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# 3D bioprinting: Materials, processes, and applications

Paulo Bartolo (1)[a,b,*], Ajay Malshe (1)[c], Eleonora Ferraris (2)[d], Bahattin Koc[e]

[a] Singapore Centre for 3D Printing, School of Mechanical and Aerospace Engineering, Nanyang Technological University, Singapore
[b] Department of Mechanical, Aerospace and Civil Engineering, University of Manchester, UK
[c] School of Mechanical Engineering, Purdue University, USA
[d] Department of Mechanical Engineering, Catholic University of Leuven, Belgium
[e] Faculty of Engineering and Natural Science, Sabanci University, Turkey

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ABSTRACT

Ageing population and new diseases are requiring the development of novel therapeutical strategies. 3D bioprinting an novel application domain of additive manufacturing emerged as a potential transformative strategy for tissue engineering and regenerative medicine. This paper introduces the concept of 3D bioprinting, discussing in detail key requirements of bio-inks and main materials used to encapsulate cells. Recent advances related to the use of smart materials and the concept of 4D printing is also discussed. Main 3D bioprinting techniques are described in detail and key limitations highlighted. Successful cases, demonstrating the relevance of 3D bioprinting are also presented. Finally, the paper addresses the main research challenges and future perspectives in the field of 3D bioprinting.



## 1. Introduction

The main objective of tissue or organ engineering is to reconstruct a damaged or diseased tissue or organ with cells, biomaterials, and bioactive molecules [1–4]. The basic concept of traditional tissue engineering methods is to seed living cells, and/or biologically active molecules into a highly porous scaffold to fix and/or regenerate damaged tissues [5]. Various additive manufacturing methods have been used to produce scaffolds with controlled micro-architecture and geometry in a wide range of materials [6–14]. This topic has been extensively discussed at CIRP. Firstly, through a Collaborative Working Group on Biomanufacturing (2009–2012) joining academics and industrialists from all over the world, aiming to contribute to a coherent strategy for the development, dissemination, and exploitation of biomanufacturing [13,15], and after through the biannual CIRP Biomanufacturing Conferences.

However, scaffold-based approaches still face some challenges such as difficulty in seeding different cells spatially in a scaffold, limited vascularization, and weak cell-adhesion to scaffold material. Hence, living cells alone or in combination with biomolecules and biomaterials need to be assembled in three-dimension for a successful tissue or organ engineering. This paper focuses on the concept of 3D bioprinting, a special sub-set of additive manufacturing (AM), that can be defined as the manufacturing of complex biological constructs from living cells, biomolecules, and biomaterials. Like binder jetting AM techniques, 3D bioprinting is also developed from 2D ink-jet printers by replacing the ink in the cartridge with a biological material (bio-ink) and the paper with biodegradable support material. Currently, only few AM techniques are suitable for bioprinting applications (Fig. 1).

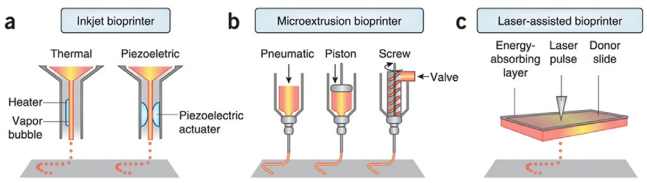


**Fig. 1.** The three main bioprinting techniques [16].

3D bioprinting is a prolific research topic (Fig. 2) and highly relevant from an economical point of view. According to a report from the Grand View Research, the 3D bioprinting market valued $1.4 billion in 2020, and it is expected to grow with a compound annual growth rate of 15.8% between 2021 and 2028 [17]. In 2024, the 3D bioprinting market will represent around 10% of the total 3D printing market. Moreover, the global Tissue Engineering market valued $2 billion in 2019 and is projected to reach $7billion by 2027 [18].

The market growth of 3D bioprinting has been driven by medical, cosmetics, and pharmaceutical sectors. The large demand for organ transplantation and the lack of organs available is one of the main drivers for the development of 3D bioprinting strategies aiming to eliminate the waiting time, need for immunosuppression and a donor-matched organ. This is expected to increase due to the worldwide large population above 60 years, who have low immunity levels and are more prone to suffer from accidents and diseases. Examples of bioprinting applications for tissue engineering/regenerative

* Corresponding author at: The University of Manchester, C4, Pariser Building, School of Mec, Manchester M13 9PL, United Kingdom.
*E-mail address:* paulojorge.dasilvabartolo@manchester.ac.uk (P. Bartolo).

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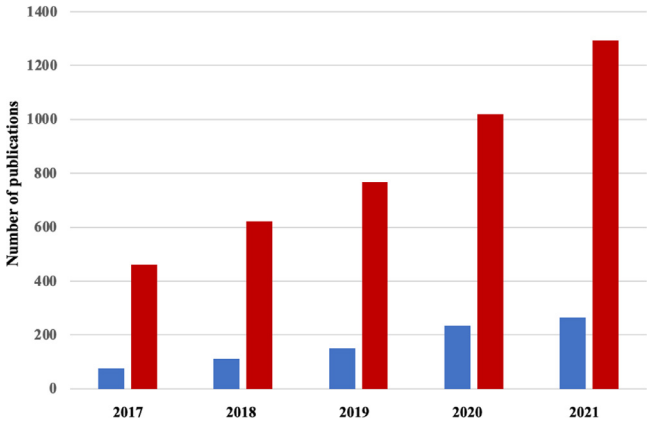


**Fig. 2.** Publications on bioprinting (red bars) and bio-inks (blue bars) in the period 2017–2021. Source: Scopus.

medicine are provided in Fig.3, and some of the most developed areas are discussed in detail in this paper.

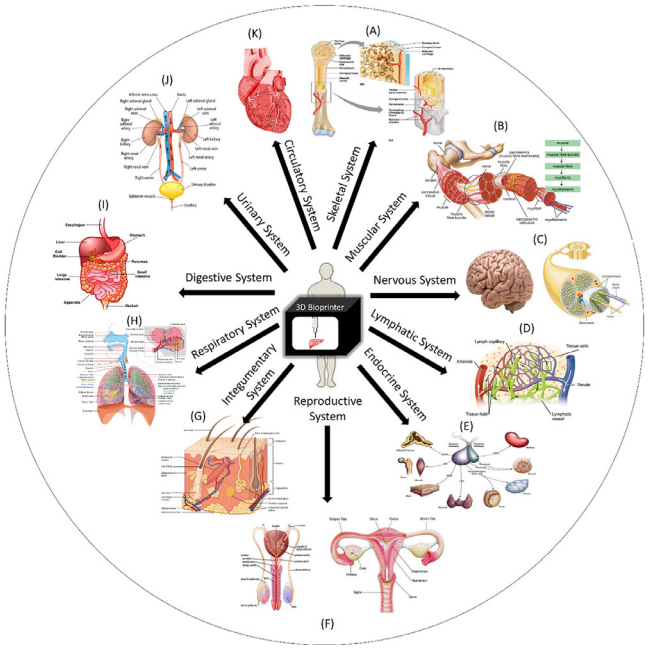


**Fig. 3.** Tissue engineering and regenerative applications of bioprinting [19].

Klebe [20] was the first demonstrating the concept of bioprinting using a Hewlett-Packard (HP) inkjet printer and two cartridges filled with a fibronectin solution and a solution containing monoclonal antibodies. Later, Wilson and Boland [21] successfully demonstrated the cell printing concept using an HP660C inkjet printer with a modified dispensing system to print mammalian cells. Since then, the field of bioprinting rapidly expanded with several printing technologies being developed, each presenting advantages and limitations. More recently, multi-modal printers were developed by integrating different types of printing heads and printing technologies into one system. These different systems are discussed in detail in this paper, which also highlights the key research challenges of this field and expected contributions from the CIRP community. Moreover, the concept of bio-ink, key requirements and main natural derived and synthetic hydrogel materials commonly used as bio-inks are also detailed.

## 2. Bio-inks

Bio-ink is the bio-printable material. It is composed of cells with biomaterials like hydrogel or cell aggregates and spheroids [22–26].

Hydrogels are 3D hydrophilic polymeric materials formed by chemical (irreversible hydrogels) or physical crosslinks (reversible hydrogels) (Fig. 4) that exhibit a remarkable ability to absorb large volumes of water without dissolution [27–31]. They are soft and elastic materials, generally used above their glass transition temperature and highly permeable to oxygen, nutrients, and other water-soluble metabolites [25,26].

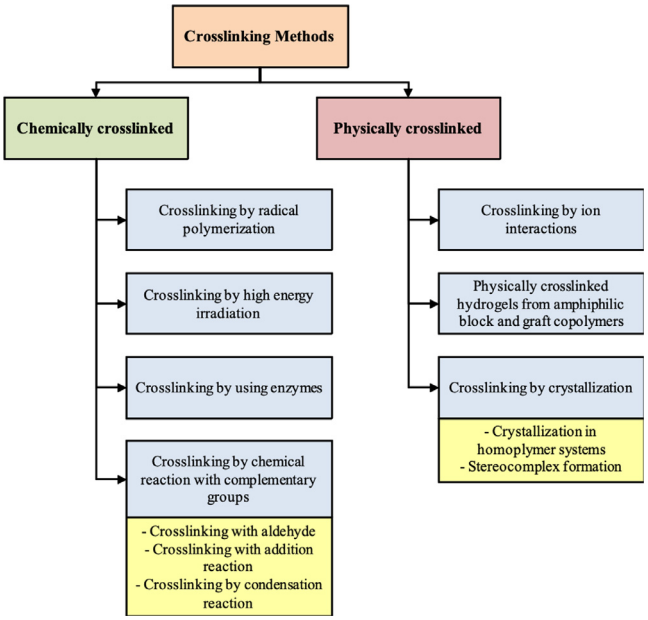


**Fig. 4.** Crosslinking methods used in hydrogels. Adapted from [31].

Bio-inks can be prepared using hydrogels from natural or synthetic origin [27,32,33]. Naturally derived polymers (e.g. collagen, gelatine, hyaluronic acid, alginate, chitosan) present biochemical similarities with the natural extracellular matrix (ECM) but limited mechanical properties, potential immunogenicity, batch to batch variability and usually require complex purification processes [34,35]. On the other hand, synthetic hydrogels (e.g. poly(ethyleneglycol) (PEG), poly(butylene oxide) (PBO), poly(vinyl acetate), poly(vinyl alcohol) (PVA), propylene fumarate (PF), poly(ethylene oxide) (PEO)) present controllable properties and higher mechanical properties, but exhibit limited biocompatibility, biodegradability and cell interaction properties [36].

### 2.1. Requirements

Bio-inks should present high printability, determined by rheological characteristics, gelation kinetics and crosslinking nature, to allow the fabrication of tissue constructs with high shape fidelity and resolution. They should be biocompatible and present adequate biomechanical, bioactive clues and degradation characteristics [37]. Moreover, bio-inks should present sub-micrometric porosity to guarantee adequate mass transport [27]. Fig. 5 summarises the key characteristics of a bio-ink and relevant interdependencies between material and the bioprinting process. The correlations between key mechanical and biological considerations and the bio-ink properties are presented in Fig. 6.

Printing resolution, shape fidelity, gelling speed, material viscoelasticity and rheological properties are crucial parameters in determining the printability of bio-inks and final mechanical and biological properties of printed constructs [40,41]. Developing advanced bio-inks also requires the incorporation of biological functional groups and crosslinking sites. Moreover, it is important to take into consideration that cell behaviour and functional properties of the new tissue depend not only on the bioprinting parameters, but also on the cell microenvironment provided by encapsulating material.

Depending on the biofabrication process and material properties, the bio-inks will exhibit different chemical and physical characteristics that will determine the corresponding application. These

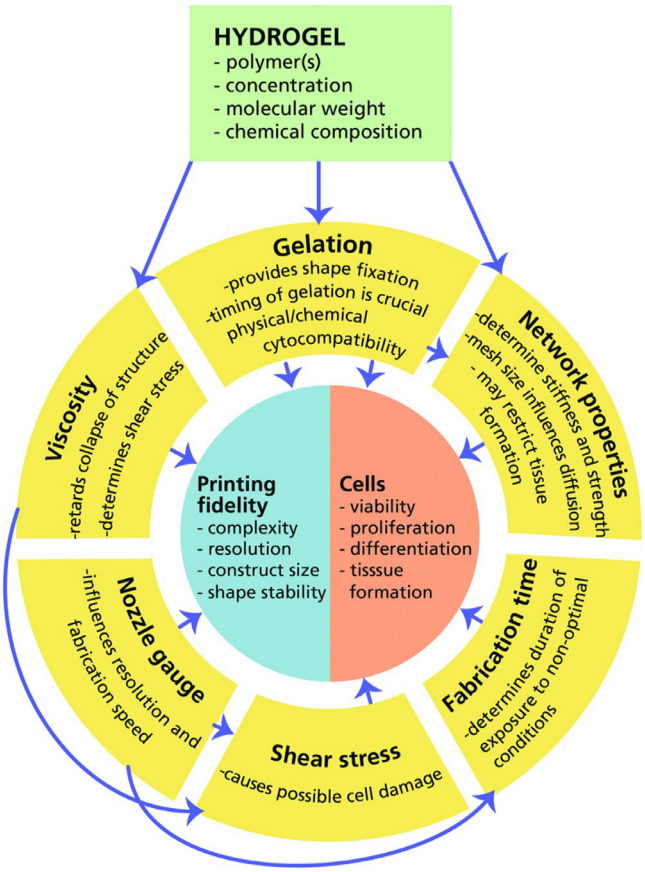


**Fig. 5.** Key bio-ink properties and inter-correlation between material properties and bioprinting [38].

properties can be determined by rheological characterization, mechanical assessment, and crosslinking properties of the hydrogel. Shear-thinning, a decrease in the viscosity due to an increase in shear rate, is a critical property as it allows to reduce shear stresses during the printing process thus increasing cell viability [42]. The viscoelastic behaviour and in particular the shear recovery characteristics of bio-inks need to be optimized to guarantee good shape fidelity [43].

### 2.2. Natural derived bio-inks

Natural hydrogels are highly biocompatible and present a structure that resembles the structure of biological tissues. However, they exhibit limited mechanical properties, batch to batch variations, and difficult to tailor degradation mechanisms.

Collagen is the most abundant natural-derived polymer [41]. Rather than a single protein, collagen is a family of 29 different proteins all of them presenting a characteristic triple helix structure [27]. Collagen types I, II, and III are the most abundant collagen types in our body. Collagen bio-inks are biocompatible and highly bioactive presenting cell-adhesion sites. Main disadvantages include poor print fidelity, which can be improved by increasing viscosity but compromising cell viability, slow gelation that compromises shape fidelity and induces cell sedimentation, fast degradation, and poor mechanical properties [27,44]. Methacrylated collagen mixed with proper photo-initiators are commonly used for 3D bioprinting applications. Dual cross-linking methods, combining photo-chemical cross-linking and chemical cross-linking methods have been also reported [45].

Gelatine is a biocompatible and biodegradable water-soluble protein derived from collagen via hydrolysis [37,46]. Like collagen, gelatine is characterised by a triple helix structure, but presents high mechanical and biological properties and low antigenicity [41]. It is also non-cytotoxic and exhibits low immunogenicity [47,48]. Gelatine gels are thermo-sensitive and undergo a reversible sol-gel transition at body temperature. Main limitations include fast degradation and poor mechanical properties [49]. Crosslinking is usually required to improve mechanical properties and to guarantee shape fidelity [50–54]. Therefore, gelatine is usually functionalysed (e.g., methacrylation) and used in the form of gelatine methacryloyl (GelMA) [55–57].

Agarose is a hydrophilic polysaccharide extracted from seaweed, composed of alternating $\beta$-D-galactopyranosyl and 3,6-anhydro-$\alpha$-L-galactopyranosyl units [58,59]. Agarose gels exhibit a thermo-reversible gelling behaviour, high mechanical properties, and bioactivity [60]. They can be easily prepared by dissolving agarose in deionized water or buffer solution at temperatures above the sol-gel-transition temperature followed by colling [59,61]. Agarose gels are biocompatible but not biodegradable in the human body [22].

Hyaluronic acid (HA), one of the major constituents of the ECM, is a biocompatible and degradable high molecular weight linear anionic polysaccharide composed of D-glucuronic acid and 2-acetamino-2-deoxy-D-glucose [62,63]. HA presents high hydrophilicity, non-thrombogenic and non-immunogenic properties and usually requires the incorporation of cell adhesion sites to increase cell-affinity [47,64]. HA gels can be obtained via esterification or crosslinking [64]. Shape stability is a major limitation of HA gels [47].

Alginate is an anionic block copolymer composed of 1,4-linked $\beta$-D-mannuronic (M blocks) and $\alpha$-L-guluronic acid (G blocks) [65]. The mechanical properties of alginate depend on the G-block/M-block ratio, which is determined by the alginate source. Gels made of M-rich alginate exhibit low mechanical properties [27,66]. The presence of carboxyl groups make alginate gels to be pH responsive [67]. Gelling occurs when divalent or trivalent ions take part in the

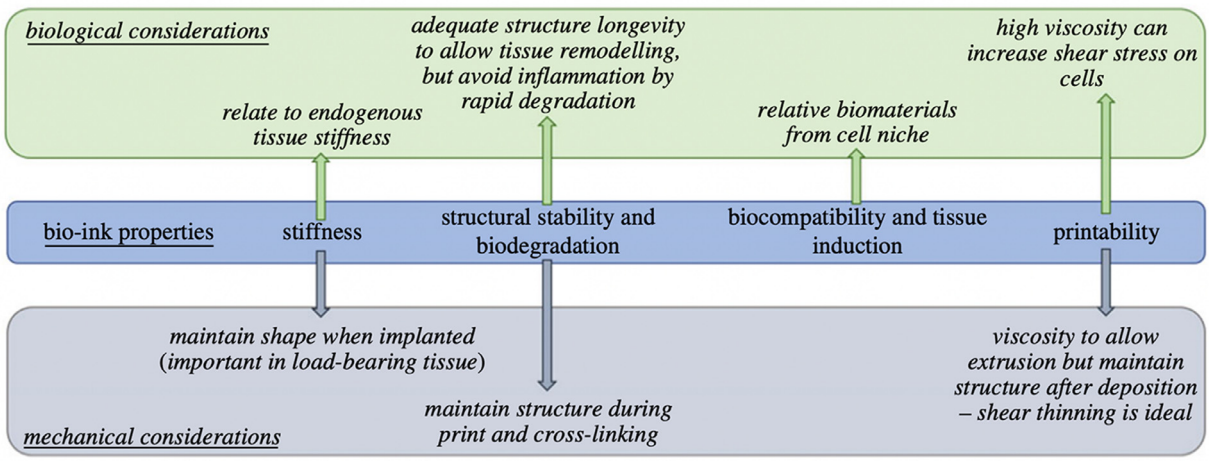


**Fig. 6.** The primary properties of bio-inks. Correlation between biological and mechanical considerations [39].

interchain ionic binding between G-blocks creating a three-dimensional network (egg-box model) [68]. However, these reactions result in inhomogeneous gels with limited long-term stability [69]. Therefore, alginate methacrylate hydrogels have been proposed [70]. Limited degradation and lack of cell adhesion sites are major limitations of alginate hydrogels [71–73].

Chitosan is a highly abundant cationic polysaccharide derived from chitin through deacetylation [62,64,74]. It is a copolymer composed of $\beta$-(1–4) linked 2-acetamino-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose [75]. Chitosan gels are biocompatible, biodegradable, low cost, and present good anti-bacterial properties [47]. However, chitosan gels also exhibit low mechanical properties, acidity, and slow gelling characteristics [27,41].

Silk is a naturally occurring material mainly composed of sericin and fibroin proteins [64,74]. Silk fibroin, obtained by boiling silk in sodium carbonate, presents high mechanical properties and low immunogenicity [64,74]. Sericin gels exhibit non-immunogenic, antioxidant, antibacterial and anticoagulation properties.

Cellulose is a biocompatible, biodegradable and hydrophobic polymer extracted from plants or bacteria (*Acetobacter xylinum* or *Gluconacetobacter xylinus*) being one of the most abundant natural-derived polymer [62,73]. Cellulose gels present excellent mechanical and cell adhesion properties.

### 2.3. Synthetic bio-inks

A range of synthetic hydrogels such as polyvinyl alcohol (PVA), polylactic acid (PLA) and polyethylene glycol (PEG) have been used for bioprinting applications. PVA is a semi-crystalline hydrophilic biodegradable and biocompatible polymer, presenting good mechanical and swelling properties [76]. PLA is a semi-crystalline biodegradable and biocompatible polymer made from $\alpha$-hydroxy acid [77]. PEG is a biocompatible and non-immunogenic [78,79]. Photocrosslinking PEG diacrylate and PEG dimethacrylate are commonly used but they are not degradable thus requiring further modification [27,61,79].

### 2.4. Decellularised bio-inks

The extracellular matrix (ECM) is a biological network that provides natural support to cells and cell-cell communication, giving physical shape to all biological tissues. It is composed of collagen, glycoproteins such as fibronectin and laminin, proteoaminoglycans, and glycosaminoglycans such as HA [37]. However, each biological tissue presents a specific ECM composition [80]. Cells interact with the ECM through proteins present in the cell membrane such as integrins, attaching to adhesion sites present in the ECM (Fig. 7), allowing cell proliferation and differentiation [81,82].

Decellularized ECM (dECM) has been investigated as a potential material for bio-ink preparation. To prepare dECM, different chemical, physical and enzymatic methods have been proposed allowing to remove all cellular content with minimal damage. Inks are prepared by dissolving dECM in phosphate buffer solutions or culture medium [83]. dECM inks are usually highly viscous and present low mechanical properties [37]. However, they exhibit excellent biochemical properties.

### 2.5. Advanced bio-inks and tissue spheroids

Stimuli-responsive bio-inks have been developed to produce constructs mimicking native tissues. These bio-inks are able to respond to different stimuli such as temperature (e.g. methacrylated poly[N-(2-hydroxypropyl) methacrylamide mono/dilactate] (pHPMA-lac)/polyethylene glycol (PEG); methylcellulose hydrogels), pH (e.g. polyethylene glycol diacrylate), electrical and magnetic fields [84–86]. A wide range of self-assembling peptides have been also investigated [87,88]. However, most of these materials present low biocompatibility, bioactivity or degradability properties [37].

