Methods 206 (2022) 1–7

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Methods for biomaterials printing: A short review and perspective

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ARTICLE INFO

Keywords:
3D printing
4D printing
Bioprinting
5D printing
Biomaterials
Printing technology

ABSTRACT

Printing technologies have opened larger windows of innovation and creativity to biomaterials engineers by providing them with the ability to fabricate complex shapes in a reasonable time, cost, and weight. However, there has always been a trouble with function adjusting in printing technologies in view of the multiplicity of materials and apparatus parameters. 3D printing, also known as additive manufacturing, revolutionized biomaterials engineering by the conversion of a digital subject into a printed object (implants, scaffolds, or diagnostics and drug delivery devices/systems). Inspired by the lessons learned from 3D printing, the concept of 4D printing (better called shape-morphing fabrication) was conceptualized and put into practice to reply on the need for responsiveness of the printed platforms to a stimulus (light, pH, temperature, voltage, humidity, etc.) in a programmable manner. Later, the next milestone in printing technology was reached by 5D printing, by which the desired objects could be printed from five axes compared to the upward one-point printing by 3D printers. 5D printers use ≈20-30% fewer materials comparatively, enabling the printing of curved surfaces. Nevertheless, all bioprinters need a bio-ink with qualified characteristics for the biomedical applications. Thus, we discussed briefly the cell viability, scaffold biomimicry, scaffold biodegradation and affordability.

1. Biomaterials printing methods

The story of 3D printing can be traced back to 1982 in Japan, when Hideo Kodama from the Nagoya Municipal Industrial Research Institute photopolymerized UV-curable resins layer-by-layer under UV light. This prototyping system, later named 3D printing/additive manufacturing, was defined as 2D printing or prototyping over and over again by taking a digital command from a computer [1]. In this process, a 3D printer recalls a digital image it has received from a computer and then transforms it into a standard triangulated language (STL) file format for printing. 3D printing technology was indeed revolutionary in the materials engineering realm, mainly because of the ability to fabricate complex geometries in the absence of hazardous chemical solvents with minimal production wastes and at a reasonable price. Quickly after, 3D printing entered biomaterials fabrication companies to advance some techniques such as surgical devices, fractured skull repairs, bone repairs, and implants [2]. However, the non-responsive character of 3D printing was a constraint in biomimicry. The programmable 4D printing was later amended fabrication of smart biomaterials that could morph into different forms for self-repairing, self-assembly, multi-functionality, and shape-shifting purposes by transforming over time. Accordingly, 4D printing technologies have undergone continued developments in response to physical (light, temperature, electricity, magnetic field, humidity, acoustic waves, and multi-stimulus combination), chemical (material/ion concentration and pH) and biological stimuli (cell traction force, enzymes, and biomolecule) fluctuations in the biological environments [3]. In parallel with accelerated progress in 4D printing technologies, more bio platforms were developing fast, but their physical and mechanical properties were not practically defendable. Thus, the 5D printing idea was introduced to print objects from five axes (to create curved surfaces) instead of one-way deposition by 3D printing that creates flat surfaces.

In principle, multi-dimensional printers are not necessarily nominated based on the fabrication techniques. For instance, 5D printing, better-called five-axis printing, should not be taken as the next episode of 4D printing story. In 5D printing technology, the print head (it moves) and the printable object (it prints) enjoy from five degrees of freedom. This superiority enables one to create curved layers with high resolution, accuracy, and strength (≈3-5 times stronger than 3D printed objects). In a short paper, Haleem and Javaid emphasized the potential of 5D

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https://doi.org/10.1016/j.ymeth.2022.07.016
Received 1 June 2022; Received in revised form 10 July 2022; Accepted 27 July 2022
Available online 30 July 2022

printing for the fabrication of orthopedic implants and artificial bones [4]. It is apparent that the inability to stimuli responsiveness was identified as the main shortcoming of the present generation of 5D bioprinting. In this sense, the idea of 6D printing has recently been established [5]. The 6D printing benefits from the combination of 4D and 5D printers to make the resulting biomaterials responsive. Similar to inspiration of 5D printing from 3D printing, one can postulate that 6D printers should be the next generation of intelligent 4D printing methods. In a detailed comparative view, fabrication methods, materials used, flexibility to biomaterials printing/responsiveness, and biomaterials application of all kinds of such printing methods are summarized in Table 1.

