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Review of the 3D bioprinting methods and materials applicable in 4D bioprinting

Masukur RAHMAN [1], Salman Al-hossain PRODHAN [1], Rayhanul ISLAM [1], Ashik MAHMUD [1], Khan Rajib HOSSAIN [2,*]

[1] Faculty of Engineering, University of Rajshahi, Rajshahi, Bangladesh
[2] Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou, China
*Corresponding author: apexlabbd2@mails.ucas.ac.cn

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Abstract

The advent of 4D bioprinting, a technique that integrates time dimensions into the conventional 3D method, substantially changed tissue engineering. By providing a time dimension to conventional 3D printing, the innovative process of 4D bioprinting is revolutionising tissue engineering. This development allows the creation of dynamic tissues, allowing structures to change and adapt over time to reflect the complexity of natural tissues. Specifically, shape memory polymers enable customised scaffold fabrication with improved mechanical strength and biomimicry for use in bone tissue engineering. An essential additional use for soft tissue regeneration is the development of injectable thermosensitive hydrogels that react to body temperature. Within asymmetrical defect regions, these hydrogels gel, offering a flexible cure for healing injured soft tissues. In addition to structural considerations, the potential applications of 4D bioprinting include organisms, vascularisation and dynamic disease models. Despite the advancements, issues like suitable biocompatibility and long-term stability still need to be resolved. However, by creating complex, adaptable and patient-specific designs in the realm of tissue engineering, 4D bioprinting has enormous potential to transform personalised medicine and regenerative therapies. This review provides a comprehensive investigation into the future of regenerative medicine by exploring the various uses and potentially revolutionary effects of 4D bioprinting in tissue engineering.

1. Introduction

Charles Hull was the first to focus on three-dimensional (3D) printing, also known as additive manufacturing (AM) in 1986 [1-2]. In 1976, David E.H. Jones introduced the idea of 3D printing [3], which is now a standard technology in various engineering and biomedical fields [4]. This technology gives a boundless advantage, as it can provide biomedical equipment to meet the needs of patients. This technology has significantly developed in tissue engineering, such as in the nerve, kidney, cartilage, wound dressings, bone, trachea, muscles and tendons, liver and other tissues [5]. This technology has been applied to medication screening and transplantation in addition to the development of functional tissue and organs. Though there are such advantages, a considerable drawback of 3D printing technology is that it remains the same as the initial state of the printed object and is considered static. To overcome this issue, time is integrated with the previously improved 3D technology as a fourth dimension to get a four-dimensional technology called 4D printing. Here, the integrated time does not mean that the time of printing will be prolonged; instead, printed 3D biocompatible objects or living cellular constructs will change their shape over time. In this technology, printed objects change their shapes through external stimuli such as pH, temperature, swelling

behaviour, humidity, light, Ca^{2+} concentration, electric field and magnetic field [6]. This creative notion was presented by Prof. Tibbits and Prof. Jerry in 2013 [7]. 4D printing technology is still in its early stages and not widely known by researchers.

4D printing is a promising new bioprinting technology that can be applied extensively in the field of regenerative medicine [8]. Chronic disease, organ transplantation and shortage of donors are various things that cause the emergence of 4D bioprinting in this medical field [9]. The 4D bioprinting method stands based on digital light processing (DLP). This method uses hydrogels, thermoplastics, ceramics and polymers to imitate the tissue mechanism [10]. Four-dimensional (4D) bioprinting has incredible prospects for various biomedical applications, for example, tissue engineering, actuators, biosensors and robotics [11]. Tissue engineering is a remarkable field within regenerative medicine that focuses on harnessing the power of in vitro and in situ techniques to rejuvenate and revive targeted tissues, thereby re-establishing their natural biological capabilities [12]. 4D bioprinting concerns scaffolding, dentistry, cell growth and artificial fabrication structures that will help tissue engineering simultaneously in the future [13].

The review article focuses on 4D printing technology that still needs to be sufficiently developed and its application in bioprinting could be more convenient. However, this technology has the efficiency to overcome the lack of 3D printing technology applications in bioprinting. This review paper discusses 3D printing techniques, bioprinting, materials used in bioprinting, 4D bioprinting and its applications in tissue engineering. In the end, it will be discussed the significant obstacles to this approach and try to find potential solutions to provide future perspectives on this technology.

2. 3D bioprinting techniques

3D bioprinting is a growing 3D printing technology that uses biological materials (bioinks) to build 3D tissues or 3D cell culture environments [14]. When researchers decide to bioprint tissue models, they choose based on their research goals and the results they hope to achieve [15]. This process allows great flexibility to adjust the final model to specific goals. The bioink may be selected to mimic the tissue composition that the researchers hope to print, but it can also be a synthetic formulation to mimic similar functions. They can also print the environment for 3D culture cells and choose the bioink or hydrogel suitable for this purpose. Bioprinting can be applied to countless fields, from drug discovery to personalised medicine [16]. 3D bioprinting is a state-of-the-art additive manufacturing technique that uses biomaterials to create 3D structures layer by layer. The structures can be inserted into compatible biomaterials to form tissues or organs [17]. Many researchers have researched the expansion of 3D bioprinting techniques. The primary 3D bioprinting modalities, in general, can be classified as fused deposition modelling (FDM), laser-assisted bioprinting (LAB), inkjet bioprinting/droplet bioprinting, extrusion-based bioprinting, stereolithography (SLA) and vat polymerisation (Fig. 1) [18-20].