The combined use of additive manufacturing (3D printing) and smart materials is known as 4D printing and attracted the attention of researchers from different fields [89–91]. The most commonly

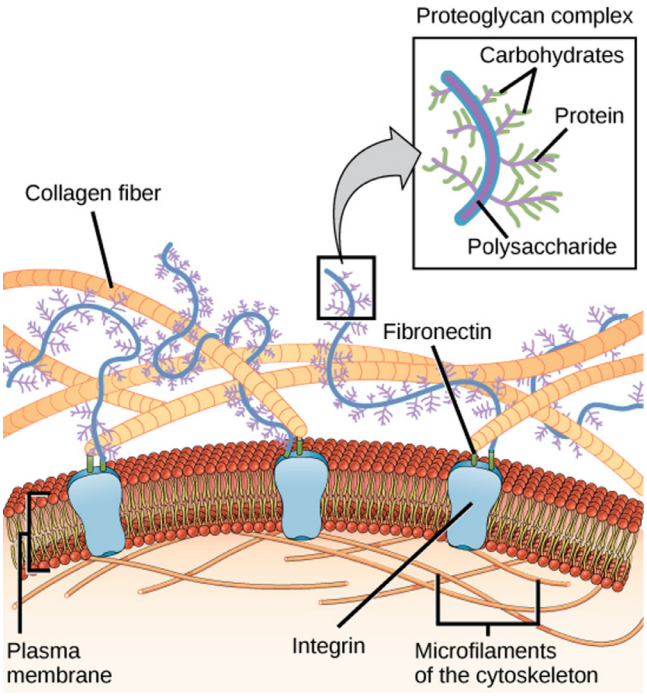


**Fig. 7.** Interactions between a cell and the extracellular matrix [82].

used materials are shape changing materials, i.e. materials that change their shape upon external stimulation returning to the original configuration by removing the stimulation conditions. The simplest way to achieve shape changing is using the intrinsic swelling characteristics of hydrogels. Jamal et al. [92] developed a bi-layered hydrogel consisting of photocrosslinkable PEG of varying molecular weights (Fig. 8). The hydrogel responds to aqueous stimuli, when placed in an aqueous environment changing its shape to predesigned configurations through differential swelling of the bonded hydrogel layers. Similarly, Mirani et al. [93] developed a directly activated drug delivery system. In this case, alginate fibres were mixed with a pH-responsive dye and printed in an array of porous sensors. Gentamicin-loaded alginate fibres were used to prepare the drug eluting construct. Then both the array and the drug eluting construct were combined to produce a patch (Fig. 9), able to detect bacteria and release high doses of antibiotic in the detection site. For wound applications, Miao et al. [94] designed a shape changing closed nerve conduit with the ability to open during the surgical operation of impanation and then closing again. The conduit was fabricated using graphene mixed with soybean oil epoxidized acrylate and showed good electrical conductivity and enhanced nerve regeneration. Self-folding polymers encapsulating different cells were also used for the fabrication of vascular-like structures [95].

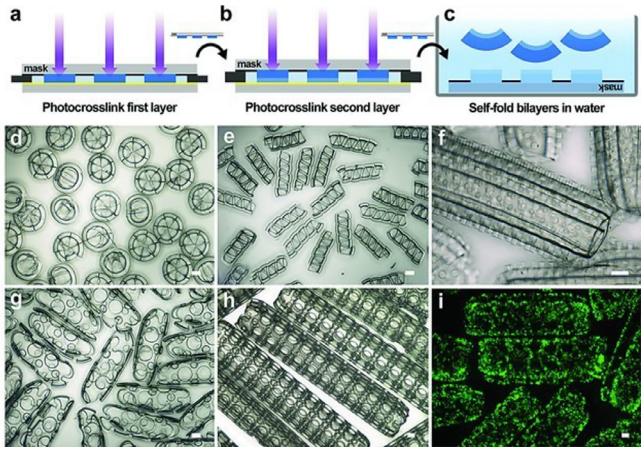


**Fig. 8.** a-c) Schematic representation of the three basic PEG bilayer photocrosslinking steps and (d-i) examples of self-folded hydrogel geometries [92].

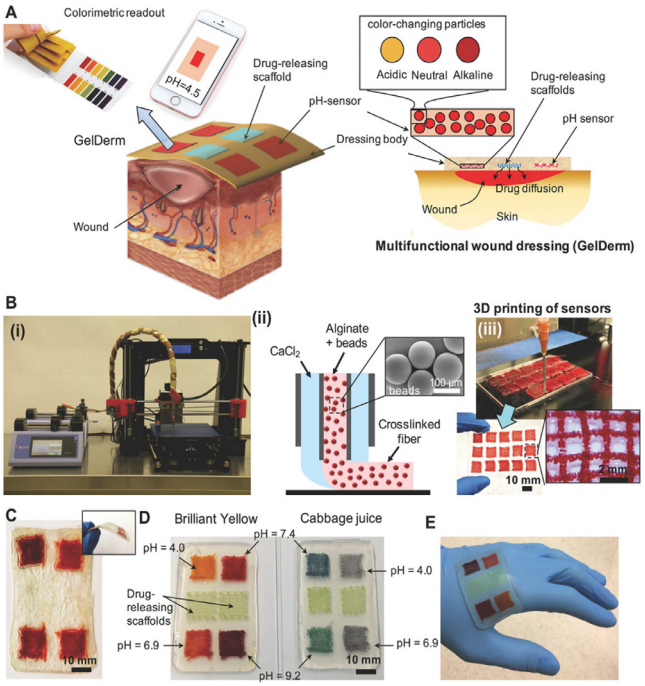


**Fig. 9.** A) Schematic representation of the GelDerm treatment of epidermal wounds, with pH-sensitive and drug-eluting components. B-(i) The porous sensors were fabricated using a 3D bioprinter equipped with a co-axial microfluidic nozzle. B-(ii) Schematic representation of the fiber deposition process using the co-axial system. B-(iii) 3D printed sensor array [93]. C) Dressings can be lyophilized and sterilized for storage and transportation. D) Synthetic brilliant yellow and naturally derived cabbage juice were used as model pH indicators for the fabrication of the sensors. Sensor arrays enable detecting spatial variations of pH on the wound site. Drug-eluting constructs release high doses of antibiotics at the wound site to eradicate the bacteria. E) The flexible GelDerm patch can be applied on curved surfaces [93].

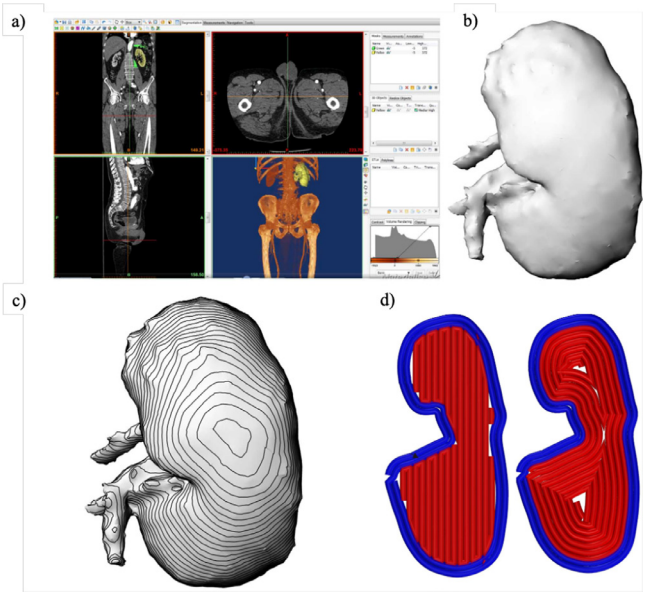


**Fig. 10.** 3D bioprinting information flow a) Medical imaging and segmentation, b) surface fitting CAD modelling, c) slicing, d) path planning.

Spheroids are multicellular self-assembled spherical clusters able to fuse and create a tissue once printed in close contact with each other [96–101]. They can mimic the physicochemical environment of tissues, with great cell viability and proliferation, supporting cell-cell and cell-matrix interactions [102]. However, larger spheroids suffer from hypoxia due to insufficient diffusion to the core which limits their size [100].

## 3. 3D bioprinting processes

### 3.1. Process chain

Bioprinting processes can be categorized according to several criteria, including the principle used to assist the deposition of bio-inks or the mode of ejection from the reservoir (orifice-free or orifice-based). In this paper, the classification of bioprinting technologies is based on the mechanism employed to assist the printing process.

The bioprinting process chain starts with biomodeling the targeted tissue or organ using medical images obtained by various data acquisition methods such as X-ray computed tomography, magnetic resonance imaging, ultrasound echoscopy, single-photon gamma rays and bioluminescence imaging (Fig. 10.a) [103]. A segmentation software is then used to capture the 3D model [104,105]. Then, the 3D surface geometry is transformed into a tessellated mesh file (STL file), which could be fitted with parametric free-form surfaces for further processing in a CAD software (Fig. 10.b). The CAD model of the target tissue or organ is then sliced to form the layers (Fig. 10.c). After slicing, deposition path planning is generated at each layer using zig-zag or contour-offsetting techniques (Fig. 10.d).

After bioprinting paths are determined, bio-inks must be prepared. For patient specific tissue/organ bioprinting, primary cells could be harvested from a patient. In addition to primary cells from different tissues, stem cells such as mesenchymal stem cells (MSCs), induced pluripotent stem cells (iPSCs) or embryonic stem cells could also be used for bio-ink [106,107]. Cells needs to be cultured and expanded to reach enough cell concentration. Biochemical factors or small molecules (growth factors, signalling and differentiation molecules) can be added to help cell growth, differentiation, and maturations. Bio-inks are then loaded on a bioprinter and the bioprinting process can then be started. Finally, constructs can be printed in situ or in the machine platform under sterile conditions being then cultured in an incubator for further maturation. Different types of bioreactors could be used for maturation of the tissue constructs. After maturation, the constructs can then be implanted in the patient or can be used as a disease model.

For efficient clinical deployment, bioprinters must be compact to be able to operate in a laminar flow unit, presenting high accuracy, printing speed and resolution. The type of tissue to be printed strongly determines the bioprinter selection.

### 3.1. Material jetting processes

Material jetting is a droplet-based technique that ejects material droplets out of a nozzle and comprises droplet-on-demand (DOD) and continuous jetting processes [108–110]. DOD systems use thermal, acoustic, or piezoelectric actuators to generate pressure pulses producing small droplets (5–50 $\mu$m) [16,111]. Thermal-based systems use heating elements to rapidly increase the temperature of the material in the close vicinity of the heating system, vaporizing the material, thus generating bubbles that expand and eject droplets onto a substrate [112]. Studies showed that the use of short heating pulses (~2 $\mu$s) only increase the temperature in few degrees not affecting cell viability [113]. However, material jetting presents some limitations in printing high density of cells, as cells settle at the bottom of the material reservoir, resulting in clogging the printing head and non-homogeneous distribution of cells. Therefore, inks must present viscosities lower than 15 mPas and cell density should be also lower than $10^6$ cells/ml [42]. In the case of acoustic systems, cell viability is limited by the level of frequencies used. Resolution ranges between 20 and 100 $\mu$m [114].

Aerosol jet printing (AJP) (Fig. 11) is a relatively novel ink deposition technology used to produce electronics such as transistors, batteries, supercapacitors, and sensors [115–118]. The printing process comprises five main stages including atomization/nebulization, aerosol gas transportation, collimation, focusing and impaction. The suspension of liquid or solid particles is atomized into 1–5 μm droplet size by using either pneumatic or ultrasonic methods depending on

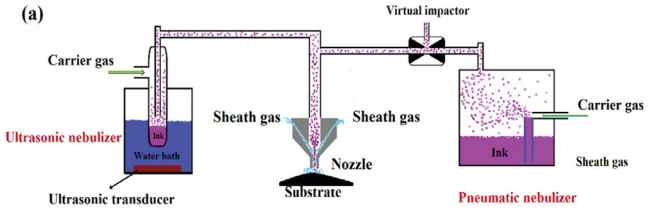


**Fig. 11.** Aerosol jet printing [115].

the ink viscosity. The generated droplets are transported by the carrier gas (i.e. nitrogen), reaching the bottom of the printing nozzle which is surrounded by a sheath gas flow. The interaction between the sheath gas and the carrier gas result in the collimation of the ink stream at the centre of the deposition nozzle. The droplet velocities at the exit range from 10 to 100 m/s within 1–5 mm of nozzle-substrate distance. AJP can print materials with a wide range of viscosities (1–100 mPa.s) and high resolution (10 μm) on planar and non-planar substrates [119,120].

However, the use of AJP for 3D bioprinting is not fully explored. Most studies focused on the fabrication of 2D biosensors [121], cell patterning on complex surfaces [122], and hydrogel inks [123].

Process optimisation is a complex task in AJP. Ultrasonic nebulization creates more uniform ink droplets, but it is restricted to low viscosity inks while pneumatic nebulization can print inks with a wide range of viscosities but form polydispersed streams. Small droplets with insufficiently controlled flow rate tend to impinge on the wall during the transport step, affecting the collimation and focusing and hence causing nozzle clogging and reducing printing resolution [124]. Larger droplets tend to hit the wall due to the gravity settling, compromising transport efficiency, and leading to flow instability.

### 3.2. Extrusion-based technologies

Extrusion-based bioprinting systems comprise a computer-numerically control (CNC) system (gantry or robotic-arm), which controls the motions of pneumatic (compressed air), mechanical (piston or screw) or solenoid (electrical pulses) driven printheads, and a platform that collects the printed material [108, 125-127]. Some systems also include an optical fibre to irradiate the deposition area inducing photo-crosslinking reactions. Extrusion-based bioprinting allows to print a wide range of materials with different mechanical properties and viscosities ($10–10^{13}$ mPa.s) and bio-inks with high cell densities (up to $10^7$ cells $mL^{-1}$) [42,108]. Pneumatic systems are usually used to process low viscous materials, while mechanical systems are more suitable for highly viscous materials. Printed materials become solid through thermal, physical, or chemical methods.

Printing resolution (200–2000 $\mu$m) is one of the main limitations of this technique compared with other bioprinting processes and requires proper optimization of processing conditions (e.g. flow rate and deposition velocity) and bio-ink properties (e.g. rheological properties – a shear thinning behaviour is required – wettability, surface tension and cell density) [108,128]. Besides, extrusion-based processes present some limitations in precisely controlling the volume of printed material during the lag time when the pressure is switched on and off, and potential risk of nozzle clogging [128]. This technique is also characterized by high pressure drops and for inducing high mechanical stresses on the materials during the printing process, which can compromise cell viability [129]. New developments include extruding the material in a coagulation bath that induces the gelation of the printed material, printing the material in a granular or colloidal bath or co-axial extrusion [130–132].

To guarantee a homogeneous composition of multi-component bio-inks, Bhattacharyya et al. [133] developed a twin-screw extruder (Fig. 12) successfully used to print an alginate-based bio-ink containing tricalcium phosphate nanoparticles and osteoblasts. The system presents variable screw pitches and two separate inlets reducing the exposure of cells to high shear stresses. Bioprinted constructs

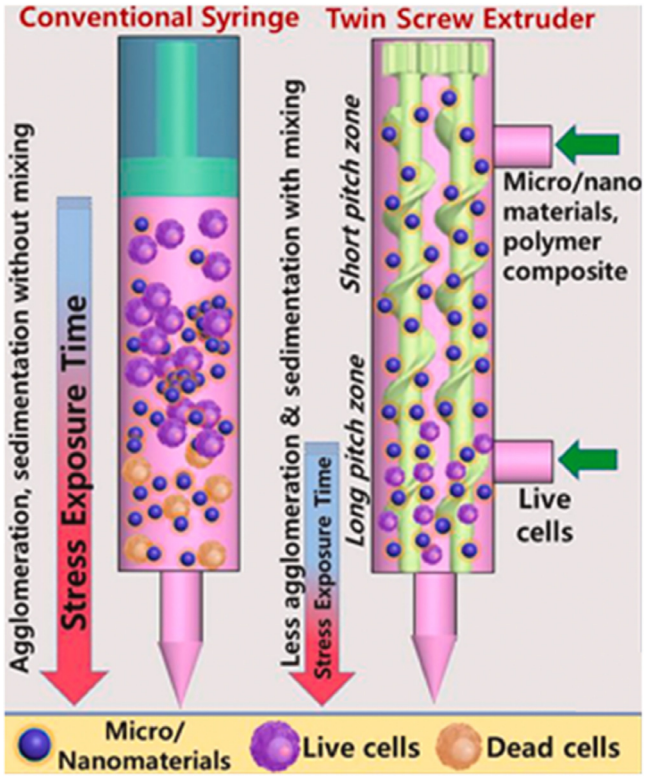


**Fig. 12.** Twin screw extruder versus conventional printing system [133].

exhibited superior mechanical and biological properties than conventionally (single screw or serynge-type) bioprinted samples.

The Bioplotter (Fig. 13a) is an extrusion-based system developed by Landers and Mülhaupt [134] and commercialized by Envisiontec (Germany). This was one of the first bioprinting systems introduced

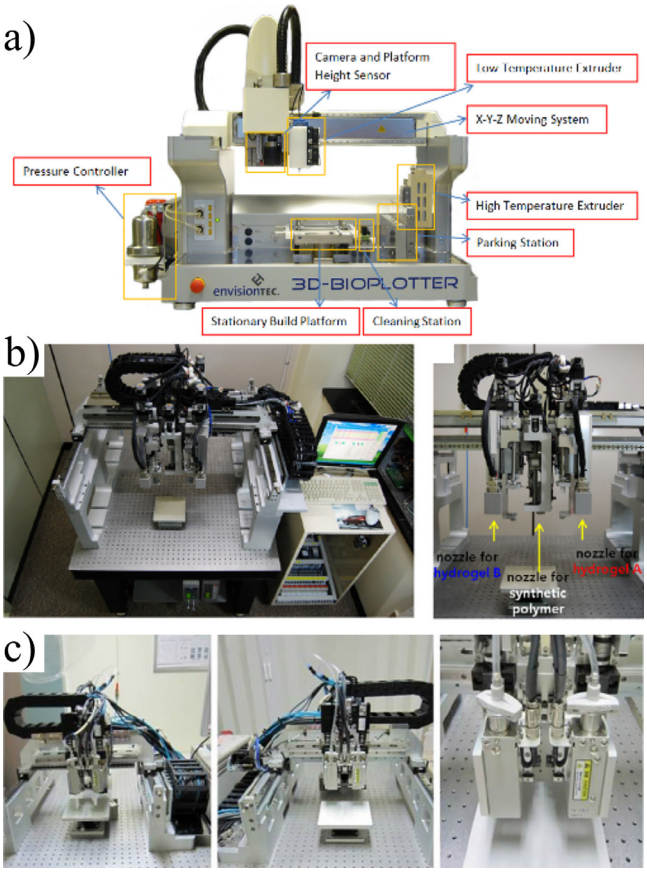


**Fig. 13.** Multi-printhead systems. a) The 3D bioplotter developed by EnvisionTEC (Germany); b) The MtoBS system [135]; c) The multi-head deposition system (MHDS) [136].

into the market and comprises multiple pneumatic printing heads allowing for multi-material printing. The dispensing printing heads move in three dimensions and the fabrication platform is fixed.

Shim et al. [135] developed a multi-head tissue printing system (MtoBS) comprising six printing heads with an accuracy of ± 2.4 μm and repeatability of ± 1.0 μm (Fig. 13b). Two temperature-controlled pneumatic printing heads were used to process thermoplastic biomaterials. The other four printing heads were used to print bio-inks.

Ozbolat et al. [137] developed a multi-arm bioprinter (MABP) comprising pressure-assisted and piston-assisted printing heads. The system was used to print a wide range of materials including cell spheroids [87,138,139]. Similar systems were developed by Jung et al. [136] (Fig. 13c), Kang et al. [140] and Organovo (San Diego, USA).

Recently, Ma et al. [141] developed a six-degree-of-freedom robotic-assisted system for cartilage in situ printing composed of an air compressor, an extrusion-based printing unit, a workbench and a control cabinet (Fig. 14). An off-line programming software was used for setting the printing parameters and a laser tracker was included for calibration. The system was successfully used to treat rabbits' osteochondral lesions with a diameter of 5 mm and a height of 4 mm.

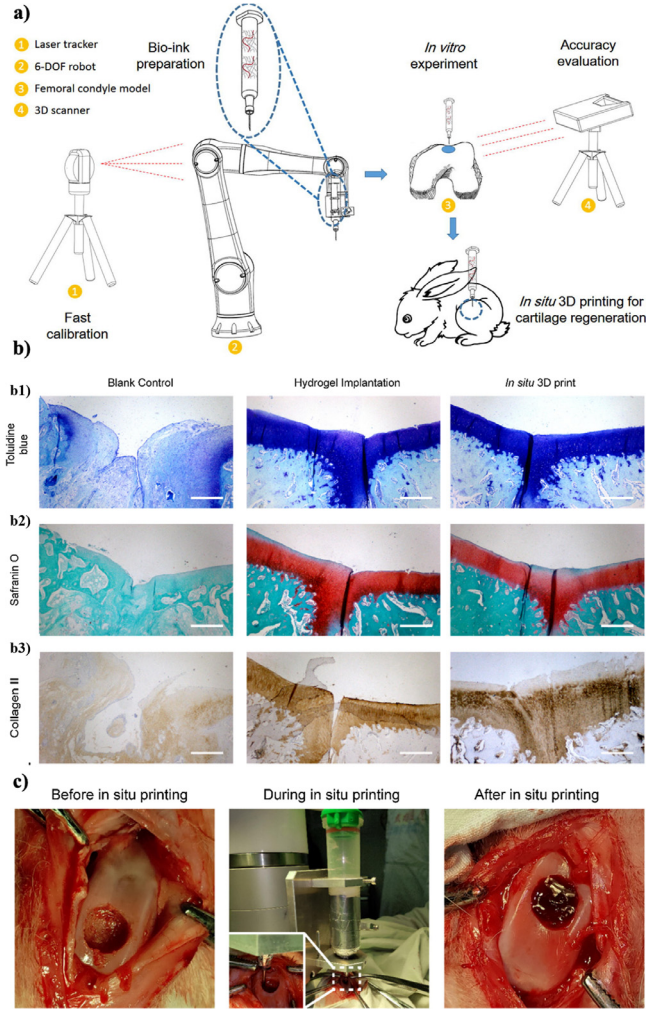


**Fig. 14.** Robotic-assisted in situ printing for cartilage regeneration. a) Schematic illustrations of in situ printing for cartilage defects of rabbit models using robotic-assisted device. b) Histological characterisation of in situ printed, hydrogel implantation and control groups, with toluidine blue, Safranin O, and collagen II staining respectively (Scale bar: 500 μm). c) The osteochondral defect with a diameter of 5 mm and a height of 4 mm created before and during in situ printing, and the defect fulfilled by hydrogel bio-ink after in situ printing [141].

