PERSPECTIVE ARTICLES

3D bioprinting processes: A perspective on classification and terminology

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Abstract: This article aims to provide further classification of cell-compatible bioprinting processes and examine the concept of 3D bioprinting within the general technology field of 3D printing. These technologies are categorized into four distinct process categories, namely material jetting, vat photopolymerization, material extrusion and free-form spatial printing. Discussion will be presented on the definition of classification with example of techniques grouped under the same category. The objective of this article is to establish a basic framework for standardization of process terminology in order to accelerate the implementation of bioprinting technologies in research and commercial landscape.

Keywords: additive manufacturing; 3D bioprinting; material jetting; material extrusion; vat photopolymerization; bioassembly

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1. Introduction

Biofabrication, bioprinting, and bioassembly are three terms that have received great attention in recent years[1,2]. The definition of biofabrication has been highlighted as "the automated generation of biologically functional products with structural organization from living cells, bioactive molecules, biomaterials, cell aggregates such as microtissues, hybrid cell-material constructs through bioprinting or bioassembly"[1]. Defining terminologies and technologies in the field of biofabrication is much needed to establish a framework and provide a reference map to the research community. Recently, researchers proposed a refined working definition of Biofabrication, including Bioprinting and Bioassembly as complementary strategies within Biofabrication. While Moroni *et al.* established a guide for terminology in biofabrication and defined the efficiency of the respective biofabrication technologies using a metric system called spatial resolution/time for manufacturing (RTM) ratio as a quantitative characterization of the processes[2]. Several reviews have also highlighted current bioprinting technologies and compared the print resolution between the different technologies[2–5]. These published articles have provided the necessary basic clarity on the definition of biofabrication. However, much work is still needed to further categorize the 3D bioprinting process in terms of processes and terminology.

The field of 3D bioprinting started from the idea of combining additive manufacturing (AM) or 3D printing which uses layer-by-layer fabrication techniques with living organisms and biomaterials to produce complex tissues *in vitro*[6]. While there is much advancement in this field, the academia and industry have seen a lower adaptation rate of 3D bioprinting compared to AM due to the lack of clarity in processes, materials and applications. There exist some form of mismatch between these three.

This article aims to provide a classification of 3D bioprinting processes that have been reviewed and described in detail in numerous reviews[3,7,8]. The details

of these processes are not the focus of this perspective article. Instead, the paper examines the concept of 3D bioprinting within the general technology field of 3D printing (Figure 1). Discussion presented include the definition of classification, example of techniques grouped under the same process, the range of materials and resolutions obtained and a summary on the application strategies of these techniques.

2. Classification of Cell-compatible 3D Bioprinting Technologies

In Groll *et al.*[1] and Moroni *et al.*[2], bioprinting refers to the use of computer-aided transfer processes for patterning and assembling living and non-living materials with a prescribed 2D or 3D organization to produce bio-engineered structures. In this article, we proposed that these technologies can be categorized into four distinct process categories (Figure 2), namely material jetting, vat photopolymerization, material extrusion and bioassembly. The classifications are based on the classification methods established for standardization of terminology in additive manufacturing with reference to the standard document ISO/ASTM 52900:2015-12 Standard Terminology for Additive Manufacturing.

2.1 Material Jetting

Material jetting is a process in which droplets of build material are selectively deposited onto a build bed to develop a three-dimensional object[9,10]. The process

