

Applications of 3D Printing in Cancer

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ABSTRACT

The tumour heterogeneity and interindividual variability is a major problem when treating cancer as every patient responds in a different way to the current drug therapies. 3D printing is a tool that can hamper the issues faced in cancer patients allowing for individualisation of treatment by the production of *in vitro* models with micro-environments mimicking more closely real cancer conditions facilitating complex therapies. Further improvements are required, for example the development of biocompatible bioinks or need for vascularisation. The journey from bench to bedside is challenging from the regulatory point of view where the establishment of manufacturing guidelines, quality systems and safety of use and administration of personalised medicines remains unclear. This review will provide an insight in the major applications of 3D printing in cancer both in the development of *in vitro* cancer models as well as personalised medicines for cancer patients focused on hydrogels and therapeutic implants.

Keywords: 3D printing, cancer, *in vitro* studies, therapeutic implants, 3D bioprinting, hydrogels, controlled release, FDM, SLA, PAM

PERSONALISED THERAPIES AND 3D PRINTING

Cancer is the second leading cause of death in the world, being responsible for one of every six deaths that occur annually [1]. Unfortunately, new cases are predicted to increase approximately by 70% in the next 20 years [1]. Thus, it is critical to develop more sensitive technologies for early diagnosis and targeted therapies that are more active and with less adverse effects [2].

Tumour heterogeneity and interindividual variability is a major problem when treating cancer as every patient responds in a different way to current drug therapies. Dose adjustments are frequently based on empirical methods leading to higher chance of undesirable side effects. Conventional drug manufacturing, in order to maintain the costs low, does not allow for tailoring the dose to individual patients leaving a clear unmet need.

Drug manufacturing has moved forward from traditional dosage forms to targeted biopharmaceuticals and nanomedicines that have an increased specificity and reduced toxicity. Novel manufacturing technologies based on Rapid Prototyping, also known as 3D printing or additive manufacturing, involve the fabrication of 3D physical models prepared based on computer-aided design, are proposed towards the manufacture of personalised therapies [4]. Rapid prototyping was first introduced in 1980 by the Japanese Dr. Hideo Kodama that filed a patent for Rapid prototyping technology, which was denied by authorities as he missed the one-year deadline to file the full patent requirements. Four years later, the American Charles Chuck Hull filed the first patent on stereolithography [5] and in the early 90s, at the Massachusetts Institute of Technology, Sachs et al. filed a patent on another 3D technology known as inkjet printing where a binder solution was deposited onto a powder material bed [6]. Nevertheless, several decades have passed until these techniques were applied in the production of medicines. Aprelia Pharmaceuticals in the 2016 was the first company to get approval by the FDA and launch to the market the first 3D printed medicine, Spritam[®], containing levetiracetam indicated in epileptic attacks. This medicine is produced by using a Zip Dose Technology (inkjet printing) where the layers are deposited one on top of the other resulting in a 3D highly porous tablet with a very fast disintegration [7]. In 2012, Scott Crump filed a patent on another well-extended 3D printing technology, currently known as Fused Deposition Modeling [8], which has been applied latter on in the development of solid dosage pharmaceutical forms. This technique is based on printing using drug-excipient filaments produced by hot melt extrusion that are fed into a extruder nozzle that heats the filaments and deposits the semi-solid material onto a platform layer by layer till harden into a 3D object [9].

Nowadays, 3D printing is a tool that can hamper the issues faced in cancer patients allowing for individualisation of treatment by the production of *in vitro* models with micro-environments mimicking more closely real cancer conditions facilitating complex therapies. This review will provide an insight in the major applications of 3D printing in cancer both in the development of *in vitro* cancer models as well as 3D printed personalised medicines for cancer patients focused on hydrogels and therapeutic implants.

3D BIOPRINTING OF *IN VITRO* CANCER MODELS

Executive summary

- There are several types of 3D printing techniques but not all of them are suitable for bioprinting as they should be relatively mild and cell friendly.
- Bioinks have to possess certain properties in order to ensure the quality of the 3D printed scaffolds such as: printability, functionality, and mechanical strength.
- The evolution from simple 2D to complex 3D bioprinted models allows to address many of the challenges associated with cancer models such as disease progression, metastasis, spatio-temporally evolving cell-matrix interactions, hypoxic cores, leaky unorganised vasculature and presence of host signaling molecules.

