

**Universidade de Lisboa  
Faculdade de Farmácia**



**3D & 4D Printing for Health Application:  
New Insights into  
Printed Formulations and Bioprinted Organs  
Is the Topical Route on this way?  
Topical Printed Formulations and Skin Bioprinting  
3D and 4D Printing**

**Rafaela Alexandra Dias Quitéria**

Monografia orientada pela Professora Doutora Andreia Patrícia  
Henriques Ascenso, Professora Auxiliar e coorientada pelo Professor  
Doutor Rúben Filipe Brás Pereira, Professor Auxiliar.

**Mestrado Integrado em Ciências Farmacêuticas**

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apresentado à Universidade de Lisboa através da Faculdade de Farmácia**

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## Resumo

A impressão 3D é uma tecnologia inovadora com um vasto campo de aplicações. Engloba um grupo de tecnologias versáteis capazes de revolucionar várias áreas ao oferecer soluções a certos desafios. A sua versatilidade é devida à natureza de *additive manufacturing*, fácil acesso, vasta variedade de técnicas e materiais que podem ser utilizados. Aqui as técnicas de impressão 3D e 4D e de bioimpressão são revistas com o objectivo de providenciar as mais recentes aplicações na área da saúde.

Tanto a indústria médica como a farmacêutica partilham desvantagens, tais como baixa adesão à terapêutica devida à polimedicação, falta de formulações farmacêuticas para populações específicas que levam a um baixo sucesso terapêutico, escassez e falta de dadores de órgãos, próteses à medida do doente, modelos de estudo insuficientes e o decréscimo no processo de cicatrização de feridas. Com a tecnologia de impressão 3D, a formulação de medicação personalizada que inclui comprimidos, hidrogeles, entre outras formulações farmacêuticas contendo uma ou mais substâncias activas, é possível com o uso de tintas específicas onde as substâncias activas e os excipientes são combinados. Evita o uso de vários equipamentos enquanto providencia uma variedade de perfis de libertação através da manipulação da forma das formulações.

Juntamente com a impressão 3D, novas tecnologias surgem, tais como a impressão 4D e a bioimpressão 3D, uma evolução da primeira. A técnica de bioimpressão permite a impressão de componentes biológicos ao usar *bioinks*, abrindo novos caminhos na engenharia de tecidos e medicina regenerativa. A impressão de tecidos e órgãos tornou-se uma realidade com uma taxa de sucesso promissora. Em particular, a bioimpressão de pele é um dos investimentos mais promissores para o melhoramento do tratamento e cicatrização de lesões e queimaduras, ao oferecer uma solução à medida com uma taxa de sucesso aumentada e com menos sofrimento.

Em resumo, esta revisão sobre o uso de impressão 3D e bioimpressão no desenvolvimento de formulações farmacêuticas e bioimpressão claramente evidencia o impacto positivo desta tecnologia de ponta nesta área e os seus resultados promissores no bem-estar.

**Palavras-chave:** Impressão 3D/4D; Formulações Farmacêuticas Impressas; Bioimpressão; Bioimpressão de Pele; Bioink

# Abstract

3D printing is an innovative technology with a broad range of applications. It encompasses a group of versatile technologies capable of revolutionizing various fields by offering new solutions for major challenges. Its versatility is due to the additive manufacturing nature, easy access, wide variety of techniques, and a vast range of processable materials. Hereby, the 3D and 4D printing and bioprinting techniques are overviewed aiming to provide a comprehensive state of the art of their main applications in the health field.

Both medical and pharmaceutical industries share some drawbacks such as low patient compliance due to the poly-medication, lack of appropriate pharmaceutical formulations for specific populations leading to a poor therapeutic success, scarcity and shortage of organ donors, patient-specific prosthetics, insufficient study models, and decreased success in wound healing. With 3D printing technology, the formulation of personalized medication including tablets, hydrogels, among other pharmaceutical formulations containing single or multiple drugs, is possible by using specific inks where a defined composition of drugs and excipients are combined. It avoids the use of much equipment while providing a variety of release rates through the shape manipulation of those formulations.

Alongside 3D printing, newer technologies arise, such as 4D printing as well as 3D bioprinting, an evolution of the first one. The bioprinting technique allows the fabrication of biologically functional tissue constructs and in vitro tissue models by the controlled deposition of bioinks, thus opening new avenues in tissue engineering and regenerative medicine. Tissue and organ printing have become a reality with promising rate of success. In particular, skin bioprinting is one of the most promising investments to improve the treatment and healing of skin injuries, including chronic wounds and burns, by providing a tailored solution with greater chance of success and less suffering.

In summary, this overview about the use of 3D printing and bioprinting on the development of pharmaceutical formulations and skin bioprinting clearly evidenced the positive impact of this cut edge technology in this crucial field and the promising outcomes in wellness.

**Keywords:** 3D/ 4D Printing; Printed Pharmaceutical Formulations; Bioprinting; Skin  
Bioprinting; Bioink

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# Abreviaturas

<b>2PP</b>	Two-photon polymerization
<b>AFA-LIFT</b>	Absorbing film-assisted laser induced transfer
<b>AM</b>	Additive manufacturing
<b>Ang2</b>	Angiopoietin-2
<b>BioPL</b>	Biological laser printing
<b>CAD</b>	Computer aided design
<b>CIJ</b>	Continuous inkjet printing
<b>CJP</b>	Color jet printing
<b>CLIP</b>	Continuous liquid interface production
<b>CNC</b>	Computer numerical control
<b>CNTs</b>	Carbon nanotubes
<b>CT</b>	Computerized tomography
<b>dECM</b>	Decellularized extracellular matrix
<b>DED</b>	Direct energy deposition
<b>DIW</b>	Direct ink writing
<b>DLP</b>	Digital light processing
<b>DOD</b>	Drop-on-demand
<b>EBM</b>	Electron beam melting
<b>EBAM</b>	Electron beam additive manufacturing
<b>EC</b>	Endothelial cells
<b>EPCs</b>	Endothelial progenitor cells
<b>ECM</b>	Extracellular matrix
<b>FDM</b>	Fused deposition modelling
<b>FGF</b>	Fibroblast growth factor

<b>GelMA</b>	Methacrylate gelatin or gelatine
<b>HMVECs</b>	Human microvascular endothelial cells
<b>HSCs</b>	Hematopoietic
<b>HUVECs</b>	Human umbilical vein endothelial cells
<b>iPSCs</b>	Induced pluripotent cells
<b>iPSC-ECs</b>	Induced pluripotent stem cell-derived endothelial cells
<b>IR</b>	Infrared
<b>LCST</b>	Low critical solution temperature
<b>LENS</b>	Laser engineering net shaping
<b>LGDW</b>	Laser guided direct write
<b>LIFT</b>	Laser induced forward transfer
<b>LOM</b>	Laminated object manufacturing
<b>MAPLE-DW</b>	Matrix-assisted pulsed laser evaporation direct write
<b>ME</b>	Material extrusion
<b>MJ</b>	Material jetting
<b>MRI</b>	Magnetic resonance imaging
<b>MSCs</b>	Mesenchymal cells
<b>NIR</b>	Near infrared
<b>PAM</b>	Pressure assisted micro-syringe
<b>PBF</b>	Powder bed fusion
<b>PEG</b>	Polyethylene glycol
<b>PEGDA</b>	Poly(ethylene glycol) diacrylate
<b>PEGTA</b>	Poly(ethylene glycol)-tetra-acrylate
<b>PI</b>	Photo-initiator
<b>PNIPAM</b>	Poly(N-isopropylacrylamide)

<b>SA</b>	Surface area
<b>SLA</b>	Stereolithography
<b>SLM</b>	Selective laser melting
<b>SLS</b>	Selective laser sintering
<b>SMPs</b>	Shape memory polymers
<b>SSE</b>	Semi-solid extrusion
<b>UAM</b>	Ultrasound additive manufacturing
<b>UV</b>	Ultraviolet
<b>VEGF</b>	Vascular endothelial growth factor
<b>VP</b>	Vat photopolymerization
<b>WAAW</b>	Wire arc additive manufacturing

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# 1 Introduction

3D printing is a popular technology which allows the production of customized parts in various industries, such as aerospace, automotive, electronic, fashion, food, and health, among others. Its various technologies are under continuous development allowing the processing of a wide range of materials from synthetic and natural sources (1,2). More recently, 4D printing has arisen as an evolution of 3D printing, enabling the fabrication of stimuli-responsive constructs using “smart” or stimuli-responsive materials (3).

3D printers were once out of reach of most households. Due to all advances achieved so far, more complex, accurate, and precise constructs and objects are now possible to obtain at lower cost and with a higher speed rate at the same time.

In particular, the health sector has made major investments on 3D and 4D printing considering their clear advantages. For example, usual prosthetics need to be readjusted several times before becoming a perfect fit for the patient besides further adjustments throughout the prosthetic lifetime. In addition, some implants and orthodontics present the same problem. A way to tackle this challenge is the use of 3D printing, since it uses all the gathered information obtained from medical imaging, measurements, casts, etc., combining it to create prosthetics that fit to the patient (4). The same principle has been applied to create organ models to assist in surgery planning and evaluate whether tailor-made prosthesis fit into complex defects printed in patient-specific models.

Other important applications of this type of technology correspond to the development of drug delivery systems and tissue engineering/ organ bioprinting (5,6). In fact, the 3D printing technology has allowed to surpass many challenges in both medical and pharmaceutical industries. For instance, the development of personalized medicines has been a major challenge for both paediatric and geriatric populations since most drugs have a specific dosage, not to mention that an individual might need an intermediate dosage that is not available in the market. On the other side, the lack of compatible organ donors leads to a decrease of patient’s life expectancy (6–9). Thus, 3D printing and 3D bioprinting are emerging as a solution for these struggles. Accordingly, 3D printing has allowed the printing of patient-specific drugs on demand, alongside the development of complex pharmaceutical formulations where incompatible drugs can be present in the same formulation, among other innovations (7–9). In turn, 3D

bioprinting affords the fabrication of cell-laden scaffolds in a fast and precise manner, opening the door to organ bioprinting, thus tackling the shortage of organs for transplantation as well as reducing organ rejection since cells derived from the patient can be used. Bioprinted skin is one of the possibilities with a great impact in burn injuries that need skin grafts, especially when major damage with loss of superficial and deep skin layers are observed. Moreover, it is emerging an alternative to create in vitro skin models that surpass limitations and restrictions associated to the use of synthetic and animal models for cosmetic and pharmaceutical testing (6,10–12).

## **2 Objective**

This review purposes to appraise and demonstrate how the innovations obtained in the field of 3D printing and 3D bioprinting can be applied and developed towards the pharmaceutical and medical fields to resolve old, if not ancient, and newer challenges. These developments and innovations are now being combined to create new solutions to address common bottlenecks in the pharmaceutical and medical fields. This premiss gives uniqueness to this embracing review since most research is only pointed towards to one of these fields. In fact, only by combining efforts and think of the problem as a one big web with connecting points in various fields, it might be possible to resolve challenges which are transversal to various areas.

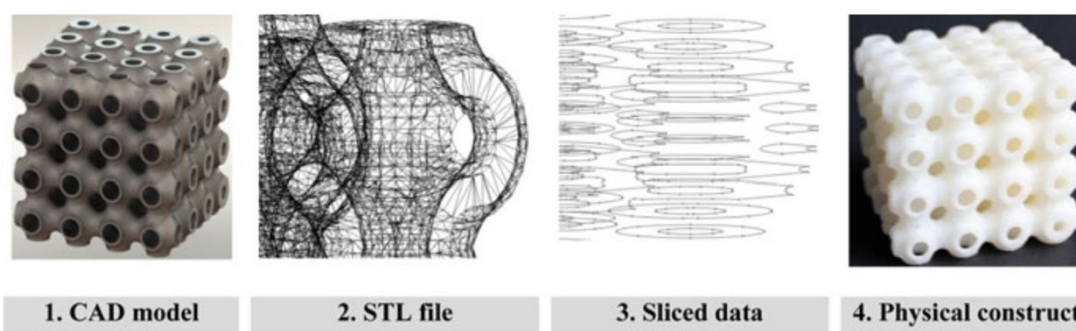
### 3 Research Methodology

The research for this literature overview was entirely based on electronic resources mainly Science Direct, PubMed and Google Scholar databases. The search was performed between January and June 2021, nonetheless most of the research was made between January and April. Overall, 86 articles were selected for reference from a total of 183 articles. The excluded and unincluded articles were rejected either by title, abstract, or the full article. The included articles were published between 2011 and 2021. Reliable information published in indexed scientific journals was strictly followed in this research methodology.

The keywords used during research were *3D printing; 4D printing; 3D bioprinting; 4D bioprinting; bioprinting; organ printing; tablets; nanoparticles; hydrogels; topical printed formulations; pills; oral printed formulations; Spritam<sup>®</sup>; bioinks; bioprinting materials; vascular printing; skin bioprinting; 3D printing technologies; applications; advantages; disadvantages; biomaterials; tissue engineering; challenges; opportunities.*

## 4 3D and 4D printing

3D printing is a type of digital technology, also known as additive manufacturing (AM), which enables the production of a three-dimensional (3D) object or part by the layer by layer deposition of a material(s) (1). It is a form of rapid prototyping due to the use of a computer aided design (CAD) software that allows developing models for 3D printing, and thus, the deposition of the materials into 3D objects with intricate designs (13,14). In Figure 1 are represented the main steps involved in additive manufacturing.