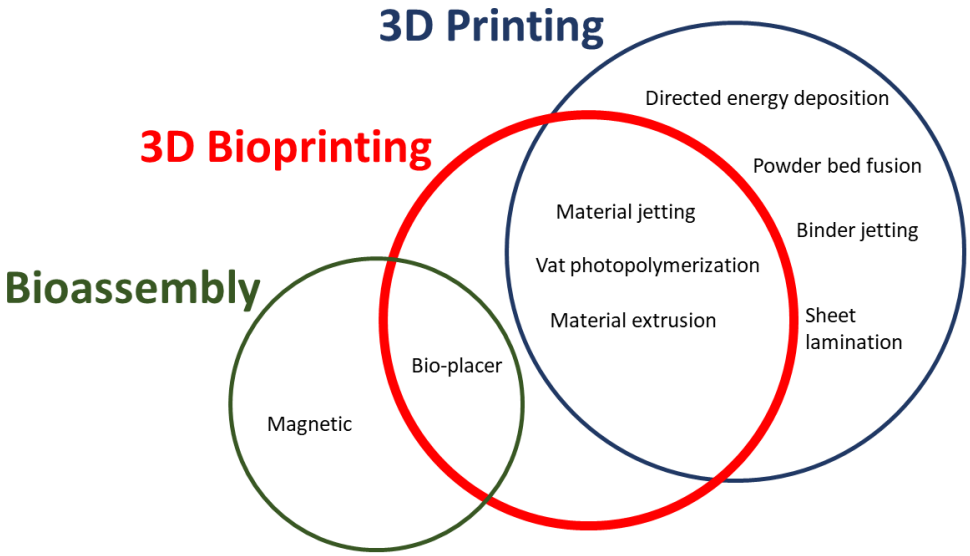

Figure 1. Process categories of 3D printing and proposed 3D bioprinting process categories

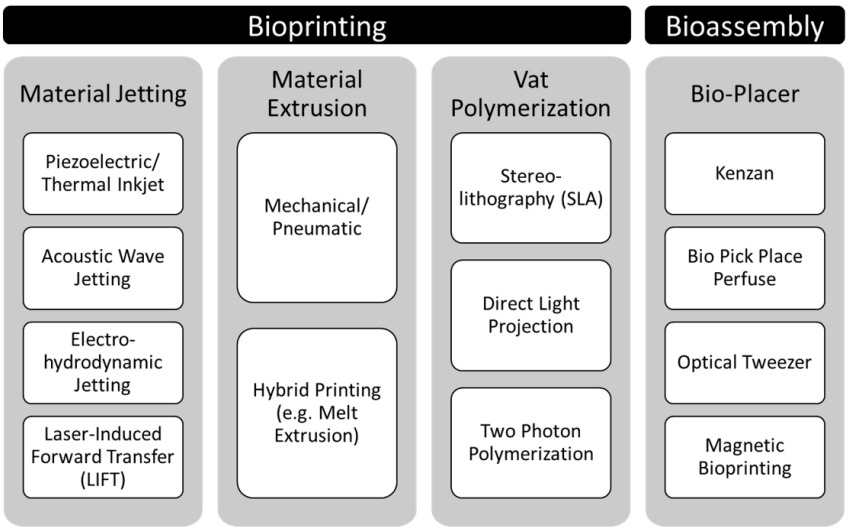

Figure 2. Categorizing biofabrication technologies and differentiating cell-compatible technologies

involves formation and deposition of droplet material and displacement of material with or without cells onto a desired spatial position. Bioprinting technologies that form constructs based on droplet deposition include piezoelectric/thermal ink jetting, acoustic wave jetting, electrohydrodynamic jetting and laser-induced forward transfer (LIFT)[11–17]. Piezoelectric/thermal ink jetting uses piezoelectric/thermal force, which generates pressure pulsed in the nozzle to expel droplets (Figure 3A). In contrary, acoustic wave jetting employs acoustic radiation force generated by acoustic actuator to produce droplets (Figure 3B) and electrohydrodynamic jetting applies an electric voltage to form droplets (Figure 3C). In the case of LIFT which is nozzle-free jetting technique, a focused laser hits an absorbing layer generating a high-pressure bubble that propel droplets towards the desired build bed (Figure 3D).