Although 6D printing idea is just introduced, the main focus is placed on more well-known 4D- and 5D printing methods for the fabrication of biomaterials. Theoretically, combination of 4D and 5D printing methods in a complementary manner should end in 6D printing, but practically it is far beyond experience and fabrication. The main difficulty would be the selection or finding the best candidate material possessing rigidity at the same time responsiveness to one or more stimuli. From a more realistic point of view, the association between printing mode and biomaterials' mission can be demonstrated by Fig. 1.

Indeed, all of the mentioned printing types rely on some common and specific needs. In general, they are highly sensitive to the rheological (shear rate, temperature, and ingredient concentration) and physiochemical (printability, viscoelasticity, *in-situ* gelation, biocompatibility and permeability) properties of the bioinks as well as the printing parameters (printing speed, extrusion rate, nozzle diameter, moving speed and height) [6,12]. Unlike 3D and 4D printers, 5D printing machines are programmable. In particular, 5D printing has the capability to create more complicated/anomalous rigid structures in various axes and angles (fixed in the *z* axis, but rotates reciprocally on the *x* and *y* axes), unlike one-way 3D printing (z axis). Although the fabrication steps in 5D printers are almost the same as those of 3D printers, the physical quality of the obtained structures is by far superior to the ones created by using 3D printers. For instance, a 5D-printed scaffold can resist a pressure four times more than a 3D-printed object because of the curved slices of layers formed in the former. Thus, the applicability of 5D printers for hard and complicated structured tissues like bone tissue engineering could be understood [4]. From an application point of view, however, the current optimization and standardization protocols are still far from ideal. All in all, 4D printers are the only ones taking the credit for bioprinting up to now because of being able to change the shape and the function of 3D printed platforms with time in response to a change in the surrounding environment. Table 2 illustrates the potent applications of 3D, 4D and 5D bioprinting in biomedical engineering field [14].

2. Bioinks

During the past few years, bioprinting technology has provided scientists with a great opportunity to print biologically active platforms, technically called biological inks or cell-laden inks (Fig. 2). Bioinks are liquid or semi-solid forms of biomaterials pertaining to two main classes: Scaffold-based ones, which chiefly exist in the form of microcarriers, gels as well as decellularized tissue, and Scaffold free platforms such as cell pellets [23]. Such dynamic structures must endorse cell growth, adhesion, and proliferation, enabling cells to secrete the extracellular matrix resembled the host tissue characteristics [24,25]. In this regard, one query may arise: What would be an ideal biomaterial providing the cardinal biological properties while possessing suitable mechanical properties/printability, enduring the applied physical stress and offering an integrated structure for the ultimate printed scaffold?

Natural biopolymers are the best possible options among the existing ones because of possessing good biocompatibility, cytocompatibility, strong hydrophilicity, efficient interconnectivity (neither exaggerated missing the cells signals nor less than the appropriate value supporting cell migration, nutrient diffusion as well as cell/matrix bio interactions) as well as producing no or very few toxic by-products during the degradation process [29,30]. Additionally, natural biopolymers (polysaccharides and proteins [31]) not only have the capability to be mechanically enriched after being combined with chemical or physical crosslinkers, but also provide the experts with the opportunity to perform post-printing modifications [32]. However, some of their serious limitations are undeniable. For instance, immunogenicity, structural instability, slow gelation time, unoptimized rheological properties (e.g., undesirable viscosity), weak protein absorption capacity as well as low printability have caused concerns finding more practical biomaterials [33]. Apart from some few categories of synthetic biomaterials (e.g., silicones, poly ethylene glycols and polyurethanes), the rest of them are comparatively not useful for usage as bioinks because of not being biologically supportive and producing toxic byproducts [34]. In this section, we tersely encode some of the main prerequisites of suitable bioinks to have a broader view of qualified and

Table 1
Comparison of 3D, 4D and 5D printing technologies in terms of fabrication method, materials used, flexibility to printing, and main applications in biomaterials fabrication.