**Figure 1: Main steps involved in additive manufacturing. The image illustrates the modelling, processing and bioprinting of a 3D scaffold for tissue engineering (taken from (15)).**

4D printing can be seen as an evolution of 3D printing by the addition of the fourth dimension – time –, owing to the ability of shape change overtime by using smart materials. Those materials are mainly polymers that respond to different stimuli of physical or chemical nature, such as pH, temperature, humidity, and light. This incredible cut edge technology allows producing self-assembled objects with the use of smart active materials (such as polymers) and shape programming (3).

### 4.1 3D and 4D Printing technologies

There are many types of 3D and 4D printing technologies. Regarding 3D printing, the technology types can be subdivided in seven major groups including subgroups.

A scheme of some of these technologies is presented in Figure 2.

#### **4.1.1 3D™ Printing**

A three-dimensional structure is obtained in a platform with a layer of powder particles by spraying a liquid bonding agent on a selective way which will act as glue and bind the particles together. A new layer of powder is spread by a delivery platform placed adjacently to the lowered building platform (16,17). This technology follows the nomenclature mentioned in the International Society of Biofabrication (18,19).

#### **4.1.2 Direct energy deposition (DED)**

A motion-controlled nozzle deposits and directs material, usually powder, into a small region where a high-power laser beam is focused, thus resulting in the heating, and melting of the substrate. The object is usually formed in a motion-controlled building platform able to move in multiple axes. As this versatile technique is not limited to build on a successive and flat way, enables its use in repairing existing objects. Although similar in concept, DED differs from powder bed fusion as a directed continuous stream of powder is used instead of a pre-deposited layer of powder. This is the main reason why different and various materials can be used in this printing technique. The resolution can be controlled by intensity of the energy source (i.e., laser > electron beam > arc due to heat input) and quantity of material deposited. However, an additional surface-finishing step is often needed (16,17).

#### **4.1.3 Fused Deposition Modelling (FDM)**

Material in the form of filaments/ wires is melted into a semi-liquid state as it is pushed out through a hot nozzle at a constant pressure and deposited in ultra-fine filaments at a specific speed along the building platform that is lowered after each layer. The object is built in layers that must cool down and bind with the previous one. A support material is required for the fabrication of complex parts with overhanging regions, which is latter removed (1,16,17,20).

#### **4.1.4 Material jetting (MJ)**

In a building platform, droplets of photopolymerizable materials are selectively deposited/ jetted and solidified by a UV light source. The layers linking is possible due to the softening of the previous layer created by the deposition of a new one, enabling the construction of the object. This technique allows obtaining a smooth surface finish that is directly related with the volume of each droplet. However, sacrificial materials are required when overhanging structures and channels are desired in the final product (1,17,20).

#### **4.1.5 Powder bed fusion (PBF)**

Powder bed fusion uses a high-power thermal source which irradiates the powder bed on a selective way. The layer of linked particles is obtained through their partial or full fusion followed by another layer of powder added by a roller or a blade recoater. Two distinct source powers can be applied, such as a laser or an electron beam, depending on the use of a selective laser sintering (SLS), selective laser melting (SLM) or an electron beam melting (EBM). The sintering method can be used for various materials where the particles only partially fuse at the surface, thus resulting in inherent porosity. In the melting method, the resulting product is a highly dense and low porous one, due to the full melting of the particles. This method is commonly used in the production of metal parts. No support structure made out of sacrificial materials is here needed since the surrounding powder works as the “support structure” (17,20).

#### **4.1.6 Sheet lamination**

Materials in the form of sheets are used for sheet lamination instead of filaments and powders. In this process, each sheet is cut by laser and bond together by either thermal bonding or clamping bonding in laminated object manufacturing (LOM) or even by ultrasounds in ultrasound additive manufacturing (UAM). This last technique is considered a hybrid process as it includes ultrasonic metal welding and computer numerical control (CNC) milling. It is commonly used in metal construct productions (16,17).

#### 4.1.7 Vat photopolymerization (VP)

This technique is based on the principle of photopolymerization where small monomers are crosslinked into chain-like polymers by reaction with catalysts (optional) and photoinitiators. Photopolymers are cured within a vat using either visible or ultraviolet (UV) light. The number of crosslinks must be sufficient and strong enough to maintain the structural integrity during application. Various techniques are available such as stereolithography (SLA), digital light processing (DLP), and continuous liquid interface production (CLIP). In this case, layers are stacked and solidified one upon the other on a building plate. Two-photon polymerization (2PP) enables the polymerization on a focused area with a higher resolution and volumetric 3D printing, allowing to obtain a construct without layering by using a rotating tub and an index-matching resin (1,17,20).

4D printing uses the same techniques above described for 3D printing, nevertheless these techniques may require some adaptation to process smart materials. An example of an important adaption is the addition of the air circulation system that helps cooling the material below its glass transition temperature in the FDM process (3).

The advantages and disadvantages as well as equipment, materials, and resolution of 3D and 4D techniques are listed in Table 1.

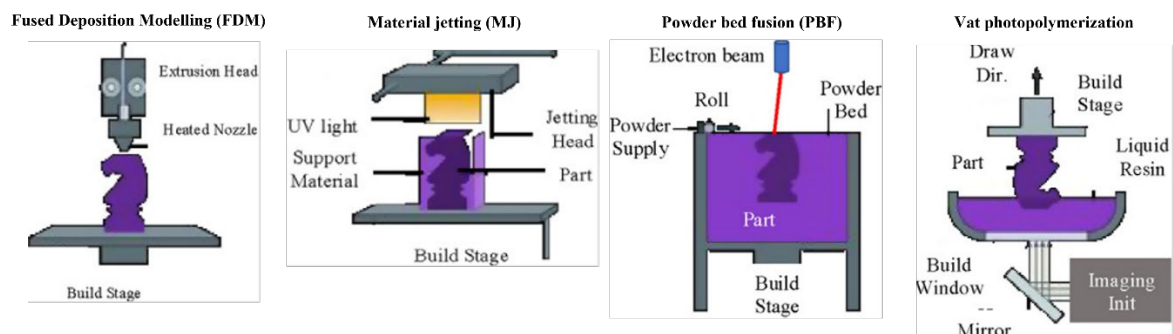


Figure 2: Scheme of 3D printing technologies (Taken from (20))

**Table 1: Equipment, materials, resolution, and advantages versus disadvantages of 3D and 4D printing techniques.**

	<b>Binder jetting</b>	<b>Direct energy deposition (DED)</b>	<b>Fused deposition modelling (FDM)</b>	<b>Material jetting (MJ)</b>	<b>Powder bed fusion (PBF)</b>	<b>Sheet lamination</b>	<b>Vat photopolymerization</b>
<b>Advantages</b>	<ul style="list-style-type: none"> <li>• Free support</li> <li>• Design freedom</li> <li>• Large build volume</li> <li>• High print speed</li> </ul>	<ul style="list-style-type: none"> <li>• Allows repairing of existing objects</li> <li>• Not limited to build in a flat manner</li> <li>•</li> </ul>	<ul style="list-style-type: none"> <li>• Low cost (desktop printers)</li> <li>• Build fully functional parts of products</li> <li>• Operating at room temperature</li> </ul>	<ul style="list-style-type: none"> <li>• Better surface quality</li> <li>• Overhanging structures and channels using sacrificial materials</li> <li>• Wide range of materials</li> </ul>	<ul style="list-style-type: none"> <li>• Overhanging structures without the need of sacrificial material</li> <li>• Fast speed</li> <li>• Structures with bulk-like material</li> <li>• Fast solidification</li> <li>• High accuracy</li> </ul>	<ul style="list-style-type: none"> <li>• Full colours print</li> <li>• Relatively inexpensive</li> <li>• Easy material handling</li> <li>• Excess material can be recycled</li> <li>• More complex geometrical forms can be obtained at lower cost of fabrication and less operational time</li> </ul>	<ul style="list-style-type: none"> <li>• High resolution</li> <li>• Fast process</li> <li>• High quality surface</li> </ul>
<b>Disadvantages</b>	<ul style="list-style-type: none"> <li>• Low Resolution</li> <li>• Less choice of materials</li> <li>• Poor Surface finish</li> <li>• High Porosity</li> </ul>	<ul style="list-style-type: none"> <li>• Additional finishing-surface step is often needed</li> </ul>	<ul style="list-style-type: none"> <li>• Less choice of materials</li> <li>• High Porosity Thermoplastics and thermoplastics composites: limitations on mechanical and chemical properties</li> <li>• Difficulty in making voids an overhanging structure; sacrificial material needed</li> <li>• Coating, polishing, and sandpapering may be required</li> </ul>	<ul style="list-style-type: none"> <li>• Expensive printers and resins</li> </ul>	<ul style="list-style-type: none"> <li>• High temperatures limit the materials choice</li> <li>• Surface finish-rough (related to porosity and powder particle size)</li> </ul>	<ul style="list-style-type: none"> <li>• High material waste</li> <li>• Difficult to remove support trapped in internal cavities</li> <li>• Thermal cutting produces noxious fumes</li> <li>• Possible deviation of shape as a result of heat of laser</li> </ul>	<ul style="list-style-type: none"> <li>• Photosensitive materials</li> <li>• Photoinitiator and UV light irradiation</li> </ul>

<b>Equipments(®)</b>	<ul style="list-style-type: none"> <li>• ExOne</li> <li>• Voxeljet</li> <li>• Color Jet Printing (CJP)</li> </ul>	<ul style="list-style-type: none"> <li>• Optomec’s Laser Engineered Net Shaping (LENS)</li> <li>• InssTek DMT 3D Metal Printers</li> <li>• BeAm machines</li> <li>• Sciaky’s electron beam AM (EBAM)</li> <li>• Wire+arc AM source (WAAM)</li> </ul>	<ul style="list-style-type: none"> <li>• Stratassy’s Fortus Production Series</li> </ul>	<ul style="list-style-type: none"> <li>• Stratasys’s production series Polyjet machines</li> <li>• 3D System’s Multi Jet Printing machines</li> </ul>	<ul style="list-style-type: none"> <li>• Aconity3D</li> </ul>	<ul style="list-style-type: none"> <li>• MCor’s 3D printers</li> </ul>	<ul style="list-style-type: none"> <li>• Lumen X+™ DLP Bioprinter</li> </ul>
<b>Materials</b>	<ul style="list-style-type: none"> <li>• Ceramic</li> <li>• Metal</li> <li>• Glass</li> <li>• Sand</li> <li>• Polymer</li> </ul>	<ul style="list-style-type: none"> <li>• Stainless steels</li> <li>• Copper</li> <li>• Aluminium</li> <li>• Titanium</li> <li>• Nickel</li> <li>• Cobalt</li> <li>• Tin</li> </ul>	<ul style="list-style-type: none"> <li>• Thermoplastics</li> </ul>	<ul style="list-style-type: none"> <li>• Photopolymer</li> <li>• Polymers</li> <li>• Ceramics</li> <li>• Composite</li> <li>• Biological and hybrid</li> </ul>	<ul style="list-style-type: none"> <li>• Titanium</li> <li>• Stainless steel</li> <li>• Aluminium</li> <li>• Cobalt-chrome</li> <li>• Nickel based alloys</li> </ul>	<ul style="list-style-type: none"> <li>• Paper</li> <li>• Plastic sheets</li> <li>• Metal sheets</li> </ul>	<ul style="list-style-type: none"> <li>• VP photopolymers <ul style="list-style-type: none"> <li>○ Monomers/oligomers</li> <li>○ Photo-initiators</li> <li>○ Additives <ul style="list-style-type: none"> <li>▪ Stabilizers</li> <li>▪ Flexibilizers</li> <li>▪ Reactive diluents</li> <li>▪ Solvents</li> </ul> </li> </ul> </li> </ul>
<b>Resolution</b>	<ul style="list-style-type: none"> <li>• High</li> </ul>	<ul style="list-style-type: none"> <li>• Low</li> </ul>	<ul style="list-style-type: none"> <li>• Dependent on the nozzle size, viscosity, and flow characteristics of the filament</li> <li>• Surface finish and porosity depends on the z-axis movement control and the adherence between layers</li> </ul>	<ul style="list-style-type: none"> <li>• High</li> </ul>	<ul style="list-style-type: none"> <li>• Low</li> </ul>	<ul style="list-style-type: none"> <li>• High</li> </ul>	<ul style="list-style-type: none"> <li>• High</li> </ul>

## **4.2 Applications**

3D and 4D printing have been used for applications in various fields, including aerospace, automotive, electronic, fashion, food, and health industries among others, thus showing how versatile, useful, and innovating these technologies can be. In particular, their application on healthcare, medical, and pharmaceutical industry will be here discussed in detail.

In fact, this is one of the most innovating and useful tools in drug developing, medical research and design of new pharmaceutical forms. On one hand, it allows the printing of customized drugs with high reproducibility and complex release profiles. On the other one, the organ printing, such as skin, bone, and cartilage, represent a great advance in medicine (1,2).

### **4.2.1 Innovative Solutions for Biomedical Applications**

#### **4.2.1.1 Surgical planning**

Although being very useful and important for pre-operation planning, Computerized Tomography, and Magnetic Resonance Imaging (CT and MRI, respectively) could be not enough for all interventions, especially when certain complications are only known after the procedure has begun. However, if CT and MRI are combined with 3D printing technology, an accurate model of the patient organ could be built, thus allowing a complete understanding of its anatomy and physiology. The printed model provides an identical representation of the vascular anatomy and a deeper understanding of spatial relationships between elements of anatomy, enabling the rerun of different scenarios and the creation of back-plans. As a consequence, the diagnosis, assessment, and evaluation will be more accurate, reducing complications and operation time, hospital stay, and patient loss, and thereby, the healthcare costs (4).