Material jetting has been widely utilized in tissue engineering using a range of hydrogels including alginate, agarose, collagen, fibrinogen and thrombin, gelatin methacryloy (GelMA) *etc*. Xu *et al*. utilized piezoelectric ink jetting to fabricate vascular-like tubes using alginate material, which mimicked vascular constructs[18,19]. Coppi *et al*. employed thermal ink jetting to embedded human amniotic fluid-derived stem (AFS) cells in an alginate/collagen scaffold. The printed construct incubated *in vitro* in osteogenic medium before implantation into immunodeficient mice[20]. Michael *et al*. utilized LIFT to create a fully cellularized skin substitute. This construct implanted into mice and formed a tissue similar to simple skin after cultivation[21]. Demirci and Montesano demonstrated encapsulation of single or a few cells ejected from an open pool using acoustic droplet ejection, and showed the potential of using this technology of printing cells in various biological fluids and hydrogels[22].

Material jetting, as a droplet-based technique, provides a high-throughput method with great advantages due to its agility to precise control on displacement of biologicals and to the ability to provide high resolution. Recent developments of those aforementioned techniques have reported that the general size of jetted droplets is in the range of 1 pL to 7000 pL in volume[23]. Further, smaller volume enables higher resolution (lower to 10 μm)[7,24]. Another advantage of material jetting is that it allows to print cells or materials with a gradient concentration throughout the 3D structure by varying droplet densities or sizes[25]. It also provides great promise enabling "scaffold free" bioprinting by direct

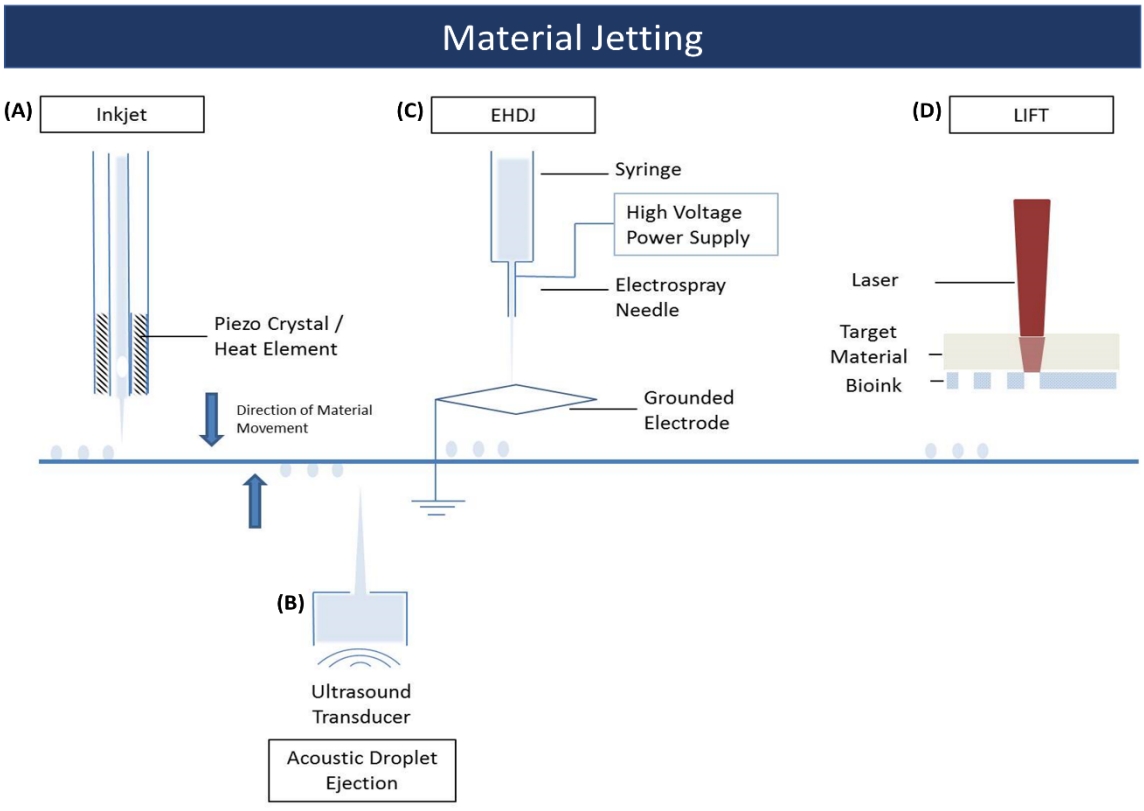

Figure 3. Materials jetting: (**A**) Piezoelectric/thermal ink jetting. (**B**) Acoustic wave jetting. (**C**) Electrohydrodynamic jetting. (**D**) Laser-induced forward transfer (LIFT).

deposition of cells.