Navid et al. [142] developed a micro-capillary multi-material extrusion-based system for a wide range of tissue engineering applications. Authors developed a computational algorithm to generate continuous deposition path plan while changing material and internal composition spatially and hierarchically based on assigned functionalities (Fig. 15.a). A range of biodegradable synthetic polymers were used as reinforcing material together with bio-inks with different biological functions (Figs. 15.b and 15.c). The simultaneous incorporation of multiple printing heads and the novel path planning system allowed to print complex constructed with graded properties (Fig. 15.d).

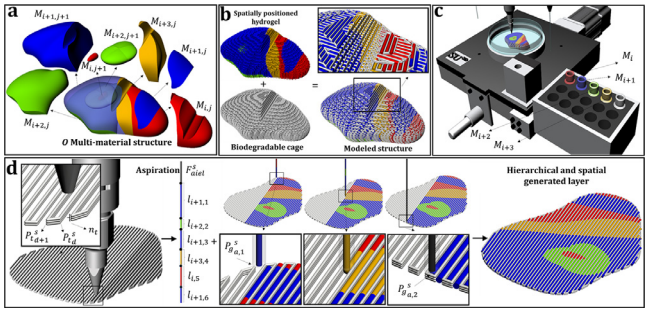


**Fig. 15.** Bio-additive manufacturing procedure. a) multi-material object; b) modelled structure; c) bioprinting; d) fabrication procedure in one layer [142].

### 3.3. Light-based technologies

Light-based technologies comprise a group of techniques that use light energy to trigger a series of chemical reactions on a photosensitive hydrogel system (vat photopolymerisation) or light energy to generate radiation forces or local heating to promote the ejection of bio-inks towards a substrate (laser-assisted cell-printing).

Photocrosslinking reactions include free radical-initiated chain polymerization and bio-orthogonal click reactions (Fig. 16). Free radical reactions based on methacrylate functionalised pre-polymers are the most common strategy but presents some limitations such as poor control over the curing kinetics, vitrification and uni-molecular termination, oxygen inhibition, and heterogeneous network formation [143–146].

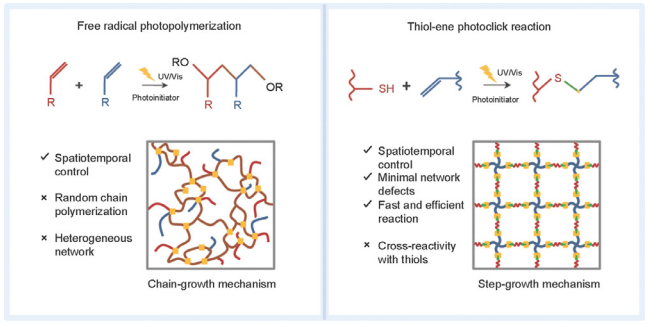


**Fig. 16.** Photo-polymerization mechanisms for 3D bioprinting [26].

Contrary to free radical photocrosslinking reactions, bio-orthogonal click reactions are step-growth reactions not sensitive to water/humidity or oxygen, allow the formation of uniform hydrogel networks and can be conducted under mild conditions [143–149]. Among the different bio-orthogonal click reactions, thiol-ene photoclick reaction has been successfully used in the field of 3D bioprinting allowing to effectively encapsulate a variety of cells in a wide range of hydrogels [25,146,148-157].

Vat photopolymerization allows the fabrication of 3D cell-laden constructs through selective photo-curing reactions that transform a liquid material into a solid one [158–160]. Two different irradiation approaches have been explored: masque-based and direct writing methods. In the masque-based method an image is transferred to a liquid polymer by irradiating through a patterned masque (liquid crystal display (LCD) system, or digital micromirror devices (DMDs)) (Fig. 17) [161]. In the direct writing approach, a focused beam is used to start the polymerisation process. The curing process can be induced via single-photon or two/multi-photon polymerisation

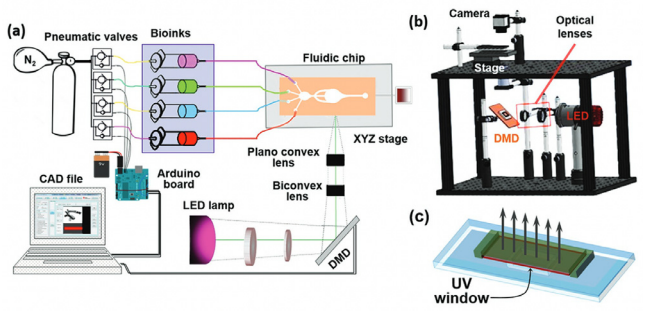


**Fig. 17.** Vat photopolymerization system suitable for bioprinting applications, which comprises a UV lamp (385 nm), optical lenses and objectives, a DMD chip, and a microfluidic device [164].

[161]. These two methods share the same chemical principle being the main difference the number of absorbed photons necessary to initiate the curing reaction. Contrary to single-photon polymerisation systems that use ultraviolet or visible light, two-photon polymerisation systems use femtosecond infrared lasers [162,163]. For bioprinting applications, the curing reaction must occur under mild conditions (low light intensity, short irradiation time, physiological temperature, and low organic solvents).

Several researchers modified vat photopolymerization system adapting them for bioprinting of multimaterial and multicellular constructs. Chan et al. [165] used vat photopolymerisation to process poly(ethylene glycol) diacrylate (PEGDA) bio-inks. To avoid cell settling at bottom of the vat, each new layer of bio-ink was manually added. A similar approach was used by Zorlutuna et al. [166] to produce spatially organized multicellular constructs. Lu et al. [167] used a dynamic masking system to produce biological constructs using a bio-ink made of PEGDA dissolved in phosphate buffered saline and 0.1 wt% of Irgacure 2959, containing murine OP-9 marrow stromal cells. Results showed an encapsulation efficiency of 73% and high cell viability after 24 h. The authors also demonstrated the feasibility of the system to produce constructs with entrapped multiple biochemical factors with precise pre-designed and spatially patterned layers (Fig. 18). For multi-material applications, Bartolo developed a masque-based system (multi-material stereo-thermal-lithographic system) comprising multiple vats (Fig. 19) [168,169].

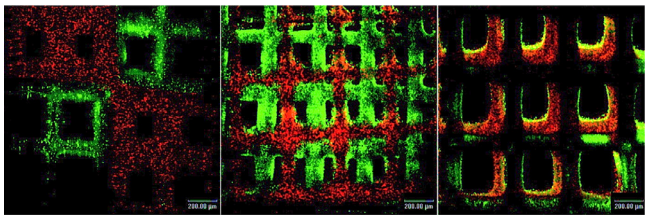

**Fig. 18.** Fluorescence confocal microscopy of PEGDA-based bio-ink constructs produced with pre-defined spatial patterns [167].

Laser-assisted bioprinting comprises a range of techniques such as laser-guided direct writing (LGDW), laser-induced forward transfer (LIFT) and modified laser-induced forward transfer (modified-LIFT) processes that allow the deposition of cell solutions with high concentrations of cells at high velocity (≥10 m/s) [170–172]. Typically, a laser-assisted bioprinting system consists of a pulsed laser source (near-infrared or near-ultraviolet radiation), a "ribbon" or donor slide comprising a light-absorbing layer and a bio-ink layer, and a collector. Upon irradiation, light is locally absorbed by the light-absorbing layer inducing its vaporization, which results in a high gas pressure that ejects the droplets of bio-ink towards the collector (Fig. 20) [171–174]. As described by Guillemot et al. [173], the droplet formation is a complex mechanism that depends on the light intensity, laser pulse duration, laser spot size, distance between the ribbon and the collector and the thickness of the bio-ink layer. This technique allows printing droplets of bio-inks with high printing resolution and

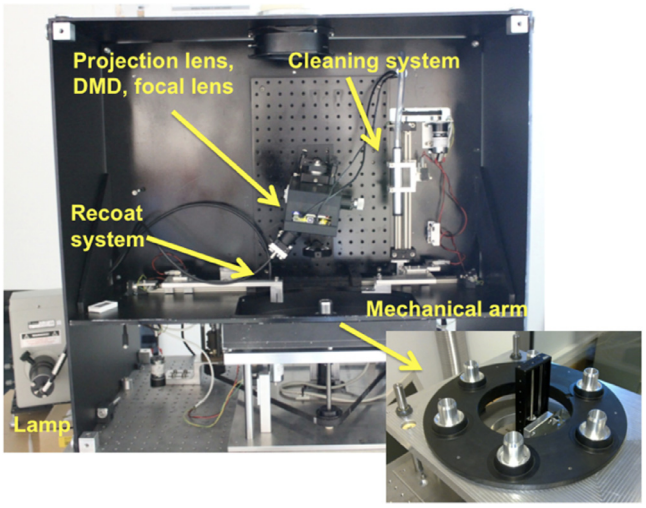


**Fig. 19.** Multimaterial stereo-thermal-lithographic system.

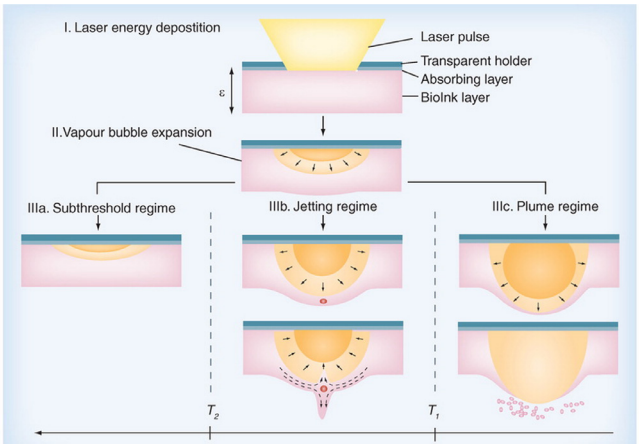


**Fig. 20.** Jetting regimes in laser-assisted bioprinting. Subthreshold regime (IIIa): the bubble expansion is very weak, and no jetting is formed; jetting regime (IIIb): the bubble expands, collapses and a jet is formed; plume regime (IIIc): splashing mechanism due to excessive bubble expansion overcoming surface tension [174].

high cell viability. However, the heat generated by the laser might have a negative impact on cells.

The LGDW process employs radiation pressure to guide and deposit cells over a distance of ~300 μm with micrometre resolution [175]. Cell transport over distances up to 7 mm are also possible using a hollow optical fibre. This process has been used to deposit multiple cell types including embryonic chick spinal cord cells [176], human umbilical vein endothelial cells [177] and mouse embryonic stem cells [178]. However, LGDW presents low cell throughputs (2.5 cells/min) and poor reproducibility.

LIFT-based techniques make use of a high-energy pulsed laser to induce the local melting of a liquid suspension leading to its ejection towards the substrate [179,180]. The droplet formation depends on both laser and bio-ink properties (the laser fluency, bio-ink viscosity, film thickness). The LIFT process has been mainly used for the direct writing of metals but can be also highly relevant for biomedical applications due to its unique resolution and patterning capabilities.

Modified-LIFT processes comprise two main techniques [171,175,181-183]: the matrix-assisted pulsed laser evaporation direct write, or matrix assisted pulsed laser direct write (MAPLE DW) (Fig 21a) and the biological laser printing (BioLP) or absorbing film-assisted LIFT (Fig. 21b). MAPLE DW uses a low-powered pulsed laser operating in the UV or near-UV wavelength region, focused by a microscope objective at the interface print ribbon/optical absorbing material, heating and vaporizing the biomaterial. In the BioLP the droplet formation and ejection are achieved by focusing a high-powered laser pulse (usually a near-IR laser) onto a biocompatible laser

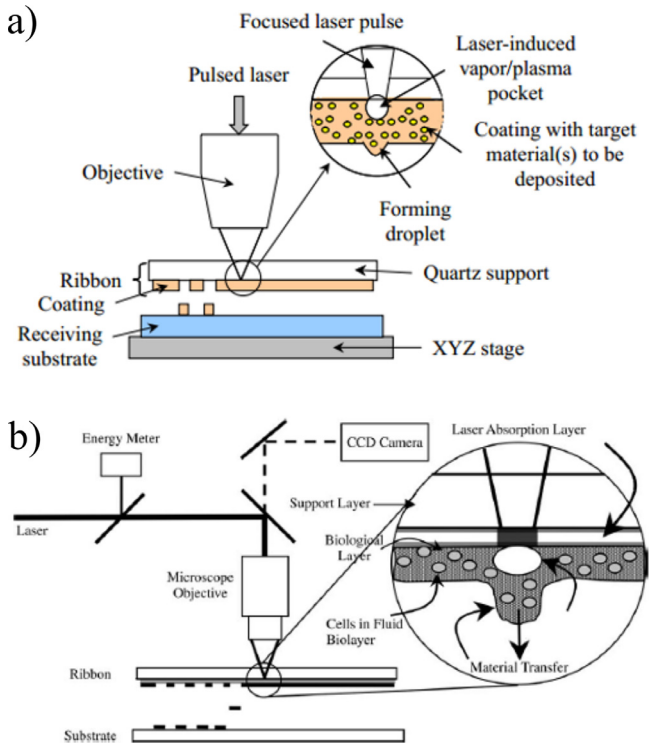


**Fig. 21.** a) The MAPLE DW system [184]; b) The BioLP system [185].

absorption interlayer (1–100 nm) placed between the ribbon and the cellular layer, allowing more efficient droplet ejection and high reproducibility.

LIFT-based processes have been used to print viable mammalian cells, including human osteoprogenitors cells human osteosarcoma cells [183], human endothelial cells [172,180], rabbit carcinoma cell and human umbilical vein endothelial cells [186]. *In situ* printing have been also reported. Keriquel et al. [187] successfully used a BioLP system for the in situ printing of a composite material to treat mouse calvaria defects of critical size (Fig 22).

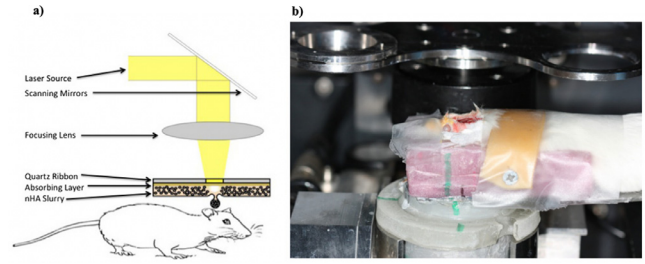


**Fig. 22.** In vivo bioprinting using a BioLP system. a) Setup; b) Mice place in a specific holder [187].

### 3.4. Other systems

Each printing method described in the previous sections present advantages and disadvantages (Table 1) and, as a consequence, different research groups started developing multi-modal systems by integrating multiple printing principles into a single machine. Xu et al. [188,189] developed a system combining material jetting and solution electrospinning (Fig. 23) The electrospinning unit was used to create nanoscale polymeric meshes supporting bio-inks printed through the material jetting unit. Some commercially systems (e.g. RegenHu, Switzerland) also integrate extrusion-based systems and melt-electrospinning. To increase printing resolution, Zhang et al. [190] developed a system called E-FDM based on electrohydrodynamic (EHD) jet printing and filament-based extrusion. The allowed high printing resolution (10 μm) and fast printing speed of 40 mm/s.

The BioScaffolder (SYS+END, Salzgitter-Bad, Germany) (Fig 24) allows to process a large variety of natural and synthetic materials

**Table 1**
Main advantages and disadvantages of bioprinting processes [16,108-110,129,191-193].

| | Advantages | Disadvantages |
|---|---|---|
| Material jetting | Low cost; high print speed, high resolution (20–100 μm), printing speed and printing efficiency; high cell viability (>86%) | Limited bio-ink viscosities (1–200 mPa.s); low cell density (up to $10^6$ cells/ml); nozzle clogging |
| Extrusion-based systems | High versatility; ability to print materials with a wide range of viscosities; systems can comprise multiple printing heads allowing for multi-material/cellular printing; high printing efficiency | Low printing resolution (200–2000 μm); low cell viability and cell deformation with small printheads; nozzle clogging; slow printing speed |
| Laser-assisted bioprinting | High printing resolution (80–140 μm); high cell density; good spatio-temporal control; controllable droplet size; nozzle-free technique, so no prone to clogging problems | Only able to print one cell type at a time; low printing efficiency; limited range of viscosities (1–300 mPa. s); potential transfer of cytotoxic materials from the ribbon; expensive systems |

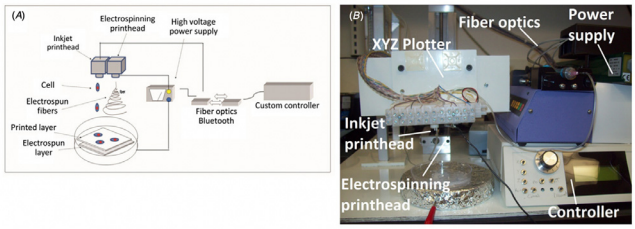


**Fig. 23.** Multi-modal system comprising material jetting and solution electrospinning. a) Schematic representation; b) Set-up [188].


**Fig. 24.** The Bioscaffolder system [194].

with up to four independent printing heads [194]. The printing unit comprises a variety of printing technologies including pneumatic, piston, and screw-assisted extrusion, filament-based extrusion, and melt electrospinning. The system is being improved by incorporating syringe-based printing head to print bio-inks and an atmospheric plasma pen for surface modification. Similarly, Liu et al. [10,195-197] developed a plasma-assisted bioextrusion system (PABS) (Fig. 25), able to produce multi-material/functionally graded tissue constructs using a multi-head extrusion system (two pressure-assisted and one screw-assisted printing heads) and a low temperature plasma jet unit for surface modification. Other systems such as the one developed by Dikyol et al. [1] combine microfluidic, coaxial material-extrusion and laser-based multimaterial bioprinting approaches (Fig. 26).

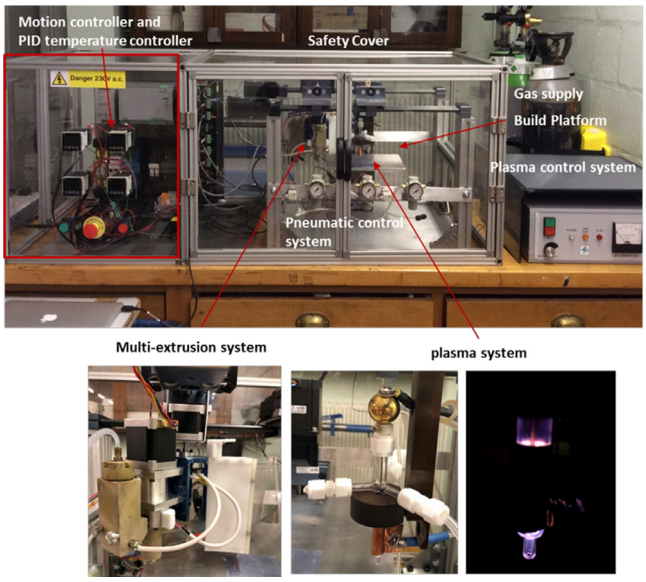


**Fig. 25.** The PABS system. a) Set-up of plasma-assisted bio-extrusion system; b) multi-extrusion system; c) plasma modification system.

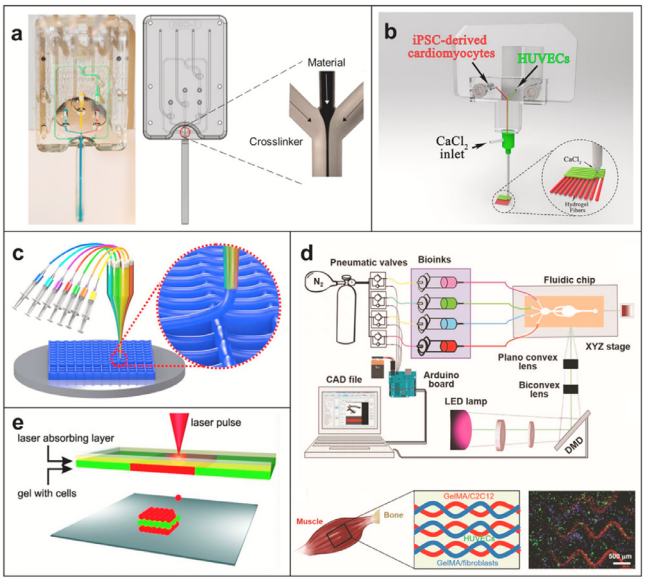


**Fig. 26.** Different biofabrication platforms employing microfluidic and laser-based multimaterial bioprinting approaches: a) Microfluidic printhead with on-the-fly multi-material switching capability. b) Combination of coaxial needles with microfluidic chips, where bioink solutions are delivered from the microfluidic printhead and then crosslinked by $CaCl_2$ supplied from the coaxial needle. c) Capillary-based microfluidic printhead, in which bio-inks are flowed through separate capillaries without any contact with each other till the end of the nozzle. d) Setup of DMD-based, microfluidics-enabled multimaterial bioprinting platform (up) and a skeletal muscle model fabricated with this platform e) LIFT setups also include multimaterial bioprinting capability [1].

## 4. Applications

### 4.1. Skin

Skin is the largest organ of the human body and has a wide variety of functionalities such as maintaining homoeostasis, regulating body temperature, providing a protective barrier from external agents, and acting as a sensory organ [198,199]. Therefore, a loss in skin functionality may result in serious health problems.

Several researchers used different bioprinting techniques to produce epidermal, dermal, and dermo-epidermal constructs [41,157,200,201]. Most studies, focused on the use of UV or visible light photocrosslinking hydrogels [25,26,202]. To take advantage of specific attributes of other chemistries, photopolymerization has also been combined with physical and chemical reactions, yielding dual-crosslinked hydrogels exhibiting specific characteristics such as the adhesion to the wound bed, self-healing and drug release ability [203,204].