Bioprinting challenges

3D bioprinting can accurately build highly complex architectures and tissue models in a layer-by-layer manner and hence, advances on this field are key to mimic what is happening in real tissues [10]. When developing *in vitro* cancer models, highly porous scaffold materials as used as a template to induce the growth and differentiation of cells creating ideal microenvironments mimicking the *in vivo* conditions for drug testing. When printing tumour organoids, several issues have to be considered such as vascularization, innervation and safety of biomaterials used before their used in drug screening and development [11]. Conventional techniques such as injection molding, electrospinning and porogen-leaching have a limited control over the cell distribution within the scaffold, its final architecture, pore size and composition [12]. Herein, it is a necessity to be able to reproduce the complexity of alive tissues and in order to do that, a highly precision in the scaffold construction is required. A major limitation in this field is to design suitable bioinks that allow printing of living cells. Therefore, printing at elevated temperatures is not an option as cells can be easily damaged. However, most polymers utilised in 3D printing require the use of high temperatures or solvents in order to either melt or dissolve the polymer such as polycaprolactone or polylactic acid [13]. The development of suitable

bioinks and the use of an appropriate bioprinting method are key to ensure the success of 3D bioprinting.

3D Bioprinting methods

There are several types of 3D printing techniques but not all of them are suitable for bioprinting as they should be relatively mild and cell friendly. Droplet-based, extrusion-based and laser-based are the most commonly used methods. The two most typical droplet-based methods are: continuous inkjet printing (CIJ) and drop-on-demand (DOD). CIJ consists on a high pressure pump that directs the liquid ink towards an orifice (50-80 μm) creating a continuous ink flow. The liquid ink is broken down into small drops of a specific size (usually between 10-50 μm equivalent 1 to 70 pL) due to the action of the piezoelectric crystal. Droplets are electrostatically charged and hence, they are directly deposited on the platform due to the electrostatic field that is generated (Fig. 1). The DOD printing method employs multiple heads and uses two types of translators (piezoelectric crystal or thermal head). The thermal head can reach temperatures of up to 300°C, which can induce the degradation of bioactive compounds and cells. For this reason, CIJ methods are more suitable for bioprinting [4].

Regarding the extrusion-based methods, the ink is passed through a nozzle that originates layer by layer the 3D structure. Extrusion-based methods can be divided in two different types of printing depending on if the ink has to be melted (in the case of Fused Deposition Modeling, FDM) or not (Pressure-Assisted Microsyringes, PAM). FDM allows to produce complex scaffolds with high mechanical strength; however this technique is not suitable for bioprinting as requires melting of the materials that can provoke cell degradation. The fundamental principle of FDM consists on filaments that are arranged in rolls in a way that go through an extruder nozzle whose temperature is above the melting temperature of the material which melts and deposits, layer by layer in the form of very fine filaments that pretty fast solidify (Fig. 2). This technique is broadly used in the manufacturing of solid dosage forms due to its ability to produce complex geometries with high quality and at good speed but it is less suitable for bioprinting [3,14]. In contrast, PAM technology is a good option for bioprinting in which a viscous and semi-solid material is deposited layer by layer by means of a pressurised air piston and a syringe extruder (Fig. 3). The major advantage of this technique is the fact that can work at room temperature using aqueous based materials with a continuous flow. In order to print accurately complex scaffolds, it is necessary to investigate in advance the viscosity, viscoelastic properties and apparent elastic limits of the printed materials as they are key elements to ensure the adequate deposition of the bioinks on the substrate [15].

Laser-based printing methods are characterised by the use of an ultraviolet light beam in the form of a laser that transfers the energy into a liquid photopolymerizable resin. In order for the photopolymerization to occur, radicals have to be released after the interaction between the photoinitiator and the UV light resulting in solidification of the material. When a layer solidifies, the building platform goes down so a new layer of liquid resin will receive the laser beam and will solidify leading to the final tridimensional geometry (Fig. 4). The major advantages of this technique are its high resolution as well as the low heating required during the printing. Hence, this technique could potentially be employed for bioprinting as long as a cell-laden prepolymer formulation is used and the photocuring process takes place in cell friendly conditions [16]. Laser-induced forward transfer can also be used in bioprinting. In this case, ink solution is deposited on a glass slide which is coated with a laser absorption layer such as a metal oxide towards which is directed the laser, creating a local pressure to eject the ink layer to the substrate [10,17]. However, the lack of GRASS (Generally Recognised as Safe) excipients is one of the limitations of these techniques.

