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1 **4D Printing and Stimuli-responsive Materials in Biomedical Aspects**

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Abstract

Three-dimensional (3D) printing has revolutionized the world manufacturing production. In biomedical applications, however, 3D printed constructs fell short of expectations mainly due to their inability to adequately mimic the dynamic human tissues. To date, most of the 3D printed biomedical structures are largely static and inanimate as they lack the time-dependant dimension. To adequately address the dynamic healing and regeneration process of human tissues, 4D printing emerges as an important development where “time” is incorporated into the conventional concept of 3D printing as the fourth dimension. As such, additive manufacturing (AM) evolves from 3D to 4D printing and in the process putting stimulus-responsive materials in the limelight. In this review, the state-of-the-art efforts in integrating the time-dependent behaviour of stimulus-responsive materials in 4D printing will be discussed. In addition, current literatures on the interactions between various types of stimuli (categorized under physical, chemical and biological signals) with the associated stimulus-responsive materials will be the major focus in this review. Lastly, potential usage of 4D printing in biomedical applications will also be discussed, followed by technical considerations as well as outlook for future discoveries.

Keywords: 4D printing, responsive materials, biomedical engineering

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1. Introduction

Firstly introduced by Charles Hull in the 1980^[1], 3D printing has been deemed to revolutionize the world manufacturing production with economic impact of USD 0.2 trillion to 0.6 trillion in 6 years' time as predicted by McKinsey & Company^[2]. This technology involves depositing materials precisely in layer-by-layer manner using computer-aid controls, and thus resulting in complex 3D geometries with desired spatial arrangement^[3]. It has allowed ideas to develop faster than ever by allowing instant 3D print of concept into prototypes, hence helping researcher to stay one step ahead of the competition. In the healthcare industry, 3D printing garnered tremendous attention due to its capability in offering on-demand and highly patient-specific treatments through printing of patient-specific biomedical devices based on computerised 3D model data manipulated from individual patient's medical images^[4, 5].

In spite of the advantage of achieving biomedical devices with precise geometry, 3D printed constructs are unable to mimic human tissues closely due to their static and inanimate characteristic^[6, 7]. In other words, living tissues in the human body are often subjected to dynamic living environment but these static printed artefacts with no functional variation across directions or time generally fail to capture many of the intricacies in nature's designs. For instances, during tissue healing, dynamic and extensive remodelling of the cellular microenvironment continually imposes intra- and extracellular contractile forces on cells which further trigger changes in gene expression and cell behaviour, through a process known as mechanotransduction^[8]. This dynamism is essential for modulating all important functions in body^[9-12]. Therefore, the ideal printed construct should have the ability to respond to such dynamism in order to achieve integration with the human body.

In view of this, 4D printing emerges as an important concept which emphasizes the need to consider the dynamic tissue healing and regeneration processes within the human body when

designing biomedical devices ^[13-15]. In this review, the fourth dimension in 4D printing is defined as the “time” that is integrated into the conventional 3D printing whereby the transformation of a material or 3D construct over time is taken into account after being printed. By considering this transformation over time as an additional aspect, it provides dynamism into the construct to create smarter and more relevant devices especially in biomedical applications. This is made possible by cleverly manipulating the characteristics of certain materials that can be significantly transformed over time by external stimuli such as temperature, liquid, light, magnetism, electrical field, pH, ionic concentrations, glucose concentration and enzymes. For revolutionary enhancement in 4D printing, the time dependent transformation should be predictable and also programmable. Therefore, vast knowledge of how stimuli responsive materials behave over time when triggered by external stimuli, is necessary and will be discussed in this review.

2. From 3D printing to 4D printing - typical approaches and their biomedical applications

Both 3D printing and 4D printing use AM as the fundamental approach to create constructs. AM is a production technique in which materials are printed layer-by-layer to form constructs with desired spatial arrangement under computer control. There is a range of process technologies under AM and these technologies vary in their method of layer manufacturing. According to the American Society for Testing and Materials group, “ASTM F42-Additive Manufacturing”, these process technologies can be classified into seven categories, namely material extrusion, vat polymerisation, powder bed fusion, material jetting, binder jetting, sheet lamination and directed energy deposition ^[16]. Out of these seven standard AM process categories, material extrusion, vat polymerisation, material jetting and powder bed fusion are the typical processes for printing of biomedical devices as illustrated in **Table 1** ^[17].

Table 1: Comparison of common printing techniques for biomedical applications: material extrusion, vat polymerisation, powder fusion and material jetting. Reproduced with permission from^[18, 19].