Pereira et al. [157,205] developed a single-component methacrylate modified pectin bio-ink with a controlled density of cell-adhesive ligands, tunable mechanical and rheological properties for extrusion bioprinting of 3D dermal constructs. The bio-ink was designed to allow the incorporation of cell adhesion motifs and the formation of hydrogels by a dual crosslinking mechanism based on an ionic gelation mechanism, to slightly increase the bio-ink viscosity guaranteeing high printability, followed by UV photopolymerization (Fig. 27). Initial studies based on the use of encapsulated fibroblasts showed that the printed constructs provided a suitable microenvironment for the formation of a matrix, rich in collagen and fibronectin (Fig. 28). To improve the properties of the printed bio-inks, increasing biocompatibility and producing more homogeneous hydrogel networks, authors also developed novel protease-degradable hydrogels obtained by UV photoinitiated thiol-norbornene click chemistry [157]. In vitro results showed that this bio-ink was able to support the formation of full-thickness skin with morphological resemblance to human skin (Fig. 29).

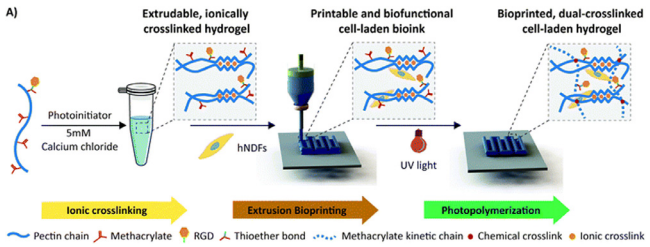


**Fig. 27.** Dual-crosslinking printing approach of a functionalized pectin bio-ink [205].

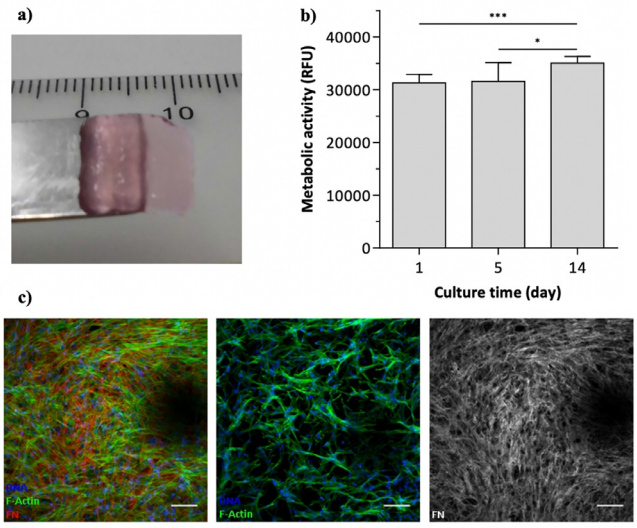


**Fig. 28.** a) Printed 3D construct ($8 \times 8 \times 0.9$ mm$^3$) at day 14 post-printing; b) metabolic activity of entrapped dermal fibroblasts showing that the printed constructs can support cell growth; c) Morphology of entrapped dermal fibroblasts and fibronectin network within the construct, stained for nuclei (blue), F-actin (green) and fibronectin (FN). The scale bars correspond to 50 $\mu$m [204].

Despite the success of these mono or bilayer constructs they were not able to recapitulate key characteristics of skin such as pigmentation and the presence of appendages such as hair follicles.

Pigmentation, an important feature of the human skin, has been investigated by several researchers. Gledhil et al. [206] developed 3D pigmented skin equivalents using a collagen type I bio-ink containing induced pluripotent stem cells (iPSCs)-derived fibroblasts printed

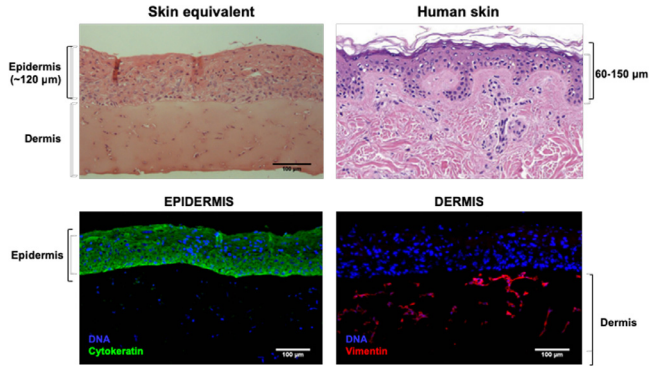


**Fig. 29.** Comparison between the 3D printed skin construct and human skin (top) and morphological characterization of the equivalent skin after 14 days of cell differentiation at the air-liquid interface. Adapted from [157].

and crosslinked on a polyethylene terephthalate substrate and incubated for 7 days in culture medium. Then, iPSC-derived keratinocytes and iPSC-derived melanocytes were seeded on top of the previously created layer and incubated for 7 days. Finally, the construct was submitted to an air-liquid interface stratification process. Results showed that the iPSC-derived melanocytes were able to produce and transfer melanin. Similarly, Min et al. [207] used a multi-printhead pressure-assisted extrusion system to print a collagen bio-ink containing fibroblasts to form the dermal layer upon crosslinking with sodium bicarbonate. Melanocytes and keratinocytes were printed on top of the dermal layer and the construct was then submitted to air-liquid inducing stratification and skin pigmentation. Ng et al. [208] used a material jetting technique to produce a bilayer pigmented 3D skin, depositing keratinocytes, melanocytes, and fibroblasts according to predefined patterns.

Huang et al. [209] used an extrusion-based system to fabricate a 3D skin construct that promotes epidermal progenitor differentiation into sweat gland cells using a gelatine/sodium alginate bio-ink containing mouse plantar dermis and epidermal growth factors. The developed 3D constructs with 300 $\mu$m pore size supported the cellular self-organization and formed sweat gland tissues. In vivo studies in burnt mice demonstrated the ability to functionally restore sweat glands. Recently, Vahav et al. [210] demonstrated the ability of induce hair follicle formation in reconstructed skin by incorporating human neopapillae spheroids constructed from expanding and self-aggregating of dermal papillae cells into the dermis of reconstructed skin.

Vascularisation is a key feature required by a skin construct. The lack of vascularisation compromises the wound healing process [211]. Pre-vascularisation strategies based on the incorporation of growth factors and angiogenic potent cells in the printed skin construct is a promising approach [212,213]. Other relevant strategy involves the use of sacrificial inks (e.g. Pluronic F-27), removed after the printing process, which allows the formation of a network of interconnected hollow channels within the 3D construct that can be used for the perfusion of endothelial cells [214,215]. Another relevant strategy is the incorporation of silicate-based biomaterials into the skin construct. This was recently demonstrated by Ma et al. [216], which used a gellan gum-sodium alginate-methyl cellulose bio-ink containing strontium-silicon microparticles, fibroblasts and endothelial cells. In vivo blood vessel formation was observed considering three animal models. Moreover, Abaci et al. [217] used 3D bioprinting to spatially micropattern a vasculature network using primary and induced pluripotent stem cells-derived endothelial cells. The printed skin structure was able to form a barrier function and facilitated neovascularisation upon implantation on mice.

*In situ* skin bioprinting on small and large-size animal models have been reported, demonstrating the potential of 3D bioprinting for future clinical applications. Albanna et al. [218] proposed a system comprising an imaging system for wound scanning and a material jetting system to print bio-inks containing autologous and allogeneic dermal fibroblasts and epidermal keratinocytes (Fig. 30). Studies on

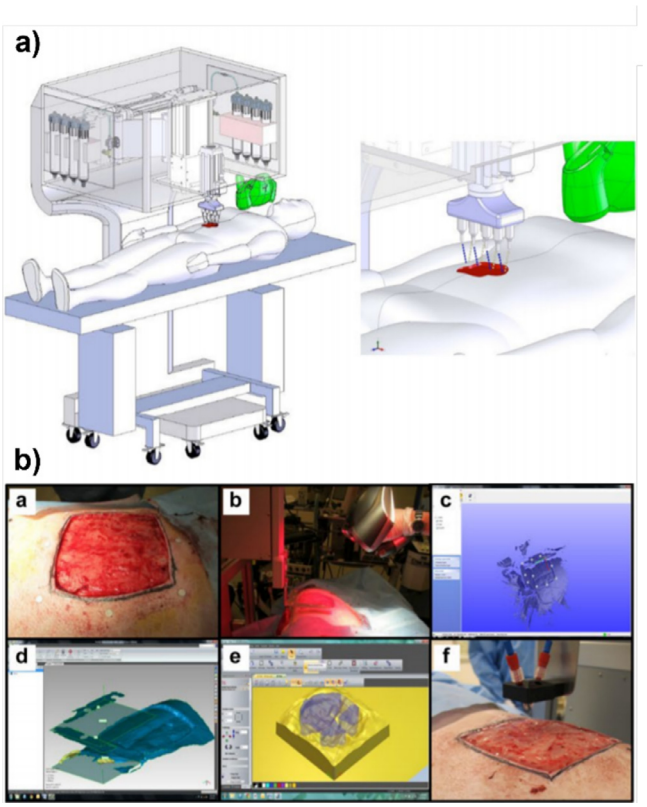


**Fig. 30.** Skin bioprinter. a) Integrated wound scanning and material jetting printing system; b) scanning, design and printing steps [218].

mice and porcine models showed rapid wound closure, reduced contraction, and fast re-epithelialization.

### 4.2. Cartilage

Current cartilage therapies include non-surgical and surgical treatment. Non-surgical treatments can be used for relieving the pain and slow down the progression of osteoarthritis. However, these only suppress or reduce the pain but not cure the joint disease. Cartilage defects increase in size and depth as the grade of injury increases. Therefore, a surgical intervention will eventually be required. Marrow stimulation such as microfracture and implantation of autograft and allograft are frequently chosen for surgical treatments. However, these techniques usually form fibrocartilage instead of the original hyaline cartilage to fill the defect. Therefore, they may show inferior mechanical properties and longevity compared to nature cartilage tissue [219]. Tissue engineering strategies utilising biomaterials, cells, and biomolecules have been explored to regenerate damaged cartilage tissue.

For in situ cartilage printing a portable handheld device called Biopen was developed enabling the bioprinting of bio-inks with high cell viability [220,221]. The handheld device (Fig. 31) is flexible and allows the deposition of bio-inks in a small area during the operation procedure. It is also easy to sterilise due to its small size and less costly than robotic-assisted bioprinters. The device can be equipped with a light source allowing to process photo-curable bio-inks. A coaxial nozzle has been also developed to allow the fabrication of constructs with enough structural stability [222]. The device allows fast crosslinking (less than 1 s) and the fabrication of constructs with Young's modulus around 0.5 MPa, slightly lower than native cartilage (0.5–8 MPa). However, it requires several improvements. For example, the single size extruder nozzle limits the selection of bio-inks. It is also challenging to achieve thermal gelation by the handheld device without an alternative temperature system [220].

Potential next steps in the levels of autonomy and miniaturisation of in situ printing using robotics systems include, first, higher degrees

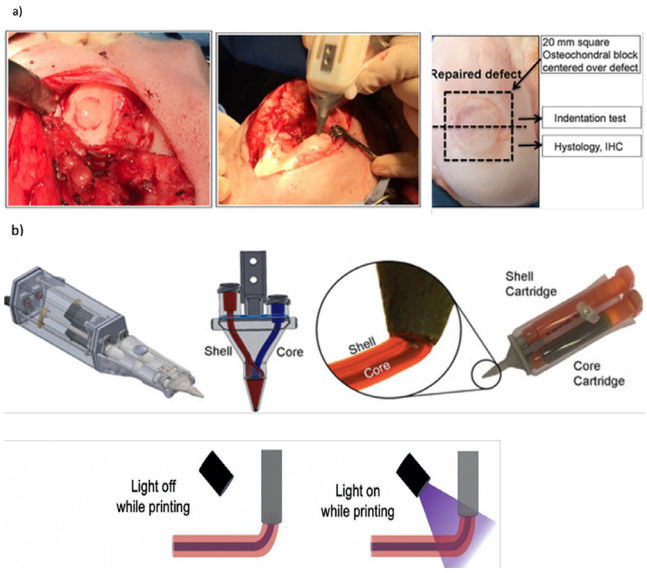


**Fig. 31.** Handheld biopen developed for in situ cartilage printing. a) *In situ* printing to repair a full-thickness chondral defect in a sheep; b) The co-axial nozzle to produce core-shell filaments via thermal of photocrosslinking methods [221–223].

of autonomy of robotic systems in conjunction with artificial intelligence (AI) based control systems; second, the creation of fully implantable robots which enable the restoration and replacement of physiological processes; third, the realisation of micro- and nanoscale robotic devices [224]. Impressive progresses in micro- and nano-robotics, have been achieved, with a good example being the stem cell laden micro-robots for knee cartilage defects (Fig. 32) [225]. The micro-robot was fabricated using a PLGA micro-scaffold and equipped with an electromagnetic actuation system for targeted cell transport of the micro-robot. It also was subsequently immobilised to the defect by a permanent magnet after the injection of micro-robots. The micro-robot showed strong magnetic properties and ensured degradation, viability, proliferation, and chondrogenic differentiation of the stem cells.

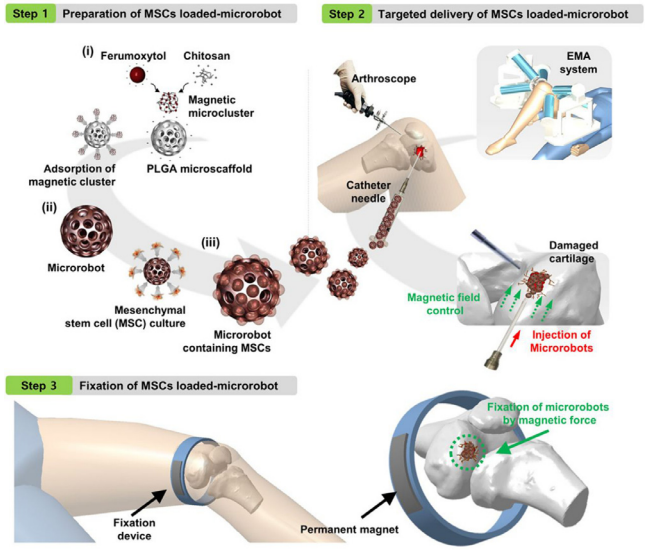


**Fig. 32.** Schematic representation of the use of magnetic microrobot-mediated stem cells delivery system for cartilage defects [226].

### 4.3. Nerve

Like other tissues, nerve exhibits limited regeneration capabilities, especially beyond a critical defect size (>5 mm) [227]. Current clinical treatments are based on nerve sutures (neurorrhaphy or coaptation) and autologous nerve transplantation. However, due limited number of donors and morbidity at the donor site, new clinical strategies are required. The advent of tissue engineering allowed the development of artificial nerve conduits using biomaterials able to guide the regeneration of nerve fibres (Fig. 33).

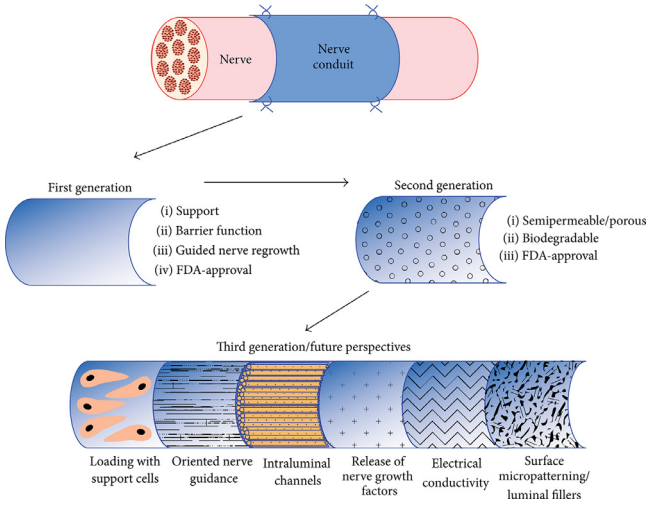


**Fig. 33.** Three generations of nerve conduits and corresponding characteristics [228].

Ideal nerve conduits should be biocompatible, biodegradable, electrically conductive, flexible, and containing internal micro-channels to promote nerve regeneration with minimal scar and also to preventing the formation of neuromas [229,230].

Ning et al. [231] used a material extrusion system (3D Bioplotter, Envisiontec, Germany) to print a nerve construct using a bio-ink composed of alginate, hyaluronic acid, fibrin, RGD peptide and Schwann cells (cell density of 1 × 106 cells/ ml). The bio-ink was printed on a container including a calcium/thrombin crosslinking solution. Post-printing analysis showed that after 10 days encapsulated cells were alive and proliferating.

Researchers are also focusing on the in situ strategies to reconstruct neural tissue by combining medical imaging and robotic-assisted 3D bioprinting systems (Fig. 34) [232]. However, there are several technical problems such as the complex surgical procedure, the level of detail of the printed neural structure and some problems related to the biological guidance conduit that needs to be addressed.

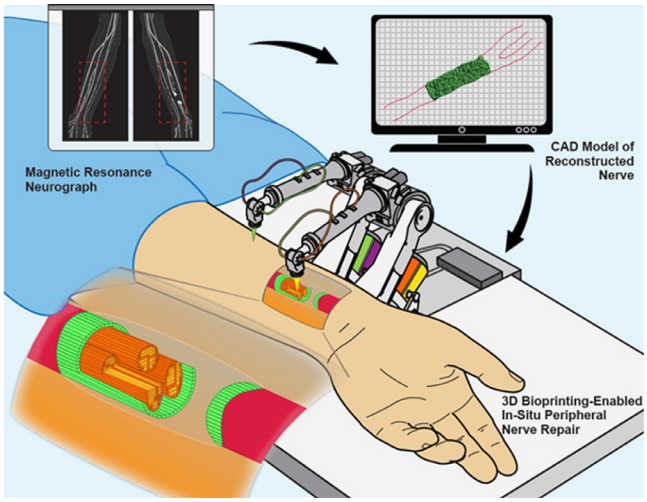


**Fig. 34.** *In situ* printing to repair peripheral nerve injuries [232].

### 4.4. Vascular tissues

Vascularisation is a critical aspect in 3D bioprinting as oxygen diffusion is only effective at short distances (100–200 $\mu$m) posing significant limitations regarding the fabrication of large and thick

constructs [233]. Attempts to fabricate vascularized heterogeneous structures have been based on the use of various multimaterial bioprinting approaches incorporating microfluidic and coaxial bioprinting, multi-head 3D bioprinting systems or dual printing platforms. Among them, coaxial multimaterial bioprinting is commonly employed to produce constructs containing stacked perfusable tubular networks [234]. The lumen is commonly supported with a sacrificial fugitive bio-ink to provide physical support during the bioprinting process until sufficient crosslinking is provided to the inner wall of the vessel, being then washed out. The sacrificial fugitive ink can be supplemented with a crosslinker to increase the mechanical strength of the inner wall of the vascular structure. The development of all-in-one coaxial nozzle or microfluidic coupled systems enabled the fabrication of vascularised heterogeneous structures by using a single nozzle system. Sing et al. [235] successfully used a triple coaxial nozzle bioprinting system for fabrication of a perfusable renal tubular tissue (Fig. 35).

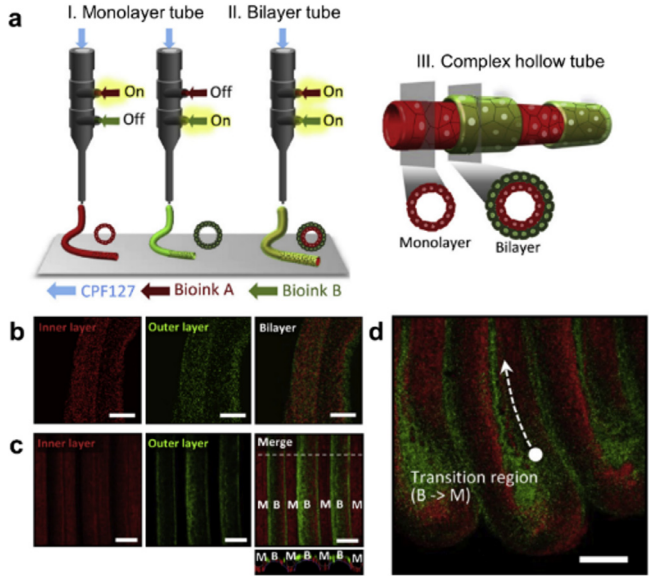


**Fig. 35.** a) Schematic diagram of microfluidic co-axial nozzle system for monolayer (M), bilayer (B) and complex hollow tube bioprinting and representative views. b) Confocal images of bi-layered hollow tube, c) complex hollow tube with monolayer and bilayer structures and d) their transitional region. Inner and outer shells were demonstrated with red and green fluorescent beads embedded in the ink, respectively [235].

Kucukgul et al. [236] developed biomimetic and self-supporting scaffold-free macrovascular constructs (Fig. 36). In this study, scaffold-free bioprinting of aortic tissue constructs was conducted based on medical images of real human aorta, employing a capillary-based extrusion of mouse embryonic fibroblast (MEF) aggregates and agarose support structures from two separate printheads according to a developed toolpath planning.

### 4.5. Other tissues

Cardiovascular disease is the most common cause of death in developed countries and the only treatment option for end-stage heart failure is a heart transplant. The limited number of suitable donors stimulated the use of 3D bioprinting to develop cardiac patches and whole hearts. Noor et al. [237] successfully fabricated millimetre thick cardiac patches using an extrusion-based bioprinting system to print a decellularised adipose tissue derived hydrogel containing cardiomyocytes and endothelial. The vascular channels were produced by printing endothelial cells in a sacrificial gelatine bioink within the patch which was liquified at 37 °C leaving a lumen lined with endothelial cells. In vitro and in vivo studies showed lumen formation and significant sarcomeric actinin striation in the patches. Furthermore, a proof-of-concept small-scale heart was

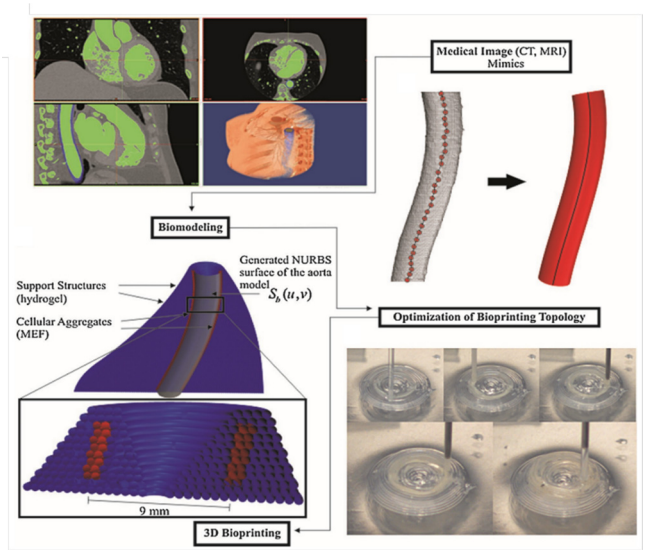


**Fig. 36.** Scaffold-free multimaterial bioprinting of biomimetic and self-supporting macrovascular constructs allows fabrication of vascular constructs by dispensing cells without encapsulating them within any exogenous material [236].

printed with major blood vessels using a support bath during the printing process (Fig. 37).

