continuous droplets, that are controlled by the charge of droplets and an electric field, while in DOD method the droplets are obtained only when reaching the ejection signal – a controlled pressure pulse causes an ejection of a single droplet through a nozzle. Due to the complexity of continuous inkjet printing, only DOD technique has found its application in 3D bioprinting up to this date. Drop-on-demand inkjet bioprinting allows high efficiency and high precision of geometry of the printout, but at the same time, its main limitation is the right selection of bioink, which must be characterized by low concentration and suitable, restricted value of viscosity to avoid clogging and incorrect geometry of the printout. Due to those reasons, the variety of possibly used materials is much narrower in contrast to extrusion 3D bioprinting [20]. Stereolithography 3D bioprinting technique is the last of the 3 most popular 3D processing methods. Contrastingly to previous techniques, SLA requires the preparation of a prepolymer solution that is photosensitive. To obtain a scaffold using this technique, a previously prepared image is used as a mask and projected onto bioink containing photosensitive solution mixed with cells. The bioink is then photocrosslinked by a chemical reaction initiated by a photoinitiator. SLA allows to combine high precision of the geometry of the printouts (even highly complex ones) with a high speed of

fabrication, but at the same time, selection of materials suitable for SLA bioink is very limited. Only a few hydrogels containing acryloyl or alkenyl functional group meet the requirements of biocompatibility, cytotoxicity and photosensitivity, mostly hydrogels based on modified polymers, such as PEGDA (poly(ethylene glycol) diacrylate) or GelMA (gelatin methacryloyl) [21].

Hydrogels used in bioprinting of cancerous tumors

The series of hydrogels properties made them the most prominent materials in 3D cancer bioprinting. Hydrogels are well-studied and widely used biomaterials, not only in additive manufacturing. The adjustability of mechanical, biodegradation, and optical properties made them one of the obvious choices in tissue model preparation. On top of that, hydrogels offer a superior ability to hold live cells, which is essential in tumor studies [22]. The main characteristics of hydrogels for bioprinting applications obtained from polymers of natural, semi-synthetic, and synthetic origin are compared in Table 1. It is important to note that the properties of polymers may vary depending not only on the type of polymer used but also on its concentration, as well as the method of cross-linking applied. Formerly most hydrogels used for tissue engineering applications were based on polymers of natural origin, such as alginate, agarose, collagen, gelatin, and hyaluronic acid [23]. As shown in Table 1, all of the aforementioned materials exhibit satisfactory biocompatibility and promote high cell viability and proliferation. Despite this great advantage, the bioprintability and reproducibility of natural-origin hydrogels can be highly challenging, due to their rather poor mechanical properties (Fig. 2d) [24]. Therefore, hydrogels made of semi-synthetic and synthetic polymers have emerged into the spotlight of 3D bioprinting research. Semi-synthetic polymers, such as the most commonly used GelMA, are obtained by modifying natural polymers. As shown in Table 1. below, in comparison with natural polymers, semi-synthetic ones have improved stability and tunability, while maintaining their biocompatibility [25]. While hydrogels made from synthetic polymers, such as PEG often exhibit high shape fidelity and excellent bioprintability, they are typically associated with poor biocompatibility and cell viability as compared to other hydrogel scaffolds. The interaction of these materials with cells can be improved by modifying their structure or by combining synthetic with natural polymers [23]. Apart from the type of polymer used, crosslinking of hydrogels' structures affects the properties of bioink and obtained 3D printouts equally. The main methods of crosslinking that can be distinguished are chemical (including enzymatic) and physical crosslinking or a combination of the above. Both chemical and physical methods of crosslinking can be used for natural, semi-synthetic, and synthetic polymers. Hydrogel networks formed by chemical methods of crosslinking are made by nonreversible covalent bonds created by chemical reactions, photo-crosslinking, or the addition of crosslinking agent. Although hydrogels produced through this method display remarkable shape fidelity and high mechanical properties, the crosslinking agents used are often toxic, which is highly undesirable for biomaterials intended for cell interactions [26,27]. On the other hand, physically crosslinked hydrogels are reversible, and their networks are formed by noncovalent bonds, among others we can distinguish thermal crosslinking, hydrophobic or ionic interactions [28]. Physical crosslinking is more cell-friendly but leads to obtaining hydrogels with worse mechanical properties than chemical crosslinking methods [29].