Fused deposition modelling is a widely adopted method for building structures. It involves melting filaments or wires of thermoplastic polymers such as acrylonitrile butadiene styrene (ABS), polylactic acid (PLA) and polycarbonate (PC) through a hot nozzle, which then naturally cools and solidifies [21-22]. The laser-assisted bioprinting technique employs a laser to deposit bioink onto a receiving substrate directly. A laser beam interacts with a bioink solution, creating a pressure wave that propels tiny droplets onto the target surface. This process is repeated layer by layer to build the desired structure [18]. This technique was

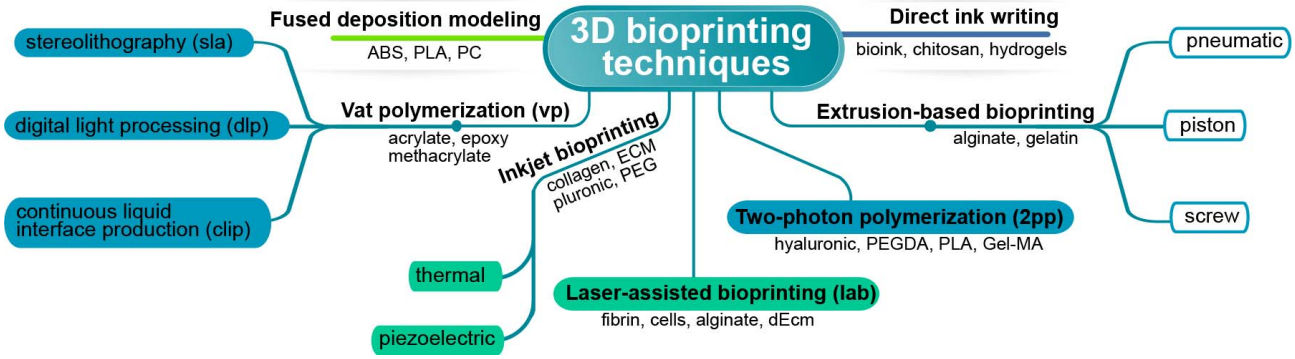

Figure 1. Diagram of the 3D bioprinting techniques

developed for writing metals after researchers successfully applied it to bioprinting nucleic acids such as DNA or organ cells. Inkjet bioprinting or multicolour multi-material jetting can print photopolymerisable resins with multiple printheads using ultraviolet (UV) light, which is more modern than FDM [23]. Extrusion-based 3D bioprinting is a printing technique used for multicolour and multi-layer printing techniques used to produce plastics, food and living cells. Vat photopolymerisation is a process in additive manufacturing used to selectively cure liquid photopolymer in the vat by light-activated polymerisation layer by layer to form the target entity by using specific light (e.g. UV light) [24]. However, among the superior principal techniques, other associated methods are widely utilised in 3D bioprinting, particularly magnetic [25], acoustic and plotting [26].

3D bioprinting is a process that uses a computer to control the deposition of living cells, extracellular matrix (ECM) components and biochemical factors in a specific pattern to create 3D tissue constructs [27]. This innovative technology promises to produce various transplantable soft tissues like skin, bone and cartilage. 3D bioprinting employs the following main process methods: inkjet, extrusion, laser-assisted bioprinting, direct ink writing and stereolithography.

2.1 Inkjet 3D bioprinting

Inkjet 3D printing was used in paper printing for a long time. Inkjet 3D bioprinting was first introduced in 2003 [28] by Wilson and Boland. This technology allows tissue scaffolds to be created using bioink [29]. Here, the biomaterials are low-viscosity suspensions of living cells, while it is a noncontact computer-based process [30]. The main advantages of inkjet 3D bioprinting are its printing speed and the low price of devices. Inkjet 3D printing can be categorised as continuous inkjet 3D printing and drop-on-demand (DOD) inkjet 3D printing.

In continuous inkjet 3D printing, ink flows from the microscopic nozzle at a constant pressure to form a single column. Because of the Plateau-Rayleigh instability of the fluid, this column can split into individual droplets. Even though droplets are not needed, the ejection of droplets cannot be controlled. In the DOD method, the droplets are ejected from the nozzle by creating a pressure pulse when needed. A DOD inkjet 3D printing device can have multiple print heads; each of the heads contains a chamber that contains multiple nozzles connected to it. Based on the droplet actuation mechanism, the DOD method can be classified as thermal inkjet 3D bioprinting or piezoelectric inkjet 3D bioprinting [31]. In thermal inkjet 3D bioprinting, a heat actuator is in the chamber. That heat actuator increases the temperature for a microsecond, which vaporises the bioink and produces a heat bubble. These heat bubbles create a pressure pulse on the nozzle orifice, which is the driving force for ejecting ink droplets. The diameter of the produced droplets is around 30 – 80μm and the cell viability is about 90 %. The diameter of the droplets can be controlled by maintaining the viscosity of the ink [32]. In piezoelectric inkjet 3D bioprinting, there is a piezoelectric transducer in the chamber. When this transducer receives a voltage pulse, it is extended. For a sudden volume change, it ejects a droplet. Here, the diameter of the droplets is 50 – 100μm and the cell viability is about 70 – 90 %, depending on the printing cell type. Hydrogels, binders, powders, polymers and small molecules are biomaterials in inkjet 3D bioprinting.

2.2 Extrusion-based 3D bioprinting (EBB)

Extrusion-based 3D bioprinting is the most widely used technique for 3D bioprinting (Fig. 2). A new method in tissue engineering called 3D bioprinting is gaining popularity as an innovative method to create scientifically reasonable tissue structures [33]. It draws inspiration from AM, which layers materials onto a substrate to create intricate 3D objects. In contrast to traditional AM, bioprinting builds living biological models using hydrogel materials called bioinks that are loaded with cells [34]. The biomaterials are loaded into cartridges and forced through a nozzle using mechanical or pneumatic pressure [35]. One of the many benefits of extrusion 3D bioprinting is its capacity to extrude bioinks with high cell densities, which enables the creation of heterogeneous models with varying cell types and quantities. It has become relatively affordable and customisable for specific applications. Commercially available extrusion 3D bioprinters can be adapted for various advanced techniques.