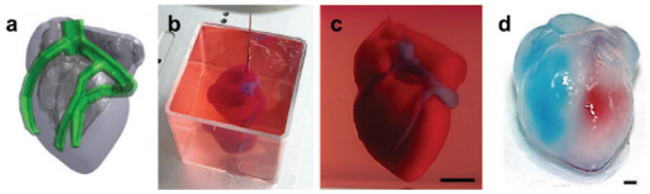


**Fig. 37.** Cardiac patches bioprinting. a) CAD model for human heart, b) printed heart within a support bath, c) after removal of support medium and d) after perfusion of red and blue dyes [237].

An alternative approach explored by Lee et al. [238] was the printing of collagen only heart tissue (Fig. 38). Collagen is the main component of the ECM, however, it is difficult to print by itself typically requiring viscosity modifiers and chemical modification. The bioprinting approach utilised by the authors was freeform reversible embedding of suspended hydrogels (FRESH). Gelatine microparticles were used as a support matrix for the extruded collagen which could have a filament diameter as low as 20 µm. The collagen printed constructs exhibited a microporosity, due to the removal of the gelatine microparticle support, which aided in vascularisation. The high-resolution printing of collagen allowed the fabrication of a cardiac ventricle model with two separate layers of collagen encapsulating a central bio-ink consisting of cardiomyocytes. The collagen model was cultured for up to 28 days whilst maintaining structural integrity and showed visible contraction with interconnected and striated cells. Additionally, a functional tri-leaflet heart valve, multiscale vasculature, and neonatal-scale human heart were all demonstrated using this technique. Furthermore, the research group has further developed the method to bioprint a full-size heart model using alginate rather than collagen [239].

Chronic kidney disease is a major worldwide health issue with end-stage patients requiring life-long dialysis unless a suitable transplant is found. Subsequently, biofabrication and tissue engineering approaches are being actively explored to regenerate kidney function or replace the kidney entirely [240,241].

A perfusable tissue chip model of the renal proximal tubule was developed by Homan et al. [242]. A silicone gasket was initially printed to form the chip boundary followed by casting of a gelatine-fibrin hydrogel, then printing of a fugitive pluronic ink which was connected to the perfusion system, and finally further hydrogel was

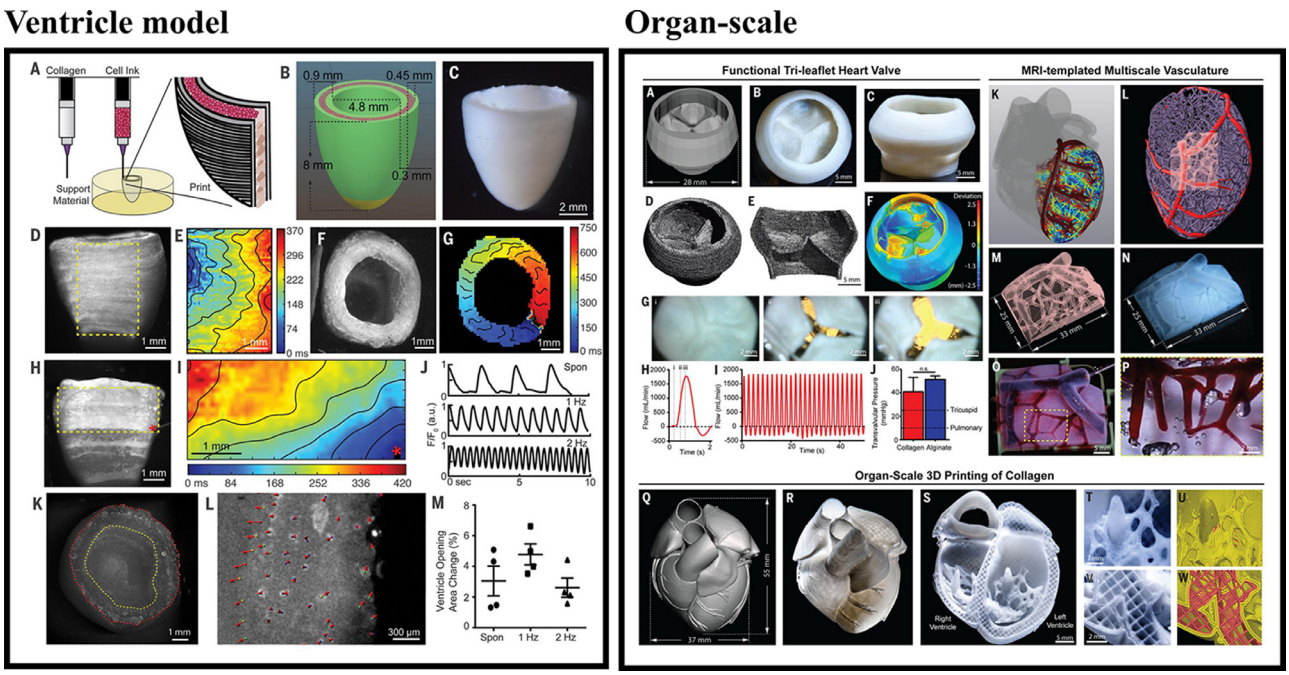


**Fig. 38.** FRESH bioprinting of cardiac tissue using collagen. **Ventricle model.** A) Schematic of the bioprinting process, B) CAD design, C) and actual bioprinted ventricle model. Staining with calcium sensitive dye showing D) side view of the construct with E) spontaneous directional calcium wave propagation with F) and G) showing the top view. H) Point stimulation (red dot) of the construct showing I) anisotropic calcium wave propagation and J) calcium transient traces during spontaneous contractions (top), 1-Hz (middle) and 2-Hz field stimulation (bottom). K) Top view of the inner (yellow) and outer (red) walls of the ventricle with L) inner and outer wall motion during field stimulation (arrows indicate magnitude and direction) and the M) interior chamber cross-sectional area at peak systole. **Organ-scale.** Functional tri-leaflet heart valve A) CAD model, B) top and C) side view of the bioprinted construct, D) full and E) cross-sectional μCT reconstruction, and F) construct deviation from the CAD model. G) Opening sequence of valve during flow and H) doppler flow velocimetry of a single and I) multiple cycles. J) Maximum pressure of valves compared to native tissue. K) MRI based human heart model with L) left ventricle, M) highlighted sub-region (pink), and N) bioprinted vascular network. O) and P) show perfusion of the vascular network. Q) Neonatal sized heart model and R) bioprinted construct with S) cross-sectional and T-W) high magnification images showing precise reproduction of the model and in-fill pattern [238].

casted on top. The pluronic was removed by cooling to 4 °C and the hollow tubular channel remaining in the hydrogel was perfused with media containing proximal tubule epithelial cells. The cells formed a functional epithelial barrier. Subsequently, Lin et al. [243] further developed the model by printing adjacent epithelial tubules and vascular endothelial vessels that showed solute exchange by active reabsorption through tubular–vascular exchange comparable to native kidney tissue (Fig. 39). This bioprinted model provides a platform for disease modelling, pharmaceutics, and understanding tissue physiology. Similarly, Similarly, Gao et al. [244] used co-axial bioprinting to directly print tubular ephitelial and endothelial cells using a hybrid bio-ink composed of decellularized kidney tissue and alginate with a pluronic fugitive ink in the core. This enabled the development of a vascularised and perfusable proximal tubule-on-a-chip model.

Kidney organoids are a promising development in disease modelling but are limited by variability, throughput, and scale. Lawlor et al. [245] used an automated extrusion-based bioprinting system to deposit a cell only paste that self-organises into a kidney organoid. This approach replaces manual organoid preparation with a significant increase in throughput and reproducibility with precise control of organoid properties including size, cell number, and conformation.

Muscle has considerable capacity for self-regeneration but large-scale loss due to trauma, disease, or surgery results in fibrotic healing and debilitating scar formation. In vivo bioprinting of muscle has been demonstrated by Quint et al. [246] using a gelatine methacrylate bio-ink and Laponite nanoclay for controlled release of vascular endothelial growth factor (Fig. 40). A handheld bioprinting device allowed the in situ deposition and crosslinking of the bio-ink enabling the formation of an adhesive scaffold that fills the area of tissue loss. In vivo studies in a mouse model showed improved muscle recovery and a decrease in fibrosis. Similarly, Kim et al. [247] used an extrusion-based system to print a collagen bio-ink containing gold nanowires and myoblasts. An external electrical field was also used to guarantee high alignment of the gold nanowires

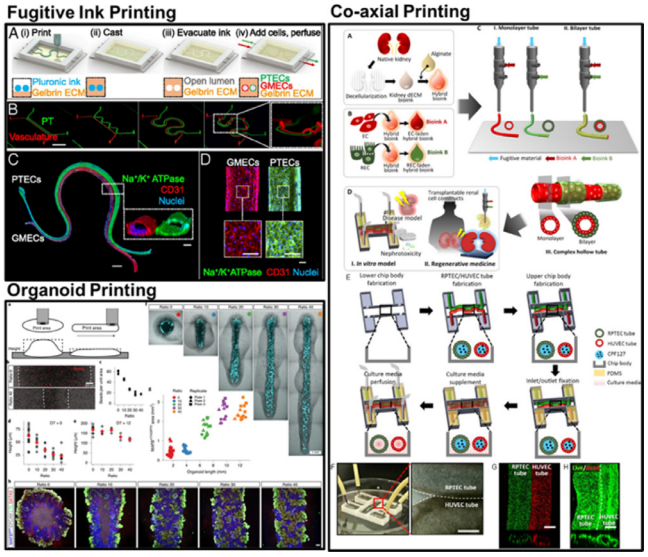


**Fig. 39.** Kidney bioprinting. **Fugitive ink printing**. A) Schematic of the printing process and B) different model architectures. C) Fluorescence imaging of the glomerular microvascular endothelial cell (GMEC) and proximal tubule epithelial cell (PTEC) channels and (inset) cross-section of the lumens. D) High magnification images of the channels. **Co-axial printing**. Schematic of the A) decellularisation and production of the hybrid bioink with B) loading of endothelial (EC) and renal epithelial cells (REC). C) Co-axial printing of mono- and bilayer tubes. D) Application of complex bioprinted hollow tubes for in vitro models and regenerative medicine. E) Process to produce vascularised kidney proximal tubule-on-a-chip using co-axial bioprinting and F) actual chip with G) fluorescence and D) live/dead imaging of the tubes. **Organoid printing.** a) Organoid profile depending on deposition ratio with b) cell paste spread, c) bead density quantification, and tissue height at d) day 0 and e) 12. f) Fluorescence imaging of organoids printed at different ratios with blue showing glomerular area and g) quantification of area versus length. h) Immunofluorescence of bioprinted organoids at different conformations [243].

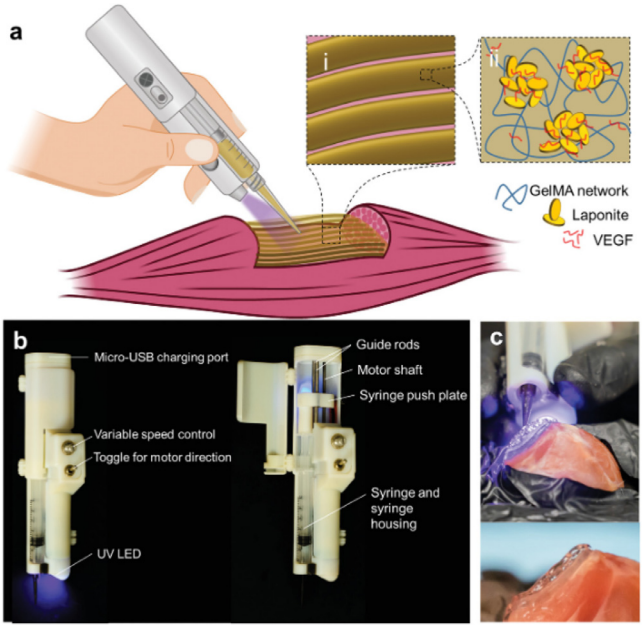


**Fig. 40.** a) Schematic of the in vivo muscle printing process using a GelMA, Laponite nanoclay, and vascular endothelial growth factor. b) Main features of the handheld bioprinting device. c) *In situ* bioprinting of volumetric muscle loss (top) and adhering to tissue (bottom) [246].

promoting cell alignment and mimicking the organisation of muscle tissue engineering.

Large-scale bone defects resulting from trauma and disease have limited capacity to spontaneously regenerate. 3D bioprinting approaches are being developed to fabricate scaffolds that can fill these defect areas and promote mineralisation, osteogenesis, and vascularisation.

A promising approach by Jakus et al. [248] is the development of highly loaded ink composed of 90 wt% hydroxyapatite and 10 wt% polycaprolactone or poly(lactic-co-glycolic acid) dissolved in a trisolvent system (Fig. 41). The graded and controlled evaporation allows rapid deposition of hyperplastic scaffolds with high particle loading (melt extruded scaffolds typically have lower particle loading and are more brittle). The scaffolds promoted osteogenic differentiation of mesenchymal stem cells without additional osteogenic factors. In vivo studies showed high biocompatibility, tissue integration, vascularisation, mineralisation, and minimal immune response.

An alternative approach to 3D printing a ceramic based scaffold proposed by Romanazzo et al. [249] is freeform direct writing of a calcium phosphate biomaterial ink into a cell-laden gelatine microgel support (Fig. 41). The ink rapidly solidifies in situ and bone apatite nanocrystals form. The printed structures could direct mesenchymal stem cell organisation and promote osteogenesis depending on proximity to the deposited scaffold structure. The use of non-harsh and biocompatible solvents also enabled the loading of drugs such as dexamethasone and ibuprofen.

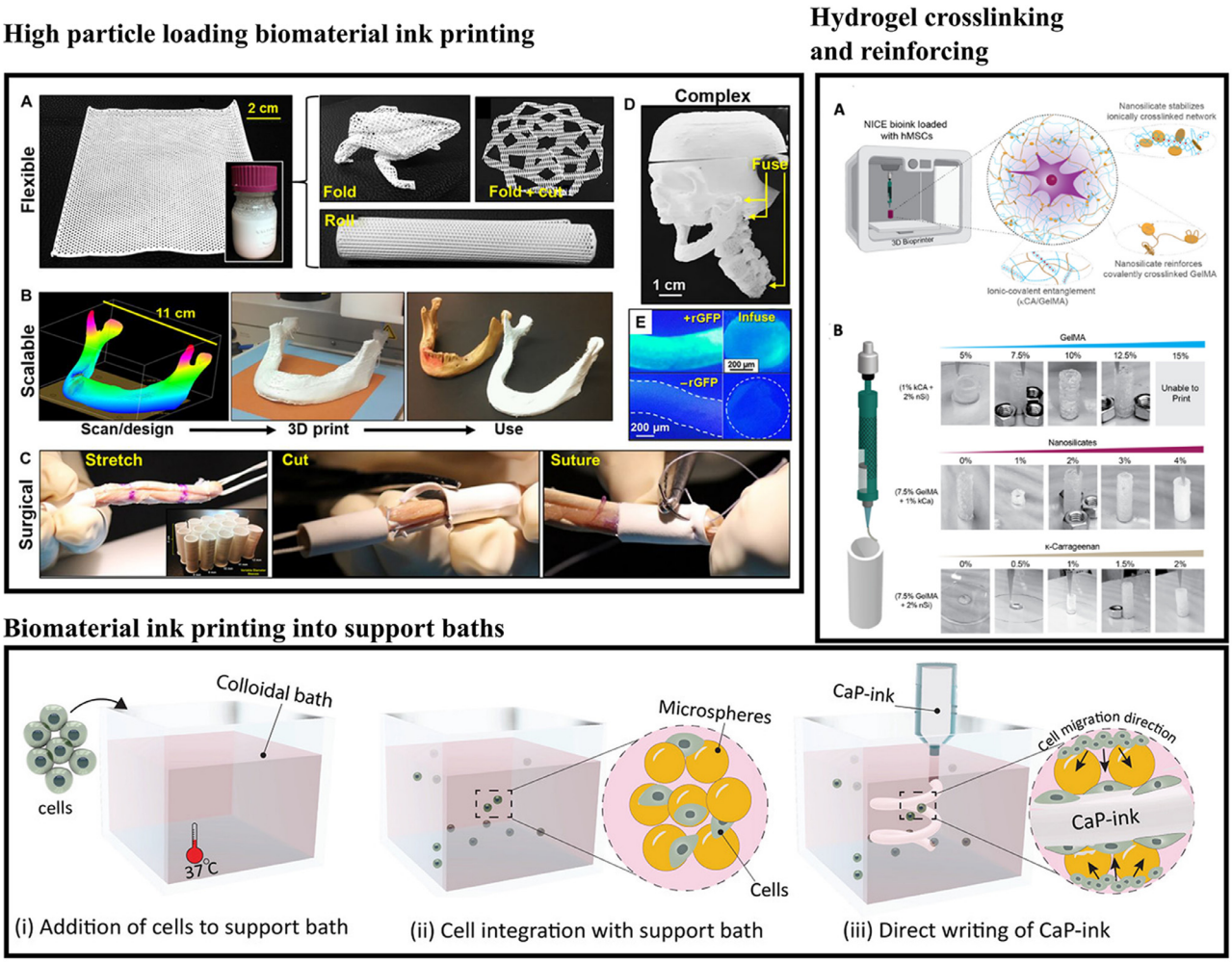


**Fig. 41.** 3D bioprinting of bone scaffolds. **High particle loading biomaterial ink printing**. The hyperelastic bone biomaterial ink shows A) flexibility, B) scalability, C) suitability for surgical handling, D) utilisation in complex applications, and E) allows incorporation of biomolecules. **Biomaterial ink printing into cell-laden support baths**. Schematic of the printing process. **Hydrogel crosslinking and reinforcement**. A) Schematic of the nanoengineered hydrogel network and B) printability studies [248–250].

Although typically ceramics and bioglasses are utilised in bone tissue engineering, a different class of materials, nanosilicates, have been explored by Chimene et al. [250] in a bio-ink (Fig. 41). The bio-ink containing GelMA and kappacarrageenan (kCA) forms an entangled ionic-covalent network after crosslinking which is reinforced with nanosilicates (laponite), allowing the hydrogel to reversibly dissipate energy and increase the mechanical strength and toughness. The presence of nanosilicates promotes osteogenic differentiation of the encapsulated mesenchymal stem cells without additional osteogenic factors. The cells also induce matrix remodelling and deposition of ECM proteins within the hydrogel.

## 5. Challenges and opportunities

As a rapidly developing technique, 3D bioprinting faces many challenges. Biological tissues are complex hierarchical and, in many cases, zonal structures with a functional gradient in composition and properties, and 3D bioprinted constructs must recapitulate these characteristics. Therefore, to repair/regenerate biological damaged tissues, the composition and structure of printed constructs must be considered as key parameters. However, currently both in vitro and in situ bioprinting studies present difficulties in recapitulating the complexity of native tissues or promoting long-term phenotypically stable new-formed tissues. Successful bioprinting will require multiple advancements to become a viable clinical approach, and the CIRP research community can play a pivotal role in addressing some of these challenges. The following are key challenges:

- The ideal bio-inks should satisfy several requirements including controllable printability, cytocompatibility to maintain cell viability and to direct cell behaviours after in situ fabrication [108,251]. Rheological characterisation such as viscosity, which can be highly modulated by material formulations (e.g. molecular weight, concentration, and curing process), can directly influence the printability of bio-inks and cell distribution during the printing. Additionally, the biocompatibility of materials, cells, and biomolecules guarantees positive cell viability. In the case of the photopolymerisation process the selection of photoinitiators is also critical and the combination of multiple photo-initiators sensitive to different wavelengths an important aspect to create multi-material functionally graded structures. Light intensity and exposure time are also critical to avoid potential cytotoxicity [252]. To guide cellular responses after fabrication, printed constructs must have sufficient mechanical properties to withstand the forces of native tissues. Therefore, other post-printing activities, for example, a faster curing process and double crosslinking networks, can provide an improved mechanical and biological properties. Furthermore, the degradation rate of printed materials must match the rate of new tissue formation, which can be achieved by using advanced materials. CIRP has a long research tradition in the field of additive manufacturing, contributing to significant developments to the current state-of-the-art and a major role on the industrial adoption of this technology. Our expertise ranges a wide range of materials, including in recent years materials for biological applications. As CIRP has significant expertise in designing novel material for additive manufacturing, pre- and post-processing characterisation of these materials and in establishing proper correlations between process conditions-morphological development-final physical and chemical properties, our community is well positioned to contribute to development of novel materials for bioprinting applications. Collaborations between CIRP members and researchers from other communities (e.g. biologists, chemical engineers) can significantly contribute to address some of the current major challenges related to advanced materials for 3D bioprinting.
- Considering the prolonged fabrication time required to print complex and dimensionally suitable tissues and organs, an important disadvantages of encapsulating living cells in hydrogels is that bio-inks need to be stored for considerable periods of time in the machine reservoirs. This compromises cell viability and limits their bioactivity. Therefore, more stable conditions to store the bio-inks and a more automated way of loading and ejecting the bio-ink are required for scale-up 3D bioprinting.
- An important challenge in robotic surgical bioprinting is the innovation and integration of imaging, robotics, and bioprinting techniques. Current imaging scan techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) may have limited resolution, thus the constructs created may differ from the actual native defects. Therefore, new systems allowing for high quality data and real-time data are required to achieve high speed, resolution, and minimal errors. Moreover, most current bioprinting equipment uses a 3-axis motion system, which limits the complexity of printed structure. Therefore, it is expected that the use of multi-axis robots such as 4-, 5-, and 6-axis will significantly increase, enabling more range of freedom and motion to improve surgical dexterity and construct complexity. Some surgical tools also can be scaled down to enable the precise manipulation of surgeons within complex or small tissue areas [253].
- The next generation of robotic surgical systems should be integrated with the enhanced imaging, sensing and feedback (i.e. force and haptic feedback; thermal, pressure, and positioning sensors), faster digital communication, and improved bioprinting systems. The use of imaging techniques and position sensor allows the understanding of the surrounding environment. The better imaging system can also provide 3D high-resolution real-time video, which enables the monitoring of the bioprinting process. Wearable eyeglasses with recording capabilities are an alternative that can be potentially used for surgery as well [253]. At the stage of bioprinting, another main challenge for robotic surgery is the lack of real-time position feedback, which may cause collisions between surgical tools and tissues. Recently, surgical systems equipped with advanced positioning sensing and feedback systems have been used to improve real-time correction and minimise error [254,255] (e.g. Teskan, www.teskan.com; and Azorobotics, www.azorobotics.com). Furthermore, AI has been developed for surgical robotics and to may provide faster and better solutions to solve complex bioprinting.
- This paper presented several success studies highlighting the relevance of 3D bioprinting for clinical usage. However, the successful clinical translation requires robust regulatory protocols and quality assurance standards covering all stages of the printing process (e.g. cell biopsy/expansion, bio-ink preparation, bioprinting system and operating conditions, culture and manipulation).
- Digitisation of the entire production process is also critical for process optimisation and to improve the quality and performance of printed constructs. This will require new quality prediction tools for bioprinting to predict the quality of both built and untried printed constructs through predictive modelling (prediction within experimental domains – class or family of biological constructs) and prescriptive modelling (to predict the quality of new and untried printed constructs beyond the experimental scope). Relevant will be also the development of 3D bioprinting digital twin considering design, fabrication and evaluation steps and integrating computer modelling and simulation, visualisation tools, AI and data analytics.