These models can be also used for teaching and training of medicine students being more realistic, affordable and available than traditional ways (e.g. corpses and simulators) (4).

#### **4.2.1.2 Medical devices**

Tailor made medical devices can be fabricated without the need of further fitting, which is a great advantage (4). Several medical devices are obtained by printing technique as follows, hearing aids, prosthetics limbs, stents, medical utensils, among others.

##### ***4.2.1.2.1 Orthodontics, Implants and Prosthetics***

For example, dental scaffolds and surgical drill guides can be obtained by 3D printing, resulting in a more comfortable and shorter treatment (4).

Conventional moulding techniques have limitations concerning prosthetics or implant fabrication. Highly specific CAD models can be created by using patient's biometric scans obtained through CT, MRI, and negative scans of the negative plaster mould, resulting in a perfect fit implant/ prosthetic. This method provides several advantages such as flexible production at a low cost and with reduced waste of materials, besides allowing the implementation of drug release mechanisms (4).

##### ***4.2.1.2.2 Wound dressings***

3D printed wound dressing can fit to the wound shape and topography, besides other medical applications, resulting in faster healing with less complications in a personalized manner (4).

#### **4.2.1.3 Tissue engineering and Organ Bioprinting**

Functional tissue structures and organs have been printed using biomaterials, living cells, and biochemicals. This technology tackles the problematic lack of compatible donors and chronic immunosuppressant treatment, by using cells provided by the own patient (4).

#### **4.2.1.4 Drug Delivery Systems**

Pills and tablets can be printed on demand and fit every patient individually. Specific dosage, shape and structure are achievable, allowing a higher control and efficiency.

Other pharmaceutical formulations as well as electronic devices can be obtained by printing, thus enabling control and monitoring of drug release, which will lead to a perfect therapy option (4,5).

#### **4.2.2 Applications and Market Demands in the Pharmaceutical Field**

3D printing enables the printing of various pharmaceutical formulations, such as polypills, dose specific formulations (i.e., tablets) with or without complex release profiles, personalization, and individualization of medication, among others, not to mention its ability for on demand production, decreased need of various machinery and excipients, culminating in a decrease of cost (7–9). These arise as solution for some of market demands and old challenges, such as low compliance of poly-medication, “one size fits all” industry mentality, lack of formulations for paediatric and geriatric populations, and so on. Thus, resulting in a more sustainable way of producing new medication (7–9).

The factors responsible for the tailoring of the release rate of these pharmaceutical formulations are common throughout all techniques. The factors in question are shape and surface area/ ratio.

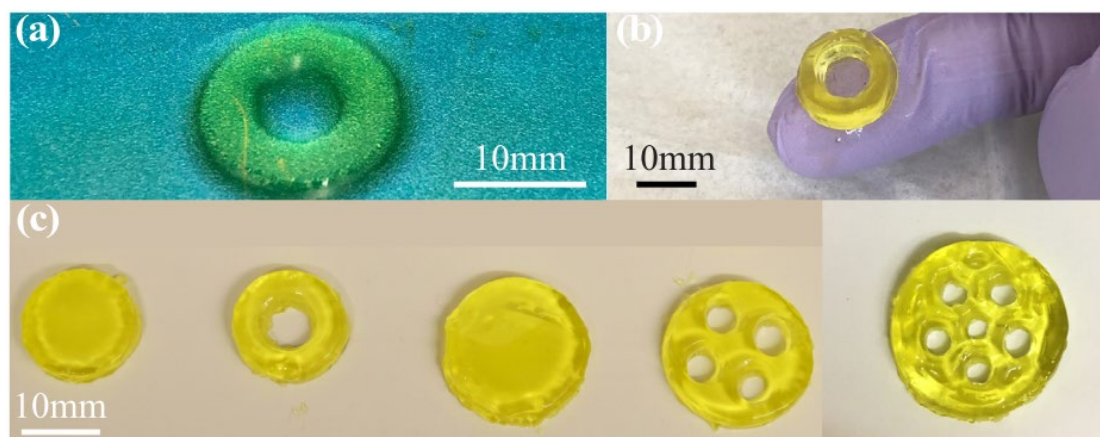
##### **4.2.2.1 Hydrogels**

###### **4.2.2.1.1 Stereolithography (SLA)**

SLA is an orifice-free printing technology capable of producing pharmaceutical formulations, such as hydrogels, by photopolymerization. High resolution and surface finish can be achieved in a short time and be extremely useful when working with thermolabile drugs. The main issues are the reduced number of photocurable resins (21,22) and the potential toxicity of photo-initiator (PI) needed for the curing process (21,23). SLA has become a useful tool in customized drug delivery systems with high control and flexibility, enabling the production of complex of personalized medicines (21).

Hydrogels containing hydrophilic vitamins have been produced by this technique using poly(ethylene glycol) diacrylate (PEGDA) as the encapsulating polymer network, and

ascorbic acid as the PI. Various vitamins release rates were achieved mostly due to the changes in geometry while keeping a basic composition. Multi-layer polypills up to six drugs and hydrogels containing paracetamol have been also successfully produced by SLA (21,23). Figure 3 is an example of a printed hydrogel.



**Figure 3: Hydrogel tablets printed in various sizes and shapes (taken from (21)).**

#### 4.2.2.2 Solid/Oral Forms

##### 4.2.2.2.1 *Semi-solid extrusion (SSE)*

This technology allows the use of thermolabile and heat-sensitive drugs and excipients, due to the ability of rapid printing at room temperature of gels and pastes (24).

Lipid solid tablets were already printed by using emulsion gels containing lipid-based formulations capable of delivering fenofibrate, a poorly water-soluble drug. The mass and dose uniformity, easy handling, and a strong structure with a rapid dissolution time of these tablets were comparable with those obtained by traditional technique. Thus, SSE allows an easy way of achieving dose adjustments on demand of poorly water-soluble drugs (24).

##### 4.2.2.2.1.1 **Pressure assisted micro-syringe (PAM)**

This is a semi-solid extrusion technique at room temperature where the ink is extruded through the nozzle by pressured air, instead of melting, thus being optimal for thermosensitive drugs (25,26). It requires the use of solvents, usually organic, which leads to a required drying step besides a toxicity evaluation.

For example, long-lasting and stable tablets of levetiracetam were obtained by PAM with good printing quality and without the use of organic solvents. These tablets were quite comparable to those obtained through conventional tableting (25). In another study, a multilayer orodispersible tablet with different doses was produced for paediatrics subgroups in an hospital context. It was observed that the dissolution time varied with the number of layers. The main problem relied on the tablet size for infants and especially neonates. Nonetheless, it proved to be a reproducible process and concordant with the requirements of European Pharmacopoeia (26).

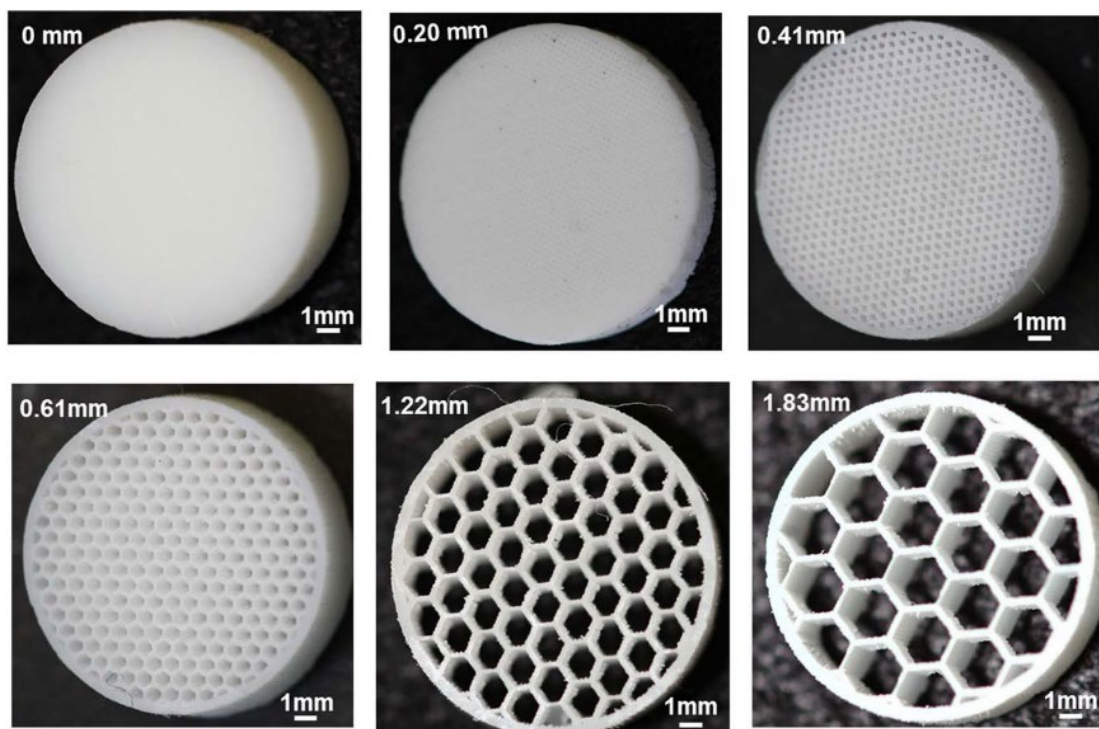
#### ***4.2.2.2.2 Screen printing***

3D screen printing allows a flexible way of printing different tablets with only one screen print by pushing a paste with the active pharmaceutical ingredient through the screen. It is possible to obtain tablets highly comparable to conventional tableting, in terms of mass and content unity, friability, and breaking force. Whereas the friability is due in part to the drying process and mostly to the formulation composition (27). 3D screen printing proved to be a high reproducible, flexible and easy technology in large-scale manufacturing tablets with different geometries, sizes and drug release rates (27). For example, paracetamol tablets designed for delayed drug release of various sizes and geometries (donut, disk, cuboid, oval, and grid) were produced by 3D screen printing (27).

#### ***4.2.2.2.3 Inkjet printing***

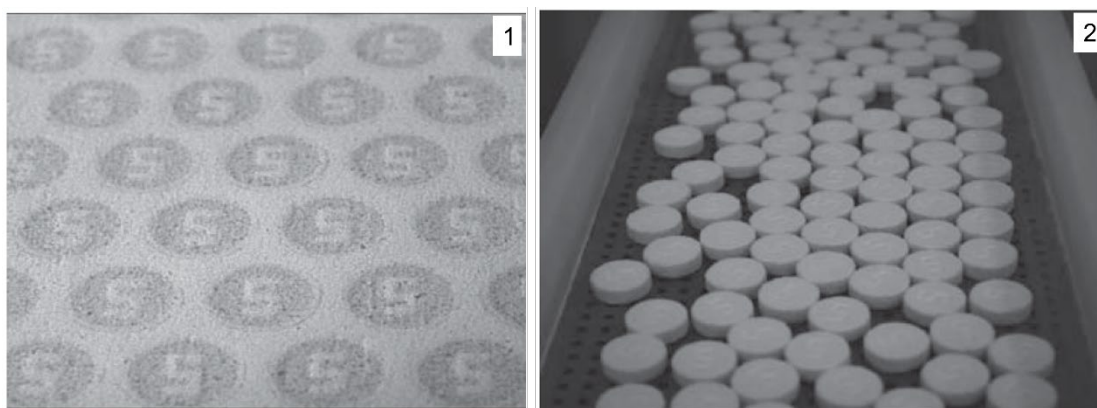
A technology that enables the deposition of multiple inks simultaneously, allowing an accurate dosing, with high spatial resolution with few formulation ink and without the need of solvents (22,25). It is very useful for paediatric therapy.

For instance, honeycomb shaped tablets were produced by inkjet printing (Figure 4), enabling personalized release rate of a single or multiple drug in one tablet (25). Tablets of poorly-soluble drugs, such as carvedilol, have been also produced with UV inkjet printing with great success and achieving different release rates by changing geometry, while using the same ink formulation (22).



**Figure 4: Printed solid tablet and honeycomb-like tablets with varying cell sizes (Taken from (22)).**

ZipDose<sup>®</sup> is an inkjet printing technology, which was used for the development and production of the first FDA approved 3D printed tablet, Aprecia's Spritam<sup>®</sup> (Figure 5). It uses the print glue approach, where binder solutions are used to consolidate API-containing powders into rapid-dispersing tablets (28). Spritam<sup>®</sup> is an orodispersible tablet of levetiracetam, which can hold higher loads of levetiracetam than conventional tablets present in the market, while presenting a smaller size due to their increased porosity. Therefore, it tackles the issue with increasing doses that usually lead to the administrations of bigger tablets at multiple times a day, compromising the medication compliance (29).



**Figure 5: |1| Spritam<sup>®</sup> tablets after printing and drying; |2| Spritam<sup>®</sup> tablets after harvesting (taken from (29)).**

#### ***4.2.2.2.4 Fused deposition modelling (FDM)***

This is a method in which higher working temperature favours the mixing and viscosity, yet it becomes a problem when working with a thermosensitive drug, leading to its degradation (30).

A multicompartment tablet with two different release rates was produced via FDM. This tablet was composed by two gel layers, where the diffusion occurred, and one porous shell, that worked as an osmotic pump (31). In another study, it was possible to produce different dose tablets while keeping the same release rate, by using filaments of different drug concentrations or the same drug concentration with a placebo filament (30).