Variable	3D	4D	5D	Comment	Refs.
Fabrication method	Layer-by-layer printing made of 2D layers by computer-aided design (fabricating flat surfaces without stimuli-responsiveness)	Layer-by-layer printing through computer-aided design (fabricating surfaces with self-transformation ability or stimuli-responsiveness)	Layer-by-layer printing by computer-aided design (fabricating curved surfaces without stimuli-responsiveness)	Structural quality of 3D and 4D printed scaffolds is not practically high	[6–8]
Materials	Thermoplastics, ceramics, metals	Stimuli responsive biomaterials such as polysaccharides; Chitosan (sensitive to enzyme, glucose, pH, and electric field), sodium alginate (sensitive to pH and temperature), hyaluronic acid (sensitive to tension and temperature), agarose (sensitive to temperature and electric field)	Highly printable materials, similar to the ones used in 3D printing	Privatization per application seems to be necessary	[9,10]
Flexibility	Inflexible	Flexible	Rigid, more than the rigidity expecting from 3D printed objects	Designing scaffolds with both flexibility and time-related changes seems to be essential, at the same time the main challenging feature	[10]
Applications	Artificial scaffolds and tissue regeneration	Targeted drug/gene delivery and smart medical implants	Dental and bone tissue engineering	Focusing on employing printing techniques is challenging and controversial areas such as cancer diagnosis and treatment	[11–13]