## 6. Summary

3D bioprinting is an emerging multidisciplinary research domain. This review detailed the most relevant materials and the most relevant technologies in the field. The concept of bio-ink is detailed, and main requirements discussed. Despite some successful examples related to skin, cartilage, nerve, bone, kidney, and cardiac tissue applications several challenges remain. As human tissues are extremely complex it is not possible to recapitulate their functional and structural characteristics using a single material. The combination of multiple materials and the development of functionally graded materials is critical, together with the development of novel materials with improved mechanical properties, biocompatibility,

biodegradability, and printability. Moreover, biological tissues and organs are multi-scale hierarchical structures undergoing physiological changes in response to external stimuli. The use of smart materials opens new possibilities for maturation of printed constructs in a programmed way. However, the limited choice of biocompatible, bioactive, and biodegradable smart materials is still a constraint. A range of 3D printing techniques have been successfully used to fabricate biological constructs. To recapitulate the multimaterial and hierarchical nature of biological systems novel multi-modal systems have been proposed. Moreover, robotic-assisted 3D bioprinting systems have been developed for in situ printing with significant success. However, further research is still required to improve printing resolution and speed. The integration of other digital tools (big data, AI, virtualisation) will contribute to further develop this research domain.

## Declaration of Competing Interest

We are submitting this original research communication entitled "3D bioprinting: materials, processes, and applications" for your consideration. We confirm that this work is original and has not been published nor is it currently under consideration for publication elsewhere.

## References


[1] Dikyol C, Altunbek M, Bartolo P, Koc B (2021) Multimaterial Bioprinting Approaches and Their Implementations for Vascular and Vascularized Tissues. *Bioprinting* 24:e00159.
[2] Akbari A, Jabbari N, Sharifi R, Ahmadi M, Vahhabi A, Seyedzadeh S, Nawaz M, Szafert S, Mohamoodi M, Jabbari E, Asghari R, Rezaie J (2020) Free and Hydrogel Encapsulated Exosome-Based Therapies in Regenerative Medicine. *Life Sciences* 249:117447.
[3] Kanetaka K, Eguchi S (2020) Regenerative Medicine for the Upper Gastrointestinal Tract. *Regenerative Therapy* 15:129–137.
[4] Hamid O, Eltaher H, Sottile V, Yang J (2021) 3D Bioprinting of a Stem Cell-Laden, Multi-Material Tubular Composite: An Approach for Spinal Cord Repair. *Materials Science and Engineering C* 120:111707.
[5] Hann S, Cui H, Esworthy T, Miao S, Zhou X, Lee S-J, Fischer J, Zhang L (2019) Recent Advances in 3D Printing: Vascular Network for Tissue and Organ Regeneration. *Translational Research* 211:46–63.
[6] Silva E, Huang B, Helaehil J, Nalesso P, Bagne L, Oliveira M, Albiazetti G, Aldalbahi A, El-Newehy M, Santamaria-Jr M, Mendonça F, Bartolo P, Caetano G (2021) In Vivo Study of Conductive 3D Printed PCL/MWCNTs Scaffolds with Electrical Stimulation for Bone Tissue Engineering. *Bio-Design and Manufacturing* 4:190–202.
[7] Nalesso P, Wang W, Hou Y, Bagne L, Pereira A, Helaehil J, Andrade T, Chiarotto G, Bartolo P, Caetano G (2021) In Vivo Investigation of 3D Printed Polycaprolactone/Graphene Electro-Active Bone Scaffolds. *Bioprinting* 24:e00164.
[8] Wang W, Bartolo P (2020) Engineering the biological Performance Of Hierarchical Nanostructured Poly(E-Caprolactone) Scaffolds for Bone Tissue Engineering. *CIRP Annals – Manufacturing Technology*. 69(1):217–220.
[9] Koc B, Acar A, Weightman A, Cooper G, Bunn G, Bartolo P (2019) Biomanufacturing of Customized Modular Scaffolds for Critical Bone Defects. *CIRP Annals – Manufacturing Technology*, 68(1):209–212.
[10] Liu F, Mishbak H, Bartolo P (2019) Hybrid polycaprolactone/hydrogel scaffold fabrication and in-process plasma treatment using PABS. *International Journal of Bioprinting* 5:1.
[11] Ceretti E, Ginestra P, Ghazinejad M, Fiorentino A, Madou M (2017) Electrospinning and Characterisation of Polymer-Graphene Powder Scaffolds. *CIRP Annals – Manufacturing Technology*, 66(1):233–236.
[12] Khani N, Nadernezhad A, Bartolo P, Koc B (2017) Hierarchical and Spatial Modeling and Bio-Additive Manufacturing of Multi-Material Constructs. *CIRP Annals – Manufacturing Technology*, 66:229–232.
[13] Bartolo P, Kruth J-P, Silva J, Levy G, Malshe A, Rajurkar K, Mitsuishi M, Ciurana J, Leu M (2012) Biomedical Production of Implants by Additive Electro-Chemical and Physical Processes. *CIRP Annals – Manufacturing Technology*, 61(2):635–655.
[14] Melchels F, Domingos M, Klein T, Malda J, Bartolo P, Hutmacher D (2012) Additive Manufacturing of Tissues and Organs. *Progress in Polymer Science* 37:1079–1104.
[15] Mitsuishi M, Cao J, Bartolo P, Friedrich D, Shih A, Rajurkar K, Sugita N, Harada K (2013) Biomanufacturing. *CIRP Annals – Manufacturing Technology* 62(2):585–606.
[16] Murphy S, Atala A (2014) 3D Bioprinting of Tissues and Organs. *Nature Biotechnology* 32:773–785.
[17] Grand View Research. *3D Bioprinting Market Size, Share, and Trends Analysis Report*, www.grandviewresearch.com.
[18] Applied Market Research. *Tissue Engineering Market by Type (Synthetic Scaffold Material, Biological Derived Scaffold Material, and Others), Application (Orthopedics and Musculoskeletal, Neurology, Cardiovascular, Skin and Integumentary, Dental, and Others): Global Opportunity Analysis and Industry Forecast, 2020-2027*, https://www.alliedmarketresearch.com/tissue-engineering-market.
[19] Vijayavenkataraman S, Yan W, Lu W, Wang C, Fuh J (2018) 3D Bioprinting of Tissues and Organs for Regenerative Medicine. *Advanced Drug Delivery Reviews* 132:296–332.
[20] Klebe R (1988) Cytoscribing: A Method for Micropositioning Cells and the Construct of Two- and Three-Dimensional Synthetic Tissues. *Experimental Cell Research* 179:362–373.
[21] Wilson W, Boland T (2003) Cell and Organ Printing 1: Protein and Cell Printers. *Anatomical Record – Part A Discoveries in Molecular, Cellular, and Evolutionary Biology*, 272:491–496.
[22] Wang Y, Li J, Li Y, Yang B (2021) Biomimetic Bioinks of Nanofibrillar Polymeric Hydrogels for 3D Bioprinting. *Nano Today* 39:101180.
[23] Lee J, Lee S, Ahmad T, Perikamana S, Lee J, Kim E, Shin H (2020) Human Adipose-Derived Stem Cell Spheroids Incorporating Platelet-Derived Growth Factor (PDGF) and Bio-Minerals for Vascularized Bone Tissue Engineering. *Biomaterials* 255:120192.
[24] Mi K, Bin L, Se-Jeong S, Jaesung P, Jinkyu L, Sung Won K, Hansoo P, Heungsoo S (2019) Fabrication of Core-Shell Spheroids as Building Blocks for Engineering Complex Vascularized Tissue. *Acta Biomaterialia* 100:158–172.
[25] Pereira R, Bartolo P (2015) 3D Bioprinting of Photocrosslinking Hydrogel Constructs. *Journal of Applied Polymer Science* 2015:42458.
[26] Pereira R, Bartolo P (2015) 3D Photo-Fabrication for Tissue Engineering and Drug Delivery. *Engineering* 1:91–113.
[27] Unagolla J, Jayasuriya A (2020) Hydrogel-Based 3D Bioprinting: A Comprehensive Review on Cell-Laden Hydrogels, Bioink Formulations, and Future Perspectives. *Applied Materials Today* 18:100479.
[28] Sharma S, Tiwari S (2020) A Review on Biomacromolecular Hydrogel Classification and Its Applications. *International Journal of Biological Macromolecules* 162:737–747.
[29] Ebhodaghe S (2020) Hydrogel – Based Biopolymers for Regenerative Medicine Applications: A Critical Review. *International Journal of Polymeric Materials and Polymeric Biomaterials* 2020:1–18.
[30] Catoira M, Fusaro L, Francesco D, Ramella M, Boccafoschi F (2019) Overview of Natural Hydrogels for Regenerative Medicine Applications. *Journal of Materials Science: Materials in Medicine* 30:115.
[31] Hamidi M, Azadi A, Rafiei P (2008) Hydrogel Nanoparticles in Drug Delivery. *Advanced Drug Delivery Reviews* 60:1638–1649.
[32] Mobaraki M, Ghaffari M, Yazdanpanah A, Luo Y, Mills D (2020) Bioinks and Bioprinting: A Focused Review. *Bioprinting* 18:e00080.
[33] Khoshnood N, Zamanian A (2020) A Comprehensive Review on Scaffold-Free Bioinks for bioprinting. *Bioprinting* 19:e00088.
[34] Pita-López M, Fletes-Vargas G, Espinosa-Andrews H, Rodríguez-Rodríguez R (2021) Physically Cross-Linked Chitosan-Based Hydrogels For Tissue Engineering Applications: A State-O-The-Art Review. *European Polymer Journal* 145:110176.
[35] Annabi N, Tamayol A, Uquillas J, Akbari M, Bertassoni L, Cha C, Camci-Unal G, Dokmeci M, Peppas N, Khademhosseini A (2014) 25th Anniversary Article: Rational Design and Applications of Hydrogels in Regenerative Medicine. *Advanced Materials*, 26:85–124.
[36] Lutolf M, Hubbell J (2005) Synthetic Biomaterials as Instructive Extracellular Microenvironments for Morphogenesis in Tissue Engineering. *Nature Biotechnology* 23:47–55.
[37] Mao H, Yang L, Zhu H, Wu L, Ji P, Yang J, Gu Z (2020) Recent Advances and Challenges in Materials for 3D Bioprinting. *Progress in Natural Sciences: Materials International* 30:618–634.
[38] Malda J, Visser J, Melchels F, Jungst T, Hennink W, Dhert W, Groll J, Hutmacher D (2013) 25th Anniversary Article: Engineering Hydrogels for Biofabrication. *Advanced Materials* 25:5011–5028.
[39] Skeldon G, Lucendo-Villarin B, Shu W (2018) Three-Dimensional Bioprinting of Stem-Cell Derived Tissues for Human Regenerative Medicine. *Philosophical Transactions B* 373:20170224.
[40] Jain S, Fuoco T, Yassin M, Mustafa K, Finne-Wistrand A (2020) Printability and Critical Insight Into Polymer Properties During Direct-Extrusion Based 3D Printing of Medical Grade Polylactide and Copolyesters. *Biomacromolecules* 21:388–396.
[41] Gao C, Lu C, Jian Z, Zhang T, Chen Z, Zhu Q, Tai Z, Liu Y (2021) 3D Bioprinting for Fabricating Artificial Skin Tissue. *Colloids and Surfaces B: Biointerfaces* 208:112041.
[42] Cui X, Li J, Hartanto Y, Durham M, Tang J, Zhang H, Hooper G, Lim K, Woodfield T (2020) Advances in Extrusion 3D Bioprinting: A Focus On Multi-Component Hydrogel-Based Bioinks. *Advanced Healthcare Materials* 9:1901648.
[43] Holzl K, Lin S, Tytgat L, Van Vlierberghe S, Gu L, Ovsianikov A (2016) Bioink Properties Before, During and After 3D Bioprinting. *Biofabrication* 8:032002.
[44] Rodriguez-Pascual F, Slatter D (2016) Collagen Cross-Linking: Insights on the Evolution of Metazoan Extracellular Matrix. *Scientific Reports* 6:1–7.
[45] Kajave N, Schmitt T, Nguyen T-U, Kishore V (2020) Dual Crosslinking Strategy to Generate Mechanically Viable Cell-Laden Printable Constructs Using Methacrylated Collagen Bioinks. *Materials Science and Engineering C* 107:110290.
[46] Gomez-Guillen M, Gimenez B, Lopez-Caballero M, Montero M (2011) Functional and Bioactive Properties of Collagen and Gelatina From Alternative Sources: A Review. *Food Hydrocolloids* 25:1813–1827.
[47] Vanaei S, Parizi M, Vanaei S, Salemizadehparizi F, Vanai H (2021) An Overview on Materials and Techniques in 3D Bioprinting Toward Biomedical Application. *Engineered Regeneration* 2:1–8.
[48] Dias J, Baptista-Silva S, de Oliveira C, Sousa A, Oliveira A, Bartolo P, Granja P (2017) In Situ Crosslinked Electrospun Gelatina Nanofibers For Skin Regeneration. *European Polymer Journal* 95:161–173.
[49] Raucci M, D'Amora U, Ronca A, Demitri C, Ambrosio L (2019) Bioactivation Routes Of Gelatina-Based Scaffolds to Enhance at Nanoscale Level Bone Tissue Regeneration. *Frontiers Bioengineering Biotechnology* 7:1–11.
[50] Broderick E, O'Halloran D, Rochev Y, Griffin M, Collighan R, Pandit A (2005) Enzymatic Stabilization of Gelati-Based Scaffolds. *Journal of Biomedical Materials Research Part B* 72:37–42.

[51] Yung C, Wu L, Tullman J, Payne G, Bentley W, Barbari T (2007) Transglutaminase Crosslinked Gelatin as a Tissue Engineering Scaffold. *Journal of Biomedical Materials Research Part A* 83:1039–1049.

[52] Ulubayram K, Aksu E, Gurhan S, Serbetci K, Hasirci N (2002) Cytotoxicity Evaluation of Gelatin Sponges Prepared With Different Cross-Linking Agents. *Journal Biomaterials Science Polymers* 13:1203–1219.

[53] Lien S, Li W, Huang T (2008) Genipin-Crosslinked Gelatin Scaffolds for Articular Cartilage Tissue Engineering with a Novel Crosslinking Method. *Materials Science and Engineering C* 28:36–43.

[54] Ming-Kung Y, Yan-Ming L, Kuang-Ming C, Niann-Tzyy D, Cheng-Che L, Jenn_-Jong Y (2011) A Novel Cell Support Membrane For Skin Tissue Engineering: Gelatin Film Cross-Linked with 2-Chloro-1-Methylpyridinium Iodide. *Polymer* 52:996–1003.

[55] Negrini N, Celikkin N, Tarsini P, Farè S, Swieszkowski W (2020) Three-Dimensional Printing of Chemically Crosslinked Gelatin Hydrogels For Adipose Tissue Engineering. *Biofabrication* 12:025001.

[56] Nguyen A, McKinney J, Miller T, Bongiorno T, McDevitt T (2015) Gelatin Methacrylate Microspheres For Controlled Growth Factor Release. *Acta Biomaterialia* 13:101–110.

[57] Billiet T, Gevaert E, De Schryver T, Cornelissen M, Dubruel P (2014) The 3D Printing of Gelatin Methacrylamide Cell-Laden Tissue-Engineered Constructs with High Cell Viability. *Biomaterials* 35:49–62.

[58] Krommelbein C, Mutze M, Konieczny R, Schonherr N, Griebel J, Gerdes W, Mayr S, Riedel S (2021) Impact Of High-Energy Electron Irradiation On Mechanical, Structural And Chemical Properties of Agarose Hydrogels. *Carbohydrate Polymers* 263:117970.

[59] Norton I, Goodall D, Austen K, Morris E, Rees D (1986) Dynamics of Molecular Organization in Agarose Sulphate. *Biopolymers* 25:1009–1029.

[60] Yazdi M, Taghizadeh A, Taghizadeh M, Stadler F, Farokhi M, Mottaghitalab F, Zarrintaj P, Ramsey J, Seidi F, Saeb M, Mozafar M (2020) Agarose-Based Biomaterials for Advanced Drug Delivery. *Journal of Controlled Release* 326:523–543.

[61] Le Goff K, Gaillard C, Helbert W, Garnier C, Aubry T (2015) Rheological Study of Reinforcement of Agarose Hydrogels by Cellulose Nanowhiskers. *Carbohydrate Polymers* 116:117–123.

[62] Gyles D, Castro L, Silva Jr J, Ribeiro-Costa R (2017) A Review of the Designs and Prominent Biomedical Advances of Natural and Synthetic Hydrogel Formulations. *European Polymer Journal* 88:373–392.

[63] Graca M, Miguel S, Cabral C, Correia I (2020) Hyaluronic Acid-Based Wound Dressings: A Review. *Carbohydrate Polymers* 241:17.

[64] Gasperini L, Mano J, Reis R (2014) Natural Polymers for the Microencapsulation of Cells. *Journal of the Royal Society Interface* 11:20140817.

[65] Pina S, Oliveira J, Reis R (2015) Natural-Based Nanocomposites for Bone Tissue Engineering and Regenerative Medicine: A Review. *Advanced Materials* 27:1143–1169.

[66] Axpe E, Oyen M (2016) Applications of Alginate-Based Bioinks in 3D Printing. *International Journal of Molecular Sciences* 17:1976.

[67] Sun J, Tan H (2013) Alginate-Based Biomaterials for Regenerative Medicine Applications. *Materials* 6:1285–1309.

[68] Braccini I, Pérez S (2001) Molecular Basis of Ca2+-Induced Gelation in Alginates and Pectins: The Egg-Box Model Revisited. *Biomacromolecules* 2:1089–1096.

[69] GhavamiNejad A, Ashammakhi N, Wu X, Khademhosseini A (2020) Crosslinking Strategies for 3D Bioprinting of Polymeric Hydrogels. *Small* 16:2002931.

[70] Hasany M, Talebian S, Sadat S, Ranjbar N, Mehrali M, Wallace G, Mehrali M (2021) Synthesis, Properties and Biomedical Applications Of Alginate Methacrylate (ALMA)-Based Hydrogels: Current Advances and Challenges. *Applied Materials Today* 24:101150.

[71] Jia J, Richards D, Pollard S, Tan Y, Rodriguez J, Visconti R, Trusk T, Yost M, Yao H, Markwald R, Mei Y (2014) Engineering Alginate as Bioink for Bioprinting. *Acta Biomaterialia* 10:4323–4331.

[72] Freeman F, Kelly D (2017) Tuning Alginate Bioink Stiffness and Composition For Controlled Growth Factor Delivery and to Spatially Direct MSC Fate within Bioprinted Tissues. *Scientific Reports* 7:1–12.

[73] Andersen T, Strand B, Formo K, Alsberg E, Christensen B (2011) Alginates as Biomaterials In Tissue Engineering. *Carbohydrates Chemistry* 37:227–258.

[74] Manoukian O, Arul M, Rudraiah S, Kalajzic I, Kumbar S (2019) Aligned Microchannel Polymer-Nanotube Composites for Peripheral Nerve Regeneration: Small Molecule Drug Delivery. *Journal of Controlled Release* 296:54–67.

[75] Levengood S, Zhang M (2014) Chitosan-based Scaffolds for Bone Tissue Engineering. *Journal of Materials Chemistry B* 2:3161–3184.

[76] Jensen B, Dávila I, Zelikin A (2016) Poly(vinyl alcohol) Physical Hydrogels: Matrix-Mediated Drug Delivery Using Spontaneously Eroding Substrate. *The Journal of Physical Chemistry B* 120:5916–5926.

[77] Basu A, Kunduru K, Doppalapudi S, Domb A, Khan W (2016) Poly(lactic acid) Based Hydrogels. *Advanced Drug Delivery Reviews* 107:192–205.

[78] Lin C-C, Anseth K (2008) PEG Hydrogels for the Controlled Release of Biomolecules in Regenerative Medicine. *Pharmaceutical Research* 26:631–643.

[79] Zhu J (2010) Bioactive Modification of Poly(Ethylene Glycol) Hydrogels for Tissue Engineering. *Biomaterials* 31:4639–4656.

[80] Trappmann B, Gautrot J, Connelly J, Strange D, Li Y, Oyen M, Stuart M, Boehm H, Li B, Vogel V, Spatz J, Watt F, Huck W (2012) Extracellular-Matrix Tethering Regulates Stem-Cell Fate. *Nature Materials* 11:642–649.

[81] Gattazzo F, Urciuolo A, Bonaldo P (2014) Extracellular Matrix: A Dynamic Microenvironment for Stem Cell Niche. *Biochimica et Byophysica Acta (BBA) – General Subjects* 1840:2506–2519.