#### **4.2.2.3 Advanced Controlled Drug Delivery Systems**

Advanced controlled drug delivery systems are a new frontier in drug delivery, allowing a target therapy due to the improvement of drug solubility, bioavailability, stability, and the capacity of overcoming biological barriers (32–36). The main techniques to obtain specific drug delivery systems, such as hydrogels with nanocrystals and nanofibers of cellulose and nanoparticles, are described as follows. Nevertheless, few reports about this application appear in literature to this date and a long way is still in progress.

#### **4.2.2.3.1 *Direct ink writing (DIW)***

DIW is a technique able to print complex shape morphing nanocellulose-based composites with antimicrobial properties by using hydrogels as an ink. Hydrogels possess physical-chemical similarities with soft tissues, allowing them to be great candidates for smart drug delivery systems and wound dressings (37). Such structures have been conceived with the use of hydrogels with nanocrystals and nanofibers of cellulose on its composition (37). The alignment of these components depend on the nanocellulose concentration and the needle diameter (37–39). The ability to change the shape is achieved with the orientation of nanocellulose reinforcements within the hydrogels creating an internal stress when swelling begins, therefore forcing a programmed deformation. This shape changing ability was based on the mechanism of swelling and shrinking of cells walls of plants, when exposed to different amounts of water. Thus, DIW has become very useful in wound healing treatments due to their capacity of modelling its shape to the water percentage, accompanying therefore the wound shape changings (37).

#### **4.2.2.3.2 *Fused deposition modelling (FDM)***

Solid state materials are heated nearly to its melting point temperature, and then extruded through a heated nozzle in the form of semi-molten strings, onto a platform that lowers itself between every layer. Single or multiple materials can be printed simultaneously, also allowing the use of sacrificial materials for removable support structures (6).

Nanoparticles could be introduced into solid pharmaceutical forms, by soaking the FDM printing tablets into a nanoparticle suspension. These tablets possessed a channelling agent which was responsible for the infill percentage and the drug loading of the tablet. It proved to be an innovating and successful mean of producing solid oral forms with nanoparticles obtained by a simple and inexpensive manner (32).

## 5 Bioprinting

3D bioprinting allows the production of cell-laden tissue scaffolds and in vitro tissue models in a fast and precise manner, by assembling living and non-living biomaterials in specific patterns (6,18,40). Both tissue constructs and in vitro models are often composed of biomaterials, growth factors and tissue-specific cells, which are printed into specific 3D locations and arrangements, towards promoting tissue modelling and regeneration (6,41–46). Therefore, 3D bioprinting enables the construction of tissues and organs, thus a possible solution for the lack of a compatible organ donor. This bioprinted construction must be able to replicate the vascular networks of the tissue and to integrate with the body vascularization system in order to maintain functions and possess sufficient mechanical properties which match the host tissue (6).

### 5.1 Bioprinting technologies/Methods

A scheme of these technologies is presented in Figure 6.

#### 5.1.1 Inkjet bioprinting

Inkjet allows the bioprinting of single or multiple materials at the same time, with high resolution patterning and no restrictions on the spatial arrangement. This technology follows the nomenclature mentioned in the International Society of Biofabrication. Essential features for cell growth and proliferations, such as perfusable channels and interconnected pores, can be produced with inkjet bioprinting (6).

Continuous inkjet printing (CIJ) and drop-on-demand inkjet printing (DOD) are two distinct operating modes. However, the most suited for bioprinting is DOD, since CIJ requires an electrically conducted ink and it has low printing resolution and high risk of contamination (6).

There are two sub-types of DOD inkjet printing, piezoelectric and thermal. The first one, uses a piezoelectric material that surrounds the ink chamber, which then applies a mechanical force leading to a sudden volume change of the ink resulting in the formation of droplets. In thermal DOD inkjet printing, a heater/resistor creates a vapour bubble, by vaporizing a small amount of ink, resulting in the volume change needed for the ejection of the droplet (6).

DOD allows reactive inkjet printing, meaning that a droplet of different inks is deposited one after other in the same spot, causing reactions and formation of product material. This becomes a solution for materials that are difficult to transform into printable inks but that can be formed through reactive printing (6).

DOD allows to achieve gradient concentrations through droplet size and frequency, precise control of deposition with high resolution, low risk of cross-contamination since no contact is established, and low material waste due to the use of quite low ink volumes which enable the printing of extremely expensive biomaterials, such as enzymes, growth factors, and hormones (6,46,47). Unfortunately, DOD also presents some disadvantages such as a nozzle size that could lead to clogging, irregular droplet size, low printing resolution, limited choices of printing materials, lowered cell and biomaterials viability due to heat and stress applied during printing, cells deposition in the ink reservoir, and slow fabrication speeds (6).

### **5.1.2 Extrusion-based bioprinting**

Extrusion-based bioprinting is capable of printing cell-laden materials with high cell density, thus allowing a large scale production of biofabricated tissues and organs (6,48,49). However, it possesses low printing resolution and the materials used require a shear thinning ability to overcome their surface tension, and in turn, the stress resulted from that process and solidification itself, can lower the viability of living cells (6,50).

Mechanical extrusion bioprinting uses bioinks in viscous or pre-crosslinked form, which are deposited through the nozzle by a piston, pneumatic pressure, or a rotating screw mechanism. Pneumatic extrusion has the slowest printing speed, while piston-driven extrusion enables a more controlled and faster printing, and screw driven extrusion allows the use of very high viscosity hydrogels, though it has not been commonly used for bioprinting cells. Then again, as mentioned before, the cell viability might be compromised due to the pressure and shear stress applied. The resolution, the physiochemical properties of the bioinks and the construct geometry will all affect the extrusion rate (6).

Extrusion bioprinting is the most popular bioprinting technology for the fabrication tissue constructs and in vitro models owing its versatility and ability to integrate

multiple modules into the bioprinter towards the processing of a broad range of biomaterials. This includes temperature control modules, light-curing systems or spray nozzles, allowing the deposition of temperature sensitive biomaterials (e.g., collagen) or the crosslinking of photosensitive (bio)inks. Multiple bioprinting strategies have been developed using extrusion bioprinting, such as the direct deposition of (bio)inks to create 3D constructs as well as the deposition of sacrificial inks (e.g., Pluronics, gelatin), which are later removed from the bioprinted construct. Bioprinting of sacrificial inks has been widely explored for the fabrication of 3D constructs with perfusable channels in an attempt to address the challenge of vascularization (6).

### **5.1.3 Stereolithography (SLA)**

Stereolithography (SLA) allows the construction of tissue scaffolds with multiple microenvironments, being quite helpful in lineage differentiation of stem cells. To create these constructs, a light source (UV, infrared or high-intensity laser) polymerizes the region when it hits and crosslinks the material, thus generating the desired structure (6).

The main limitation of this technique is the need for photo-curable materials which are biocompatible and biodegradable (6).

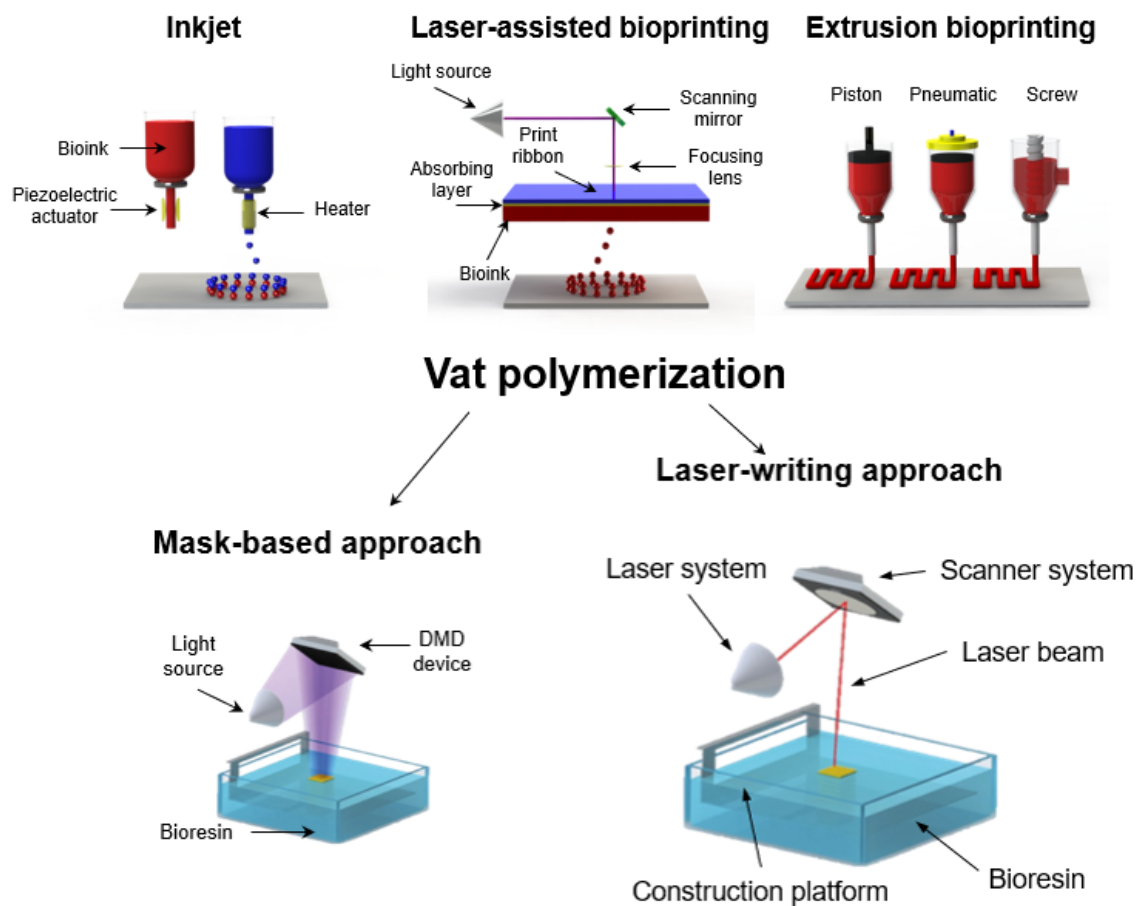
### **5.1.4 Laser-assisted bioprinting**

Although mostly used for metal and electronic components printing, laser-assisted printing has also been adapted for bioprinting. There are numerous variations, as laser guided direct write (LGDW), laser induced forward transfer (LIFT), absorbing film-assisted laser induced forward transfer (AFA-LIFT), matrix-assisted pulsed laser evaporation direct write (MAPLE-DW) and biological laser printing (BioPL), all based on different laser sources and energy absorbing layers (6).

In laser-assisted bioprinting, the laser beam is guided through mirrors onto a bioink, which is supported on a thin and transparent solid surface. The energy produced by the laser beam is absorbed by the bioink, resulting in a vapour bubble which causes a tiny volume of the bioink that leaves a film surface deposited on the receiver substrate. Cell

viability is increased with higher viscosity and thickness of bioinks while higher energy laser beam decreases the cell viability (6).

This technology has the highest resolution without nozzle clogging, and yet, it is generally limited to printing 2D constructs alongside photonic cell damage, cytotoxicity induced by using metal energy absorbing layer in LIFT and AFA-LIFT and limited scalability. However, there is an increased interest for its use on the construction of vascular structures since hollow and overhanging structures can be printed with higher viscosities bioinks (6).



**Figure 6: Scheme of bioprinting technologies (Taken from (51,52)).**

## 5.2 Applications

3D bioprinting has been used for several purposes in tissue regeneration, regenerative medicine, and other biomedical applications (10).

Some medical and surgical materials present a small size, personal differences depending on patient anatomy (i.e., hearing aid), leading to higher costs. 3D bioprinting tackles these issues, allowing a cheaper and patient specific manner of fabrication (10).

Tissue regeneration is one of the main applications of 3D bioprinting, alongside with organ bioprinting. Bone, vascular systems, and organs, such as liver and skin, have been successfully bioprinted. For example, *Cubo et al.* demonstrated the generation of human bilayered skin constructs, and *Dvir et al.* bioprinted a fully personalized contracting cardiac patches using patient's cells, which matched the anatomical, biochemical, cellular, and immunological properties of the patient. However, these biomimetic structures are still simple, and yet complex enough to perform basic function of the organ/structure in question. Developments have been made to improve and conceive the most complex, realistic, and patient specific organs and tissues, thus being an alternative for skin grafts and organ donations, which could lead to organ rejection (10,11,53). In fact, this technique allows adjusting the presentation and concentration of growth factors, cell adhesion, and degradation in different regions of the biomaterials and loading of different types of cells in specific locations. Nevertheless, some drawbacks, such as early or unwanted interactions between biological active materials that lead to the stem cell differentiation, might occur besides the limited type of cells that can be used in bioprinting (41). Depending on the type of equipment, a prolonged bioprinting time and/ or nozzle clogging might be observed as well.

**Table 2: Advantages and limitations of 3D bioprinting (adapted from (10)).**

<b>Advantages</b>	<b>Limitations</b>
<ul style="list-style-type: none"><li>• Production of biomimetic constructs</li><li>• Simultaneous production of structures with different physical and chemical properties and functions</li><li>• Adjustment of growth factors concentration, cell adhesion, and degradation in different regions of the biomaterials</li><li>• Loading of different types of cells in different regions</li><li>• Patient specific, therefore increased compliance</li></ul>	<ul style="list-style-type: none"><li>• Biological active materials might interact with each other leading to early or unwanted cellular interactions and stem cell differentiation</li><li>• Prolonged production time for highly complex constructs</li><li>• Nozzle clogging (depending on the technology)</li></ul>

### **5.3 Bioinks and Composite Scaffolds**

Bioink is a formulation of material(s) and biological molecules or cells processed using bioprinting technologies (54). Appropriate mechanical properties and biodegradability to act as a supportive frame, good biocompatibility, and low cytotoxicity to facilitate cell growth, are the main properties that these inks should possess (6,55).