2.2 Material Extrusion

In material extrusion techniques, biomaterials are extruded from the nozzle producing defined structures[26–28]. Extrusion-based printing can be divided into three categories based on the mechanism for extruding materials. Mechanical extrusion uses motor to drive piston downwardly, where material extrusion is determined by rate of motor's displacement; Pneumatic dispensing system supplies air pressure into the syringe cartridge, where the difference between supplied air pressure and ambient pressure drives the flow of material; the third setup uses a rotary-screw, where the angular turn of the screw affects the amount of material extruded. In extrusion-based bioprinting, both pneumatic[29–38] and mechanical systems[39,40] have been commonly used for dispensing cell-hydrogel (Figure 4). The use of rotary screw extrusion in bioprinting has been limited only when high viscosity material such as PCL melt is used (Figure 4)[40]. Alternatively, valves can be placed at the nozzle to regulate the flow of the hydrogel[41]. Such printing method is also termed as microvalve bioprinting[42]. Droplets or strands of hydrogel can be dispensed using microvalve bioprinting[43].

With adjustable pressure setting, extrusion-based bioprinting can process material with wide range of viscosity 30–6 × 10^7 mPa.s[44]. A printing method termed as conformal printing involves deposition of Agarose hydrogel filament that act as support structures for subsequent cell-hydrogel filament[39]. This conformal printing strategy can also be observed in hybrid printing of construct with thermoplastic filament adjacent to cell-laden hydrogel filament. Hybrid bioprinting, that integrates other fabrication methods such as melt-extrusion and electrospinning with bioprinting, fabricates construct with enhanced structural fidelity due to the additional scaffolding material[45–51].

Resolution from material extrusion technology is determined by variables such as nozzle size, applied pressure on material, printing speed, substrate wettability, and printing temperature[44,52,53]. Highest resolution of 15–400 µm has been reported[30,37,39–41,45,49,54–59].

2.3 Vat Polymerization Printing

In vat polymerization printing (VPP), a container filled with cell-hydrogel suspension is subjected to selective curing of polymer to form 3D structures. The components of vat polymerization printing system used for bioprinting resembles closely of those from additive manufacturing counterparts. VPP systems comprise of an energy source that selectively initiates the polymerization process within the entire vat containing photosensitive polymer. Three dimensional constructs are formed point by point through laser curing in stereolithography (SLA) (Figure 5). Alternatively, UV light can be area-projected in digital light processing (DLP) (Figure 5) into the vat of photopolymer using digital micro-mirror device[60–62].

Hydrogels used in this printing system are light sensitive photopolymers such as polyethylene glycol–diacrylate (PEGDA) and gelatin-methylacryloyl (GelMA). Notably, there has been increase research interest in using non-UV based systems for