3D bioprinted models of the highly malignant cancers

Lung cancer

Lung cancer is the cause of the most deaths worldwide among all cancers. Up to now, 11 genetically differentiated and two main histopathological subtypes of this cancer type were classified. Small-cell lung cancer, referring to 15% of cases, is the most aggressive type in the metastatic stage [48]. One of the reasons for the high mortality rate is late diagnosis. Despite the number of cases, obtaining lung cancer model bioprinting is quite a rare approach. The main difficulties are connected with mimicking the lungs themselves – mechanical movements and fluid dynamics. However, few studies were conducted, and the ability to analyze correlations between different types of tumor-stromal elements,

Table 1
Comparison of the selected properties of different origin hydrogels for 3D bioprinting applications.

Hydrogels made of polymers			Main properties	Most common type of crosslinking used	Example of application in cancerous models
Of natural origin	Plant sourced	Alginate	• Moderate biocompatibility • Not biodegradable under mammalian cells • Mechanical properties highly depending on structure blocks ratio [30,31]	Ionic (Ca^{2+})	Breast cancer model [32,33]
		Agarose	• Moderate biocompatibility • Not biodegradable under mammalian cells • Thermosensitive, has self-gelling properties [34,35]	Thermal	Ovarian cancer model [36,37]
	Animal sourced	Collagen	• Excellent biocompatibility • Fully biodegradable and nontoxic • One of the main components of ECM (extracellular matrix) [38]	Enzymatic/ pH-mediated	Pancreatic cancer model [39]
		Gelatin	• Excellent biocompatibility and biodegradability • Gelling at a room temperature • More commonly used in its modified form [30, 40]	Thermal	Bone marrow model as an environment for cancer cell metastasize [41]
		Hyaluronic acid	• Good biocompatibility and cell adhesion • One of the main components of ECM • Can be modified to improve poor mechanical properties [22]	UV irradiation	Colorectal cancer model [42]
Of synthetic origin		PEG	• Worse biocompatibility then natural polymers • Biodegradability depends on molecular weight • Highly hydrophilic [43]	Chain-growth/step-growth polymerization	Lung adenocarcinoma model [44]
Of semisynthetic origin		GelMA	• Modified form of gelatin • Maintained biocompatibility • Biodegradable • Increased stability and tunability in comparison with unmodified form [25]	Free radical polymerization	Bladder cancer model [45]

tumor cells, and tumor-related immune cells, simulating clinical reality at the level of the whole organ. Also, 3D bioprinted models allowed studying the influence of the flow of cellular metabolites and regulatory molecules [49,50]. One of the most used cell lines in lung cancer bioprinting is A549 and NL20 cell lines. The tumor cells could be also obtained from patient biopsies, which represent a personalized preclinical model [51]. Creation of models allowed *e.g.*, analysis of DNA phenotype changes during metastasis [52], study on lung cancer impact on muscle cachexia [53], drug-dose testing [54,55] or mimicking tumor growth *in vitro* [56]. The researchers working on 3D bioprinting of lung cancer models claim that this method is faster, has lower technical and personal entry thresholds, and has higher success rates in comparison to currently used tumor organoid culture methods (Fig. 3) [55,57].

Colon and rectum cancer

Colorectal cancers are the second most frequent cause of death among all cancers. The most common colorectal cancer type (up to 95% of cases) is adenocarcinoma, which develops in the inner lining of the colon and rectum and then spreads to other layers [58]. Similar to lung cancer, colorectal cancer is also difficult to model, mainly because of interactions of colorectal cancer with gut bacteria and also layered characteristics of the gastrointestinal tract. The state-of-art in colorectal cancer modeling is the usage of organoids, which resulted in the creation