However, mammalian cell viability in extrusion-based 3D bioprinting is often lower compared to inkjet 3D bioprinting due to shear stress from high extrusion pressure. Efforts are ongoing to address this issue through the design of shear-thinning bioinks. Improving printing resolution and speed is

also a research focus to enhance model fidelity. Extrusion 3D bioprinting has applications in various fields, including creating tumour models, biomedical implants and in vitro disease models. Continued advancements in 3D bioprinting hold promise for tissue engineering and biomedical research.

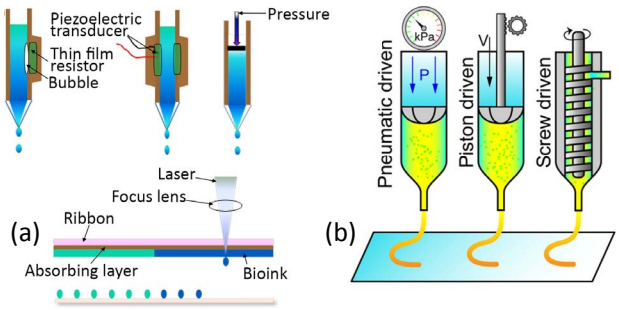

Figure 2. Common 3D bioprinting techniques: (a) inkjet printers, adapted from Li et al. [30], licensed under CC BY 4.0 and (b) extrusion-based printers, reprinted from [34], with the permission of AIP Publishing

2.3 Laser-assisted 3D bioprinting (LAB)

Laser-assisted 3D bioprinting was first developed at the University of Minnesota, USA by a research group named Dr. Odde's group. Dr. David Odde and his group mentioned this process as "laser-guided direct writing". First used for high-resolution patterning of metals for microchip fabrication. They used a cell suspension to shoot cells with a direct laser onto a surface [36]. LAB has high printing accuracy at high resolution (micron level) along with a high-frequency pulse laser, prints 5000 droplets in just one second, despite very high cell density and viscosity (1 – 8000 mPas), and also prints cells containing sols while ensuring the highest cell viability [37]. The laser beam from the laser generator passes through the ribbon and hits a bioink layer (step 1). A bubble is created in the bioink layer (step 2). Low inner pressure causes the bubble to collapse and high pressure at the bubble poles causes the bioink to form a high-speed liquid jet (step 3). The droplet touches the receiving substrate and prints in micro-nano size (step 4). LAB has three main elements: a pulsed laser source, which generates a laser beam, a ribbon and a receiving substrate (Fig. 3). The laser's wavelength varies between 90 and 1064 nm, allowing it to deposit a single cell, and the distance between the ribbon and the receiving substance is 1 nm. The ribbon is made of transparent glass attached to layers of bioink. The bioink has heat-sensitive biomaterials that react when a high-frequency laser pulses at a specific energy.

Experimentally, the deposited bioink's volume depends linearly on the energy of the laser pulse, although the droplet size can be manipulated manually. In the ribbon, an interlayer transmits the energy of the laser beam to the bioink, referred to as the laser-absorbing layer (LAL), placed between the supporting glass and the bioink layer. LAB is preferable for printing endothelial cells. In this process, scientists face two problems: one is uniformity and the other is stability. To solve these issues, they are introducing Laser-Induced Backward Transfer (LIBT), a film-free laser-induced forward transfer (LIFT). LIBT emerged by altering the position of the donor bioink layer and the substrate [38].

2.4 Direct ink writing (DIW)

In 3D bioprinting, direct ink writing (DIW) is a novel and adaptable method that has drawn a lot of interest in the field of regenerative medicine. The core idea behind DIW is that it can regulate material deposition in a way that is similar to conventional 3D printing. However, when it comes to bioprinting, the "ink" that is utilised is called a

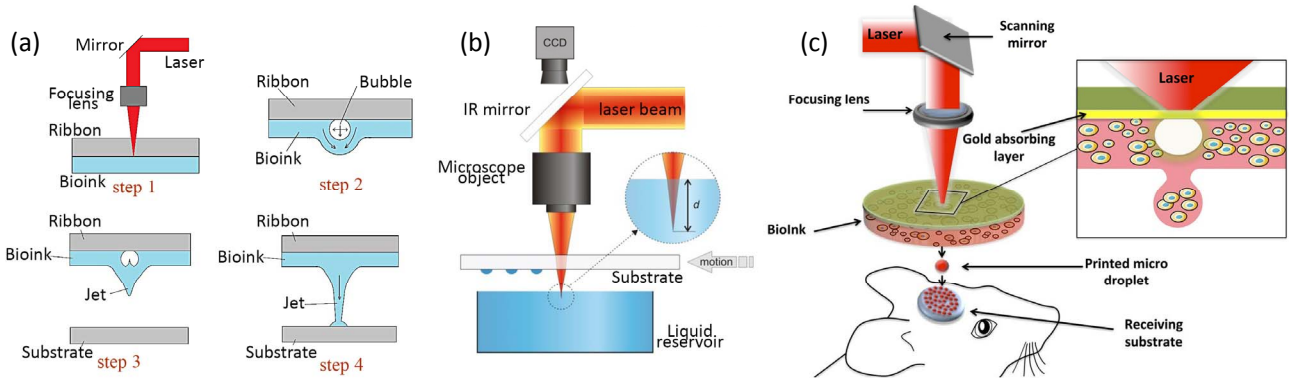

Figure 3. Laser-assisted 3D bioprinting process: (a) production of a high-speed liquid jet, reproduced from [37] with permission of Wiley, (b) laser-induced backward transfer (LIBT), reprinted from [39], with permission from Elsevier and (c) droplet deposition, reprinted from Keriquel et al. [38], licensed under CC BY 4.0

bioink, which is a material that usually consists of living cells enclosed in a matrix of support, like hydrogels or other biomaterials. A nozzle or syringe extrudes the bioink, delicately enabling precise positioning and stacking (Fig. 4a). The capacity of DIW to produce intricate, adaptable structures that resemble the architecture of genuine tissues is one of the technology's main advantages in bioprinting. This is important for regenerative medicine because it makes it possible to create precisely shaped and functioning tissues to meet each patient's demands. The microstructure of the printed constructions can be designed and controlled by researchers, which can affect tissue growth and cell activity [40].