Bioink design for 3D bioprinting

Bioinks have to possess certain properties in order to ensure the quality of the 3D printed scaffolds. These properties can be summarised basically in three: printability, functionality, and mechanical strength. Printability involves the capacity of the ink to be fed into the 3D printer and to be processed resulting in a scaffold with enough mechanical strength to hold the shape, keep the functionality and ensure living cells after printing [18]. One of the key parameters that affect the printing process is the viscosity of the bioink. In this sense, inks with high viscosity hold the shape of the scaffold for longer times post-printing; however, higher pressures are required during the printing process which maybe not be possible in certain cases limiting the achievable print size [19]. For this reason, inks with shear-thinning characteristic are better for printing. Overall, lower viscosities are recommended for droplet-based printing with values close to 10 mPa·s, while higher viscosity inks can be used in laser-based printing (ranging from 1 to 300 mPa·s) or even higher in PAM (ranging from 30 to 6×10^7 mPa·s) [20,21]. Also, bioinks should not contain substances that induce inflammation or cytotoxicity. They should be biocompatible and ensure high cell viability and keep cellular behavior such as adhesion, differentiation and migration [10]. Pressure induce differentiation in cells.

Overall, the most common bioinks are cell-laden hydrogels, decellularised extracellular matrix-based solution and cell suspensions [10]. The major advantage of the cell-laden hydrogels is their ability to mimic the cellular environment. Hydrogels can be constructed using natural

compounds (such as collagen, gelatin, fibrin, hyaluronic acid, alginate, agarose), synthetic polymers (like pluronic, poly-ethylene glycol, peptide amphiphiles [22]) or a mixture of both of them in order to combine their properties. For instance, natural compounds resemble better the extracellular matrix with inherent bioactivity whereas synthetic hydrogels even though do not promote cellular function unless made by bioactive peptide amphiphiles, possess tunable mechanical properties usually based on cross-linking reactions leading to self-supporting structures [23]. The decellularised extracellular matrix bioinks are more costly as they are obtained after removing the cells and preserving the matrix and combining it with a carrier polymer to adjust some parameters such as viscosity and solubility, but have the advantage of exhibiting better printability capacity while keeping their inherent bioactivity [24]. Cell suspension inks are also a good alternative for bioprinting. They are based on cell aggregates in suspension in the form of mono- or multicellular spheroids which undergoes a transformation after bioprinting due to cell-cell interactions. The presence of a temporary support layer can be required in certain cases [25].

***In vitro* cancer models**

In vitro cancer models have relied on last decades mostly on 2D mono-cell cultures and animal models. However, 2D models have poor translational success as they have many limitations in mimicking the tumor environment in humans. The evolution from simple 2D to complex 3D bioprinted models allows to address many of the challenges associated with cancer models such as disease progression, metastasis, spatio-temporally evolving cell-matrix interactions, hypoxic cores, leaky unorganised vasculature and presence of host signaling molecules [26]. Even though, there is no a single 3D bioprinted model able to reproduce all cancer features as above described, different approaches have been attempted in order to obtain a model that imitates as close as possible what really occurs within tumors (Table 1) [27].

Bioprinting has been successfully employed in the development of tumor spheroids which resemble the cellular heterogeneity of solid *in vivo* tumors characterised by containing cells in different proliferative and metabolic states with an external proliferating zone, an internal quiescent zone caused by limited distribution of oxygen, nutrients and metabolites and a necrotic core [28]. These models are very useful for drug testing and high-throughput screening of therapeutics. There are several examples of 3D bioprinted spheroids using MCF-7 and BT474 breast cancer cells [29,30], HeLa cells for cervical tumor [31] and stem SU3 cell line for glioma [32]. The bioink composition to construct the scaffold varied from PEG-dimethacrylate, PEG-diacrylate to gelatin-alginate-fibrinogen (Fig. 5) [26,33]. Overall, 3D

spheroid models have demonstrated that are able to reproduce typical factor and enzyme secretion according to the type of tumor, like vascular endothelial growth factor and matrix-degrading enzymes, but also have exhibited significantly higher resistance to drugs compared to 2D models mimicking closer the *in vivo* environment.