Material Extrusion: Fused-deposition modelling (FDM), Bioprinting			<ol style="list-style-type: none"> 1. Melted materials are fed through a extrusion head onto print bed; 2. Subsequent layers are deposited, fused with previous layers and solidified; 3. Process 1-2 repeat until finish; 4. Remove printed part.
	Available Printers	MakerBot Replicator+ 3D printer; Ultimaker 2+; 3D Bioscaffolder	
	Materials	Thermoplastics (e.g. ABS, PLA); photocrosslinkable hydrogels	
	Pros	Inexpensive process; wide choice of materials	
	Cons	Average accuracy, low speed	
Vat polymerization: Stereolithography (SLA); Two-Photon Polymerization (2PP); Digital light processing (DLP)			<ol style="list-style-type: none"> 1. A light source (<i>e.g.</i> laser or ultra-violet light) emitted and cured resin layer by layer; 2. Cured resin moves either further into the vat or away from the vat, leaving cured portion just under the surface; 3. Process 1-2 repeat until finish; 4. Drain vat and remove part.
	Available Printers	Formlabs Form 2; 3D Systems ProJet 1200; EnvisionTec Aria	
	Materials	Ultra-violet photopolymer resin	
	Pros	High accuracy; quick process; large build areas	
	Cons	Limited material use; toxic; expensive	

Powder Bed Fusion:

Selective laser
sintering (SLS);
Electron beam
melting (EBM);
Selective laser
melting (SLM)

1. Powders dispersed over build platform;
2. Laser melts and fused powders;
3. Build platform moves down and feeder bed rises, spreading new layer of powders;
4. Process 2-3 repeat until finish;
5. Part is left to cool before being removed; loose materials brushed away.

Available Printers	Sinterit Lisa; EOS P 500; ARCAM A2X; 3D Systems ProX300
Materials	Powder based metal (e.g. titanium, steel) or polymers (e.g. nylon)
Pros	Isotropic mechanical properties; complex geometries
Cons	High power usage; grainy surface finish

Material Jetting:

Drop on Demand
(DOD) approach

1. Droplets of liquid resin are jetted to the desired locations while the printhead travels over the build platform;
2. Deposited materials solidified either by laser or blower. Build platform moves downwards.
3. Process 1-2 repeat until finish;
4. Completed part is left to cool or laser cured and removed.

Available Printers	Mimaki 3DUJ-553 UV LED
Materials	Polymers (e.g. PS, polypropylene, PMMA, HDPE)
Pros	Smooth surface finish; high accuracy; homogeneous mechanical and thermal properties
Cons	High cost; support materials required; limited material choice

These AM systems, regardless of any process technology, are operated on the basis of forming a layer of material on x-y plane and subsequently building of layers in the z-direction. This operation is communicated to the AM printers through “Standard Triangle Language” or “Standard Tessellation Language” (both generally known as “STL file”), which contains

surface geometry information of a 3D design created from computer-aided design software [20-22].

Overall, both 3D printing and 4D printing uses AM to realise digital blueprints to physical objects by building them layer by layer. However, in contrast to 3D printing where static materials are assigned discretely at the predefined regions during the fabrication process, 4D printing holds the potential of creating parts capable of changeable properties and functions according to their environmental conditions. This is achieved through the use of smart and stimulus responsive materials and more sophisticated digital designs that are programmed to take into account the transformation in shape and size of the construct over time after printing [13, 23-25]. These intended transformations (e.g. bending, folding, twisting, expansion or contraction) require the design and selected material of the construct's components to be programmed at specific positions with scheduled sequences as illustrated in **Figure 1** [23, 26-28].

Figure 1

To achieve the desired transformations, current solutions require mathematical models which predict specific interactions for allowing shape shifting to occur in a desirable manner [29-31], AM printers which are equipped with the capability of printing multi-materials at specific positions [32, 33] and smart materials with established responsive profile for material printing [24] [34].

The advancement of 4D multi-material printing has led to increase of advanced modelling software to postulate the behaviour of materials and simulate its deposition accordingly for precise transformation. Commercial specialized software have been a great aid to simulate or control the shape-shifting sequences in a controlled manner [13]. For instances, Project Cyborg from Autodesk [35] [36], Foundry from MIT's Computer Science and Artificial Intelligence Lab

[37], CANVAS software from Mosaic Manufacturing, and Monolith multi material voxel software [37] are existing tools for self-assemblies simulation and printing parameters optimization [38]. Besides, programming tools like Origamizer and E-Origami System can help to create complex origami shapes through assigning nodes, edges, paths, polygons, vertices, and creases in the structures [39-44]. There are also numerous mathematical models being proposed and discussed to support certain designs and applications for 4D printing. For example, “spring-mass system” to understand the relationship between energy storage and release process during shape memory cycle [45-47], “four-element model” to illustrate the creep behaviour [48] while the “1D standard linear solid model” has been employed to describe polymer chain relaxation process [49] [50].