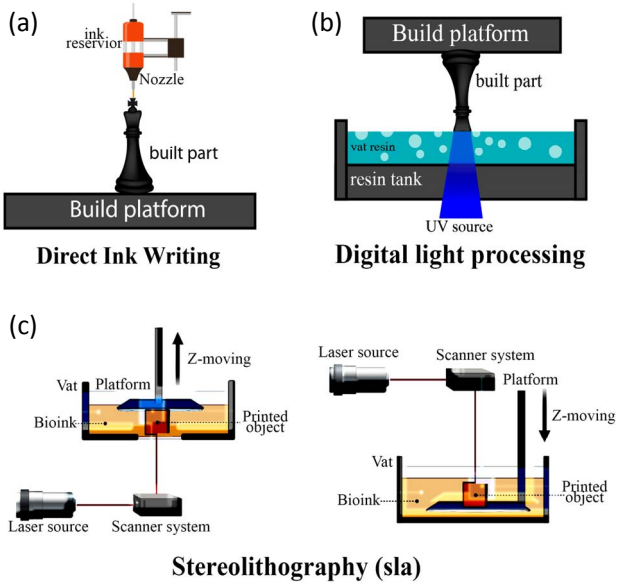

Figure 4. Schematic of: (a) direct ink writing, adapted from Hossain et al. [40], licensed under CC BY 4.0, (b) digital light processing, adapted from Hossain et al. [40], licensed under CC BY 4.0 and (c) stereolithography, reprinted from Li et al. [41], licensed under CC BY-NC-ND 4.0

Moreover, DIW enables using several bioinks in a single printing procedure. This feature allows for the printing of heterogeneous constructions, in which various cell types or biomaterials can be precisely positioned in some regions of the printed construct. This is especially useful for simulating the complexity of native tissues, which are frequently made up of different cell types arranged in specific spatial configurations [42]. Besides its adaptability, DIW in 3D bioprinting presents the possibility of high-throughput tissue construct synthesis. The technique's automated and computer-controlled design makes it possible to produce complex biological structures quickly and effectively, cutting down on the amount of time needed to create functional tissues. Despite its immense promise, there are still obstacles to achieving vascularisation inside printed tissues, guaranteeing cell survival and optimising the bioinks utilised in DIW. To overcome these obstacles, ongoing research in DIW lays the groundwork for creating bioprinted tissues for drug testing, transplantation and customised medicine [43].

2.5 Stereolithography (SLA) 3D bioprinting

In tissue engineering, stereolithography (SLA) 3D bioprinting is a state-of-the-art method that combines the accuracy of SLA with the dynamic properties of 3D printing. This novel approach uses light-sensitive polymers to selectively solidify biocompatible materials, often hydrogels that are loaded with living cells layer by layer. The Vat photopolymerisation build platform will lift (Fig. 4c, left) or move down (Fig. 4c, right) in the *z*-direction when a layer is fully bioprinted via point-by-point curing, continuing to finish the bioprinted object line-by-line and layer-by-layer [41]. With the introduction of time to the three dimensions in 3D bioprinting, printed structures can display behaviours such as shape-shifting or reactivity to external stimuli. SLA 3D bioprinting has excellent potential in tissue engineering to produce biofunctional structures with regulated, time-dependent morphologies. With the use of this technology, dynamic tissues that resemble natural biological tissues in their behaviour can be created [44]. These tissues can adapt and react to physiological stimuli. Although DLP (Fig. 4b) and SLA are very similar, the light patterning technique distinguishes the two methods. Moreover, DLP bioprinting may be divided into two subtypes based on the z-axis's travelling directions: top-down and bottom-up. The build platform is lowered to the next photopolymerisation layer in the top-down configuration after being submerged and covered in bioink. SLA 3D bioprinting is a highly effective technique for developing the synthesis of functioning, responsive tissues for regenerative medicine and tailored healthcare applications because of its exact control over structural details and capacity to induce shape changes over time. Ongoing research aims to improve printing parameters and biomaterial compositions to improve overall tissue performance and cell viability [45].

3. 4D bioprinting

4D printing represents an innovative technology with distinct benefits compared to traditional additive manufacturing (AM). It retains the fundamental qualities of 3D printing, including reducing material waste and eliminating the need for melds, dies and machining. Moreover, it introduces an additional dimension, allowing products to exhibit intelligent behaviour as time progresses. When subjected to different stimuli, these products can change shape and function [46]. These changes can happen through various means, such as applying heat or light or causing a liquid to swell. They can also be achieved through electrochemical processes and by adjusting the sensitivity to swelling in different areas of the designed structure [47]. Furthermore, other external stimuli can also influence these transformations, such as pressure or electromagnetic fields. 4D printing primarily depends on five key factors, which Nam et al. [48] proposed, and it is essential to consider all of these factors when engaging in 4D printing. These five factors include the AM process, the choice of printing materials, the stimuli involved, the interaction mechanism and the mathematical modelling aspects.