#### **5.3.1 Natural polymers**

##### **5.3.1.1 Collagen**

Collagen is the main component of extracellular matrix (ECM) being quite important for cell growth in tissue regeneration. Collagen hydrogels are mostly used for extrusion-based bioprinting due to its quick gelation rate, though it imposes some constraints to the bioprinting process (6,56). A combination of collagen and synthetic polymers is usually used with the addition of biocompatible and non-toxic crosslinkers to improve printability (6,57).

### **5.3.1.2 Gelatine**

Gelatine is a fibrous protein derived from collagen by irreversible hydrolysis and it is water-absorbing, biocompatible, non-immunogenic, and biodegradable. It is usually used as a sacrificial support material and ECM-analogue. There are two types of gelatine, i.e., aqueous type which is thermosensitive and solid form which is easily utilized in extrusion-based printing (6).

Whereas thickeners and transglutaminase are applied to improve both stability and printability, methacrylate gelatine (GelMA) has been used to improve printing resolution. This results in crosslinking reactions promoted either by heat or UV and adjusted by the density of the methacryloyl group. GelMA hydrogels promote osteogenesis and vasculogenesis while maintaining a good cell viability and proliferation. However, the cell viability needs to be evaluated as a function of UV exposure and PI (6,58).

### **5.3.1.3 Decellularized extracellular matrix**

Decellularized extracellular matrix (dECM) is obtained via the processing of tissues or organs towards the removal of cells, while preserving the main structural components of the ECM, which are responsible for the biological recognition and cell response (6,59). For achieving a greater success, it is better to use targeted tissue dECM derived from the patient as it possesses specific components of the tissue crucial for its development besides avoiding an immunological response (6). Crosslinkers can be incorporated to enable the UV-crosslinking and achieve the preservation of shape, unreachable before due to a slow gelation process (6,60,61).

### **5.3.1.4 Fibrin**

Fibrin inherently presents promotes cell-adhesion, since it takes part of the clotting process. It is usually used in wound healing and tissue modelling due to its ability to mimic the elastic behaviour of soft tissues and to the induction of growth factors, such as VEGF and FGF, leading to angiogenesis (6,62). Addition of polymers (e.g., PEGs)

and chemical modifications are used to improve the mechanical properties and to diminish the degradation rate of fibrin while maintaining the bioactivity (6,63).

#### **5.3.1.5 Alginate**

Alginate hydrogels are formed through ionic crosslinking in the presence of divalent cations. These hydrogels can hold water and possess a porous internal structure important for cellular metabolism and waste removal. They are usually used as a scaffolding material with structural properties that minimise the effect of shear stress on cells in inkjet and extrusion-based 3D bioprinting (6).

The crosslinking can be easily removed by introducing monovalent cations in the surrounding medium, leading to exchange reactions with the divalent ions crosslinkers and resulting in its release. This allows the use of alginate hydrogels as a sacrificial material to achieve perfusable tubular structures (6,64,65).

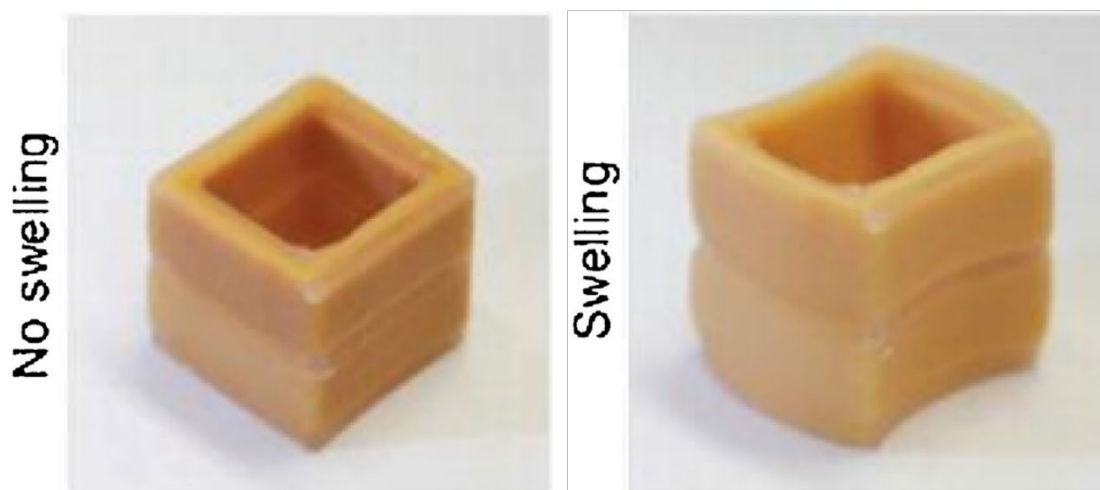
#### **5.3.2 Synthetic polymers**

PEG based polymers, such as poly(ethylene glycol) diacrylate (PEGDA) and poly(ethylene glycol)-tetra-acrylate (PEGTA), are typically employed in UV/visible light assisted bioprinting for its tailorable mechanical properties, processability, and biocompatibility. Through the molecular weight and the concentration of polymers, it is possible to control the printability. Normally, PEGDA and PEGTA are combined with GelMA to improve the cell response due to the lack of cell-adhering moieties of PEGs alone (6).

#### **5.3.3 Stimuli responsive materials**

Human tissues respond to external stimuli to properly function and the same should happen to bioprinted constructs for achieving the success of implantation. Therefore, stimuli-responsive materials use has grown and applied to 3D bioprinting, thereby creating constructs able to mimic physiological behaviours. This technology is called 4D bioprinting due to its successful integration of time as a fourth dimension (66).

4D bioprinted constructs can respond to stimuli such as water, temperature, pH, ionic concentration, light, electric, and magnetic field (Figure 7). Stimuli-responsive material need to have good printability, biocompatibility, gentle responsiveness, and proper mechanical supporting ability. However, materials with such characteristics are still limited (66).



**Figure 7: Shape change of a magnetic-responsive material (taken from (3)).**

### **5.3.3.1 Physical stimuli responsive materials**

#### ***5.3.3.1.1 Temperature-responsive materials***

Temperature-responsive materials change their shape when exposed to temperature variations from the exterior. Some hydrogels are temperature-responsive as well as shape memory polymers (SMPs). The latter possess intrinsic shape recovery capability, which allows the recovery of the primary shape under temperature stimulation. However, cells can only be incorporated after printing with SMPs (66).

On the other hand, hydrogels based on the classical poly(N-isopropylacrylamide) (PNIPAM), allow a better accommodation of living cells due to its high-water content while maintaining a switchable swelling property. Nevertheless, its low critical solution temperature (LCST) may lead to lower biodegradability, biocompatibility, and bioactivity (66).

#### **5.3.3.1.2 Humidity-responsive materials**

Humidity-responsive materials change their size, shape, and structure by shrinking and swelling depending on the surrounding humidity levels in a facile and safe manner while maintaining the cell viability. This provides the creation of cell-laden hollow tubes with internal diameters reaching to 20  $\mu\text{m}$  (66).

#### **5.3.3.1.3 Magnetic-responsive materials**

Compounds such as Fe,  $\text{Fe}_2\text{O}_4$ , and NdFeB are incorporated in other materials in nanoparticle form resulting in magnetic-responsive materials. This allows, for example, applying a straightforward magnetic field during the bioprinting and an alignment of collagen fibers. It can also be extended to shape control and soft robotics (66).

#### **5.3.3.1.4 Photo-responsive materials**

Photo-responsive materials can change their conformation and polarity stimulated by light from ultraviolet (UV) to infrared (IR) bands. Due to its relatively lower cell toxicity and higher penetration, near infrared (NIR) and IR are the preferred light sources (66).

Since the radiation produces heat, photo-responsive materials have an intrinsic temperature responsive property also known as photothermal effect. Photodegradation is another mechanism of photo-responsive materials, which induces deformation modulated by dose of radiation and the number of photodegradable moieties. These characteristics allows the development of bioinks capable of producing cell culture matrices able to mimic the degradation of ECM (66).

#### **5.3.3.1.5 Electro-responsive materials**

Electro-responsive materials can shrink, swell, or fold under an external electric field. Polyelectrolyte polymers are mostly used, and its behaviour can be regulated by both strength and direction of the electric field. Due to its biocompatibility and printability, new bioelectronics interfaces and neuroprosthetic devices can be developed. Other materials, such as carbon nanotubes (CNTs) and graphene, possess desired mechanical,

electrical, and cytocompatible properties for bioprinting that are quite suitable to control the biology and fate of stem cells and thus, for nerve tissue engineering (67).

#### ***5.3.3.1.6 Acoustic responsive materials***

Acoustic responsive materials permit the use of acoustic force patterning, which enables an accurate construction of location-controlled cell platforms in a contactless, and rapid manner. Rapid cell arrangement within gelatin methacryloyl hydrogels and multi-layered 3D brain-like construct have been already obtained using this technology. Although limited to line patterns and homogenous cell populations, this technology is non-invasive, biocompatible, and the first step on the development of methods capable of fabrication of complex heterogeneous tissue architectures (67).

### **5.3.3.2 Chemical stimuli-responsive materials**

#### ***5.3.3.2.1 pH-responsive materials***

pH-responsive materials contain chemical groups that release or accept protons due to pH variation, exhibiting a globule-to-coil transition at a critical pH value. The polymers chains go from a coil to a globule structure when the functional group of the polymer is neutralized (67).

pH-responsive hydrogels exhibit tunable stiffness, mechanical properties, adjustable morphology, and swelling characteristics according to pH changes. Thus, these hydrogels could be a promising future in injectable drug delivery and bioprinting (tissue engineering) (67).

#### ***5.3.3.2.2 Ion-sensitive hydrogels***

These hydrogels such as alginate are formed by crosslinking with multivalent ions, such as  $\text{Ca}^{2+}$  and  $\text{Ba}^{2+}$  that induce a folding mechanism, thus enabling the construction of strong scaffolds with tunable mechanical properties. The presence of chelating agents such as EDTA will extract these ions, therefore restoring the permanent shape of the printed construct (67).

Hydrogels with hydrogen bonding/  $\text{Ca}^{2+}$  ion crosslinking also exhibit shape memory, however its sensibility to the ion concentration leads to sharp volume changes. To address this issue, the combination with photo-crosslinking properties permitted cells to tolerate  $\text{Ca}^{2+}$  ions changes in the extracellular environment and the shape shift mechanism to happen (67).

Both materials are promising for tissue engineering, especially in tubular constructs for vascular bioprinting, due to its tunable properties (67).

## 5.4 Organ Bioprinting

3D bioprinting allows the fabrication of tissue-specific and biocompatible constructs (Figure 8) with hierarchical architecture similar to tissues and organs, thus constituting an alternative for traditional tissue replacement and organ transplantation as already mentioned (10,11,53,68).

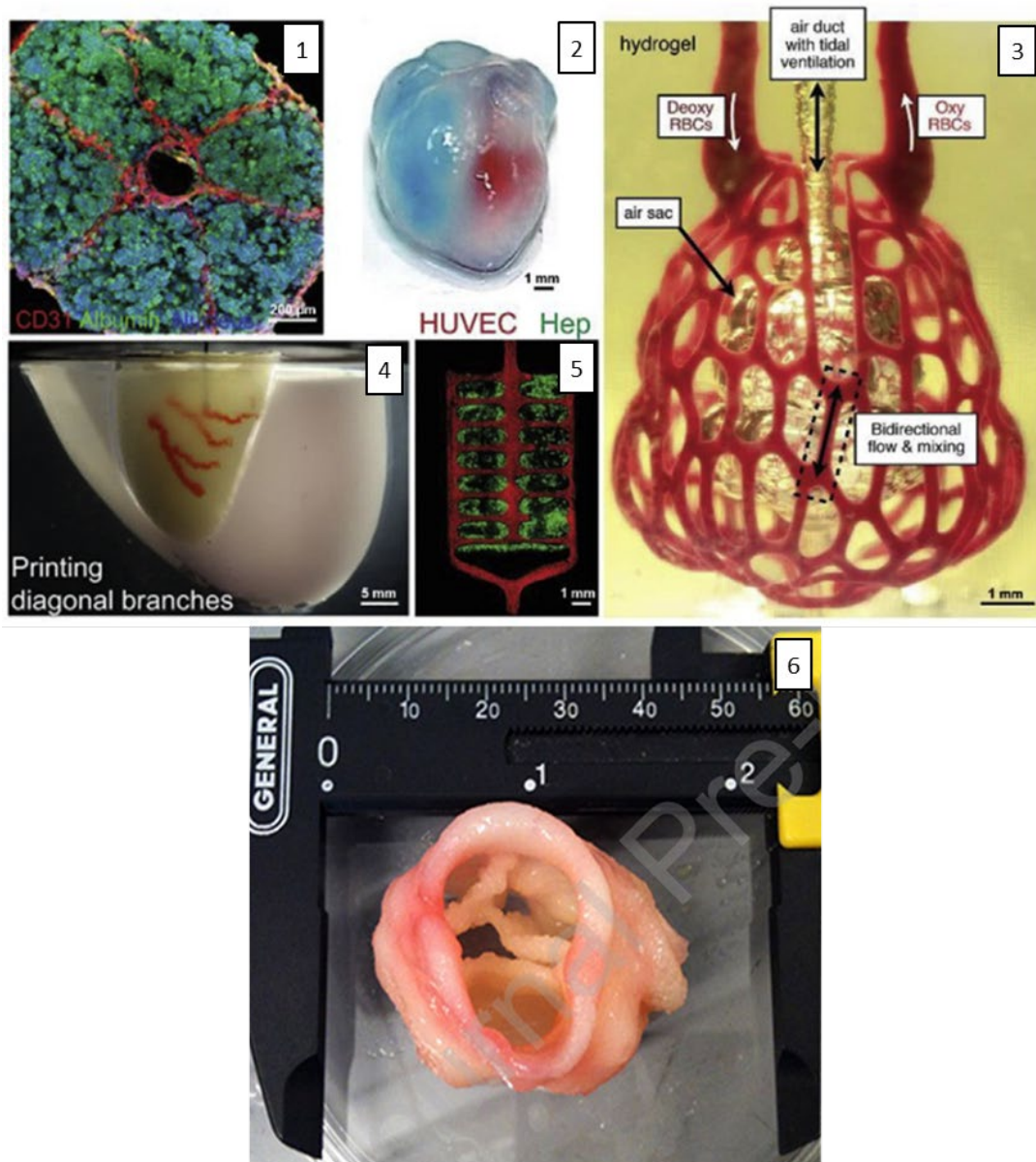
The digital model is based on clinical imaging for gross anatomical features, digital reconstruction programs based on a data base, mathematical modelling, and computer simulation to assemble macro and microanatomical structural information, resulting in a blueprint for a complete organ fabrication (69).