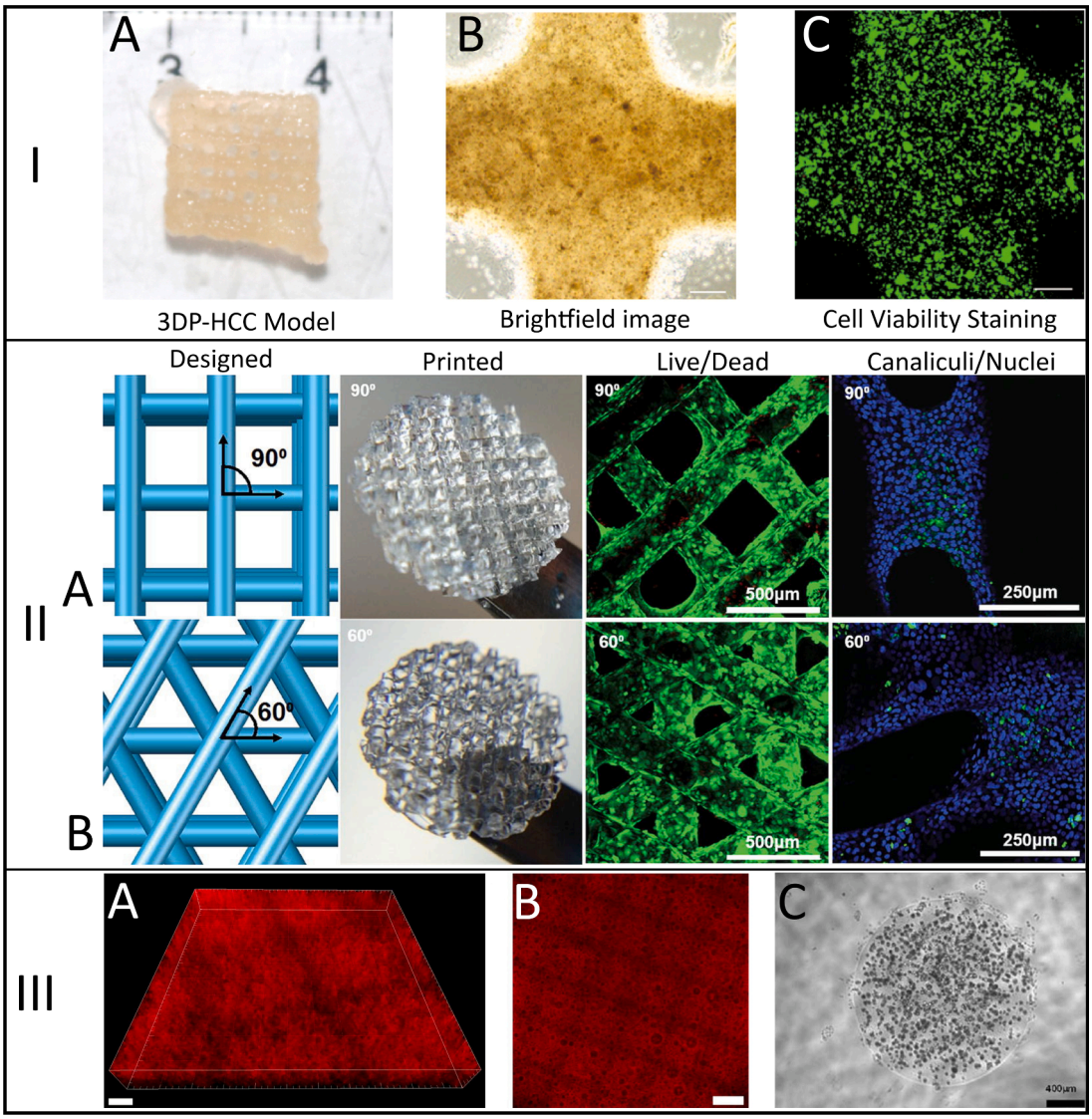

Fig. 3. The effect of designed morphology of 3D bioprinted cancer models on viability of cells. I – patient-derived three-dimensional bioprinted Hepatocellular carcinoma (3DP-HCC) model [67]. Proposed model morphology allowed for retainment of the features of parental HCCs, including stable expression of the biomarker along with maintenance of the genetic alterations and expression profiles. (A) 3DP-HCC steric grid model obtained with sodium alginate, gelatin and HCC cell suspension. (B) Even distribution of cells inside the model. (C) HCC cell viability after one month was higher than 80%. The model was stained with calcein-AM and propidium iodide after one month of culture. Living and dead cells are marked as green and red, respectively. Scale bar, 100 µm. II – The design, gelatin-based printout, HUH7 cell viability after 3 days of culture and formation of bile canaliculi in the liver cancer model printed with different angles between adjacent layers: (A) 90 or (B) 60° [63]. Strut width, 200 µm and spacing, 700 µm. Living and dead cells are marked as green and red, respectively. Canaliculi were stained with cholyl-lysyl-fluorescein and visible as green, nuclei were stained with Hoechst 33,342 and were visible as blue. The hepatocyte specific functions (albumin secretion, CYP activity, and bile transport) were the highest in more interconnected 3D-printed gelatin cultures (60°) compared to a less interconnected geometry (90°) and to 2D controls. III - 3D bioprinted lung cancer model with porous (pore size 7.08 ± 2.25 µm) microgel structure based on poly(ethene glycol), gelatin methacryloyl and A549 cell line [57]. It was found that microporous structure was suitable for simulation of lung tumor tissue due to regulation of actin cytoskeleton polymerization through the Rho-associated kinase - actin signaling pathway. (A) 3D reconstruction of microspores gel structure based on images taken with confocal fluorescence microscope. Scale bar, 10 µm. (B) Micrograph of single layer of the gel. Scale bar, 40 µm. (C) microgel cultured with A549 cells for 3 days.

of realistic models where colorectal cancer cells quickly adopted a phenotype that appeared both mesenchymal and metastatic, similarly to their *in vivo* tumor of origin [59]. Besides the successes, research points out that organoids are not the ideal models, mainly due to the limited distribution of oxygen or nutrients and waste removal operating *in vivo* [60]. However, very few works are focusing on the bioprinting of colorectal cancer. Skardal et al. prepared a metastasis-on-a-chip platform to study the colorectal cancer model using Int-407 intestine epithelial cells and HCT-116 colorectal cancer cells coupled with liver construct. Modeled colorectal cancer grew in size and entered circulation, reaching to liver model. After