The first factor is the AM process used for printing. This aspect involves choosing the specific method used to create objects layer by layer from digital information. Various AM processes, such as SLA, selective laser sintering (SLS) and FDM, can be employed to print 4D materials, provided the printing material is compatible with the chosen process. The next factor is printing material. The material used for 4D printing should be programmable or intelligent, capable of responding to stimuli. These materials can undergo shape changes when exposed to external influences, determining the type of stimuli and transformations they can achieve. The third factor is stimuli. The choice of stimuli is crucial in 4D printing. Stimuli can be physical (e.g. light, temperature), chemical (e.g. pH level, chemicals) or biological (e.g. enzymes). These stimuli trigger physical or chemical changes in the material, leading to structural deformations. The fourth and fifth factors are the mechanism of interaction and mathematical modelling. An interaction mechanism, such as mechanical loading or physical manipulation, is required to accomplish the intended transformation. This mechanism plans and sequences the shape changes that occur when a stimulus is applied to the smart material. Mathematical modelling is critical in 4D printing (Fig. 5). It determines the duration for which the stimulus will act upon the smart material. This planning ensures the material undergoes the desired transformation within a specified timeframe [49].

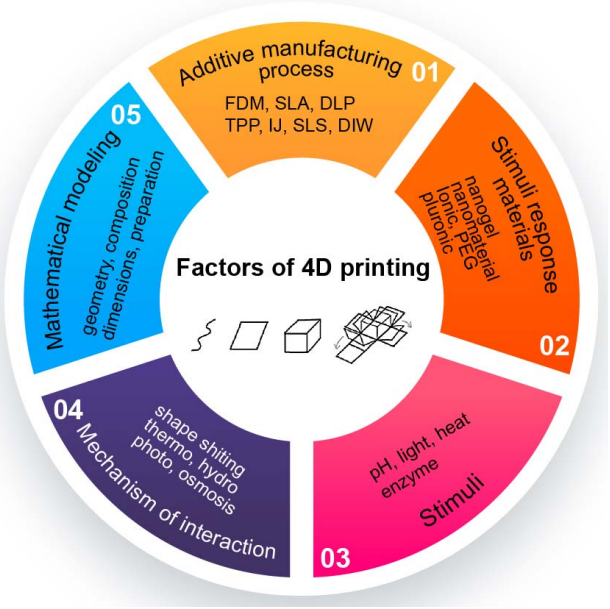

Figure 5. Illustration of the fundamental key factors of 4D printing

3.1 Bioinks and biomaterials

Bioinks are an important component of 3D bioprinting. They contain living cells and biomaterials that simulate the extracellular matrix microenvironment. They are cross-linked or stabilised during or immediately after the bioprinting process to form a final shape that simulates the expected tissue structure. The properties that an ideal bioink should have include printability, adjustable gelation and fluidity to ensure high resolution and rapid prototyping of printing, modifiable chemical structure to achieve printing of specific tissue structures, high biocompatibility to accommodate living cells and support cell attachment, proliferation and differentiation, and sufficient mechanical strength and stability to maintain structural morphology. The printability of bioinks depends not only on the viscosity, surface tension and cross-linking ability of the bioink itself but also on the surface properties of the printer nozzle. The hydrophilicity and viscosity of the bioink are key factors affecting printing reliability and living cell encapsulation [50].

The tissue and organ targets, cell types and 3D models to be printed determine the selection and modification of matching bioinks. Many biomaterials are used to formulate bioinks, which can be roughly divided into natural biomaterials and synthetic materials. Compared with synthetic materials, natural materials have advantages in composition or structure close to the extracellular matrix (ECM), self-assembly ability, biocompatibility and biodegradability. Collagen and hyaluronic acid are derived from natural ECM and are commonly used biomaterials for bioprinting 3D structures. Agarose is a natural polysaccharide extracted from seaweed with good gelation, mechanical properties and biocompatibility, but its ability to support cell growth is limited. Alginate is a natural biopolymer extracted from brown algae with advantages including low price and high flexibility. Other natural materials include fibrin, cellulose and silk (fibroin). Although natural polymers can provide the required microenvironment for cell attachment and proliferation to mimic the natural ECM, the adjustable properties of natural polymers are low. Therefore, natural polymers are often combined with synthetic materials or another natural polymer to obtain a more stable structure and enhance the tunability of 3D bioprinting. Commonly used polymers in 3D bioprinting include Pluronic block copolymers, polyethylene glycol (PEG) and polycaprolactone (PCL) [51].

The final product's ability to eliminate materials is linked to various 3D printing technologies. Bioinks are materials that are typically utilised in tissue engineering bioprinting. One bioprintable material is bioink. It comprises cells with biomaterials like hydrogel, spheroids and cell aggregates. The 3D hydrophilic polymeric materials known as hydrogels are created via physical or chemical cross-links, reversible or irreversible hydrogels, respectively. They show a unique capacity to absorb a lot of water without dissolving. Materials called hydrogels are pliable and squishy. They are often utilised above the temperature at which glass transition occurs and have a high degree of permeability to nutrients, oxygen and other water-soluble metabolites. Hydrogels can be used to prepare bioinks from synthetic and natural sources. Along the natural ECM, developed polymers like collagen, gelatin, alginate, hyaluronic acid and chitosan share biochemical characteristics. Still, they have some limitations to potential immunogenicity, mechanical properties, and batch-to-batch variableness, and normally require complex purification processes. Synthetic polymers constitute better properties concerned with mechanical and structural properties like high strength, controllable degradability and dominant microstructure. Synthetic polymers are used as widely applicable materials in 3D printing to supply these properties. However, synthetic polymers also have fewer limitations in their fabrication procedure [18]. Synthetic polymers include polyvinyl acetate, polyvinyl alcohol (PVA), propylene fumarate (PF), polyethylene oxide (PEO), polyethene glycol (PEG), polybutylene oxide (PBO) [52].