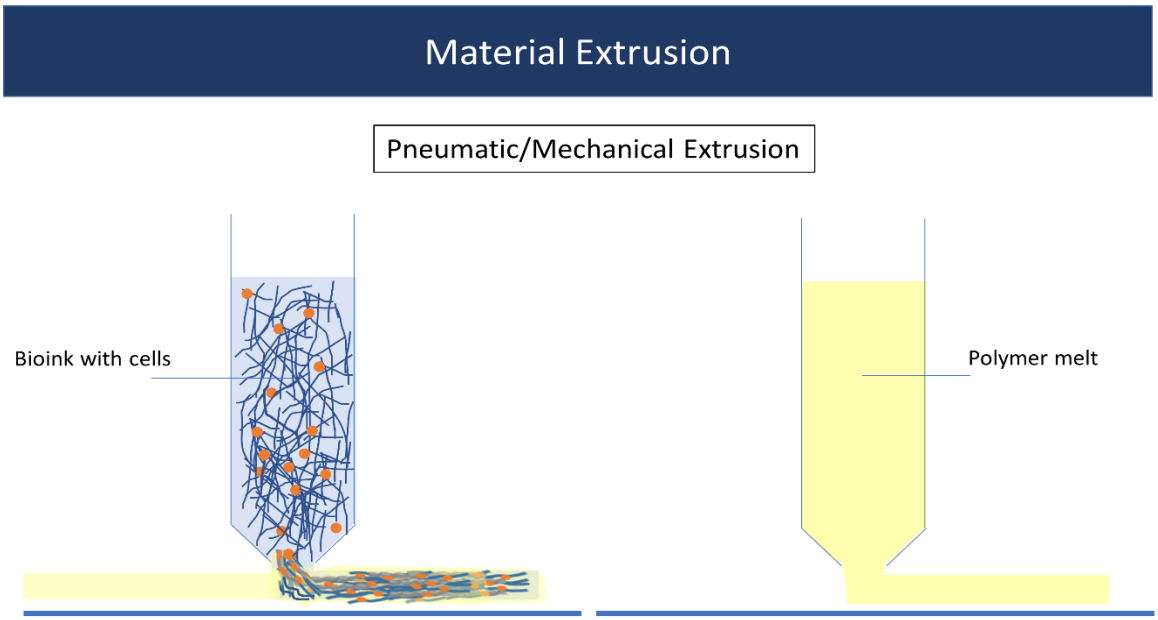

Figure 4. Illustration of Material Extrusion with/without cells.

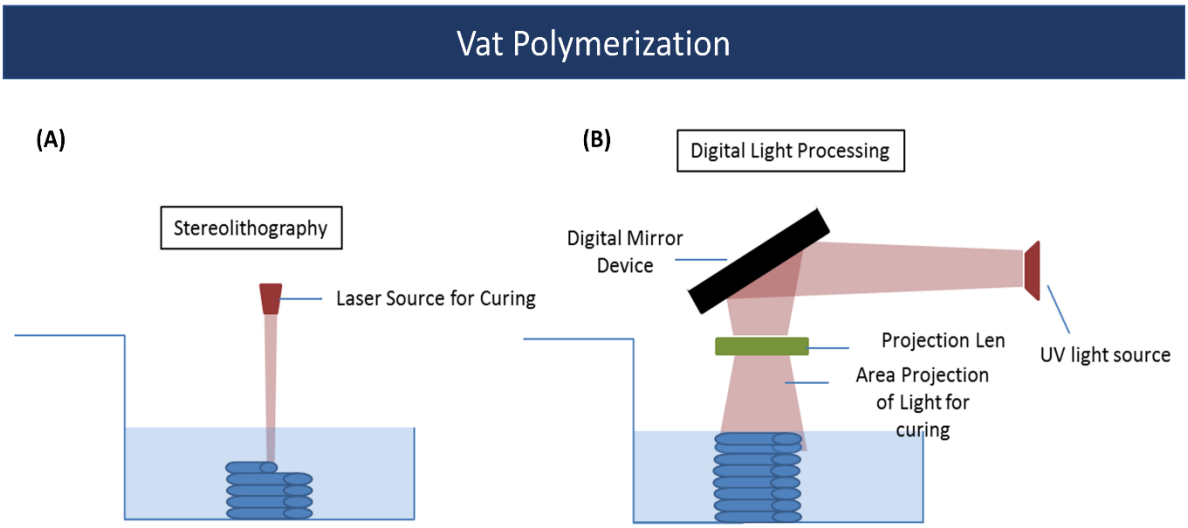

Figure 5. Vat polymerization: (**A**) Stereolithography. (**B**) Digital Light Processing.

polymerization[63,64]. UV light has been reported to damage cell DNA which may be detrimental for 3D bioprinting[65,66]. Photoinitiators such as eosin Y and lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) are used for curing photopolymers under visible-light.