Accurate organ phantoms are now achievable being an improvement on medical training, learning, and on surgeries preparation. Traditional organ models are often basic, lacking some details, and rigid. In turn, organ phantoms produced through bioprinting would possess similar characteristics to real organs, allowing a better understanding of their anatomy and function (53). In fact, it has become a clear advantage in learning, and mostly on surgery preparation, since medical imaging lacks the three-dimensional aspect tackling difficulties such as nerve and blood vessel positioning (53). This is possible through combination of all imaging data available of the patient, resulting in an organ construct equal to the original one (10,53).

Although fully functional bioprinted organs are yet to be achieved, some basic constructs have been fabricated, thus representing a good start for using bioprinted organs on transplants. Constructs, such as organ tissues, will be quite useful for organ regeneration in the case of neural tissue, pancreas, liver, bone, cartilage, and cardiac tissue. As a result, the continuous studies and developments made in these constructs

will culminate on the printing of transplantable organs, which tackle the lack of compatible organ donors, sizing, and organ rejection, since the organ is constructed with the patient's cells in a tailored manner (10,11,53,68).

Nonetheless, organ-on-a-chip is now real due to 3D bioprinting, enabling the study of new medicines in a more controlled manner with more realistic results by providing real time data. This type of technology has also been used for fabrication of cancer models and study of new treatments, since it is a fully functioning organ/tissue being more accurate than a culture of cells or even animal models (10,11,53,68).



**Figure 8: Examples of components of bioprinted organ constructs (4,6). [1] Immunostaining of printed hepatic lobule [2] Printed heart with major blood**

vessels |3| **Printed vasculature of air sack while ventilated with oxygen** |4| **Printed cardiac tissue with septal branches** |5| **Confocal microscopy images showing that hydrogel anchors physically entrap fibrin gel containing the hepatocyte aggregates** |6| **Printed artificial cardiac valve.**

## **5.5 Vascular Bioprinting**

Vascular networks are very complex, intricate, and composed of different types of cells and vessels. Blood vessels are composed of three layers, an inner layer of endothelial cells (EC), a middle layer of smooth muscle cells (responsible of structure and function in physiological and pathological conditions) and an outer layer of collagen fibres and elastic tissue, apart from capillaries that only possess the EC layer (6,70). There are different types of EC, the most used in bioprinting are human umbilical vein endothelial cells (HUVECs), human microvascular endothelial cells (HMVECs), and induced pluripotent stem cell-derived endothelial cells (iPSC-ECs). Although, HUVECs are the most frequently used, HMVECs are used to form microvascular networks, and human iPSCs due its capability of self-renewal and multi-lineage differentiation, and absence of immunological response are used for customized vascularized tissues/organs (6,71,72).

Vasculogenesis and angiogenesis are the processes of vessels formation. The first occurs via migration and differentiation of endothelial progenitor cells (EPCs) in the embryo state originating the primitive vasculature, and the latter, through growth factors, such as vascular endothelial growth factor (VEGF), angiopoietin-2 (Ang2) and fibroblast growth factor (FGF), which initiate vessel formation and growth by the ECs in already existing blood vessels, primarily in the capillaries (6,70,73).

The main challenge in vascular bioprinting is the creation of hierarchical and perfusable channels that allows access to nutrients, oxygen, and removal of wastes, with cells and biomaterials in specific locations. Without it, the production of functioning organs and tissue in vivo cannot be successfully achieved (6,74,75).

## 5.6 Skin bioprinting

The skin is the largest organ of the human body and one of the most important organs. It provides protection as a physical barrier against UV light, mechanical stress, and pathogens, hence providing immune surveillance while participating in the regulation of temperature, hydration, excretions, etc. (12). The skin is composed of three layers, epidermis, dermis, and hypodermis (76). The first one acts as a barrier providing protection due to the stratum corneum; the second one is irrigated by blood vessels and composed of a matrix of connective tissue that gives elasticity and resistance to deformation, and the latter, also known as subcutaneous fat tissue, provides support to the previous two, mechanical protection, and thermal isolation (76). Therefore, an intricate and functional multi-layered structure would be desirable in a bioprinting model.

Traditional and silver-containing dressings, grafts (such as auto-, allo- and xenografts), skin substitutes, cell therapy, and cytokine therapy, are the current approaches in wound healing (77). However, these traditional approaches, if combined may cover a large repair area, other limitations such as few donors for skin grafting, probable immune rejection of the graft, prolonged healing time, high costs, and lack of vasculature that can be reconnected with the host vascular system (76). Skin bioprinting emerges as a solution for most of these problems as it allows an accurate cell positioning, high production capacity, controllable tissue structure preparation, and wide size range. Since the cells can be derived from the patient, there is a minimal chance of immune rejection while creating a tailored and functional skin construct that follows the topography of the wound. It also allows the printing of vasculature that will connect itself with the host vasculature through angiogenesis (76–78). Beside cells, growth factors, cytokines, and chemokines are added to the bioink to promote the signalling network (77,78).

The most used bioprinting technologies are inkjet, laser assisted, and extrusion bioprinting permitting the deposit of living cells, factors (i.e., growth factors), and biomaterials (76–78). Printing can occur *in vitro* or *in situ*, meaning that it can be printed on a surface and then transferred to a bioreactor, or it can be printed directly on the patient, where maturation will occur (12,79,80). The latter one possess a challenge since the patient motion produced by breathing could compromise the construct printing (12).

### 5.6.1 Biomaterials

Biomaterials used in skin bioprinting should be biocompatible, printable, biodegradable, promote cell adhesion and proliferation, and possess enough mechanical properties of skin tissue. The bioink composed of these materials should support and enhance cell viability, proliferation, differentiation and formation of appropriate tissue, and distribution/ migration of cells. The incorporation of other materials and active agents for functional or structural support of the construct should permit both alongside cell-cell adhesion and paracrine signalling (76–78,81).

Materials such as collagen, gelatine, alginate, fibrin, hyaluronic acid, chitosan, and pectin have been often blended in different combinations to offer a more suitable and viable skin construct (Table 3) (78).

**Table 3: Examples of biomaterials and their properties/ functions in skin bioprinting (adapted from (78,82)).**

<b>Biomaterial</b>	<b>Properties/ Functions</b>
Collagen	<ul style="list-style-type: none"><li>• Main component of the extracellular matrix that influences the adhesion and cellular functions of fibroblasts alongside the cellular functions of keratinocytes (shape, differentiation, and migration).</li><li>• Often used mixed with other biomaterials due to its weakness.</li></ul>
Gelatine	<ul style="list-style-type: none"><li>• Derived from collagen and with higher mechanical strength.</li><li>• Capable of accelerating the healing process by hemostatic effect that leads to macrophage activation.</li><li>• Versatile.</li><li>• Thermo-sensitive (the viscosity optimization of the gelatine solution is difficult to achieve).</li><li>• Blending with other materials.</li></ul>

Alginate	<ul style="list-style-type: none"> <li>• Biocompatible</li> <li>• Able to undergo fast gelation when exposed to <math>\text{Ca}^{2+}</math> ions under physiological pH and temperature conditions.</li> <li>• Blending with other materials (not used alone due to its lack of cell adhesion sites.</li> <li>• Low cost.</li> </ul>
Fibrin	<ul style="list-style-type: none"> <li>• ECM component and promoter of cell differentiation, proliferation, and vascularization, thus providing numerous cell adhesive sites.</li> <li>• The reaction between fibrinogen and thrombin leads to its spontaneous gelation.</li> <li>• Capable of reducing cell contraction (used for wound closure).</li> </ul>
Hyaluronic acid	<ul style="list-style-type: none"> <li>• Biodegradable, biocompatible, and non-immunogenic.</li> <li>• Able to regulate the transport and diffusion of electrolytes alongside the blood vessel permeability.</li> <li>• Allows water retention in the skin and promotion of wound healing.</li> </ul>
Chitosan	<ul style="list-style-type: none"> <li>• Often used on wound dressings due to its antibacterial and anti-inflammatory properties.</li> <li>• Slow gelation</li> <li>• Promote proliferation and adhesion of keratinocytes and fibroblasts</li> </ul>
Pectin	<ul style="list-style-type: none"> <li>• Biocompatible and biodegradable.</li> <li>• Gelation process like alginate.</li> <li>• Inherent lack of cell-proteolytic and endogenous cell-adhesive sites.</li> <li>• Biochemical molecules can be introduced in the bioinert polymer backbone.</li> </ul>

Besides the natural materials mentioned above, synthetic materials such as polyethylenoglycol (PEG) and poly(vinyl alcohol) (PVA) are also another option. These materials can be modified by biofunctionalization using peptides and/or other materials to tune the bioink properties for skin bioprinting (76–78,81).

Combinations of natural and synthetic materials are often used to develop hybrid bioinks with mechanical properties closer to natural skin. The majority of bioinks are in the form of hydrogel precursors or pre-crosslinked hydrogels, due to their viscoelastic properties, porosity, low immunogenicity, and permeability, thus providing a hydrated and highly permeable microenvironment which is vital for cell proliferation, migration, and maturation (76–78).

### **5.6.2 Cells used in Skin Bioprinting**

Cell selection is of utter importance for the success of 3D bioprinted and tissue-engineered constructs. This is the first step to assure both function and cell survival of the biofabricated skin (12,76–78,81).

Autologous cells derived from the patient are the gold standard, yet cell lines, primary cells or stem cells (pluripotent or multipotent) can be also used in skin bioprinting (12,76,77). Pluripotent cells, such as induced pluripotent cells (iPSCs), are genetically tailored to the patient being versatile and ethically well accepted (76).

Keratinocytes and fibroblasts are the two-base type of cells used for this purpose. Keratinocytes from the outer epidermal layer can be easily cultured and maintained in the laboratory (12,76–78). Fibroblasts are responsible for the production of connective tissue that forms the dermal layer of the skin. Therefore, they will provide support and maintain the tissues, thus overcoming the long-term fragility of the keratinocytes (12,76,77). However, fibroblasts possess different functional properties and different response to the healing phase depending on the donor site (77).

Melanocytes, endothelial cells, and stem cells such as sebaceous, mesenchymal (MSCs), hair follicle, sweat, hematopoietic (HSCs), and neural cells can be also used to fabricate bioprinted skin. Due to the importance of vascularization for skin survival, endothelial cells alongside HSCs become the key element for angiogenesis. Moreover,

melanocytes, sebaceous glands, and hair follicles allow the protection from the exterior and material exchange, besides offering a natural look to the construct (12,76–78,81).

### **5.6.3 Desirable Properties of Bioprinted Skin**

To serve its purpose, whether is wound healing, in vitro models, pharmaceutical testing, or disease models, bioprinted skin constructs must possess certain properties. Due to the skin complexity, a variety of factors need to be put into account to achieve this success (76,77,81).

Thickness and mechanical strength are crucial for the skin architecture, shape, and ability to overcome stress derived from movement and possible aggressions. Consequently, the placement and deposition of the biomaterials, such as collagen and cells, on the construct must be precise to achieve the wound depth (for wound healing) as well as the skin heterogeneity (76,77).

As mentioned in the previous topic, the cell selection will influence both skin architecture and functionality. Cell placement and density throughout the skin surface are vital for cell-cell communication, thus allowing nutrients and waste exchange between them. However, this is only possible if enough cell adhesion sites and the appropriate biochemical composition/ environment are present in the construct (76,77,81). The vascularization (responsible for those exchanges) is fundamental for cell and skin viability, and most important, cell survival. Therefore, a differentiated system of numerous perfusable channels must be included to assure the construct blood supply and survival (76,77,81), yet not big enough for dehydration to occur (77).

At least, Merkel and Langerhan cells, melanocytes, and skin appendages (sweat and sebaceous glands and hair follicles) offer a more functional and complete skin construct. For example, the incorporation of melanocytes will enable skin pigmentation and protection from UV-light (12,76,77,81).