As mentioned previously, multi-material printing is one of the ways to achieve 4D printing. When multi-material 4D printing is conducted, it is important to note that materials orientation and distribution (*e.g.* uniform distribution, gradient distribution and special patterns, as shown in **Figure 2**) has to be calculated and programmed, so as to control the expansion or contraction occurring at desirable rates and directions. In terms of material distribution, it has been shown that through controlling the specific joint designs, self-evolving structures which either fold, curl, twist or expand could be achieved [51, 52]. Whereas, for material orientation, Gladman *et al.* have demonstrated anisotropic stiffness and swelling caused by shear-induced cellulose fibrils alignment in ultra-violet crosslink-able hydrogel composites for creating plant-inspired architectures [53]. It should be noted that these transformations arise from varying material structure internally and need multi-material printers for realization. In view of this, several multi-material printing systems have been proposed. For instance, Espalin *et al.* modified and designed a multi-material, multi-technology FDM system for discrete fabrication [54]. Another hybrid manufacturing system designed by Lopes *et al.* through integrating SLA and direct print technologies has enabled

the fabrication of monolithic 3D structures with embedded electronic circuits^[55]. Dunn *et al.* has also developed a novel 4D printer which combines aerosol, inkjet, direct ink write and extrusion-based printing techniques together, and it can handle multiple elastic and stiff materials simultaneously^[56]. Commercially, Objet260 Connex3 has been designed to create multi-color, multi-material parts as well^[57-59].

Figure 2

Besides multi-material printing, 4D printing also covers single material printing when it comes to fabrication of smart/stimulus-responsive materials with precise geometry for applications which require changes in the properties of the printed construct in a controlled fashion over time. To achieve successful 4D printing of such materials, it is important to have a deep understanding on how external stimuli such as temperature, moisture, light, magnetic field, electric field, pH, ionic concentration or chemical compounds will affect the characteristics of these materials. This understanding lays the essential foundation to 4D print stimulus-responsive materials with programmed transformation for desired application. In the next section, different types of external stimulus and the corresponding stimulus-responsive materials for biomedical applications will thus be discussed.

3. Stimulus and responsive materials - pursuit of smart materials for biomedical applications

In biomedical applications which includes tissue regeneration, drug delivery and diagnostics, there is a great demand for smart-responsive materials which are sensitive to external triggers, biological signals or pathological abnormalities for the development of next-generation

1 precision medicine. In addition, worldwide competition among global enterprises has fuelled
2 the search for smart materials with unprecedented properties.

3 There are various types of stimuli and they can be categorized into physical cues, chemical
4 cues, biological cues, and combinations of different stimuli. In this section, the interaction
5 mechanism between these stimuli and their corresponding responsive material will be
6 discussed with examples from scientific studies, followed by concerns which might need to
7 be taken into considerations for future explorations.

9 **3.1. Physical Stimuli**

10 Physical stimuli such as temperature, liquid/moisture, light, magnetic field and electrical
11 stimulation generally transform the chain dynamics or internal atomic packing arrangements
12 of stimuli-responsive materials, resulting in shape-shifting behaviour from these materials.
13 These kinds of shape shifting behaviour has imparted materials with unprecedented
14 properties which can respond to physical change in environmental conditions, hence
15 attracting large interest in their potential use for biomedical applications.

17 *3.1.1. Temperature*

18 Most experimental studies focused on the use of temperature as the external stimulus in
19 developing shape-deforming bio-printed parts. Generally, shape-memory materials are
20 printed and then undergo deforming step at appropriate conditions (*e.g.* mechanical loading
21 imposed and held above critical temperature such as glass transition temperature, T_g and
22 followed by cooling down) in order for them to change into their metastable temporary shape.
23 Subsequently, recovery step occurs during application, the temporary deformed structure will
24 transform to desired shape when sufficient transformation energy is applied^[60, 61]. This type
25 of shape recovery properties could be extremely useful for self-fitting small bone defect