Apart from developing microtumor environments through 3D bioprinted spheroids, the study of cell-cell communication is also key to understand the regulatory pathways controlling the progression of the disease, adhesion, migration and metastatic behavior. For example, cancer cell interaction with immune cells like macrophages through paracrine communication is known to play a crucial role in tumor cell extravasation and hence, metastasis development [34]. 3D bioprinting has been successful in biomimetic vessel-like microenvironment containing macrophages in the core and MDA-MB-231 breast cancer cells in the sheath using peptide conjugated alginate fibers as support [35]. Another model to study the disease progression and the regulatory feedback mechanisms was based on a human ovarian cancer cells (OVCAR-5) co-cultured with normal fibroblasts overlaid on a Matrigel™ (a gelatinous protein mixture secreted by Engelbreth-Holm-Swarm mouse sarcoma cells) which spontaneously formed a multicellular acini. The acinar growth kinetics recapitulate features of ovarian cancer micronodules *in vivo* [36].

Also, several attempts have been performed to reproduce cancer cell migration behavior and hence, metastasis. For example, laser-based printers have been able to reproduce artificial bone microenvironment combining MDA-MB-231 breast cancer cells co-cultured with human fetal osteoblasts forming multi-cellular spheroids with hydroxyapatite nanoparticles, PEG and PEG-diacrylate. This biomimetic model showed the migration capacity of cancer cells within bone-like structures [37]. Stereolithography has also been employed to bioprint breast cancer cells (BrCa) co-cultured with bone stromal cells (fetal osteoblasts or bone marrow mesenchymal stem cells) encapsulated in gelatin methacrylate hydrogel with nanocrystalline hydroxyapatite. The 3D culture exhibited an increased vascular endothelial growth factor secretion compared to monocultured BrCa cell models leading to an enhanced migration of BrCa cells into the stromal cell-laden bioprinted matrix [38].

Overall *in vitro* cancer models based on 3D bioprinting have evolved with great sophistication being capable of recapitulating the extremely complex and heterogeneous cancer microenvironment. However, further improvements are required like for example the need for vascularisation, innervation, financial cost and safety of bioinks used for the construct. [11]. A promising strategy to overcome these challenges is the fabrication of 3D printed organ-on-a-

chip which allows the creation of micro-organs with heterogeneity, cellular arrangement, tissue-specific functions and cyclic movement within a microfluidic device whose construction can be easily automated for massive production and hence, reduced cost [39,40]. Further direction indicates that the hybridization of different types of bioprinting technologies will enhance the features of the *in vitro* cancer models based on the integration of extrusion-based units to print the polymeric scaffolds along with pressure-assisted microsyringes able to deposit different bioinks of interest [27,41].

3D PRINTING OF PERSONALISED CANCER TREATMENTS

Executive summary:

- 3D printing can revolutionize cancer treatments by printing of personalised hydrogels, therapeutic implants and breast prostheses.
- 3D printed hydrogels are considered to be the next generation of hydrogels able not only of facilitating physical support for cells, but also promoting cell proliferation, cell differentiation as well as controlled released of therapeutic agents and cell-regulating factors.
- 3D printed therapeutic implants using different types of materials like metals and polymers have revolutionised the personalised medicine allowing a great flexibility in implant design to obtain a wide range of shapes and, a much better fit than conventional implants leading to greater results in patient health care.
- Using 3D printed flexible breast molds is a simple and inexpensive solution owing to its flexible nature which allows the de-epithelialized DIEP flap placed in the mold changes into a shape symmetrical to the contralateral breast with only minor adjustments.

Extensive work has been performed in the area of 3D printed polypills where more than one active ingredient is combined within a single dosage form. However, many polypills are targeting polymedicated patients with hypertension, diabetes and cholesterol and for this reason this review will focus on 3D printed hydrogels and therapeutic implants for cancer therapies.