Of the current vat polymerization-based printing system, digital light processing technique prints constructs with higher resolution and at a fast printing speed than optical projection systems. An *in vitro* triculture liver tissue model made of complex 3D honeycomb pattern was printed using dynamic optical projection stereolithography[67]. The features printed using the DLP-based system mimics the *in vivo* features of the liver. The triculture model with biomimetic liver lobule features presents a physiologically relevant model with great potential in pathophysiological studies and drug screening applications. Other than higher resolution and faster fabrication speed, DLP-based system can produce complex designs with lumen-like features to support maturation of tissue construct. For instance, a pre-vascularized tissue with an intricate network mimicking the branching system of vasculature was printed using DLP-based system[61]. Resolution from VPP technology is determined by variables such as curing time, curing depth, wavelength of light source. The highest resolution reported in the literatures can be in the range of 5–100 μm[60,61,68].

2.4 Bioassembly

In many reports, bioassembly is defined as the fabrication of hierarchical constructs in prescribed organization through automated assembly of pre-formed cell-containing fabrication untis[1]. In this article, we propose the name Bio-Placer, which include processes such as pick and place of spheroids and magnetic bioprinting (Figure 6). These pre-formed modular units of cells may exist as spheroids[69–71], cylindrical rods[70], and even sheets (toroids and honeycombs)[72]. The relationship of bioassembly with other 3D bioprinting proceses will depend on further evolution and development of the definition as well as the bioassembly technology.

2.5 Pick and Place of Microtissues

Other than the methods that requires delivery of spheroids through suspensions, robotic arms have been designed to directly manipulates spheroids in a pick and place manner. One of such pick and place method is described as Kenzan method[73,74]. The setup comprises of needles that are arranged in arrays for placement of cell spheroids (Figure 6A). A suction is used to pick and transfer spheroids from the well plates and onto the needle arrays. The spheroids are placed in a pre-defined configuration. Of which, the spheroids on needle arrays are incubated for several days prior to removal from the array platform. Another approach that directly picks microtissues using a custom-made device that grips the microtissues through suction (Figure 6B)[72,75]. Microtissues are first formed through seeding cells onto agarose mold. Spheroids, toroids and honeycomb sheets of microtissues are lifted from the mold using a gripper. Toroids microtissues were aligned vertically through stacking microtissues with aligned lumens.

2.6 Magnetic Bioprinting

Another method for microtissue assembly uses magnetic forces to control positioning of cells (Figure 6C). Magnetic bioprinting is a contactless technique for