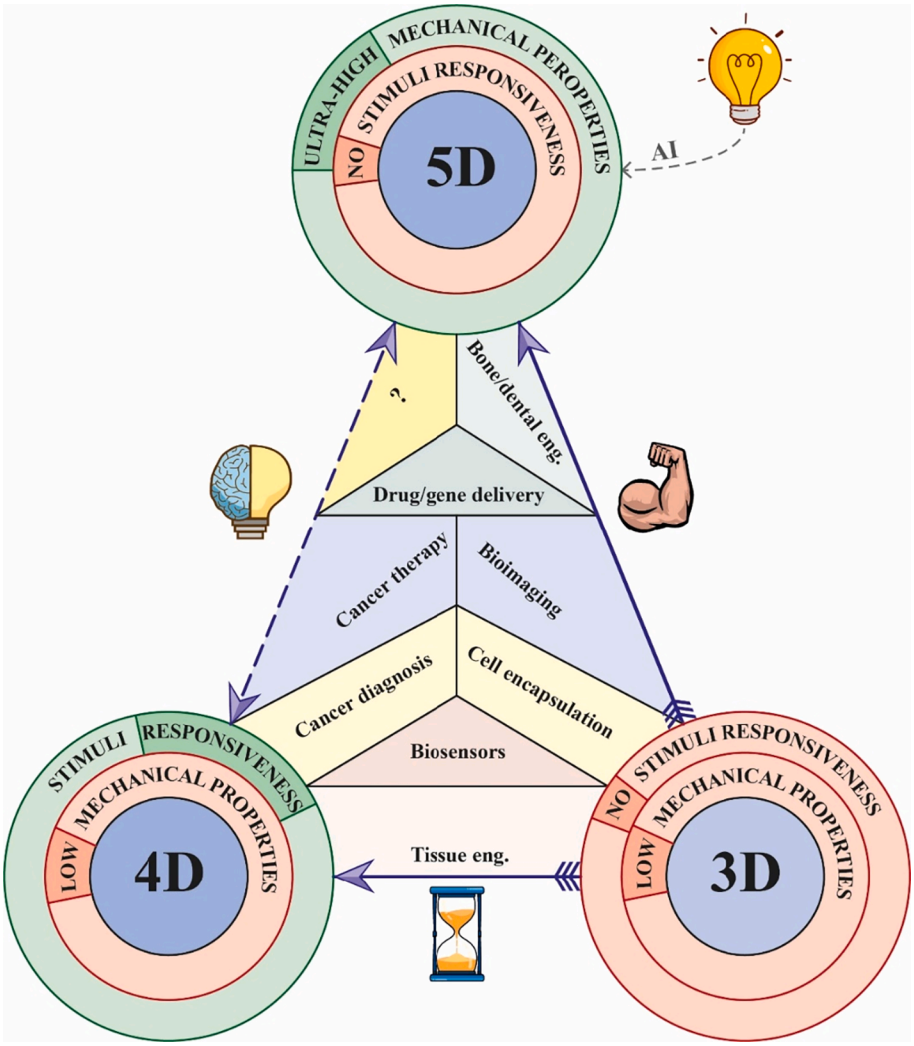


Fig. 1. The brainstorming in biomaterials printing technologies: The advent of 4D and 5D printing, inspired by 3D printing, has revolutionized biomaterials engineering. The triangular brainstorming puzzle provided herein attempts to highlight some weaknesses and/or advantages of printing technologies. In response to the need for smart biomaterials with stimuli responsiveness, 4D printing was born from 3D printing concept by giving the time function as a new dimension to the 3D printers. On the other hand, higher mechanical strength was required for hard tissue engineering, which provided scientists a ground for further innovations leading to the advent of 5D printing. We believe that the concepts of 4D and 5D printing technologies can impart stimuli-responsiveness and mechanical strength in a complementary manner to a more robust and versatile bioprinting technique. Nevertheless, fine adjustment of materials and printer parameters is a *priory* in making biomaterials remoteable.

non-qualified biomaterials.

2.1. Cell viability

The first and foremost biological feature offered by a dynamic printed scaffold is extracellular matrix remodeling and stimulating cellular mobility after being encapsulated inside the scaffold. Notably, the structural properties of the scaffold govern the composition of the cells' secreted proteins and morphogens finally adjusting the cells' migration patterns. The molecular dynamics and orientations of the biomaterials as well as the geometrical characteristics leave essential traces on cellular internal and external signaling activities [35]. Furthermore, there are some structures among polymers (e.g., integrin and dopamine), which play key roles in cellular adhesion to the matrix [36]. Cell viability is also adversely affected by the shear stress applied by ultra-viscous bioinks. Typically, the higher the viscosity, the more the damage exerted to the cell membrane leading to cell necrosis [37]. On the other hand, high viscosity entails the higher quality of the printed scaffold, which is another desired aspect. One resort to print exceptionally hydrated gels is to moderating the applied stress to the cells while preventing microenvironment dryness and diminishing the cell necrosis [38].

Noteworthily, the preparation method is another facet affecting the cell viability. For instance, preparation of hydrated gels *via* photocuring procedure adversely affects the cellular metabolic activity due to the light exposure. On contrary, some efficient techniques have been suggested to accelerate the cytocompatibility [39]. For instance, some used a decellularized tissue as bioinks and surface chemical modification with some biomolecules (e.g., chitin) to enhance the cell viability [40]. However, one main concern associated with using decellularized tissue as bioinks is the degradation of scaffold after exposure to matrix metalloproteinases secreted by the seeded cells [41,42].

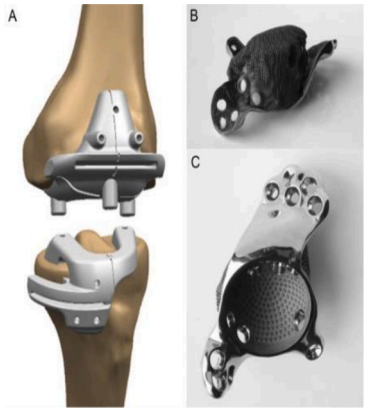
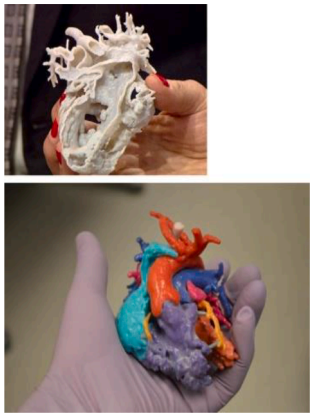
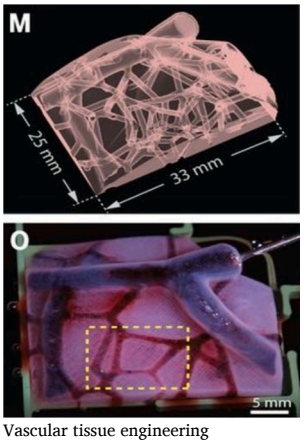
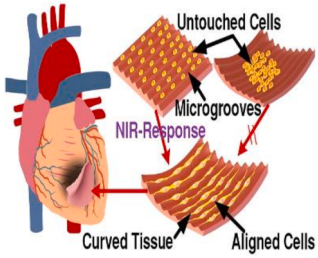
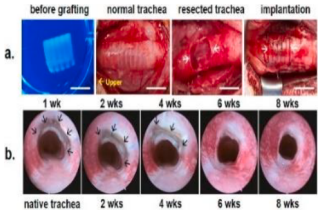
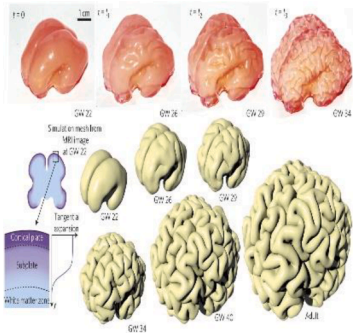
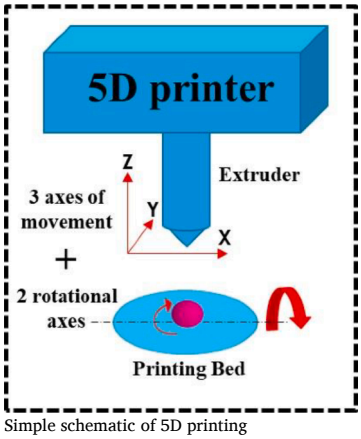
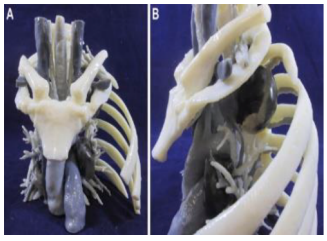
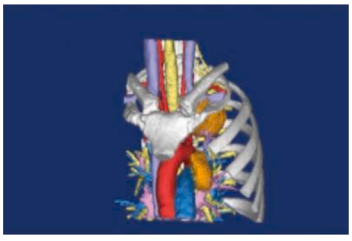
2.2. Scaffold biomimicry