Bioinks should have excellent printability, defined by rheological properties, cross-linking nature and gelation kinetics, to produce tissue structures with high shape fidelity and resolution. In addition, they must exhibit sufficient biomechanical, bioactive and degrading qualities and be biocompatible. Furthermore, to ensure sufficient mass movement, bioinks must have submicrometric porosity [53]. Appropriate materials for bioprinting depend on printability, biocompatibility, mechanical properties, biodegradability and sterilisation stability [54]. Inkjet bioprinting has been used with various bioinks, including adipose-derived decellularised ECM (dECM) and bovine fibrinogen for the hypodermal compartment, skin-derived dECM and bovine fibrinogen for the dermal compartment and gelatin hydrogel for channels that can be pushed through and have blood vessels in them inside the skin tissue model. A DIY extrusion and inkjet 3D bioprinter was equipped with these bioinks [55]. Some examples of commonly used materials for laser-based bioprinting include collagen, fibrin, hyaluronic acid, alginate, gelatin, matrigel, chitosan, PEG and poly lactic-co-glycolic acid (PLGA). These materials can be altered to have distinct mechanical, chemical and biological characteristics appropriate for particular tissue engineering applications [56].

3.2 Application in tissue engineering

Recently, there has been a surge in the application of innovative and fascinating concepts within the field of 4D bioprinting. Novel techniques are emerging to multiply their applications in biomedicine and enhance printing precision. Notably, recent advancements in 4D bioprinting

have successfully addressed challenges that were previously considered formidable, such as creating microscale vascular models, establishing drug delivery systems in the gastric environment and developing muscle actuators. Major applications include bone tissue, cell regeneration, cancer treatment and biomimicry. With a more profound understanding of 4D bioprinting, significant attention is now directed toward its role in tissue regeneration, biomedical devices, smart actuators and healthcare products. Also, there are some applications in soft robotics and space solar systems. Researchers acknowledge that 4D bioprinting is well-suited to align with the physiological features of the body [57]. Polymers that are responsive to specific stimuli change when exposed to them. This makes it possible to build complex structures that behave like living tissues. Tissue engineering and regenerative medicine have made significant strides in overcoming challenges related to constructing intricate tissue and organ shapes and managing tissue microarchitecture with the aid of 3D printing. There is a growing demand for tissue mimics and scaffolds that can sense the dynamic tissue microenvironment and adjust their shape or chemistry accordingly. Stimuli-responsive materials and 3D printing should improve how the body responds to pathology. This will allow for minimally invasive surgeries and implant placement in places that would not be possible otherwise [58].

Biomimetic 4D printing has the potential to address the national organ scarcity in implantable organ development by providing options for research, transplants, repairs and medication testing. Research on its clinical applicability is still needed [59]. Tissue engineering offers a potential treatment for disorders associated with skin fragility, severe burns and surgical wounds. Printed skin provides faster healing times, less pain and possibly a more pleasing aesthetic outcome than skin transplants taken from parts of the patient's body that are not damaged. Additionally, patient skin grafts might only sometimes be possible, particularly in cases where there have been severe burns. Because the idea of self-healing is so ubiquitous, the material science community is very interested in this topic. The nerves bundled together to form the peripheral nervous system (PNS) are called axons and encased within. The nerve is the basic unit of the nervous system and is in charge of sending electrical impulses. One fantastic idea is to use 4D bioprinting to restore injured nerve tissue. Leng et al. [60] developed a conduit that is initially sealed but can be temporarily opened and secured to facilitate surgical procedures during conduit installation. Additionally, the printing material is made of a mix of graphene and soybean oil-epoxidised acrylate because it is very good at conducting electricity and helps nerves grow back. The 4D-manufactured conduits aid nerve regeneration by providing superior chemical and physical signals. Bioprinted skin may eventually be clinically available thanks to additive printing techniques' quick production, high volume capability and precision. A prototype skin printer is presented, targeting utilising a microfabricated cartridge to produce a skin substitute packed with cells [61-63].

4D bioprinting has great promise for the field of bone reconstruction. 4D printing is used in bone tissue engineering to produce injectable hydrogels, biomimetic microenvironments and shape-changing structures promoting healing (Fig. 6). Composed of 65 % inorganic material, 25 % organic material and 10 % water, bones can mend themselves. The key to solving biomaterial problems is to have high mechanical strength, porosity, biodegradability and a structured framework that looks like natural tissue. All transplantation using metallic fixators is the traditional method; however, shape memory polymers like polylactide (PLA), polycaprolactone (PCL) and polyurethane (PU) are becoming more and more popular due to their higher biocompatibility and biodegradability when used as scaffolds made of polymers. Enhancing bone regeneration may be possible through integrating time-dependent intelligent structures, a novel and little-studied idea [59]. With 4D bioprinting, a novel method for producing dynamic, compact-sized stents is presented, providing a customised, 3D-printed medical solution. Biocompatibility and alignment with human biological properties are issues addressed in various ways, such as shape-morphing biopolymer hydrogels, self-expanding/shrinking tubular lattices and shape-memory stents [64].

SLA-printed lattice structures have benefits for the biomedical field. Xu et al. [65] used photo-cross-linkable poly(trimethylene carbonate) resins with different amounts of hydroxyapatite (HA) to make a controlled scaffold. Putting HA nanoparticles in bone marrow stem cells made them more osteogenic and sped up the rate at which living things' bones healed. Twenty percent

of the HA showed the best results. This method uses SLA-enabled, hierarchically ordered biomaterials and optimised interfacial interactions to show customised biological and medical functions. However, reinforcement fillers may cause problems like higher viscosity, UV light scattering and overheating in some areas.