3D printed hydrogels for controlled drug release

Hydrogels are based on polymeric networks consisting on hydrophilic macromonomers able to: (i) retain large amounts of water which makes them biocompatible with most soft biological tissues, (ii) minimise inflammatory reactions of the surrounding cells and (iii) construct

scaffolds due to good mechanical properties as well as facilitating drug encapsulation [42]. In order that hydrogel is formed, it is required to cross link the macromonomers either by physical noncovalent interactions or chemically covalent bonds or a mixture of both. *In situ* forming hydrogels are preferred over preformed hydrogels in order to avoid surgical interventions and also because drugs and other biological components can be incorporated by simple mixing with the precursor polymer solution. Upon injection, the initial fluidic nature of the precursor solution ensures proper shape adaptation within the cavity where it is administered and then gelation takes place under physiological conditions increasing the viscosity and ensuring controlled drug release [43].

Amongst all different types of hydrogels, those denominated “smart” or “stimuli response hydrogels” have gained in popularity as they are able to change their volume (swell or shrink), degrade, release their drug cargo or exhibit a sol-gel phase transition in response to environmental stimuli like pH, pressure, light, ionic strength, temperature and concentration of specific biomolecules such as enzymes [44].

3D printed hydrogels are considered to be the next generation of hydrogels able not only of facilitating physical support for cells, but also promoting cell proliferation, cell differentiation as well as controlled released of therapeutic agents and cell-regulating factors (Table 2). For example, poloxamer-based hydrogels with a solid-disk shape (12 x 1 mm) containing paclitaxel and rapamycin have been successfully 3D printed using an extrusion-based method achieving a 99% encapsulation efficiency. Disks absorbed water and swelled up by 2-fold in one hour maintaining controlled drug release over 24 h. Upon intraperitoneal implantation of a single disk carrying paclitaxel/rapamycin at 20/20 mg/kg in ES-2-luc human ovarian cancer-bearing xenograft mice, tumor burden decreased substantially from 100 to 30% one day post-surgery and mean survival was increased from 20 to 30 days [45].

Laser-based printing methods (SLA) have also been successful in 3D printing of drug-loaded hydrogels. A 10% w/w ibuprofen-loaded hydrogels of cross-linked polyethylene glycol diacrylate containing up to 30% water were printed using a Formlabs 1+ SLA printer. Ibuprofen release rate was controlled based on the amount of water. Hence, the larger the water content, the higher the dissolution rate of the drug [46]. The same principle can be applied for cytostatic drugs. As a proof of concept, stereolithography has been also utilised for 3D printing of self-assembling thermoresponsive nanoemulsions into hierarchical mesostructured hydrogels [47]. Nanoemulsions are being investigating widely for potential application in cancer therapy as they can solubilise and target poorly soluble drugs [48]. However, to print a nanoemulsion-

based ink with rheological and photoreactive properties satisfying the requirements of SLA printers is challenging. Hsiao et al, have developed a thermoresponsive ink consisting of poly(dimethyl siloxane) droplets suspended in an aqueous phase with a surfactant, sodium dodecyl sulphate, and a crosslinker, poly(ethylene glycol) dimethacrylate (PEGDMA). Control of the hydrogel microstructured was achieved due to the fast structural recovery of the nanoemulsion after large strain rate yielding along with a shear thinning behaviour that allows the ink to conform to the build platform of the printer. The PEGDMA molecules possess two main functions in the nanoemulsion-based ink, on one hand, they induce colloidal gelation which can be tuned depending on the temperature conditions and on the other hand, provide photochemically cross-linked hierarchical mesostructured hydrogels [47].

Hydrogels with nanoscopic dimensions, known as nanogels, have properties similar to hydrogels, except to the fact that nanogels can reach areas of the body with difficult access, being proficiently internalised by the target cells, reducing accumulation in non-target tissues and hence toxicity, being this the reason why they are becoming major contenders in the intracellular administration of chemotherapy drugs [49]. 3D printed nanogels have been used to be implanted in postoperative tumor cavities with the ability to release DNA nanocomplexes to eliminate residual glioma cells. The nanogel was constructed by laser-based printing technology using a gelatin methacrylamide scaffold incorporating DNA nanocomplexes which were composed of pVSVMP (a plasmid DNA encoding matrix protein of the vesicular stomatitis virus able to eradicate cancer cells after transfection and induce anticancer immunity response) and degradable heparin-polyetherimide nanogel particles [50]. To improve the transfection efficiency of particles, Pluronic F127 was also incorporated to the DNA complexes. Insertion of the nanogels into the glioblastoma resection cavity delayed tumour recurrence and significantly prolonged the overall survival in mice [50].