Biomimicry is a crucial feature to be considered, particularly for cell-laden bioinks. Such platforms chiefly suffer from lack of dynamic cellular interactions as well as heterogenous distribution of the cells leading to inaccurate recapitulation of the native modelled tissue [43]. Furthermore, conservating cellular morphology, cellular stability as a function of time, cell–cell and cell-matrix interactions resembled to the real tissue as well as optimum cellular density (neither too much to narrow the cells' media to reproduce nor too little to jeopardize cellular exchanges) are the other important factors centered to scaffold biomimicry. Noteworthily, apart from printing method, biomaterials concentration and viscosity strongly affect the biomimicry [44].

2.3. Bioinks' biodegradation

Bioinks degradation profile (either in terms of time or the percentage of the remained mass) is not only dependent on the external factors such as temperature, pH, the presence of enzymes and vibration, but also

Table 2
Application of 3D, 4D, and 5D printing methods in biomaterials engineering. Accordingly, 3D bioprinting is utilized in orthopedics, heart and vascular tissue engineering. 4D bioprinting is well-known for being utilized as adjustable curvature for myocardial regeneration, trachea transplantation as well as tangential cortical expansion. Moreover, 5D printers are utilized to fabricate complicated bones and clear representation of tumor invasion to surrounding structures.

Printing type	Applications			Refs.
3D	 Orthopedics	 Heart tissue engineering	 Vascular tissue engineering	[15–17]
4D	 Myocardial regeneration	 Trachea transplantation	 Cortical expansion	[18–20]
5D	 Simple schematic of 5D printing	 Lung tumor invasion to surrounding structures	 Anatomic model of lung tumor invasion	[21,22]

relies on the chemical composition of the printed scaffold, the existence of nanoparticles or nanofibers, polymer chain length, as well as scaffold surface modification. This is why evaluation of *in vitro* degradation of the printed scaffold is usually performed within a phosphate buffer saline (PBS) solution with a specific pH of 7.4 in a shaker incubator at 37° C as a function of time and biomaterials compositions [45]. Importantly, biodegradation pattern needs to be smartly tuned considering the fact that soon degradation leads to the deformation of the cells' media and late degradation causes cell congestion and death [46]. This becomes more critical when drug is loaded inside the polymer chains and the release time is short.

2.4. *Affordability*

The costs associated with the preparation of bioinks can be classified in three main categories, biomaterials-related expenses, the opted technique as well as the storge costs. However, bioinks derived from natural biopolymers are mostly available and cost-effective [47]. Additionally, natural bioinks incorporated with synthetic nano/microcarriers are reasonably priced considering the fact that the weight proportion of nanostructures (nanoparticles or nanofibers) in the matrix is usually below one percent. From fabrication standpoint, the bioprinting process is not quite cheap because of being prolonged and requiring expert performers. Financial issues become more demanding when it comes to extracellular matrix-based bioinks derived from the decellularized tissue, which are associated with exorbitant expenses [45].