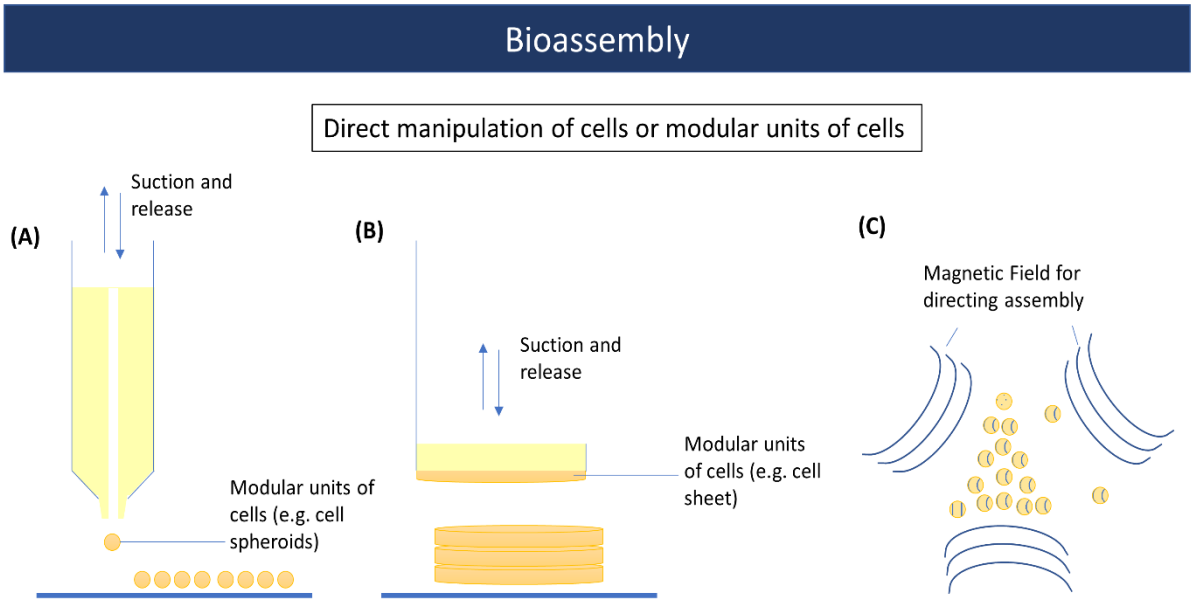

Figure 6. Direct manipulation of cells or modular units of cells: (**A**) Pick and place spheroids with needles. (**B**) Pick and place spheroids using a custom-made device. (**C**) Pick and place using magnetic forces.

manipulating and assembling cells into different shapes. Two distinct methods are used in this setup. Firstly, in label-free diamagnetophoretic printing, cell-medium was mixed with a paramagnetic buffer and exposed to an external magnetic field[76]. Cells suspended in the medium moved towards a region of lower magnetic field strength. The shape of 3D cell assemblies was controlled through changing the magnet configuration. In the second approach, cells are magnetized through incubating with magnetic nanoparticles overnight[77,78]. The magnetized cells were seeded in a low-adherent plate, forming cell aggregates through levitation. Thereafter, the magnetized cell aggregates were re-suspended in medium and patterned using a ring-shaped magnet. Spatial patterning of the cell aggregates into desired morphology are controlled through varying variation in the shape of magnetic template used[79]. Limitation in the magnetic field strength in constructing larger construct requires further improvisation for miniaturization[76]. Nevertheless, cytotoxicity and plausible internal stresses of the engulfed magnetic nanoparticles may have detrimental effects on the cells. A summary of bioprinting and bioassembly technologies is given in Table 1.

3. Conclusions and Outlook

3D Bioprinting has become an enabling fabrication tool in various applications using different material systems and spanning across micro- and macro-scales. There have been numerous works in making 3D bioprinting more adoptable for real applications. One of these examples include the use of multimaterials to create 3D microchannels to enable vascularization. This multimaterials 3D bioprinting system can be applied similarly like a fused deposition modelling (FDM), which is a material extrusion AM technique[80]. Hybrid bioprinting is another future trend that combine natural and synthetic materials. This hybrid system can use strong biodegradable polymer as support and bioactive hydrogel as model materials to create exterior of 3D scaffolds[51]. As 3D bioprinting advances and more techniques start to emerge, standardized classification of technology using consistent terminology is necessary to serve as a baseline towards development of standards for 3D Bioprinting. The proposed classification here will also promote knowledge and helps to stimulate new research by defining the processes based on the physical principles of the technologies.

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Table 1. Summary of different bioprinting and bioassembly technologies

Bioprinting Techniques	Materials	Resolution	Advantages	Reference
Material Jetting	Hydrogel: e.g. Alginate, collagen, fibrin, agarose, gelatin methacryloyl, polyethylene glycol	<10 μm to 200 μm diameter, Droplet volume of 1 pL to 7000 pL	Good resolution, Ultrafine droplets availability, Precise deposition of materials High speed printing of droplet	[18–21]
Material Extrusion	Hydrogel: e.g. Alginate, collagen, fibrin, agarose, gelatin methacryloyl, polyethylene glycol	15–400 μm	Relatively good resolution, Easy to implement, Wide range of hydrogel materials, Potential for multi-material bioprinting	[29–38]
Vat Polymerization Printing	Light sensitive photopolymers (e.g. GelMA, PEGDA)	5–100 μm	Good resolution, Fast printing speed, Nozzle-free, Potentially free from support-structure	[60,61,67]
Bioassembly or Bio-placer	Microtissues (cell spheroids, cell sheet)	Depends on the size of microtissues	Direct cell manipulation, Scaffold-free Multi-cellular construct for complex tissue	[69–71]

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