3D printed therapeutic implants

3D printed therapeutic implants using different types of materials like metals and polymers have revolutionised the personalised medicine allowing a great flexibility in implant design to obtain a wide range of shapes like screws, joints, and flat substrates and, a much better fit than conventional implants leading to greater results in patient health care [51].

3D metal printing has been used successfully in manufacturing implants mainly for dental prosthesis and bone fractures [52]. Actually, fracture is one of the most common complications of bone cancer caused by a primary cancer starting in the bone or a secondary cancer

consequence of metastasis of cancer cells localised at other body sites. Bone cancer is mostly treated with parenteral administration of chemotherapeutics. High drug doses are necessary in order to achieve effective concentration at the bone site leading to high toxicity in the rest of the body and resulting in therapeutic failure in many cases [53]. One alternative strategy to hamper these issues is to utilise drug-loading bone implants able to release the chemotherapeutic drug directly at the affected site and reducing toxic concentrations in the rest of the body.

Using a 3D metal printer (selective laser melting), titanium implants loaded with two types of anticancer drugs, doxorubicin and apoptosis-inducing ligand have been successfully prepared for the treatment of bone cancers [54]. The 3D-printed titanium wafers were manufactured by forming a layer of powder material that was selectively melted using a laser (Fig. 6). The process was repeated till the desired thickness of the implant was achieved. The implant was electrochemically anodized in order to generate an anodic layer featuring unique microparticles and nanosurface (consisting of vertically aligned nanotubes) topography to enhance biointegration. Doxorubicin and apoptosis-inducing ligand were loaded at 188 and 25 $\mu\text{g}/\text{cm}^2$ respectively by depositing a solution containing both drugs onto the surface of the 3D wafers and using vacuum forces to remove potential air gaps inside the nanotubes. Drug release occurred in two phases: an initial fast release within 6 h (40 and 70% of the chemotherapeutics was released respectively) followed by a slow release phase of 16 days for doxorubicin and 4 days for the apoptosis-inducing ligand. The drug loaded therapeutic implant showed also strong *in vitro* anticancer efficacy against cancer cells (MDA-MB-231-TXSA)[54].

Magnetic hyperthermia can be also used as potential treatment in cancer taking into account that induced hyperthermia (raising the temperature up to 45 °C) can cause tumour cell death [55]. Implantable anticancer magnetocaloric polycaprolactone (PCL)/ Fe_3O_4 nanoparticulated mats have been created using electrohydrodynamic jet (E-Jet) 3D printing technology [56]. When subjected to an alternating magnetic field due to the conversion of magnetic energy to heat induced by the external magnetism, the temperature of the magnetic particles raises leading preferentially to death of cancer cells over non-cancer cells [57].

E-Jet printing consists on the printing of a liquid solution driven by an electric field. Due to the exposure to the electric field, mobile ions in a polarizable liquid accumulate at the liquid surface. The coulombic repulsion of the ions makes the meniscus of the liquid located at the nozzle of the printer deforms into a conical shape (known as Taylor cone). A drop of fluid is emitted from the apex of the nozzle towards the substrate when the electric field exceeds a

critical limit and hence, the stress from the surface charge repulsion at the cone apex exceeds the surface tension of the liquid [58]. A 7% (w/v) solution of PCL in solvents (DMF and DCM) was mixed with Fe₃O₄ magnetic starch nanoparticles producing composite solutions that were E-jet printed resulting in PCL/Fe₃O₄ mats [56]. PCL was employed due to its optimal biocompatible and biological stability properties which was combined with the anticancer magnetocaloric properties of the Fe₃O₄ nanoparticles. The advantage of 3D printing the mats is that significantly improves their specific surface area enhancing the contact with the cancer cells and then the efficacy of the therapy. The mats could be potentially placed after surgery in the vicinity of tumours avoiding frequent intravenous administration of anticancer drugs. *In vivo* experiments (based on studying the efficacy of the mats after implantation on tumour bearing mice) showed significantly tumour growth inhibition as well as prolongation of survival time after 4 weeks of treatment consisting of the application of external alternating magnetic fields over 45 min every other day [56].