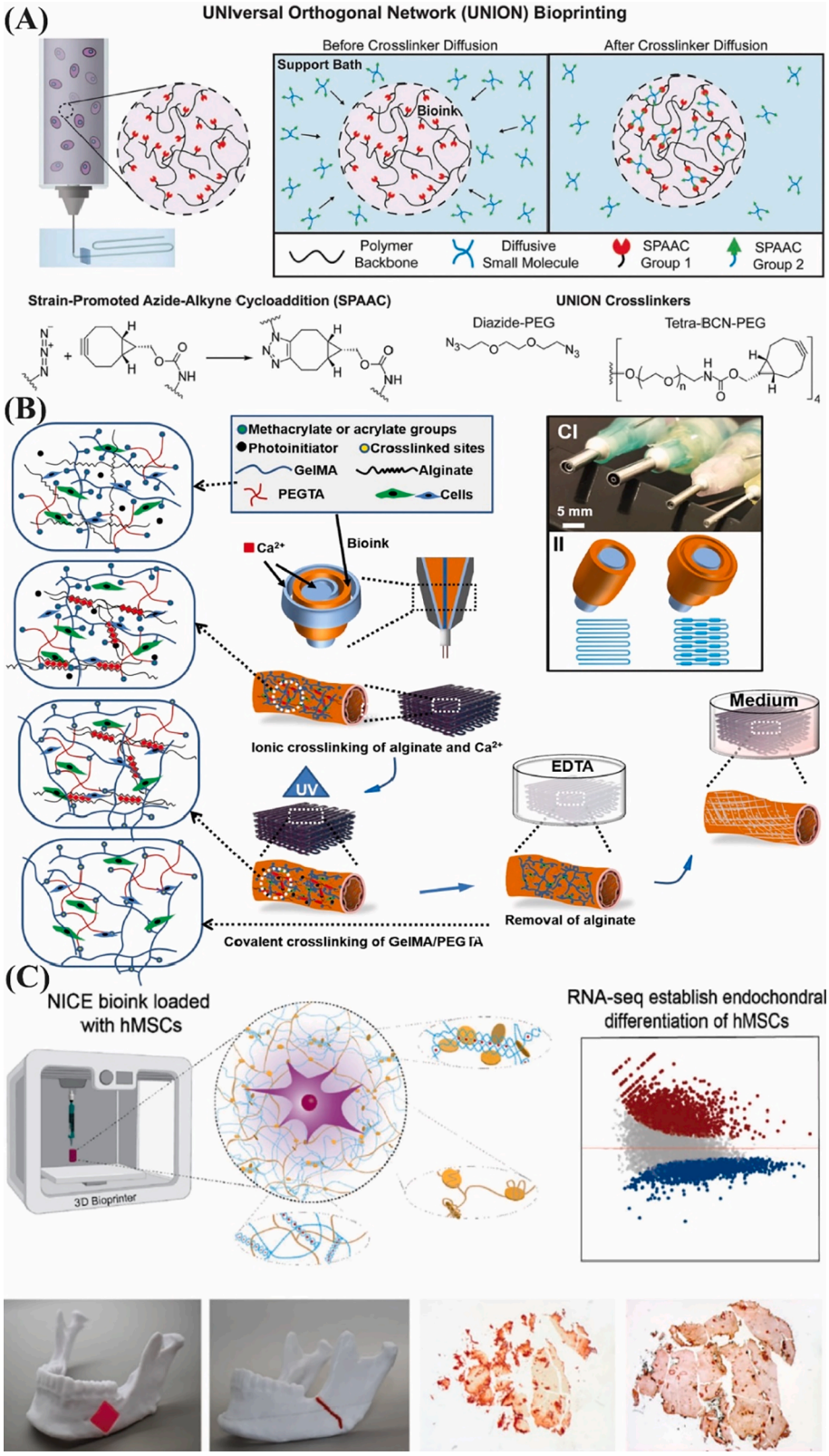


Fig. 2. Schematic illustration of some instances of bioinks used for XD bioprinting: (A) A cell seeded polyethylene glycol-based ink extruded into a gel support bath and mechanically improved after the addition of crosslinker [26]. (B) Covalently crosslinked alginate-based ink utilized for printing hollow tubes after being exposed to $CaCl_2$ solution and UV light [27]. (C) Gelatin/carrageenan osteoconductive ink seeded with human mesenchymal stem cells used for bone tissue engineering [28].