[82] Bio libretexts. *Extracellular matrix and intracellular junctions*, https://bio.libretexts.org/@go/page/68572.

[83] Saldin L, Cramer M, Velankar S, White L, Badylak S (2017) Extracellular Matrix Hydrogels from DECELLULARIZED TISSUES: STRUCTURE AND FUNCTION. *Acta Biomaterialia* 49:1–15.

[84] Cochis A, Bonetti L, Sorrentino R, Negrini N, Grassi F, Leigheb M, Rimondini L, Faré S (2018) 3D Printing of Thermo-Responsive Methycellulose Hydrogels For Cell-Sheet Engineering. *Materials* 11:579.

[85] Mouser V, Abbadessa A, Levato R, Hennink W, Vermonden T, Gawlitta D, Malda J (2017) Development of a Thermosensitive HAMA-Containing Bio-Ink for the Fabrication of Composite Cartilage Repair Constructs. *Biofabrication* 9:015026.

[86] Apsite I, Stoychev G, Zhang W, Jehnichen D, Xie J, Ionov L (2017) Porous Stimuli-Responsive Self-Folding Electrospun Mats for 4D Biofabrication. *Biomacromolecules* 18:3178–3184.

[87] Yu Y, Moncal K, Li J, Peng W, Rivero I, Martin J, Ozbolat I (2016) Three-Dimensional Bioprinting Using Self-Assembling Scalable Scaffold-Free "Tissue Strands" as a New Bioink. *Scientific Reports* 6:28714.

[88] Loo Y, Hauser C (2016) Bioprinting Synthetic Self-Assembling Peptide Hydrogels for Biomedical Applications. *Biomedical Materials* 11:014103.

[89] Lai J, Ye X, Liu J, Wang C, Li J, Wang X, Ma M, Wang M (2021) 4D Printing of Highly Printable and Shape Morphing Hydrogels Composed of Alginate and Methylcellulose. *Materials & Design* 205:109699.

[90] Ahmed A, Arya S, Gupta V, Furukawa H, Khosla A (2021) 4D Printing: Fundamentals, Materials, Applications and Challenges. *Polymer* 228:123926.

[91] Hann S, Cui H, Nowicki M, Zhang L (2020) 4D Printing Soft Robotics for Biomedical Applications. *Additive Manufacturing* 36:101567.

[92] Jamal M, Kadam S, Xiao R, Jivan F, Onn T-M, Fernandes R, Nguyen T, Gracias D (2013) Bio-Origami Hydrogel Scaffolds Composed of Photocrosslinked PEG Bilayers. *Advanced Healthcare Materials* 2:1142–1150.

[93] Mirani B, Pagan E, Currie B, Siddiqui M, Hosseinzadeh R, Mostafalu P, Zhang Y, Ghahary A, Akbari M (2017) An Advanced Multifunctional Hydrogel-Based Dressing For Wound Monitoring and Drug Delivery. *Advanced Healthcare Materials* 6:1700718.

[94] Miao S, Cui H, Nowicki M, Xia L, Xuan Z, Lee S-J, Zhu W, Sarkar K, Zhang Z, Zhang L (2018) Stereolithographic 4D Bioprinting of Multiresponsive Architectures for Neural Engineering. *Advanced Biosystems* 2:1800101.

[95] Khoo Z, Teoh J, Liu Y, Chua C, Yang S, An J (2015) 3D Printing of Smart Materials: A Review on Recent Progresses in 4D Printing. *Virtual and Physical Prototyping* 10:103–122.

[96] Frueh F, Menger M, Lindenblatt N, Giovanoli P, Laschke M (2017) Current and Emerging Vascularization Strategies in Skin Tissue Engineering. *Critical Reviews in Biotechnology* 37:613–625.

[97] Koudan E, Gryadunova A, Karalkin P, Korneva J, Meteleva N, Babichenko I, Volkov A, Radionov S, Parfenov V, Pereira F, Khesuani Y, Mironov V, Bulanova E (2020) Multiparametric Analysis of Tissue Spheroids Fabricated from Diferente Types of Cells. *Biotechnology Journal* 15:e1900217.

[98] Parfenov V, Koudan E, Krokhmal A, Annenkova E, Petrov S, Pereira F, Karalkin P, Nezhurna E, Gryadunova A, Bulanova E, Sapozhnikov O, Tsysar S, Liu K, Oosterwijk E, Van Beuningen H, Van Der Kraan P, Granneman S, Engelkamp H, Christianen P, Kasyanov V, Khesuani Y, Mironov V (2020) Biofabrication of a Functional Tubular Construct from Tissue Spheroids using Magnetoaccoustic Levitational Directed Assembly. *Advanced Healthcare Materials* 9:e2000721.

[99] Petrenko Y, Sykova E, Kubinova S (2017) The Therapeutic Potential of Three-Dimensional Multipotent Mesenchymal Stromal Cell Spheroids. *Stem Cell Research and Therapy* 8:94.

[100] Ryu N, Lee S, Park H (2019) Spheroid Culture System Methods and Applications for Mesenchymal Stem Cells. *Cells* 8:1620.

[101] Sano K, Usui M, Moritani Y, Nakazawa K, Hanatani T, Kondo H, Nakatomi M, Onizuka S, Iwata T, Sato T, Togari A, Ariyoshi W, Nishihara T, Nakashima K (2000) Co-Cultured Spheroids of Human Periodontal Ligament Mesenchymal Stem Cell and Vascular Endothelial Cells Enhance Periodontal Tissue Regeneration. *Regenerative Therapy* 14:59–71.

[102] Mironov V, Khesuani Y, Bulanova E, Kouda E, Parfenov V, Knyazeva A, Mitryashkin A, Replyanski N, Kasyanov V, Pereira F (2016) Patterning of Tissue Spheroids Biofabricated From Human Fibroblastos on the Surface of Electrospun Polyurethane Matrix Using 3D Bioprinter. *International Journal of Bioprinting* 2:45–52.

[103] Rengier F, Mehndiratta A, vo Tengg-Kobligk H, Zechmann C, Unterhinninghofen R, Kauczor H-U, Geisel F (2010) 3D Printing Based on Imaging Dara: Review of Medical Applications. *Computer Assisted Radiology and Surgey* 5:335–341.

[104] Virzi A, Muller C, Marret J-B, Mille E, Berteloot L, Grévent D, Boddaert N, Gori P, Sarnacki S, Bloch I (2020) Comprehensive Review of 3D Segmentation Software Tools for MRI Usable for Pelvic Surgery Planning. *Journal of Digital Imaging* 33:99–110.

[105] Zhou T, Ruan S, Canu S (2019) A Review: Deep Learning for Medical Image Segmentation Using Multi-Modality Fusion. *Array* 3-4:100004.

[106] Moghaddam A, Khonakdar H, Arjmand M, Jafari S, Bagher Z, Moghaddam Z, Chimerad M, Sisakht M, Shojaei S (2021) Review of Bioprinting in Regenerative Medicine: Naturally Derived Bioinks and Stem Cells. *ACS Applied Bio Materials* 4:4049–4070.

[107] Eswaramoorthy S, Dhiman N, Joshi A, Rath S (2021) 3D Bioprinting of Mesenchymal Stem Cells and Endothelial Cells in an Alginate-Gelatin-Based Bioink. *Journal of 3D Printing in Medicine* 5:23–56.

[108] Morgan F, Moroni L, Baker M (2020) Dynamic Bioinks To Advanced Bioprinting. *Advanced Healthcare Materials* 9:e1901798.

[109] Retting K, Nguyen D (2018) Additive Manufacturing in the Development of 3D Skin Tissues. in Marques A, Pirraco R, Cerqueira M, Reis R, (Eds.) *Skin models for regenerative medicine*, Academic Press, .

[110] Stojic M, Lopez V, Montero A, Quílez C, De Aranda Izuzquiza G, Vojtova L, Jorcano J, Velasco D (2019) Skin Tissue Engineering. in Garcia-Gareta E, (Ed.) *Biomaterials for Skin Repair and Regeneration*, Elsevier, .

[111] Costantini M, Colosi C, Swieszkowski W, Barbetta A (2019) Co-Axial Wet-Spinning in 3D Bioprinting State of the Art and Future Perspective of Microfluidic Integration. *Biofabrication* 11:012001.

[112] Gudapati H, Dey M, Ozbolat I (2016) A Comprehensive Review on Droplet-Based Bioprinting: Past, Present and Future. *Biomaterials* 102:20–42.

[113] Dodoo C, Stapleton P, Gaisford S (2016) Effect on Thermal Ink-Jet Printing on Bacterial Cells. *NIP & Digital Fabrication Conference* : 402–405.
[114] Mandrycky C, Wang Z, Kim K, Kim D (2016) 3D Bioprinting for Engineering Complex Tissues. *Biotechnology Advances* 34:422–434.
[115] Abdolmaleki H, Kidmose P, Agarwala S (2021) Droplet-Based Techniques for Printing of Functional Inks for Flexible Physical Sensors. *Advanced Materials* 33:2006792.
[116] Hong K, Kim S, Mahajan A, Frisbie C (2014) Aerosol Jet Printed p- and n-type Electrolyte-Gated Transistors with a Variety of Electrode Materials: Exploring Practical Routes to Printed Electronics. *ACS Applied Materials & Interfaces* 6:18704–18711.
[117] Gockeln M, Glenneberg J, Busse M, Pokhrel S, Madler L, Kun R (2018) Flame Aerosol Deposited $Li_4Ti_5O_{12}$ Layers for Flexible Thin Film All-Solid-State Li-ion Batteries. *Nano Energy* 49:564–573.
[118] Deiner L, Reitz T (2017) Inkjet and Aerossol Jet Printing of Electrochemical Devices for Energy Conversion and Storage. *Advanced Engineering Materials* 19:1600878.
[119] Behera D, Cullinan M (2021) Current Challenges and Potential Directions Towards Precision Microscale Additive Manufacturing – Part I: Direct Ink Writing/Jetting Processes. *Precision Engineering* 68:326–337.
[120] Salary R, Lombardi J, Tootooni M, Donovan R, Rao P, Borgesen P, Polikis M (2016) Computational Fluid Dynamics Modelling and Online Monitoring of Aerosol Jet Printing Process. *Journal of Manufacturing Science and Engineering* 139:021015.
[121] Goh G, Agarwala S, Tan Y, Yeong W (2018) A Low Cost and Flexible Carbon Nanotube pH Sensor Fabricated Using Aerosol Jet Technology for Live Cell Applications. *Sensors and Actuators B: Chemical* 260:227–235.
[122] De Silva E, Paulsen J, Renn M, Odde D (2006) Two-Step Cell Patterning on Planar Complex Curved Surfaces by Precision Spraying of Polymers. *Biotechnology and Bioengineering* 93:919–927.
[123] Phuah E, Hart W, Sumer H, Stoddart R (2020) Patterning of Biomaterials by Aerosol Jet Printing: A Parametric Study. *Bioprinting* 18:e00081.
[124] Secor E (2018) Principles of Aerosol Jet Printing. *Flexible and Printed Electronics* 3:035002.
[125] Forget A, Derme T, Mitterberger D, Heiny M, Sweeney C, Mudili L, Dargaville T, Shastri V (2019) Architecture-Inspired Paradigm for 3D Bioprinting of Vessel-Like Structures Using Extrudable Carboxylated Agarose Hydrogels. *Emergent Materials* 2:233–243.
[126] Luo W, Liu H, Wang C, Qin Y, Liu Q, Wang J (2019) Bioprinting of Human Musculoskeletal Interface. *Advanced Engineering Materials* 21:1900019.
[127] Placone J, Engler A (2018) Recent Advances in Extrusion-Based 3D Printing for Biomedical Applications. *Advanced Healthcare Materials* 7:e1701161.
[128] Chen D (2019) *Extrusion Bioprinting of Scaffolds For Tissue Engineering Applications*, Springer.
[129] Blaeser A, Campos D, Puster U, Richtering W, Stevens M, Fischer H (2016) Controlling Shear Stress in 3D Bioprinting is a Key Factor to Balance Printing Resolution and Stem Cell Integrity. *Advanced Healthcare Materials* 76:321–343.
[130] Pimentel C, Ko S, Caviglia Wolf A, Emneus J, Keller S, Dufva M (2018) Three-Dimensional Fabrication of Thick and Densely Populated Soft Constructs with Complex and Actively Perfused Channel Network. *Acta Biomaterialia* 65:174–184.
[131] Suntornnond R, Tan E, Na J, Chua C (2017) A Highly Printable And Biocompatible Hydrogel Composite for Direct Printing of Soft and Perfusable Vasculature-Like Structures. *Scientific Reports* 7:16902.
[132] Jin Y, Chai W, Huang Y (2017) Printability Study of Hydrogel Solution Extrusion in Nanoclay Yield-Stress Bath During Printing-Then-Gelation Biofabrication. *Materials Science and Engineering C* 80:313–325.
[133] Bhattacharyya A, Janarthanan G, Tran H, Ham H, Yoon J, Noh I (2021) Bioink Homogeneity Control During 3D Bioprinting of Multicomponent Micro/Nanocomposite Hydrogel for Even Tissue Regeneration Using Novel Twin Screw Extrusion System. *Chemical Engineering Journal* 415:128971.
[134] Landers R, Mulhaupt R (2000) Desktop Manufacturing of Complex Objects, Prototypes and Biomedical Scaffolds by Means of Computer-Assisted Design Combined with Computer-Guided 3D Plotting of Polymers and Reactive Oligomers. *Macromolecular Materials and Engineering* 282:17–21.
[135] Shim J, Lee J, Kim J, Cho D (2012) Bioprinting of a Mechanically Enhanced Three-Dimensional Dual Cell-Laden Construct For Osteochondral Tissue Engineering Using a Multi-Head Tissue/Organ Building System. *Journal of Micromechanics and Microengineering* 22:85014.
[136] Jung B, Kim J, Kim Y, Roh T, Seo A, Park K-H, Shim J-H, Yun I (2019) Ideal Scaffold Design for Total Hear Reconstruction Using a Three-Dimensional Printing Technique. *Journal of Biomedical Materials Research* 107B:1295–1303.
[137] Ozbolat I, Chen H, Yu Y (2014) Development of 'Multi-Arm BioprinterFor Hybrid Biofabrication of Tissue Engineering Constructs. *Robotics and Computer-Integrated Manufacturing* 30:295–304.
[138] Moncal K, Ozbolat V, Datta P, Heo D, Ozbolat I (2019) Thermally-Controlled Extrusion-Based Bioprinting of Collagen. *Journal of Materials Science: Materials in Medicine* 30:55.
[139] Yu Y, Zhang Y, Ozbolat IT (2014) A Hybrid Bioprinting Approach for Scale-Up Tissue Fabrication. *Journal of Manufacturing Science and Engineering, Transactions of the ASME* 136:61013.
[140] Kang H-W, Lee S, Ko I, Kengla C, Yoo J, Atala A (2016) A 3D Bioprinting System to Produce Human-Scale Tissue Constructs with Structural Integrity. *Nature Biotechnology* 34:312–319.
[141] Ma K, Zhao T, Yang L, Wang P, Jin J, Teng H, Xia D, Zhu L, Jiang Q, Wang X (2020) Application of Robotic-Assisted In Situ 3D Printing In Cartilage Regeneration with HAMA Hydrogel: An In Vivo Study. *Journal of Advanced Research* 23:123–132.
[142] Navid K, Nadernezhad A, Bartolo P, Koc B (2017) Hierarchical and Spatial Modeling and Bio-Additive Manufacturing of Multi-Material Constructs. *CIRP Annals – Manufacturing Technology* 66(1):229–232.
[143] Azagarsamy M, Anseth K (2012) Bioorthogonal Click Chemistry: An Indispensable Tool to Create Multifaceted Cell Culture Scaffolds. *ACS Macro Letters* 2:5–9.
[144] Lin C-C, Ki C, Shih H (2015) Thiol–Norbornene Photoclick Hydrogels for Tissue Engineering Applications. *Journal of Applied Polymer Science* 2015:41563.
[145] Tibbitt M, et al. (2013) Mechanical Properties and Degradation of Chain and Step-Polymerized Photodegradable Hydrogels. *Macromolecules* 46:2785–2792.
[146] Hoyle C, Bowman C (2010) Thiol–ene click chemistry. *Angewandte Chemie International Edition* 49:1540–1573.
[147] Ma H, Caldwell A, Azagarsamy M, Rodriguez A, Anseth K (2020) Bioorthogonal Click Chemistries Enable Simultaneous Spatial Patterning of Multiple Proteins to Probe Synergistic Protein Effects on Fibroblast Function. *Biomaterials* 255:120205.
[148] DeForest C, Polizzotti B, Anseth K (2009) Sequential Click Reactions for Synthesizing and Patterning 3D Cell Microenvironments. *Nature Materials* 8:659–664.
[149] Mao D, Zhang C, Liu J, Wang X, Li B, Yan H, Hu F, Kong D, Wang Z, Liu B (2020) Bio-Orthogonal Click Reaction-Enabled Highly Specific In Situ Cellularization of Tissue Engineering Scaffolds. *Biomaterials* 230:119615.
[150] Tytgat L, Van Damme L, Van Hoorick J, Declercq H, Thienpont H, Ottevaere H, Blondeel P, Dubruel P, Van Vlierberghe S (2019) Additive Manufacturing of Photo-Crosslinked Gelation Scaffolds for Adipose Tissue Engineering. *Acta Biomaterialia* 94:340–350.
[151] Kaur G, Singh G, Singh J (2018) Photochemical Tuning of Materials: A Click Chemistry Perspective. *Materials Today Chemistry* 8:56–84.
[152] Jiang Y, Chen J, Deng C, Suuronen E, Zhong Z (2014) Click Hydrogels, Microgels and Nanogels: Emerging Platforms for Drug Delivery and Tissue Engineering. *Biomaterials* 35:4969–4985.
[153] Tasdelen M, Yagci Y (2013) Light-Induced Click Reactions. *Angewandte Chemie International Edition* 52:5930–5938.
[154] Fairbanks B, Schwartz M, Halevi A, Nuttelman C, Bowman C, Anseth K (2009) A Versatile Synthetic Extracellular Matrix Mimic via Thiol-Norbornene Photopolymerization. *Advanced Materials* 21:5005–5010.
[155] Kyburz K, Anseth K (2013) Three-Dimensional hMSC Motility within Peptide-Functionalized PEG-Based Hydrogels of Varying Adhesivity and Crosslinking Density. *Acta Biomaterialia* 9:6381–6392.
[156] Shih H, Lin C-C (2013) Visible-Light-Mediated Thiol-Ene Hydrogelation Using Eosin-Y as the Only Photoinitiator. *Macromolecular Rapid Communications* 34:269–273.
[157] Pereira R, Barrias C, Bartolo P, Granja P (2018) Cell-Instructive Pectin Hydrogels Crosslinked Via Thiol-Norbornene Photo-Click Chemistry for Skin Tissue Engineering. *Acta Biomaterialia* 66:282–293.
[158] White B, Meenakshisundaram V, Feller K, Williams C, Long T (2021) Vat Photopolymerization of Unsaturated Polyesters Utilizing a Polymerizable Ionic Liquid as a Non-Volatile Reactive Diluent. *Polymer* 223:123727.
[159] Wilts E, Gula A, Davis C, Chartrain N, Williams C, Long T (2020) Vat Photopolymerization of Liquid, Biodegradable PLGA-Based Oligomers as Tissue Scaffolds. *European Polymer Journal* 130:109693.
[160] Meenakshisundaram V, Sturm L, Williams B (2020) Modeling a Scanning-Mask Projection Vat Photopolymerization System For Multiscale Additive Manufacturing. *Journal of Materials Processing Technology* 279:116546.
[161] Bartolo P, Gibson I (2011) History Of Stereolithographic Processes. in Bartolo P, (Ed.) *Stereolithography – materials, Processes and Applications*, Springer, .
[162] Huang X, Zhang Y, Shi M, Zhang L-P, Zhang Y, Zhao Y (2021) A Highly Biocompatible Bio-Ink for 3D Hydrogel Scaffolds Fabrication in the Presence of Living Cells by Two-Photon Polymerization. *European Polymer Journal* 153:110505.
[163] Thompson J, Worthington K, Green B, Mullin N, Jiao C, Kaalber E, Wiley L, Han I, Russell S, Sohn E, Guymon C, Mullins R, Stone E, Tucker B (2019) Two-Photon Polymerized Poly(Caprolactone) Retinal Cell Delivery Scaffolds and Their Systemic And Retinal Biocompatibility. *Acta Biomaterialia* 94:204–218.
[164] Miri A, Nieto D, Iglesias L, Hosseinabadi H, Maharjan S, Ruiz-Esparzo G, Khoshakhlagh P, Manbachi A, Dockmeci M, Chen S, Shin S, Zhang Y, Khademhosseini A (2018) Microfluidics-Enabled Multimaterial Maskless Stereolithographic Bioprinting. *Advanced Materials* 30:1800242.
[165] Chan V, Zorlutuna P, Jeong J, Kong H, Bashir R (2010) Three-Dimensional Photopatterning of Hydrogels Using Stereolithography for Long-Term Cell Encapsulation. *Lab on a Chip* 10:2062–2070.
[166] Zorlutuna P, Jeong J, Kong H, Bashir R (2011) Stereolithography-Based Hydrogel Microenvironments to Examine Cellular Interactions. *Advanced Functional Materials* 21:3642–3651.
[167] Lu Y, Mapili G, Suhali G, Chen S, Roy K (2006) A Digital Micro-Mirror Device-Based System for The microfabrication of Complex, Spatially Patterned Tissue Engineering Scaffolds. *Journal of Biomedical Materials Research Part A* 77:396–405.
[168] Bartolo P, Mitchell G (2003) Stereo-Thermal-Lithography: A New Principle for Rapid Prototyping. *Rapid Prototyping Journal* 9:150–156.
[169] Edited by Bartolo P (2011) Stereolithographic Processes. in Bartolo P, (Ed.) *Stereolithography – Materials, Processes and Applications*, Springer, . Edited by.
[170] Kalaitz A, Makrygianni M, Theodorakos I, Hatziapostolou A, Melamed S, Kabla A, de la Vega F, Zergloti I (2019) Jetting Dynamics of Newtonian and Non-Newtonian Fluids Via Laser-Induced Forward Transfer: Experimental and Simulation Studies. *Applied Surface Science* 465:136–142.
[171] Edited by Ringeisen BR, Othon CM, Barron JA, Wu PK, Spargo BJ (2009) The Evolution of Cell Printing. in Meyer U, Meyer T, Handschel T, Wiesmann HP, (Eds.) *Fundamentals of Tissue Engineering and Regenerative Medicine*, Springer, . Edited by.
[172] Hon K, Li L, Hutchings I (2008) DIRECT WRITING TECHNOLOGY – ADVANCES and Developments. *CIRP Annals – Manufacturing Technology* 57(2):601–620.
[173] Guillemot F, Souquet A, Catros S, Guillotin B, Lopez J, Faucon M, Pippenger B, Bareille R, Rémy M, Bellance S, Chabassier P, Fricain J, Amédée J (2010) High-Throughput Laser Printing of Cells and Biomaterials for Tissue Engineering. *Acta Biomaterialia* 6:2494–2500.