3D printed models in breast reconstruction

Breast cancer remains to be one of the most malignant diseases in women. The plastic surgery community has investigated which methods for breast reconstruction after mastectomy are the best leading to the least donor-site morbidity. The autologous DIEP (Deep Inferior Epigastric Perforator) flap reconstruction is one of the first choice methods, where the skin and abdominal fat along with the artery and inferior epigastric vein is extracted and transplanted to the same patient in the breast area. Although this technique of reconstruction tends to imitate quite well the form and the roundness of the original chest, still are required new methods of aesthetically progress [59,60].

The complexity that surgeons face when designing a breast for a specific patient with its form and its own projection, from a flap, can be facilitated using extrusion-based 3D printers like Fuse Deposition Modeling printer (FDM). Several reports have indicated that 3D printed personalised prostheses provide superior esthetics compared to the traditional wax-based handcrafted ones [61]. It is necessary to scan both the region of the affected breast and the not damaged one. The mirror image of the undamaged breast superimposed in the affected region can be printed three-dimensionally to be used both in the preoperative planning and intraoperative development.

3D surface imaging (eg. MRI, CT-scan) gives reliable estimates of the required tissue volume, which is especially useful when a large volume of tissue is required to reconstruct a

symmetrical breast. Otherwise, flap type is merely subjective and underestimation of tissue volume would lead to an insufficient breast volume. Also, shaping flat adipocutaneous tissue into a rigid 3D mold can be challenging. Using 3D printed flexible breast molds is a simple and inexpensive solution owing to its flexible nature which allows the de-epithelialized DIEP flap placed in the mold changes into a shape symmetrical to the contralateral breast with only minor adjustments [60,62,63] (Fig. 2).

The fabrication of 3D printable smart materials has resulted in four-dimensional printing that basically is the combination of 3D printing and time. 4D printing allows a printed object to be programmed to carry out shape change while adapting to its surroundings (known shape memory effect) upon certain stimuli such as temperature [64]. Last year, this 4D printing technology was successfully used in China to reconstruct a breast implant for cancer patient. The implant changed over time and patient's fibrous tissue grew into the implant until eventually replaced it altogether [65].

Future perspectives and concluding remarks

3D printing has become a potential tool when developing personalised treatments for cancer patients as well as more accurate *in vitro* tumor models. Overall, 3D printing offers numerous advantages such as increasing of accuracy when imitating physiological processes and tumor environment linked to much higher repeatability and reproducibility which can be translated in more effective anticancer drugs. 3D printed of personalised medicines such as hydrogels and implants loaded with cytostatic drugs are revolutionizing cancer treatments targeting tumor cells in a much specific way increasing efficacy and reducing the toxicity derived from a broad biodistribution in the body. However, the journey from bench to bedside is challenging especially from the regulatory point of view where the establishment of manufacturing guidelines, laws, quality systems and safety of use and administration of personalised medicines remains unclear.

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‘*’ – of interest, or ‘**’ – of considerable interest.

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Table 1. 3D printing of *in vitro* cancer models. Key: CIJ, continuous inkjet printing, CAF, cancer-associated fibroblast; PAM, pressure-assisted microsyringes.