The construct needs to be biodegradable but stable throughout the regeneration process to allow vascularization, implantation, and integration with the native tissue. Nonetheless, degradation products should not result in a major immunological response (12,76,77). In the same way that the skin construct suffers degradation, it should be able

to age as the host tissue, meaning that it should go under the same enzymatic process (12,76,77,81).

#### **5.6.4 Tissue integration, remodelling, and angiogenesis in bioprinted skin**

One of the major challenges in skin bioprinting is undoubtedly the construction of a functional vascular network for blood supply. Without it, the skin construct will perish (12,76–78,81).

Vascularization occurs under two processes, i.e., vasculogenesis during the embryonic development and angiogenesis during the adult life (77). In skin bioprinting, a vascular network composed of bigger vessels which branch out to smaller ones, must be printed with extracellular matrix (ECM)-like bioinks accompanied of endothelial cells deposited in precise spatial locations to allow angiogenesis to occur (12,76–78,81). This way, blood vessels can emerge while connecting themselves with the existing vascular system of the host, a key component of the skin construct survival (12,76–78).

Some approaches are laser-assisted, extrusion, and inkjet bioprinting by either creating perfusable channels which will be injected with a cell suspension, printing multicellular spheroids, direct patterning vascular cells onto a receiving substrate, or continuous printing of a polymeric bioink loaded with endothelial cells followed by polymer removal (12,76,77).

## 6 Opportunities vs Challenges and Conclusions

3D/ 4D printing has arisen as a problem solver for various fields including the pharmaceutical and medical fields in the health context.

The fabrication of personalized medication, a poly-pill of various drugs (incompatible or not), tablets with complex release rates which can be modified by altering the shape without changing the dose, hydrogels capable of maintaining their geometry and achieving different release rates, and target therapy by incorporating nanotechnology among the use of smart materials, are some examples of possible and great applications of 3D printing in the pharmaceutical industry nowadays (21,23,37). Whilst reducing excipients, machinery and producing steps, thus increasing sustainability, and reducing costs, it allows the printing/ preparation of medication on demand at both hospital and community pharmacies. Nevertheless, there are still some challenges, as follows: (1) the right ink characteristics are a primary challenge itself; (2) the drugs and excipients might be incompatible with the chosen techniques; (3) toxicity might be observed due to certain solvents used; (4) lack of compatible materials might occur; and (5) lack of legislation which constricts the production at hospital and community pharmacies as well as at pharmaceutical industry in which the ease of scale up and reproducibility between batches are also demanded (4,5,9,83). Overall, the acceptance of 3D and 4D printing will depend on how safe and cost-effective could be compared to traditional techniques. Anyway, future pharmaceutical forms besides tablets and hydrogels (the most common and easier to print) will certainly be developed by 3D/ 4D printing at higher extent including the advanced drug delivery systems for each route of administration.

Organ bioprinting (including the skin) is another promising technology that allows obtaining a patient specific organ construct and a built-in host matching vasculature with lower chance of rejection, thus higher chance of survival. Moreover, the bioprinting tissue and organ models could be very useful for pharmaceutical formulation testing, thus discharging animal testing following the 3R's perspective (76–78,81). This is especially important for organ transplant (since a donor is not needed), drug test and cancer models. For example, the skin bioprinting, besides becoming a new model for drug and cosmetic testing, it also offers an alternative for skin grafts and

traditional dressings in the wound healing. Nonetheless, compromised cell viability due to the printing techniques, bioinks with the desired characteristics, scale of printing, proper and functional vasculature and innervation, and the lack of appendages, represent some of the tasks to beat in the coming future to make this construct as a fully functional organ (10,12,81,84–86).

In conclusion, 3D (bio)printing is a promising technology able to resolve long-lasting challenges of both pharmaceutical and medical fields by fusing forces/ efforts from the two fields to achieve a greater good. Anyway, a constant development must be employed to battle the upcoming challenges of such technology including the regulatory and ethical issues.

## References

1. Shahrubudin N, Lee TC, Ramlan R. An overview on 3D printing technology: Technological, materials, and applications. *Procedia Manuf* . 2019;35:1286–96. Available from: <https://doi.org/10.1016/j.promfg.2019.06.089>
2. Ngo TD, Kashani A, Imbalzano G, Nguyen KTQ, Hui D. Additive manufacturing (3D printing): A review of materials, methods, applications and challenges. *Compos Part B Eng* . 2018;143(February):172–96. Available from: <https://doi.org/10.1016/j.compositesb.2018.02.012>
3. Joshi S, Rawat K, C K, Rajamohan V, Mathew AT, Koziol K, et al. 4D printing of materials for the future: Opportunities and challenges. *Appl Mater Today* . 2020;18:100490. Available from: <https://doi.org/10.1016/j.apmt.2019.100490>
4. Al-Dulimi Z, Wallis M, Tan DK, Maniruzzaman M NA. 3D printing technology as innovative solutions for biomedical applications. *Drug Discov Today* . 2020;115833. Available from: <https://doi.org/10.1016/j.drudis.2020.11.013>
5. Zhu X, Li H, Huang L, Zhang M, Fan W, Cui L. 3D printing promotes the development of drugs. *Biomed Pharmacother*. 2020;131(May):6.
6. Zhang Y, Kumar P, Lv S, Xiong D, Zhao H, Cai Z, et al. Recent advances in 3D bioprinting of vascularized tissues. *Mater Des* . 2021;199:109398. Available from: <https://doi.org/10.1016/j.matdes.2020.109398>
7. Bom S, Martins AM, Ribeiro HM, Marto J. Diving into 3D ( bio ) printing : A revolutionary tool to customize the production of drug and cell-based systems for skin delivery. *Int J Pharm* . 2021;605(June):120794. Available from: <https://doi.org/10.1016/j.ijpharm.2021.120794>
8. Tren SJ, Awad A, Goyanes A, Gaisford S, Basit AW. 3D Printing Pharmaceuticals : Drug Development to Frontline Care. *Trends Pharmacol Sci* . 2018;39(5):440–51. Available from: <https://doi.org/10.1016/j.tips.2018.02.006>
9. Pérez-ballesteros FLF, Ballesteros MP, Lalatsa A. Market Demands in 3D Printing Pharmaceuticals Products . *3D Printing Technology in Nanomedicine*. Elsevier; 2019. 165–183 p. Available from: <https://doi.org/10.1016/B978-0-12->

815890-6.00009-8

10. Matai I, Kaur G, Seyedsalehi A, McClinton A, Laurencin CT. Progress in 3D bioprinting technology for tissue/organ regenerative engineering. *Biomaterials* . 2020;226:119536. Available from: <https://doi.org/10.1016/j.biomaterials.2019.119536>
11. Koçak E, Yıldız A, Acartürk F. Three dimensional bioprinting technology: Applications in pharmaceutical and biomedical area. *Colloids Surfaces B Biointerfaces* . 2021;197:45. Available from: <https://doi.org/10.1016/j.colsurfb.2020.111396>
12. Vijayavenkataraman S, Lu WF, Fuh JYH. 3D bioprinting of skin: A state-of-the-art review on modelling, materials, and processes. *Biofabrication*. 2016;8(3):31.
13. Quanjin M, Rejab MRM, Idris MS, Kumar NM, Abdullah MH, Reddy GR. Recent 3D and 4D intelligent printing technologies: A comparative review and future perspective. *Procedia Comput Sci* . 2020;167:1210–9. Available from: <https://doi.org/10.1016/j.procs.2020.03.434>
14. Ntousia M, Fudos I. 3D Printing Technologies and Applications: An Overview. In: *3D Printing Technologies & Applications: An Overview* . Singapore; 2019. p. 243–8. Available from: 10.14733/cadconfP.2019.243-248
15. Coelho J, editor. *Potentially Applicable in Personalised Treatment Drug Delivery Systems : Advanced*. Springer Berlin Heidelberg; 2013. 421 p.
16. Madla CM, Trenfield SJ, Goyanes A, Gaisford S, Basit AW. 3D printing technologies, implementation and regulation: An overview. *AAPS Adv Pharm Sci Ser*. 2018;31:21–40.
17. Lee JY, An J, Chua CK. Fundamentals and applications of 3D printing for novel materials. *Appl Mater Today* . 2017;7:120–33. Available from: <http://dx.doi.org/10.1016/j.apmt.2017.02.004>
18. Groll J, Boland T, Blunk T, Burdick JA, Cho DW, Dalton PD, et al. Biofabrication: Reappraising the definition of an evolving field. *Biofabrication* . 2016;8(1):5. Available from: doi:10.1088/1758-5090/8/1/013001

19. Moroni L, Burdick JA, Highley C, Lee SJ, Morimoto Y, Takeuchi S, et al. Biofabrication strategies for 3D in vitro models and regenerative medicine. *Nat Rev Mater* . 2018;3(5):21–37. Available from: <http://dx.doi.org/10.1038/s41578-018-0006-y>
20. Karakurt I, Lin L. 3D printing technologies: techniques, materials, and post-processing. *Curr Opin Chem Eng* . 2020;28:134–43. Available from: <https://doi.org/10.1016/j.coche.2020.04.001>
21. Karakurt I, Aydoğdu A, Çıkrıkçı S, Orozco J, Lin L. Stereolithography (SLA) 3D printing of ascorbic acid loaded hydrogels: A controlled release study. *Int J Pharm*. 2020;584(February):1–9.
22. Kyobula M, Adedeji A, Alexander MR, Saleh E, Wildman R, Ashcroft I, et al. 3D inkjet printing of tablets exploiting bespoke complex geometries for controlled and tuneable drug release. *J Control Release* . 2017;261(June):207–15. Available from: <https://doi.org/10.1016/j.jconrel.2017.06.025>
23. Robles-Martinez P, Xu X, Trenfield SJ, Awad A, Goyanes A, Telford R, et al. 3D printing of a multi-layered polypill containing six drugs using a novel stereolithographic method. *Pharmaceutics* . 2019;11(6):16. Available from: <https://doi.org/10.3390/pharmaceutics11060274>
24. Johannesson J, Khan J, Hubert M, Teleki A, Bergström CAS. 3D-printing of solid lipid tablets from emulsion gels. *Int J Pharm*. 2021;597(December 2020).
25. Aita I El, Breitzkreutz J, Quodbach J. Investigation of semi-solid formulations for 3D printing of drugs after prolonged storage to mimic real-life applications. *Eur J Pharm Sci* . 2020;146:105266. Available from: <https://doi.org/10.1016/j.ejps.2020.105266>
26. El Aita I, Rahman J, Breitzkreutz J, Quodbach J. 3D-Printing with precise layer-wise dose adjustments for paediatric use via pressure-assisted microsyringe printing. *Eur J Pharm Biopharm*. 2020;157(May):59–65.
27. Moldenhauer D, Nguyen DCY, Jescheck L, Hack F, Fischer D, Schneeberger A. 3D screen printing – An innovative technology for large-scale manufacturing of pharmaceutical dosage forms. *Int J Pharm* . 2021;592:53–79. Available from:

<https://doi.org/10.1016/j.ijpharm.2020.120096>

28. Hsiao WK, Lorber B, Reitsamer H, Khinast J. 3D printing of oral drugs: a new reality or hype? *Expert Opin Drug Deliv* . 2018;15(1):1–4. Available from: <https://doi.org/10.1080/17425247.2017.1371698>
29. West TG, Bradbury TJ. 3D Printing: A Case of ZipDose® Technology - World's First 3D Printing Platform to Obtain FDA Approval for a Pharmaceutical Product. *3D 4D Print Biomed Appl*. 2018;53–79.
30. Gorkem Buyukgoz G, Soffer D, Defendre J, Pizzano GM, Davé RN. Exploring tablet design options for tailoring drug release and dose via fused deposition modeling (FDM) 3D printing. *Int J Pharm*. 2020;591(July).
31. Khaled SA, Burley JC, Alexander MR, Yang J, Roberts CJ. 3D printing of tablets containing multiple drugs with defined release profiles. *Int J Pharm* . 2015;494(2):643–50. Available from: <http://dx.doi.org/10.1016/j.ijpharm.2015.07.067>
32. Beck RCR, Chaves PS, Goyanes A, Vukosavljevic B, Buanz A, Windbergs M, et al. 3D printed tablets loaded with polymeric nanocapsules: An innovative approach to produce customized drug delivery systems. *Int J Pharm* . 2017;528(1–2):268–79. Available from: <http://dx.doi.org/10.1016/j.ijpharm.2017.05.074>
33. Bobo D, Robinson KJ, Islam J, Thurecht KJ, Corrie SR. Nanoparticle-Based Medicines: A Review of FDA-Approved Materials and Clinical Trials to Date. *Pharm Res* . 2016;33(10):2373–87. Available from: <http://dx.doi.org/10.1007/s11095-016-1958-5>
34. Pohlmann AR, Fonseca FN, Paese K, Detoni CB, Coradini K, Beck RC, et al. Poly( $\epsilon$ -caprolactone) microcapsules and nanocapsules in drug delivery. *Expert Opin Drug Deliv*. 2013;10(5):623–38.
35. Frank LA, Contri R V., Beck RCR, Pohlmann AR, Guterres SS. Improving drug biological effects by encapsulation into polymeric nanocapsules. *Wiley Interdiscip Rev Nanomedicine Nanobiotechnology*. 2015;7(5):623–39.