some time, metastatic cells started invading the liver construct via multicellular aggregates, which mimics the migratory *in vivo* effects [59]. Other approaches in colorectal cancer modeling by bioprinting include high-throughput chemotherapy screening [61]. The usage of hyaluronic acid and collagen to print in a gelatin bath allowed the preparation of spherical forms using immersion bioprinting. HTB-37 was used as colorectal cancer epithelial cell line. The method was found to be more consistent, reliable, scalable, and user-friendly than the usage of organoids.

Liver cancer

Liver cancer, which most often appearing cases known as hepatocellular carcinoma, is the third leading cause of cancer deaths worldwide and one of the deadliest cancers with only around 20% of 1-year observed survival rate [62]. The liver is the largest internal organ of the human body, compromising many cell types and functions, which makes it hard to estimate the influence of different factors on cancer development. Liver models are one of the most focused organ models among researchers, ranging from single-cell models (Fig. 3 II) [63] via co-cultures [64] to attempts to create functional hepatic constructs [65]. In liver cancer models three main cell lines are used: HEPG2, HEP3B, derived from young patients, MHCC97L and HCCLM3 derived from adult patients [66]. Apart from that, there is agreement that patient-derived cells should be used to realize personalized treatment for cancerous patients [67]. However, one main drawback of bioprinting hepatocellular carcinoma was spotted, namely the stiffening of structure with time, ultimately leading to the death of cells inside of printed constructs, while cells outside of structures were alive. Also, it was pointed out, that usage of patient-derived cells is easier than in other hepatocellular carcinoma model creations and overcomes the problem of low growth rates of hepatocellular carcinomacells (Fig. 4 II). This problem comes from a lack of features of epithelial stem cells in hepatocytes, the cell origin of hepatocellular carcinoma. Bioprinting allows very fast cell seeding, in comparison to weeks' time of conventional methods (Fig. 3I) [67]. Usage of this method also results in significant improvement in tumor-related gene expression *e.g.*, ALB, IL-8, or β-TGF [68]. The bioprinted HepG2 model also behaves more closely to *in vivo* conditions than conventional 2D models [68]. Hydrogel bioprinting was also successfully employed to manufacture liver models, where vascular formation and functional abilities of the liver were observed [69]. A combination of advanced liver modeling and cancer printing already results in attempts for personalized therapy, where models with functional abilities are used to test anti-cancer drug resistance and 3D bioprinted constructs show stem-like properties [70].