[174] Guillemot F, Souquet A, Catros S, Guillotin B (2010) Laser-Assisted Cell Printing: Principles, Physical Parameters Versus Cell Fate and Perspectives In Tissue Engineering. *Nanomedicine* 5:507–515.

[175] Ringeisen B, Othon C, Barron J, Young D, Spargo B (2006) Jet-Based Methods to Print Living Cells. *Biotechnology Journal* 1:930–948.

[176] Odde D, Renn M (2000) Laser-Guided Direct Writing of Living Cells. *Biotechnology and Bioengineering* 67:312–318.

[177] Nahmias Y, Schwartz R, Verfaillie C, Odde D (2005) Laser-Guided Direct Writing for Three-Dimensional Tissue Engineering. *Biotechnology and Bioengineering* 92:129–136.

[178] Raof N, Schiele N, Xie Y, Chrisey D, Corr D (2011) The Maintenance of Pluripotency Following Laser Direct-Write of Mouse Embryonic Stem Cells. *Biomaterials* 32:1802–1808.

[179] Feinaeugle M, Pohl R, Bor T, Vaneker T, Romer G-W (2018) Printing of Complex Free-Standing Microstructures Via Laser-Induced Forward Transfer (LIFT) of Pure Metal Thin Films. *Additive Manufacturing* 24:391–399.

[180] Mondal K, McMurtrey M (2020) Present Status of the Functional Advanced Micro-, Nano-Printings – A Mini Review. *Materials Today Chemistry* 17:100328.

[181] Catros S, Guillotin B, Bačáková M, Fricain J-C, Guillemot F (2011) Effect of Laser Energy, Substrate Film Thickness And Bioink Viscosity on Viability of Endothelial Cells Printed by Laser-Assisted Bioprinting. *Applied Surface Science* 257:5142–5147.

[182] Ventura R (2021) An Overview of Laser-Assisted Bioprinting (LAB) in Tissue Engineering. *Medical Lasers* 10:76–81.

[183] Barron J, Krizman D, Ringeisen B (2005) Laser Printing of Single Cells: Statistical Analysis, Cell Viability, and Stress. *Annals of Biomedical Engineering* 33:121–130.

[184] Lin Y, Huang Y, Chrisey D (2009) Droplet Formation in Matrix-Assisted Pulsed-Laser Evaporation Direct Writing of Glycerol-Water Solution. *Journal of Applied Physics* 105:093111.

[185] Barron J, Wu P, Ladouceur H, Ringeisen B (2004) Biological Laser Printing: A Novel Technique for Creating Heterogeneous 3-Dimensional Cell Patterns. *Biomedical Microdevices* 6:139–147.

[186] Guillotin B, Souquet A, Catros S, Duocastella M, Pippenger B, Bellance S, Bareille R, Rémy M, Bordenave L, Amédée J, Guillemot F (2010) LASER ASSISTED BIOPrinting of Engineered Tissue With High Cell Density and Microscale Organization. *Biomaterials* 31:7250–7256.

[187] Keriquel V, Guillemot F, Arnauld I, Guillotin B, Miraux S, Amédée J, Fricain J, Catros S (2010) In Vivo Bioprinting for Computer- And Robotic-Assisted Medical Intervention: Preliminary Study in Mice. *Biofabrication* 2:01401.

[188] Xu T, Binder K, Albanna M, Dice D, Zhao W, Yoo J, Atala A (2012) Hybrid Printing of Mechanically and Biologically Improved Constructs For Cartilage Tissue Engineering. *Biofabrication* 5:015001.

[189] Xu T, Yoo J, Atala A (2016) Inkjet Printing of Tissues and Cells. *US Patent* 9:301.925 B2.

[190] Zhang B, Seong B, Nguyen V, Byun D (2016) 3D Printing of High-Resolution PLA-Based Structures by Hybrid Electrohydrodynamic and Fused Deposition Modeling Techniques. *Journal of Micromechanics and Microengineering* 26:025015.

[191] Alblawi A, Ranjani A, Yasmin H, Gupta S, Bit A, Rahimi-Gorji M (2020) Scaffold-Free: A Developing Technique in the Field of Tissue Engineering. *Computer Methods and Programs in Biomedicine* 185:105148.

[192] Lee V, Singh G, Trasatti J, Bjornsson C, Xu X, Tran T, Yoo S, Dai G, Karande P (2014) Design and Fabrication of Human Skin By Three-Dimensional Bioprinting. *Tissue Engineering Part C* 20:473–484.

[193] Koch L, Kuhn S, Sorg H, Gruene M, Schlie S, Gaebel R, Polchow B, Reimers K, Stoelting S, Ma N, Vogt P, Steinhoff G, Chichkov B (2010) Laser Printing of Skin Cells and Human Stem Cells. *Tissue Engineering Part C* 16:847–854.

[194] Schuurman W, Khristov V, Pot M, Van Weeren P, Dhert W, Malda J (2011) Bioprinting of Hybrid Tissue Constructs with Tailorable Mechanical Properies. *Biofabrication* 3:21001.

[195] Liu F, Hinduja S, Bartolo P (2018) User Interface Tool to Design and Fabricate Functionally Graded Scaffolds Through a Novel Plasma-Assisted Bio-Extrusion System. *Rapid Prototyping Journal* 24:2018.

[196] Liu F, Wang W, Hinduja S, Bartolo P (2018) Hybrid Additive Manufacturing System for a Zonal Plasma Treated Scaffolds. *3D Printing and Additive Manufacturing* 5:218.

[197] Liu F, Wang W, Mirihanage W, Hinduja S, Bartolo P (2018) A Plasma-Assisted Bioextrusion System For Tissue Engineering. *CIRP Annals – Manufacturing Technology* 67(1):229–232. a.

[198] Pereira R, Barrias C, Granja P, Bartolo P (2013) Advanced Biofabrication Strategies for Skin Regeneration and Repair. *Nanomedicine* 8:603–621.

[199] Abdo J, Sopko N, Milner S (2020) The Applied Anatomy of Human Skin: A Model for Regeneration. *Wound Medicine* 28:100179.

[200] Ramasamy S, Davoodi P, Vijayavenkataraman S, Teoh J, Thamizhchelvan A, Robinson K, Wu B, Fuh J, DiColandrea T, Zhao H, Lane E, Wang C-H (2021) Optimized Construction of a Full Thickness Human Skin Equivalent Using 3D Bioprinting and a PCL/Collagen Dermal Scaffold. *Bioprinting* 21:e00123.

[201] Jin R, Cui Y, Chen H, Zhang Z, Weng T, Xia S, Yu M, Zhang W, Shao J, Yang M, Han X, Wang X (2021) Three-dimensional bioprinting of a full-thickness functional skin model using acellular dermal matrix gelatin methacrylamide bio-ink. *Acta Biomateria* 131:248–261.

[202] Skardal A, Murphy S, Crowell K, Mack D, Atala A, Soker S (2017) A Tunable Hydrogel System for Long-Term Release of Cell-Secreted Cytokines and Bioprinted In Situ Wound Cell Delivery. *Journal of Biomedical Materials Research B: Applied Biomaterials* 105:1986–2000.

[203] Yu R, Yang Y, He J, Li M, Guo B (2021) Novel Supramolecular Self-Healing Silk Fibroin-Based Hydrogel Via Host-Guest Interaction as Wound Dressing to Enhance Wound Healing. *Chemical Engineering Journal* 417:128278.

[204] Wang L, Yang K, Li X, Zhang X, Zhang D, Wang L, Lee C (2021) A Double-Crosslinked Self-Healing Antibacterial Hydrogel with Enhanced Mechanical Performance for Wound Treatment. *Acta Biomaterialia* 124:139–152.

[205] Pereira R, Sousa A, Barrias C, Bartolo P, Granja P (2018) A Single-Component Hydrogel Bioink for Bioprinting of Bioengineered 3D Constructs for Dermal Tissue Engineering. *Materials Horizons* 5:1100–1111.

[206] Gledhill K, Guo Z, Umegaki-Arao N, Higgins C, Itoh M, Christiano A (2015) Melanin Transfer in Human 3D Skin Equivalents Generated Exclusively From Induced Pluripotent Stem Cells. *PloS One* 10:e0136713.

[207] Min D, Lee W, Bae I, Lee T, Croce P, Yoo S (2018) Bioprinting of Biomimetic Skin Containing Melanocytes. *Experimental Dermatology* 27:453–459.

[208] Ng W, Qi J, Yeong W, Naing M (2018) Proof-of-Concept: 3D Bioprinting of Pigmented Human Skin Constructs. *Biofabrication* 10:025005.

[209] Huang S, Yao B, Xie J, Fu X (2016) 3D Bioprinted Extracelular Matrix Mimics Facilitate Directed Differentiation of Epithelial Progenitors for Sweat Gland Regeneration. *Acta Biomaterialia* 33:170–177.

[210] Vahav I, Van Den Broek L, Thon M, Monsuur H, Spiekstra S, Atac B, Scheper R, Lauster R, Lindner G, Marx U, Gibbs S (2020) Reconstructed Human Skin Shows Epidermal Invagination Towards Integrated Neopapillae Indicating Early Hair Follicle Formation In Vitro. *Journal of Tissue Engineering and Regenerative Medicine* 14:761–773.

[211] MacNeil S (2008) Biomaterials for Tissue Engineering of Skin. *Materials Today* 11:26–35.

[212] Hsu S, Hsieh P (2015) Self-Assembled Adult Adipose-Derived Stem Cell Spheroids Combined with Biomaterials Promote Wound Healing in a Rat Skin Repair Model. *Wound Repair and Regeneration* 23:57–64.

[213] Laschke M, Schank T, Scheuer C, Kleer S, Schuler S, Metzger W, Eglin D, Alini M, Menger M (2013) Three-Dimensional Spheroids of Adipose-Derived Mesenchymal Stem Cells are Potent Initiators of Blood Vessel Formation in Porous Polyurethane Scaffolds. *Acta Biomaterialia* 9:6876–6884.

[214] Kolesky D, Truby R, Gladman A, Busbee T, Homan K, Lewis J (2014) 3D Bioprinting of Vascularized, Heterogeneous Cell-Laden Tissue Constructs. *Advanced Materials* 26:3124–3130.

[215] Miller J, Stevens K, Yang M, Baker B, Nguyen D, Cohen D, Toro E, Chen A, Galie P, Yu X, Chaturvedi R, Bhatia S, Chen C (2012) Rapid Casting of Patterned Vascular Networks For Perfusable Engineered Three-Dimensional Tissues. *Nature Materials* 11:768–774.

[216] Ma Z, Song W, He Y, Li H (2020) Multilayer Injectable Hydrogel System Sequentially Delivers Bioactive Substances for Each Wound Healing Stage. *ACS Applied Materials Interfaces* 12:29787–29806.

[217] Abaci H, Guo Z, Coffman A, Gillette B, Lee W, Sia S, Christiano A (2016) Human skin Constructs with Spatially Controlled Vasculature Using Primary and iPSC-Derived Endothelial Cells. *Advanced Healthcare Materials* 5:1800–1807.

[218] Albanna M, Binder K, Murphy S, Kim J, Qasem S, Zhao W, Tan J, El-Amin I, Dice D, Marco J, Green J, Xu T, Skardal A, Holmes J, Jackson J, Atala A, Yoo J (2019) In Situ Bioprinting of Autologous Skin Cells Accelerates Wound Healing Of Extensive Excisional Full-Thickness Wounds. *Scientific Reports* 9:1856.

[219] Makris E, Gomol A, Malizos K, Hu J, Athanasiou K (2015) Repair and Tissue Engineering Techniques for Articular Cartilage. *Nature Reviews Rheumatology* 11:21–34.

[220] O'Connel C, Di Bella C, Thompson F, Augustine C, Beirne S, Cornock R, Richard C, Chung J, Gambhir S, Yue Z, Bourke J, Zhang B, Taylor A, Quigley A, Kapsa R, Choong P, Wallace G (2016) Development of the Biopen: A Handled Device for Surgical Printing of Adipose Stem Cells at a Chondral Wound Site. *Biofabrication* 8(1):015019.

[221] Di Bella C, Duchi S, O'Connell C, Blanchard R, Augustine C, Yue Z, Thompson F, Richards C, Beirne S, Onofrillo C (2018) In Situ Handheld Three-Dimensional Bioprinting for Cartilage Regeneration. *Journal of Tissue Engineering and Regenerative Medicine* 12:611–621.

[222] Duchi S, Onofrillo C, O'Connell C, Blanchard R, Augustine C, Quigley A, Kapsa R, Pivonka P, Wallace G, Di Bella C (2017) Handled Co-Axial Bioprinting: Application to In Situ Surgical Cartilage Repair. *Scientific Reports* 7:1–12.

[223] O'Connel C, Konate S, Onofrillo C, Kapsa R, Baker C, Duchi S, Eekel T, Yue Z, Beirne S, Barnsley G (2020) Free-form Co-Axial Bioprinting of a Gelatin Methacryloyl Bio-Ink by Direct In Situ Photo-Crosslinking During Extrusion. *Bioprinting* 19:e00087.

[224] Yang G, Cambias J, Cleary K, Daimler E, Drake J, Dupont P, Hata N, Kazanzides P, Martel S, Patel R (2017) Medical Robotics – Regulatory, Ethical, and Legal Considerations for Increasing Levels of Autonomy. *Science Robotics* 2(4):8638.

[225] Go G, Jeong S, Yoo A, Han J, Kang B, Kim S, Nguyen K, Jin Z, Kim C, Seo Y (2020) Human Adipose-Derived Mesenchymal Stem Cell-Based Medical Microrobot System for Knee Cartilage In Vivo. *Sci Rob* 5:eaay6626.

[226] Go G, Jeong S-H, Yoo A, Han J, Kang B, Kim S, Nguyen K, Jin Z, Kim C-S, Seo Y, Kang J, Na J, Song E, Jeong Y, Seon J, Park J-O, Choi E (2020) Human Adipose-Derived Mesenchymal Stem Cell-Based Medical Microrobot System for Knee Cartilage REGENERATION IN Vivo. *Science Robotics* 5:eaay6626.

[227] Ruijs A, Jaquet J, Kalmijn S, Giele H, Hovius S (2005) Median And Ulnar Nerve Injuries: A Meta-Analysis of Predictors of Motor and Sensory Recovery After Modern Microsurgical Nerve Repair. *Plastic and Reconstructive Surgery* 116:484–494.

[228] Gaudin R, Knipfer C, Henningsen A, Smeets R, Heiland M, Hadlock T (2016) Approaches to Peripheral Nerve Repair: Generations of Biomaterial Conduits Yielding to Replacing Autologous Nerve Grafts in Craniomaxillofacial Surgery. *BioMed Research International* 2016:3856262.

[229] Bibbo C, Rodrigues-Colazzo E, Finzen A (2018) Superficial Peroneal Nerve to Deep Peroneal Nerve Transfer with Allograft Conduit for Neuroma in Continuity. *The Journal of Foot and Ankle Surgery* 57:514–517.

[230] Won Y, Patel A, Bull D (2014) Cell Surface Engineering to Enhance Mesenchymal Stem Cell Migration Towards an SDF-1 Gradient. *Biomaterials* 35:5627–5635.

[231] Ning L, Sun H, Lelong T, Guilloteau R, Zhu N, Schreyer D, Chen X (2018) 3D Bioprinting of Scaffolds with Living Schwann Cells for Potential Nerve Tissue Engineering Applications. *Biofabrication* 10:035014.

[232] Dixon A, Jariwala S, Bilis Z, Loverde J, Pasquina P, Alvarez L (2018) Bridging the Gap in Peripheral Nerve Repair with 3D Printed and Bioprinted Conduits. *Biomaterials* 186:44–63.
[233] Malda J, Klein T, Upton Z (2007) The Roles of Hypoxia in the In Vitro Engineering of Tissues. *Tissue Engineering* 13:2153–2162.
[234] Gao G, Lee J, Jang J, Lee D, Kong J, Kim B, Choi Y-J, Jang W, Hong Y, Kwon S-M, Cho D-W (2017) Tissue Engineered Bio-Blood-Vessels Constructed Using a Tissue-Specific Bioink and 3D Coaxial Cell Printing Technique: A Novel Therapy for Ischemic Disease. *Advanced Functional Materials* 27:1700798.
[235] Singh N, Han W, Nam S, Kim J, Kim J, Kim Y, Cho D-W (2020) Three-Dimensional Cell-Printing of Advanced Renal Tubular Tissue Analogue. *Biomaterials* 232:119734.
[236] Kucukgul C, Ozler SB, Inci I, Karakas E, Irmak S, Gozuacik D, Taralp A, Koc B (2015) 3D Bioprinting of Biomimetic Aortic Vascular Constructs With Self-Supporting Cells. *Biotechnology and Bioengineering* 112:811–821.
[237] Noor N, Shapira A, Edri R, Gal I, Wertheim L, Dvir T (2019) 3D Printing of Personalized Thick and Perfusable Cardiac Patches and Hearts. *Advanced Science* 6:1900344.
[238] Lee A, Hudson A, Shiwarski D, Tashman J, Hinton T, Yerneni S, Bliley (2019) 3D Bioprinting of Collagen to Rebuild Components of the Human Heart. *Science* 365:482–487.
[239] Mirdamadi E, Tashman J, Shiwarski D, Palchesko R, Feinberg A (2020) FRESH 3D Bioprinting a Full-Size Model of the Human Heart. *ACS Biomaterials Science Engineering* 6:6453–6459.
[240] Fransen M, Addario G, Bouten C, Halary F, Moroni L, Mota C (2021) Bioprinting of Kidney In Vitro Models: Cells, Biomaterials, and Manufacturing Techniques. *Essays in Biochemistry* 65:587–602.
[241] Peired A, Mazzinghi B, Chiara L, Guzzi F, Lasagni L, Romagnani P, Lazzeri E (2020) Bioengineering Strategies Fornephrologists: Kidney was not Built in a Way. *Expert Opinion on Biological Therapy* 20:467–480.
[242] Homan K, Kolesky D, Skylar-Scott M, Herrmann J, Obuobi H, Moisan A, Lewis J (2016) Bioprinted of 3D Convoluted Renal Proximal Tubules on Perfusable Chips. *Scientific Reports* 6:34845.
[243] Lin N, Homan K, Robinson S, Kolesky D, Duarte N, Moisan A, Lewis J (2019) Renal Reabsorption in 3D Vascularized Proximal Tubule Models. *PNAS* 116:5399–5404.
[244] Gao G, Park J, Kim B, Jang J, Chao D (2018) Coaxial cell Printing of Freestanding, Perfusable, and Functional In Vitro Vascular Models for Recapitulating of Native Vascular Endothelium Pathophysiology. *Advanced Healthcare Materials* 7:1801102.
[245] Lawlor K, Vanslambrouck J, Higgins J, Chambon A, Bishard K, Arndt D, Er P, Wilson S, Howden S, Tan K, Li F, Hale L, Shepherd B, Pentoney S, Presnell S, Chen A, Little M (2021) Cellular Extrusion Bioprinting Improves Kidney Organoid Reproducibility and Conformation. *Nature Materials* 20:260–271.
[246] Quint J, Mostafavi A, Endo Y, Panayi A, Russell C, Nourmahnad A, Wiseman C, Abbasi L, Samandri M, Sheikhi A, Nuutila K, Sinha I, Tamayol A (2021) In Vitro Printing of Nanoenabled Scaffolds for the Treatment of Skeletal Muscle Injuries. *Advanced Healthcare Materials* .
[247] Kim W, Jang C, Kim G (2019) A Myoblast-Laden Collagen Bioink With Fully Aligned Au Nanowires for Muscle-Tissue Regeneration. *Nano Letters* 19:8612–8620.
[248] Jakus A, Rutz A, Jordan S, Kannan A, Mitchell S, Yun C, Koube K, Yoo S, Whiteley H, Richter C, Galiano R, Ksu W, Shah S, Hsu E, Shah R (2016) Hyperelastic "bone": A Highly Versatile, Growth Factor-Free, Osteoregenerative, Scalable, and Surgically Friendly Biomaterial. *Science Translational Medicine* 8:358.
[249] Romanazzo S, Molley T, Nemec S, Lin K, Sheikh R, Gooding J, Wan B, Li Q, Kilian K, Roohani I (2021) Synthetic Bone-Like Structures Through Omnidirectional Ceramic Bioprinting in Cell Suspensions. *Advanced Functional Materials* 31:2008216.
[250] Chimene D, Miller L, Cross L, Jaiswal K, Singh I, Gaharwar A (2020) Nanoengineered Osteoinductive Bioink for 3D Bioprinting Bone Tissue. *ACS Applied Materials Interface* 12:15976–15988.
[251] Parak A, Pradeep P, du Toit L, Kumar P, Choonara Y, Pillay V (2019) Functionalizing Bioinks for 3D Bioprinting Applications. *Drug Discovery Today* 24(1):198–205.
[252] Cadet J, Douki T (2018) Formation of UV-induced DNA Damage Contributing to Skin Cancer Development. *Photochemical & Photobiological Sciences* 17 (12):1818–1841.
[253] Thai M, Phan P, Hoang T, Wong S, Lovell N, Do T (2020) Advanced Intelligent Systems for Surgical Robotics. *Advanced Intelligent Systems* 2(8):1900138.
[254] AzoRobotics. *Medical Robotics*, www.azorobotics.com.
[255] Tekscan. *Pressure Mapping, Force Measurement and Tactile Sensors*, www.tekscan.com.