3. Present status and future portfolio

We believe that future direction in using bioprinting methods for biomaterials engineering should be looked from a need-based perspective. Indeed, brainstorming ideas widen the windows of innovation to come up with appropriate printing techniques compared to the presently available ones. Nevertheless, current devices may be provisionally reliable for target purposes. We also argue that the capabilities of 4D and 5D printers must be integrated into a sophisticated, versatile printing machine. In the meantime, our state of knowledge would be increased to propose practical guidelines in order to face penalties and shoot temporal troubles. For example, among the potential applications of 5D

printing is the cancer decoding, which facilitates surgical planning, precise decision-making in selecting margins for resection, the anticipation of possible difficulties or dangers, and more accessible instruction to learners [22]. Moreover, the anatomical models of cancerous tissue imparted by computed tomography, magnetic resonance imaging (MRI), and positron emission tomography scans before and after the treatment can provide invaluable information about the tumor and its microenvironment. Monitoring the distortion of the tumor's anatomy, tumor invasion of the surrounding structure, and aberration after neoadjuvant treatments are other factors critically determinative to the surgical programming by using 5D printers [13]. Nevertheless, sophisticated bioprinting by 5D machines requires the segmentation of images received from an anatomic model [22]. The powerfulness of the newly developed printing machines may be reflected in the future in terms of surgical anatomy, the physiology of the body, the responses of organs in the cancerous cells, and, more critically, mimicking the growing organ in a dynamic manner by revisiting data assimilation [48]. Besides the aforementioned parameters, engineering of properties of bioinks is a state-of-the-art. Overall, challenging aspects of such methodologies are most frequently viewed through the lens of cell viability and scaffold biomimicry. A versatile bio-ink fabrication method is mainly grounded on adjustment of cell viability and scaffold biomimicry, such that other requirements including scaffold biodegradation and affordability are governed the optimization of the first two factors. However, the multiplicity of parameters affecting cell viability and biomimicking character of scaffolds remains some challenges to be considered in future investigations. Bearing in mind the dimensionality of bioprinting methods from 3D to 4D and 5D, or recently introduced 6D bioprinting, necessitates continued recognition and demonstration of mechanisms underlying the efficiency of bioprinting methods.

4. Unanswered questions

To name, possible challenges of biomaterials printing can be highlighted by a few basic questions:

- Does the printing process itself govern the possible response of bioinks or seeded cells to the applied stimuli?
- Does the cell seeding process affect the responsiveness of bioinks?
- Does the dynamics of biomaterial disrupt the cell metabolic activities in a bioprinted scaffold?
- Is it possible to print scaffolds or implantable medical devices erasing the concern of size alteration?
- Does the printed tissue reveal an integrated and robust interaction with the host tissue and the native microenvironment?
- How would the printed tissue react under pathological circumstances or when surrounded by the immune system?
- How could one propose a bio-ink with multiple stimuli responsiveness capable of neuro-regulation and humoral regulation?
- How could one provide printed scaffolds with the detectability of image procedures, considering its remote controlling role in response to chemical, physical and biological fluctuations in an implanted scaffold?

Besides the aforementioned uncertainties, there are some blind spots in printing-based innovations. For example, bone repair strategies are inevitably centered to 5D printing-based biomaterials engineering. The concern about providing appropriate cell media for bone marrow cells may dial up the concerns associated with the printability of soft textures. Correspondingly, the next generations of 5D printers must be capable of capturing the stimuli responsiveness of biomaterials by online monitoring of tissue evolution, named 6D printing. In other words, the subtle balance between the stimuli responsiveness and mechanical strength, cell growth and differentiation, and tissue adaptation imparted by the 4D and 5D printing methods can significantly affect the successin the clinical phase. Along with the bioengineering considerations, efficient and professional training workshops for laboratory researchers, physicians, and device engineers, besides economic and sustainability requirements, must be orchestrated.

CRediT authorship contribution statement

Hanieh Shokrani: Writing – original draft, Investigation, Formal analysis. **Amirhossein Shokrani:** Methodology, Formal analysis. **Mohammad Reza Saeb:** Conceptualization, Supervision.

Declaration of Competing Interest

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

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