Breast cancer

Breast cancer is the most common cause of cancer-related woman deaths worldwide, mostly because of tumor metastasis and disease recurrence: the survival ratio varies from 99% for local cancer to 23% for the metastasis phase [71]. Thanks to the development of screening programs and social awareness, the survival ratio still increases [72]. breast cancer is hard to model, mostly due to interactions between tumor cells and non-cancerous cells in the stroma and progressive chances in ECM and tumor microenvironment [73]. Despite the challenges, breast cancer models are one of the most often bioprinted cancer models. breast cancer tumors have been evaluated using those models at different stages of development. Thanks to the modeling it was observed that fibroblasts stimulate the growth and aggregation of tumor cells (MDA-MB-231) by releasing soluble factors, such as matrix metalloproteinase [74]. In the later stages of breast cancer development, the process of osteomimitizing was observed, which helped promote metastasis into bones [75,76]. To promote patient-oriented treatments, human mammary-derived ECM was used for bioprinting the tumoroids. The observed behavior was similar to *in vivo* studies, where tumor cells were growing into isolated, very large masses (Fig. 4I) [77]. One of the novel approaches in the whole cancer bioprinting field is the mechanistic analysis of cancer cell redirection on the example of changing 5-hmC levels within breast cancer cells incorporated into chimeras [78]. Breast cancer models are also produced using emerging bioprinting strategies such as sacrificial methods to simulate mammary duct-like structures within a hydrogel matrix [79] or investigate lymphangiogenesis of breast cancer cells [80]. Complex breast cancer models were also employed to study T cells' influence on the tumor invasion index of immune cancers [81,82] or observe the tumor vascular bed establishing and proximity between spheroids' influence on angiogenesis and cancer invasion [83]. Another unique application of bioprinting in breast cancer modeling is the study of 13 amino acid-based flavone phosphoramidates anti-cancer activity on MCF-7 cancer cells to establish a structure-activity relationship [84]. Overall, bioprinting allows recapitulation of three different stages of breast cancer tissue morphology, which is not possible using other methods [85].

Prostate cancer

Prostate cancer is one of the leading causes of death for men. It is estimated that more than 15% of males will be diagnosed positively [86]. The survivability ratio of prostate cancer dramatically increased through the years, mostly thanks to the implementation of prostate-specific antigen (PSA) testing. There are successful primary prostate cancer treatments – the 5-year survivability ratio for local and regional prostate cancer is above 99%, whereas for the distant stage is around 30% [87]. Similarly, to breast cancer, prostate cancer prefers to metastasize to the bone microenvironment, causing woven bone formation [79]. The prostate luminal and basal epithelia are e to the breast cancer, and prostate cancer prefers to metastasize to the bone microenvironment, causing woven bone formation [88]. The prostate luminal and basal epithelia evolved from stem cells. Recent studies show that the prostate cancer does not have a single cellular source [89] and the knowledge about prostate cancer driving mechanisms is incomplete [90]. Despite the number of prostate cancer cases, the research on its 3D modeling is deficient; however, present studies provided relevant information about prostate cancer metastasizing [91,92], gene expression [93], or its interactions with mast cells [94]. Generally, researchers agree on the absence of the key factors in the vast majority of *in vitro* studies, namely tumor stroma, inflammation, and vasculature [95]. It should be also noted that commonly used prostate cancer lines, prostate cancer-3, DU 145, and LNCaP, are not suitable for prostate cancer modeling, mostly due to a lack of wild-type androgen receptor expression and osteosclerotic bone metastases [96]. Overall, prostate cancer bioprinting is still in its infancy phase, with expected development similar to breast cancer bioprinting, with a focus on bone metastasis.

Current trends and prospects

Over the last 20 years, cancer diagnostics improved substantially, especially in lesser developed regions of Asia, Africa, and Oceania, leading to a nearly two-fold increase in the number of new cases (10 million in 2000 to 18 million in 2020 – excluding nonmelanoma skin cancer) [4,97]. This increase is much higher than expected – Parkin in the 2000 cancer statistics forecasted about 15 million cases in 2020 [97].