Model	Aim	Bioink		Type of printer	REF.
		Cell (tumor type)	Matrix		
Tumor spheroids	To mimic <i>in vivo</i> tumor microenvironment, hypoxia core condition and necrosis and drug resistance	SU3 cell line (glioma)	Gelatin-alginate-fibrinogen	Extrusion-based	[32]
		Hela cell (cervical tumor)	Gelatin-alginate-fibrinogen	Extrusion-based	[31]
		BT474 cell line (breast cancer)	Poly(ethylene glycol) diacrylate (MW 700)	CIJ	[30]
		MCF-7 cell line (breast cancer)	Gelatin - PEG-dimethacrylate (MW 1000)	PAM	[29]
		MDA-MB-231 in the center (breast cancer) and CAF cells (IMR-90) at the edges	Gelatin-alginate	PAM	[33]
Cell-cell communication, migration, adhesion	To imitate the physiological environment and show regulatory pathways involved in migration, adhesion processes and metastasis	Murine RAW 264.7 Macrophages in the core and MDA-MB-231 in the center (breast cancer)	Peptide conjugated alginate fibers	Extrusion-based	[35]
		MDA-MB-231 cell line (breast cancer)- Human fetal osteoblasts	PEG (MW 300)-PEG diacrylate (MW 700)-Hydroxyapatite nanoparticles	Laser-based	[37]
		OVCAR-5 cell line (ovarian cancer) – MRC-5 cell line (normal human fibroblast)	Matrigel™ (gelatinous protein mixture secreted by Engelbreth-Holm-Swarm mouse sarcoma cells)	PAM	[36]
		BrCa cell line (breast cancer) cocultured with bone stromal cells (fetal osteoblasts or bone marrow mesenchymal stem cells)	Gelatin methacrylate hydrogel with nanocrystalline hydroxyapatite	Laser-based	[38]

Table 2. 3D printing of personalised cancer treatments.

Type of personalised treatment	Objectives	Printing method	Features	Results	REF.
Hydrogels for controlled drug release	Controlled release hydrogels for the treatment of adjunct ovarian cancer	Extrusion-based	Ploxamer-based hydrogels with a solid-disk shape (12 x 1 mm) containing paclitaxel and rapamycin.	Drug release controlled over 24 h. Tumor burden decreased from 100 to 30% one day post-surgery. Mean survival increased from 20 to 30 days.	[45]
	Self-assembling thermoresponsive nanoemulsions into hierarchical mesostructured hydrogels	Laser-based	Thermoresponsive ink consisting of poly(dimethyl siloxane) droplets suspended in an aqueous phase with a surfactant, sodium dodecyl sulphate, and a crosslinker, poly(ethylene glycol) dimethacrylate.	Control of the hydrogel microstructure due to the fast structural recovery of the nanoemulsion after large strain rate yielding a long with a shear thinning behaviour that allows the ink to conform to the build platform of the printer.	[47]
	Nanogels for eradicating the postoperative residual glioblastoma	Laser-based	Gelatin methacrylamide scaffold incorporating DNA nanocomplexes composed of pVSVMP (a plasmid DNA encoding matrix protein of the vesicular stomatitis virus), Pluronic F-127 and degradable heparin-polyetherimide nanogel particles.	Insertion of the nanogels into the glioblastoma resection cavity delayed tumor recurrence and significantly prolonged the overall survival in mice.	[50]
Therapeutic implants	Titanium implants loaded with doxorubicin and apoptosis-inducing ligand for the treatment of bone cancer	Selective fusion laser	The 3D-printed wafer (1.5 × 1.5 cm ²) was electrochemically anodized to generate an anodic layer featuring unique microparticles and nanosurface topography to enhance biointegration. Doxorubicin and apoptosis-inducing ligand were loaded at 188 and 25 µg/cm ² respectively.	Burst release within 6 h followed by a slow release phase of 16 days for doxorubicin and 4 days for the apoptosis-inducing ligand. Strong <i>in vitro</i> anticancer efficacy against cancer cells.	[54]
	Implantable anticancer magnetocaloric polycaprolactone (PCL)/ Fe3O4 nanoparticulated mats	E-jet printing	A 7% (w/v) solution of PCL in solvents (DMF and DCM) was mixed with Fe3O4 magnetic starch nanoparticles producing composite solutions that were E-jet printed resulting in PCL/Fe3O4 mats.	Significantly tumor growth inhibition and prolongation of survival time of tumor bearing mice after 4 weeks of treatment based on the application of external alternating magnetic fields over 45 min every other day.	[56]
Models for breast reconstruction	Aesthetically improve breast reconstructions	Extrusion-based	3D Fuse deposition modeling printers combined with 3D scanners	Facilitates surgeon's work when designing a breast for a specific patient with its form and its own projection, from a flap.	[62]