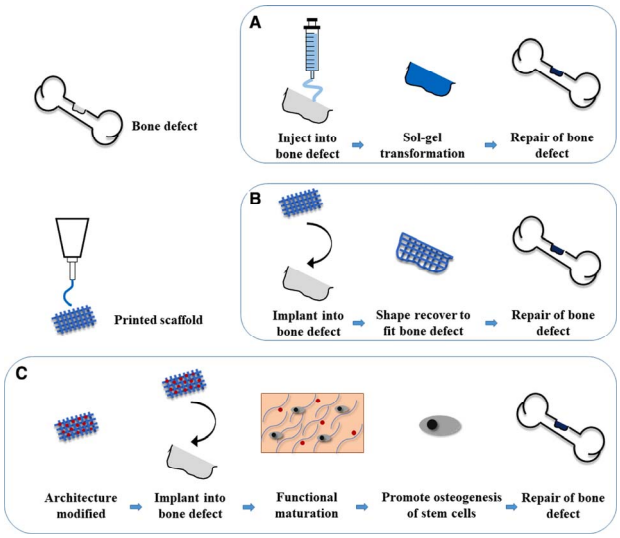

Figure 6. 4D printing in bone tissue engineering; reprinted from Wan et al. [59], licensed under CC BY-NC-ND 4.0

According to Mirani et al. [66], the prevalence of chronic illnesses has made wound care a global concern. This study presents GelDerm, a multipurpose dressing that can be used at the wound site to release antibiotics, test pH and identify infections (Fig. 7a). GelDerm exhibits accurate infection detection and is compatible with. When combined with patches, it reduces inflammation and maintains the release of antibiotics, perhaps helping to treat wounds from trauma, surgery or diabetes. Porous sensors were created using a 3D bioprinter fitted with a co-axial flow microfluidic nozzle (Fig. 7b). Sterilisation and lyophilisation of dressings are possible for storage and transportation (Fig. 7c). Design a prototype pH indicator for the sensors (Fig. 7d). GelDerm can adhere to uneven surfaces and maintain conformal contact (Fig. 7e).

The application of these technologies in bioprinting is covered along with an up-to-date comprehensive review of their positive and negative aspects and the diversity of materials used, emphasising composites, hybrids and smart materials. Elhadad et al. [67] provided an up-to-date overview of 4D bioprinting technologies, including biomaterial functionality, bioprinting materials and targeted approaches for various tissue engineering and regenerative medicine applications. Mechanical metamaterials are a class of functional materials with designability and extraordinary mechanical properties. The rapid development of 3D printing technology has provided an important means for manufacturing mechanical metamaterials with complex structures. In addition, further integration with smart material 4D printing supports the development of smart programmable mechanical metamaterials. Zhou et al. [68] progress in 3D/4D printing of mechanical metamaterials. First, single-material and multi-material 3D printing technologies and composite materials suitable for manufacturing mechanical metamaterials are discussed. Then, the structure and design of several common mechanical metamaterials in 3D printing, including 4D printing of smart programmable mechanical materials, are summarised, and the application of 3D/4D printing of mechanical metamaterials is introduced.

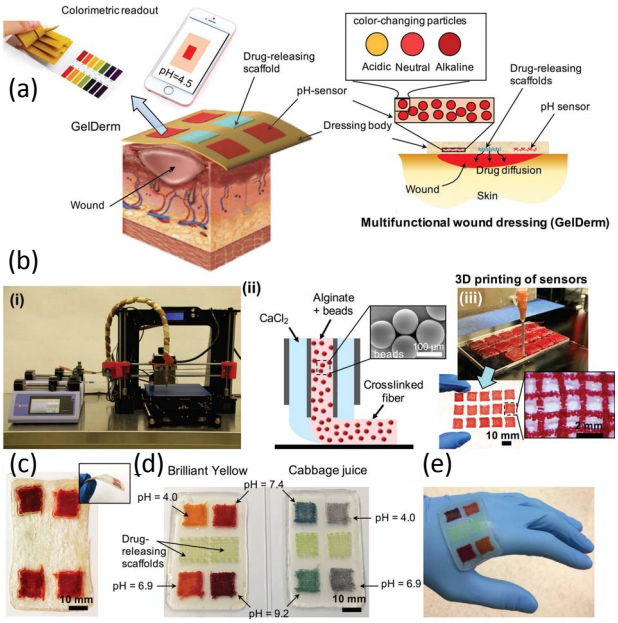

Figure 7. Multifunctional wound dressing: (a) diagram showing the pH-sensitive and drug-eluting components, (b) 3D bioprinter fitted (i), fibre deposition flow mechanism (ii) and bioprinter configured to generate porous sensor arrays (iii), (c) sterilisation and lyophilisation of dressings, (d) model creation and (e) 3D bioprinted GelDerm with conformal contact; reproduced from [66] with permission of Wiley

The composition and chemical modification of bioinks can affect their mechanical properties. Natural polymers require improved mechanical properties, which can be achieved by increasing the cross-linking density and polymer content.

Incorporating inorganic nanoparticles promotes cell adhesion, organisation and differentiation, thereby improving bioinks' mechanical and biological properties [69]. The biological activity and structure of synthetic polymers can be improved by modification. Adding methacrylate groups can increase the stiffness and mechanical stability of the polymer. Combining methacrylate polymers increases mechanical resistance. Thermoplastic fibre and hydrogel blends in printed soft tissues can improve mechanical properties and modulate degradation rates. Another way to increase mechanical strength without negative interactions is to blend polymers of different molecular weights [70].

Integrated organ printing (IOP) creates tissue-like structures with different cell types and mechanical properties. Derakhshanfar et al. [71] used IOP to fabricate a tendon unit with cell viability greater than 80 %. They used PCL to improve mechanical strength and a multi-head printer to create 3D liver tissue structures. Tan et al. [72] invented an in vitro lung-on-a-chip that mimics the mechanical and functional properties of the air-blood interface. Clinical applications will require improvements in biomaterials and bioprinting platforms, as current 3D bioprinted structures, despite improvements, still lack appropriate mechanical properties and stability. Bioink composition, construct design and printing parameters influence the ideal construct's desired architecture and mechanical properties. For tissue regeneration and surgical manipulation, mechanical strength is enhanced by adding synthetic materials such as PCL and PLA [73]. In biomedicine, shape-memory polymers (SMPs) are used in 4D printing to create bone scaffolds. Senatov et al. [74] developed porous shape-memory scaffolds for bone deformation using PLA combined with hydroxyapatite, with a shape recovery rate of 98 %. The authors used thermoplastic polymers to print shape-memory scaffolds with different fibre orientations to promote cell growth and mechanical strength. Polymers complemented with functional fillers can produce multifunctional shape-memory composites (SMCs) with excellent mechanical properties and remote controllable functions. Liquid crystal elastomers with biaxial deformation and thermally controlled reversible shape change are examples of progress [75]. Smart soft robotics and 4D-printed actuators require microrobots that can change shape and adapt. Shiblee and colleagues built thermosensitive and self-healing shape-changing actuators based on SMH. The structural transformations were triggered by temperature, water and magnetic stimulation, providing faster results [76].