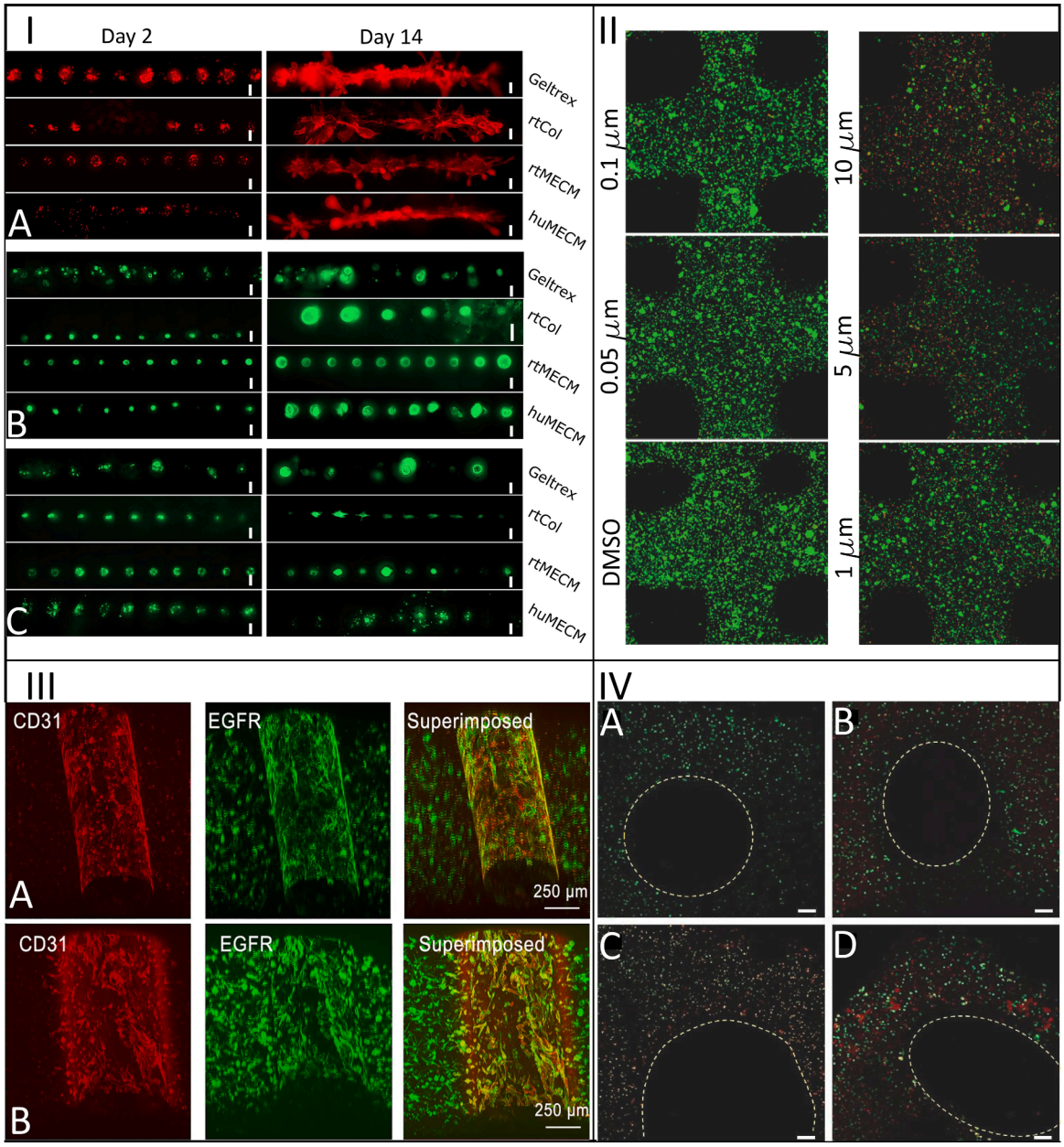

Fig. 4. The selected applications of 3D bioprinted models based on hydrogels in cancer research. I – growth of epithelial organoids and tumoroids after 2 and 14 days of culture in 3D bioprinted models obtained with selected hydrogels: Geltrex™ – commercial collagen gel, rtCol –rat-tail collagen hydrogel, rtMECM – Sprague Dawley rat mammary extracellular matrix hydrogel, huMECM - human mammary extracellular matrix hydrogel [77]. In all cases, 50±3 cells were injected in linear pattern into the hydrogel matrix at 500 μm intervals. (A) MCF-12A cells grew into large organoids in all substrates after 14 days of cultivation. After 2 days, the highest growth was observed in Geltrex. (B) MCF-7 cells after 14 days of culture grew in single spherical shapes in rtMECM and huMECM, while in rtCol they grew in bigger spherical clusters and in Geltrex in grape like clusters. (C) MB-MDA-468 cells growth behavior after 14 days showed the largest differences between hydrogels. The cells did not grow in huMECM, formed single tumoroids in rtCol and rtMECM and assembled clusters in Geltrex. Scale bars, 200 μm. Overall, ECM-based hydrogel cancer models have demonstrated pronounced *in vivo*-like reactions indicating their intrinsic functional nature. II – evaluation of the different concentrations of sorafenib effect on patient-derived three-dimensional bioprinted Hepatocellular carcinoma (3DP-HCC) models prepared using gelatin bioink [67]. Living and dead cells are marked as green and red, respectively. The sorafenib was one of four empirical targeted drugs tested in this study and was found effective for three of six patient derived models. The reproducibility of results made proposed methodology a reliable *in vitro* model system for multiple candidate drugs screening for personalized treatment of HCC patients. III – effect of vascular endothelial growth factor C (VEGF-C) on the interactions between MDA-MB-231 tumor cells and lymphatic endothelial cells (LECs) in the lymphatic vessels-impregnated breast cancer (LV-BC) model printed with gelatin methacryloyl [80]. The figure shows the reconstruction of model structure based on images taken with confocal fluorescence microscope after 20 days of culture in the (A) absence and (D) presence of VEGF-C. LECs and MDA-MB-231 breast tumor cells are visible as red and green, respectively. In the presence of VEGF-C, migration of LECs was more noticeable and the MDA-MB-231 cells were growing faster than in control environment. The proposed model behaved similarly to the processes of lymphangiogenesis and tumor invasion *in vivo*. IV – evaluation of the effect of paclitaxel on bioprinted lung cancer model prepared with A549 lung cancer cells cocultured with lung fibroblasts in hydrogel matrix made of gelatin methacryloyl [55]. Micrographs of models after 48 h of treatment with (A) 0, (B) 5, (C) 10 and (D) 20 μM of paclitaxel. Living and dead cells are marked as green and red, respectively. Scale bar, 200 μm. In the presented model, the applied drug concentration (10 μM) for creating 50% cytotoxicity in cancer cells is 10 times higher than the IC50 values obtained on 2D models, which rises the necessity to reevaluate all IC50 values previously obtained on 2D cancer models.