36. Friedrich RB, Kann B, Coradini K, Offerhaus HL, Beck RCR, Windbergs M. Skin penetration behavior of lipid-core nanocapsules for simultaneous delivery of resveratrol and curcumin. *Eur J Pharm Sci* . 2015;78:204–13. Available from: <http://dx.doi.org/10.1016/j.ejps.2015.07.018>
37. Fourmann O, Hausmann MK, Neels A, Schubert M, Nyström G, Zimmermann T, et al. 3D printing of shape-morphing and antibacterial anisotropic nanocellulose hydrogels. *Carbohydr Polym*. 2021;259(September 2020):1–11.
38. Hausmann MK, Rühls PA, Siqueira G, Läger J, Libanori R, Zimmermann T, et al. Dynamics of Cellulose Nanocrystal Alignment during 3D Printing. *ACS Nano*. 2018;12(7):6926–37.
39. Siqueira G, Kokkinis D, Libanori R, Hausmann MK, Gladman AS, Neels A, et al. Cellulose Nanocrystal Inks for 3D Printing of Textured Cellular Architectures. *Adv Funct Mater*. 2017;27(12).
40. Compaan AM, Christensen K, Huang Y. Inkjet Bioprinting of 3D Silk Fibroin Cellular Constructs Using Sacrificial Alginate. *ACS Biomater Sci Eng*. 2017;3(8):1519–26.
41. Levato R, Jungst T, Scheuring RG, Blunk T, Groll J, Malda J. From Shape to Function: The Next Step in Bioprinting. *Adv Mater*. 2020;32(12).
42. Mandrycky C, Wang Z, Kim K, Kim DH. 3D bioprinting for engineering complex tissues. *Biotechnol Adv* . 2016;34(4):422–34. Available from: <http://dx.doi.org/10.1016/j.biotechadv.2015.12.011>
43. Ng WL, Qi JTZ, Yeong WY, Naing MW. Proof-of-concept: 3D bioprinting of pigmented human skin constructs. *Biofabrication*. 2018;10(2).
44. Xu T, Zhao W, Zhu JM, Albanna MZ, Yoo JJ, Atala A. Complex heterogeneous tissue constructs containing multiple cell types prepared by inkjet printing technology. *Biomaterials* . 2013;34(1):130–9. Available from: <http://dx.doi.org/10.1016/j.biomaterials.2012.09.035>
45. Xu T, Binder KW, Albanna MZ, Dice D, Zhao W, Yoo JJ, et al. Hybrid printing of mechanically and biologically improved constructs for cartilage tissue

- engineering applications. *Biofabrication*. 2013;5(1).
46. Murphy S V., Atala A. 3D bioprinting of tissues and organs. *Nat Biotechnol* . 2014;32(8):773–85. Available from: <http://dx.doi.org/10.1038/nbt.2958>
  47. Gudapati H, Dey M, Ozbolat I. A comprehensive review on droplet-based bioprinting: Past, present and future. *Biomaterials* . 2016;102:20–42. Available from: <http://dx.doi.org/10.1016/j.biomaterials.2016.06.012>
  48. Liu T kun, Pang Y, Zhou Z zhen, Yao R, Sun W. An integrated cell printing system for the construction of heterogeneous tissue models. *Acta Biomater* . 2019;95:245–57. Available from: <https://doi.org/10.1016/j.actbio.2019.05.052>
  49. Sun W, Starly B, Daly AC, Burdick JA, Groll J, Skeldon G, et al. The bioprinting roadmap. *Biofabrication*. 2020;12(2).
  50. Miri AK, Khalilpour A, Cecen B, Maharjan S, Shin SR, Khademhosseini A. Multiscale bioprinting of vascularized models. *Biomaterials* . 2019;198(February):204–16. Available from: <https://doi.org/10.1016/j.biomaterials.2018.08.006>
  51. Pereira RF, Bártolo PJ. 3D bioprinting of photocrosslinkable hydrogel constructs. *J Appl Polym Sci*. 2015;132(48):15.
  52. Pereira RF, Bártolo PJ. 3D Photo-Fabrication for Tissue Engineering and Drug Delivery. *Engineering* . 2015;1(1):090–112. Available from: <http://dx.doi.org/10.15302/J-ENG-2015015>
  53. Wang K, Ho CC, Zhang C, Wang B. A Review on the 3D Printing of Functional Structures for Medical Phantoms and Regenerated Tissue and Organ Applications. *Engineering*. 2017;3(5):653–62.
  54. Moroni L, Boland T, Burdick JA, De Maria C, Derby B, Forgacs G, et al. Biofabrication: A Guide to Technology and Terminology. *Trends Biotechnol*. 2018;36(4):384–402.
  55. Groll J, Burdick JA, Cho DW, Derby B, Gelinsky M, Heilshorn SC, et al. A definition of bioinks and their distinction from biomaterial inks. *Biofabrication*. 2019;11(1).

56. Diamantides N, Wang L, Pruiksma T, Siemiatkoski J, Dugopolski C, Shortkroff S, et al. Correlating rheological properties and printability of collagen bioinks: The effects of riboflavin photocrosslinking and pH. *Biofabrication* . 2017;9(3):13. Available from: <https://doi.org/10.1088/1758-5090/aa780f>
57. Lee JU, Yeo M, Kim WJ, Koo YW, Kim GH. Development of a tannic acid cross-linking process for obtaining 3D porous cell-laden collagen structure. *Int J Biol Macromol* . 2018;110:497–503. Available from: <http://dx.doi.org/10.1016/j.ijbiomac.2017.10.105>
58. Gungor-Ozkerim PS, Inci I, Zhang YS, Khademhosseini A, Dokmeci MR. Bioinks for 3D bioprinting: An overview. *Biomater Sci*. 2018;6(5):915–46.
59. Jang J, Park HJ, Kim SW, Kim H, Park JY, Na SJ, et al. 3D printed complex tissue construct using stem cell-laden decellularized extracellular matrix bioinks for cardiac repair. *Biomaterials* . 2017;112:264–74. Available from: <http://dx.doi.org/10.1016/j.biomaterials.2016.10.026>
60. Skardal A, Devarasetty M, Kang HW, Mead I, Bishop C, Shupe T, et al. A hydrogel bioink toolkit for mimicking native tissue biochemical and mechanical properties in bioprinted tissue constructs. *Acta Biomater* . 2015;25:24–34. Available from: <http://dx.doi.org/10.1016/j.actbio.2015.07.030>
61. Ali M, Anil Kumar PR, Yoo JJ, Zahran F, Atala A, Lee SJ. A Photo-Crosslinkable Kidney ECM-Derived Bioink Accelerates Renal Tissue Formation. *Adv Healthc Mater*. 2019;8(7):1–10.
62. Zhao N, Suzuki A, Zhang X, Shi P, Abune L, Coyne J, et al. Dual Aptamer-Functionalized in Situ Injectable Fibrin Hydrogel for Promotion of Angiogenesis via Codelivery of Vascular Endothelial Growth Factor and Platelet-Derived Growth Factor-BB. *ACS Appl Mater Interfaces*. 2019;11(20):18123–32.
63. Murphy KC, Whitehead J, Zhou D, Ho SS, Leach JK. Engineering fibrin hydrogels to promote the wound healing potential of mesenchymal stem cell spheroids. *Acta Biomater* . 2017;64:176–86. Available from: <https://doi.org/10.1016/j.actbio.2017.10.007>
64. Lee KY, Mooney DJ. Alginate: Properties and biomedical applications. *Prog*

- Polym Sci . 2012;37(1):106–26. Available from: <http://dx.doi.org/10.1016/j.progpolymsci.2011.06.003>
65. Wang Y, Huang X, Shen Y, Hang R, Zhang X, Wang Y, et al. Direct writing alginate bioink inside pre-polymers of hydrogels to create patterned vascular networks. *J Mater Sci* . 2019;54(10):7883–92. Available from: <https://doi.org/10.1007/s10853-019-03447-2>
66. Mao H, Yang L, Zhu H, Wu L, Ji P, Yang J, et al. Recent advances and challenges in materials for 3D bioprinting. *Prog Nat Sci Mater Int* . 2020;30(5):618–34. Available from: <https://doi.org/10.1016/j.pnsc.2020.09.015>
67. Wan Z, Zhang P, Liu Y, Lv L, Zhou Y. Four-dimensional bioprinting: Current developments and applications in bone tissue engineering. *Acta Biomater* . 2020;101:26–42. Available from: <https://doi.org/10.1016/j.actbio.2019.10.038>
68. Ashammakhi N, Ahadian S, Xu C, Montazerian H, Ko H, Nasiri R, et al. Bioinks and bioprinting technologies to make heterogeneous and biomimetic tissue constructs. *Mater Today Bio* . 2019;1(February):23. Available from: <https://doi.org/10.1016/j.mtbio.2019.100008>
69. Rezende RA, Kasyanov V, Mironov V, Da Silva JVL. Organ printing as an information technology. *Procedia Eng* . 2015;110:151–8. Available from: <http://dx.doi.org/10.1016/j.proeng.2015.07.023>
70. Barrs RW, Jia J, Silver SE, Yost M, Mei Y. Biomaterials for Bioprinting Microvasculature. *Chem Rev* . 2020;120(19):10887–949. Available from: <https://doi.org/10.1021/acs.chemrev.0c00027>
71. Faulkner-jones A, King JA, Faulkner-jones A, Fyfe C, Cornelissen D, Gardner J, et al. Bioprinting of human pluripotent stem cells and their directed differentiation into hepatocyte-like cells for the generation of mini-livers in 3D Bioprinting of human pluripotent stem cells and their directed differentiation into hepatocyte-like cells for. *Int Soc Biofabrication* . 2015;7(4):13. Available from: [doi:10.1088/1758-5090/7/4/044102](https://doi.org/10.1088/1758-5090/7/4/044102)
72. Ma X, Qu X, Zhu W, Li Y, Yuan S, Zhang H, et al. Deterministically patterned biomimetic human iPSC- derived hepatic model via rapid 3D bioprinting.

- 2016;113(8):3–8.
73. Kolte D, Mcclung JA, Aronow WS. Chapter 6. Vasculogenesis and Angiogenesis . *Translational Research in Coronary Artery Disease*. Elsevier Inc.; 2016. 49–66 p. Available from: <http://dx.doi.org/10.1016/B978-0-12-802385-3.00006-1>
  74. Laschke MW, Menger MD. Prevascularization in tissue engineering : Current concepts and future directions. *Biotechnol Adv* . 2016;34(2):121–121. Available from: <https://doi.org/10.1016/j.biotechadv.2015.12.004>
  75. Novosel EC, Kleinhans C, Kluger PJ. Vascularization is the key challenge in tissue engineering ☆. *Adv Drug Deliv Rev* . 2011;63(4–5):300–11. Available from: <http://dx.doi.org/10.1016/j.addr.2011.03.004>
  76. Augustine R. Skin bioprinting: a novel approach for creating artificial skin from synthetic and natural building blocks. *Prog Biomater* . 2018;7(2):77–92. Available from: <https://doi.org/10.1007/s40204-018-0087-0>
  77. Pereira RF, Sousa A, Barrias CC, Bayat A, Granja PL, Bártolo PJ. Advances in bioprinted cell-laden hydrogels for skin tissue engineering. *Biomanufacturing Rev* . 2017;2(1):26. Available from: <https://doi.org/10.1007/s40898-017-0003-8>
  78. Xu J, Zheng S, Hu X, Li L, Li W, Parungao R, et al. Advances in the research of bioinks based on natural collagen, polysaccharide and their derivatives for skin 3D bioprinting. *Polymers (Basel)* . 2020;12(6):31. Available from: [doi:10.3390/polym12061237](https://doi.org/10.3390/polym12061237)
  79. Varkey M, Visscher DO, Van Zuijlen PPM, Atala A, Yoo JJ. Skin bioprinting: The future of burn wound reconstruction? *Burn Trauma*. 2019;7:1–12.
  80. Cubo N, Garcia M, Del Cañizo JF, Velasco D, Jorcano JL. 3D bioprinting of functional human skin: Production and in vivo analysis. *Biofabrication* . 2017;9(1):1–12. Available from: <http://dx.doi.org/10.1088/1758-5090/9/1/015006>
  81. Ng WL, Wang S, Yeong WY, Naing MW. Skin Bioprinting: Impending Reality or Fantasy? *Trends Biotechnol* . 2016;34(9):689–99. Available from:

<http://dx.doi.org/10.1016/j.tibtech.2016.04.006>

82. Yan WC, Davoodi P, Vijayavenkataraman S, Tian Y, Ng WC, Fuh JYH, et al. 3D bioprinting of skin tissue: From pre-processing to final product evaluation. *Adv Drug Deliv Rev* . 2018;132:270–95. Available from: <https://doi.org/10.1016/j.addr.2018.07.016>
83. Chen G, Xu Y, Kwok PCL, Kang L. Pharmaceutical Applications of 3D Printing. *Addit Manuf* . 2020;34(November 2019):101209. Available from: <https://doi.org/10.1016/j.addma.2020.101209>
84. Hann SY, Cui H, Esworthy T, Miao S, Zhou X, Lee S jun, et al. Recent advances in 3D printing: vascular network for tissue and organ regeneration. *Transl Res* . 2019;211:46–63. Available from: <https://doi.org/10.1016/j.trsl.2019.04.002>
85. Malyala SK, Ravi Kumar Y, Rao CSP. Organ Printing with Life Cells: A Review. *Mater Today Proc* . 2017;4(2):1074–83. Available from: <http://dx.doi.org/10.1016/j.matpr.2017.01.122>
86. Tarassoli SP, Jessop ZM, Al-Sabah A, Gao N, Whitaker S, Doak S, et al. Skin tissue engineering using 3D bioprinting: An evolving research field. *J Plast Reconstr Aesthetic Surg*. 2018;71(5):615–23.