3D printing improves mechanical engineering by using additive manufacturing, reducing material waste and enabling the creation of complex geometries. The technology uses materials such as metals, polymers and ceramics, using methods such as stereolithography (SLA), selective laser sintering (SLS) and fused deposition modelling (FDM) to produce prototypes, tools and customised parts. The advantages of this technology include high-precision customisation capability, rapid prototyping and one-time production, thereby reducing assembly work. It facilitates complex designs and enables on-demand production, reducing the time and cost associated with low-volume production [77]. In mechanical engineering, 3D printing uses FDM to produce laminated composites using materials such as PLA and PVC. Important factors like filler density, layer height and mesh orientation impact the material's tensile strength and microhardness. The advantages of this method include the ability to tailor products to specific requirements and create complex shapes. However, challenges remain to be overcome, such as optimising parameters and preventing delamination [78]. However, constraints remain, such as limited resources, reduced mechanical properties and complex post-processing. The future growth of 3D printing industries in the aerospace, automotive and healthcare industries offers the potential for improved material matching and cost-effective technologies, thereby increasing manufacturing efficiency and sustainability [79].

4D printing in mechanical engineering uses stimuli-responsive materials such as shape-memory polymers and hydrogels to create objects that change over time. Examples of applications include self-assembled structures, versatile biomedical devices and flexible robots. Advantages include complex resizing and programming capabilities. However, these problems reflect slow response times and limited carrying capacity. Methods such as modelled fused deposition and direct 4D printing have optimised these transformations, increasing mechanical efficiency and flexibility in transformation settings [80]. 4D printing is recognised worldwide for its ability to create objects that change shape, properties or

function over time in response to external stimuli. This technology offers significant geometric and functional advantages and shows great potential in biomedical engineering, electronics, robotics and photonics. 4D printing is used in mechanical engineering to create adaptive components, self-healing materials and dynamic structures. Important materials include shape-memory polymers (SMPs), liquid crystal elastomers (LCEs) and hydrogels. While this allows complex and adaptive designs, there are challenges in selecting suitable materials, increasing costs and ensuring precise manipulation of stimuli. Integrating machine learning could lead to other advances [81]. Recent advancements outside the biomedical field have highlighted the potential of 4D printing. There are wide possibilities for the applications of this technology, provided that measures are taken to reduce printing times, avoid structural degradation and discover more intelligent materials with better performance. If efforts to lower production costs continue, there may be more opportunities for 4D-printed objects to be utilised in households.

4. Conclusion and future perspectives

The development of 4D bioprinting signifies a revolutionary advance in tissue engineering, adding a temporal dimension to the manufacturing process. This innovative technique has the potential to create housing structures that can alter and adapt in response to their surroundings, which addresses the limitations of conventional 3D bioprinting. Growing across several fields, the implications of 4D bioprinting hold promise for advancements in biosensing, bioactuation, medication delivery, tissue engineering and wound healing. This revolutionary period holds the potential to completely rethink surgical techniques and scientific product development, delaying the need for scaffold replacements and bringing in a completely new era of precision technology for biomedical applications. The ability to build flexible, functional constructions that can adapt to changes in their surroundings and change their functionality signals a paradigm shift in the field of 4D bioprinting as the potential of this technology becomes more apparent. It may be possible to rethink surgical techniques, delay the need for scaffold replacements and change the face of scientific product creation by carefully replicating natural opposite numbers in artificial tissues. The capabilities of 4D bioprinting are in perfect harmony with the intricate plasticity, dynamic properties and distinct functions of human body tissues.

There are challenging circumstances along the way to fully understanding 4D bioprinting's potential. One major obstacle is the lack of advanced bioinks, which calls for materials that can meet the needs of 3D bioprinting while simultaneously demonstrating intelligent, dynamic properties for environmental adaptation. The absence of comprehensive data and systematic studies in the sector at the moment highlights the need for significant experimental work to improve the development of bioinks, mechanisms, bioprinters and structural layouts. The infancy of 4D bioprinting development highlights the need for a coordinated effort to deal with those difficult circumstances and clear the way for revolutionary advancement. In the future, 4D bioprinting's success depends on overcoming these obstacles and further improving the technology. Creating responsive materials that can self-deform in response to different physiological alarms should be the top priority for research. Enhancing printing resolution, microscale control over cellular alignment and algorithmic patterns that predict assembly progress are critical to pushing this technology to its limits. Finding the ideal balance between stiffness, biocompatibility and responsiveness in biomedical engineering will open up new possibilities. As time goes on, 4D bioprinting could reach unprecedented heights due to the convergence of material technology, printing production, software and numerical modelling. Beyond solving present tissue engineering problems, this new period holds great potential for overcoming future biomedical engineering obstacles with previously unheard-of accuracy and efficiency. The field of therapeutic innovation could witness a sea change as the era of 4D bioprinting approaches.

Finally, 4D bioprinting is an extension of 3D bioprinting and an innovation in biomedical engineering that offers a dynamic way to create live tissues. Even though it is still in its infancy, its transformational potential could change the course of tissue engineering and medical programs. The range of possibilities opens up as we navigate the difficulties and test the limits of this generation, indicating a future in which 4D bioprinting becomes an essential tool in medical advancements.

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