Despite that, the overall mortality ratio is slowly decreasing (0.61 in 2000 to 0.55 in 2022) [4,97] mostly thanks to the development of new pharmaceuticals and social breast and prostate tumor screening programs which helped to react at early phases of cancer development. In the authors' opinion, those trends will continue. Rising numbers of new cases will put pressure on governments to allocate more funds to cancer treatment programs, which will result in new and more reliable treatments and, in consequence, in a lower mortality ratio. One of the major objectives to fulfill is to understand the mechanisms of cancer development. The most promising technique is the bioprinting of cancer models. There are substantial breakthroughs in this field, resulting in a better understanding of cancer metastasis and the structure-activity relationship in pharmaceuticals. In comparison to other modeling methods, hydrogel bioprinting offers superior mimicking of the tumor microenvironment and high throughput production of models. Another interesting factor is the ability for quick screening of possible therapies based on patient-derived tumor cells. Current trends in the 3D bioprinting of cancer models involve the usage of patient-derived tumor cells and trials on high throughput testing of anti-cancer drugs. Despite the presented advantages, hydrogel bioprinting is not yet widely applied in cancer research. In the author's opinion, additive manufacturing of cancer models will soon advance to clinic usage. The most important steps to take are the standardization of protocols and their validation in clinical trials. For each type of tumor, patient tissue collection, cell isolation, and culture methods have to be established together with bioprinting and analytical protocols to ensure repeatable results. After standardization and approval by national health agencies, this method is expected to substantially accelerate research on tumor treatments with a reduction of animal testing and provide personalized therapy for each patient. However, there are three main challenges to be overcome. The first is the total cost of the method. It does not only involve the cost of the 3D bioprinter, hydrogels, and cells, but also the detailed training and necessity of employing members of interdisciplinary research teams, which should connect medical personnel, polymer scientists, technical staff and also computer scientists. On top of that, the managers have to be also specifically trained to supervise such diverse teams. The other challenge is lack of industry standard practices, which have to be yet developed by pioneer research teams and tested in clinical trials. The last of main challenges is still not perfect resemblance of tumor microenvironment, which is mainly connected to absence in currently studied models of immune microenvironment components and influence of pharmaceuticals other than anti-cancer, which are often used by tumorous patients.

Concluding remarks and future direction

Hydrogel 3D bioprinting of cancer models is a rapidly advancing technology that holds great promise for cancer research and treatment. This technique allows for the creation of complex and realistic tumor models that can be used to study the development and progression of cancer, as well as test potential therapies. Hydrogel 3D bioprinting offers precise control over the size and distribution of individual components of the cancer microenvironment, including healthy and tumorous cells, scaffolds, and other biological molecules. This level of control allows researchers to recreate the complex structure and organization of tumors, including the architecture of blood vessels and extracellular matrix, *in vitro*.

One of the most significant advantages of hydrogel 3D bioprinting is its potential for personalized medicine. By creating patient-specific tumor models, researchers can develop more effective treatment strategies that take into account the unique genetic and cellular makeup of each individual's cancer. This personalized approach to cancer treatment has the potential to significantly improve patient outcomes and reduce the risk of treatment-related side effects.

In addition to personalized medicine, hydrogel 3D bioprinting offers several other advantages over traditional cancer models. One of these advantages is improved reproducibility and accuracy. By using computer-aided design (CAD) software and precision printing techniques, researchers can create tumor models with reproducible and accurate sizes, shapes, and cell densities. This consistency enables researchers to compare and analyze different tumor models, which can lead to more meaningful results.

Another advantage of 3D bioprinting is the ability to create complex and physiologically relevant tumor microenvironments. Traditional two-dimensional (2D) cell culture methods do not accurately mimic the complex 3D architecture and heterogeneity of tumors. Hydrogel 3D bioprinting, on the other hand, allows researchers to create tumor models that more accurately reflect the *in vivo* conditions of cancer. This is particularly important when studying the interactions between the tumor and the immune system, as immune cells can be incorporated into the tumor model to better understand the complex interactions between cancer cells and the immune system.

As the technology continues to advance, 3D bioprinting is expected to become a widely used tool in cancer research and treatment. The current state of knowledge and practice enlightens a bright future ahead of this technique for cancer treatment, while one needs to face three main challenges before 3D bioprinting setups can be approved for clinical use:

- *Preparation and validation of commonly agreed testing protocols*. To smooth the pathway to the clinics, 3D bioprinted models have to be validated repeatedly to become reliable statistically and well powered based on reproducible results.
- *Certification of regulatory bodies*. Based on the developed protocols, regulatory bodies in cooperation with stakeholders from pharmaceutical industry have to propose new strategies for certification of bioprinted models. Critical design parameters and criteria have to be cross-checked and re-established before large-scale implementation.
- *Equipment and personnel cost reduction and throughput enlargement*. To commercialize the bioprinted models, the economic rationale has to be presented for pharmaceutical industry, considering that introducing new methods usually involve very high investments. The stakeholders will start to consider implementation of bioprinted models only and only when the satisfactory scale and cost efficiency are achieved.

In summary, hydrogel 3D bioprinting of cancer models holds great promise for advancing cancer research and improving patient care, and its potential to revolutionize cancer treatment should not be overlooked.

CRediT authorship contribution statement

Przemysław Gnatowski: Writing – original draft, Conceptualization, Methodology, Investigation. **Edyta Piłat:** Writing – original draft, Methodology. **Justyna Kucińska-Lipka:** Writing – original draft, Investigation. **Mohammad Reza Saeb:** Conceptualization, Supervision, Writing – review & editing. **Michael R Hamblin:** Supervision, Writing – review & editing. **Masoud Mozafari:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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