1 implant replacement as shown in Senatov's work where 98% shape recovery could be
 2 achieved after compressing the polylactide / 15 wt% hydroxyapatite scaffolds at 80 °C, as
 3 shown in **Figure 3A** ^[62]. Similar approach has been adopted in other researchers' work. For
 4 instance, Zarek and colleagues fabricated thermally-actuated methacrylated
 5 poly(caprolactone)-based tracheal stent using SLA technique ^[63]. As depicted in **Figure 3C**,
 6 the stent could expand and fit well after being deployed into the tracheal section in the body,
 7 preventing any potential injury during the insertion process. In another study, a composite gel
 8 composed of periodic stripes of poly(hydroxyl ethylacrylamide-co-Nisopropylacryl-amide)
 9 (P(HEAm-co-NIPAm) and (P(HEAmco-NIPAm)/PNIPAm hydrogels exhibited reversible
 10 planar-to-helical-to-planar transformation when the temperature was increased from 25 °C to
 11 40 °C and eventually to 65 °C. The principle behind this actuation the different extent of
 12 shrinkage/swelling of different hydrogels that could be controlled at different temperature ^[64].
 13 Besides, a large amplitude and reversible actuation when changing temperature was also
 14 demonstrated using poly(N-isopropylacrylamide)-based valves fabricated by Hippler *et al.*, as
 15 a result of material shrinkage and stiffening over their lower critical solution temperature. In
 16 addition, the research group has also demonstrated that hetero-structures with accurately
 17 programmable actuation could be efficiently realized by employing gray-tone lithography
 18 approach, rendering materials with substantially different properties in one single fabrication
 19 step. This would be of extremely useful in applications which rely on a defined actuation
 20 behaviour, for example soft robotics, microfluidics and biosciences ^[65].
 21 However, this kind of transformation is irreversible. To pursue 4D printed structures that can
 22 undergo reversible transformation, shape-changing materials are used in fabrication process,
 23 instead of the previously mentioned shape memory materials. A shape-changing material
 24 changes shape (generally limited to basic expansion or contraction) when a stimulus is
 25 applied and returns to its initial permanent state after the stimulus is removed ^[66, 67]. A typical

example could be found in Dutta's work, in which thermo-responsive hydrogels composed of Methacrylated poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) triblock copolymers exhibited reversible swelling-deswelling response when the temperature changed^[68]. This responsivity and reversibility could be useful for applications like smart valves and soft actuators. Besides single material construct, a slightly different example could be seen in Stoychev's work, where he demonstrated reversible self-folding and self-unfolding ability using bilayered construct made of poly(N-isopropylacrylamide) and poly(caprolactone)^[69]. This reversible action could probably find application in cell encapsulation and release studies. For drug delivery application, Malachowski and colleagues demonstrated controlled release when using thermo-responsive poly(N-isopropylacrylamide-co-acrylic acid) hinges in poly(propylene fumarate) based therapeutic grippers (**Figure 3B**)^[70].

Generally, the effectiveness of thermo-responsive polymeric materials for biomedical applications hinges on their thermal properties such as glass transition temperature, T_g . The parts are usually pre-formed at temperatures above the T_g such that the printed parts become smaller and more compact. These parts are then cooled to temperature below their T_g to maintain their small and compact shape for minimally invasive surgical approach^[71, 72]. These printed parts then expand into their original desired shape when implanted into body where the temperature is greater than the T_g ^{[73] [74]}. However, this method only works on materials with T_g lower or equal to body temperature. To expand the base of such materials, modification or creation of biocompatible materials with lower T_g ^{[75] [76]} or multi-responsiveness in which the material concurrently respond to more than one stimuli is warranted^[68].

Figure 3

3.1.2. Liquid / Moisture

60% of the human body contain water which exist in the form of intracellular fluid or extracellular fluid ^[77]. Thus, liquid has been explored as another stimulus of high interest for achieving smart transformation. For liquid-responsive materials, transformation is designed such that differential swelling of the different compartments occurs in spatially and temporally dependent manner. **Table 2** shows studies that make use of liquid as stimulus for different applications.

Overall, liquid responsive materials offer several useful functions such as cell encapsulation, controlled drug delivery and reversible actuation for smart valve. However, issues with regards to slow response time, weaker mechanical properties after expansion as well as possible degradation / hydrolysis after several swelling/de-swelling cycles need to be taken into consideration especially when evaluating the material's intended usage life span ^{[78] [79] [80]}.

Table 2: Water-actuated smart materials and applications.

Application	Description	Reference
Cell-resembling structures for tissue engineering and targeted drug delivery	Picoliter aqueous flower-shaped network spontaneously self-folded into hollow sphere, arising as a result of different swelling from two layers of lipid droplets (1,2-diphytanoyl-sn-glycero-3-phosphocholine) with different osmolarities (Figure 4A).	Villar <i>et al.</i> ^[81]
Cell encapsulation for insulin production	Self-folding cylindrical scaffolds made of poly(ethylene glycol)-based bi-layered hydrogel used to encapsulate cells in benign environment (Figure 4B).	Jamal <i>et al.</i> ^[82]
Tissue regeneration or smart valve application	Water-actuated reversible natural hydrogel construct composed of carboxymethyl cellulose hydrocolloid (Figure 4C).	Mulakkal <i>et al.</i> ^[83]
Smart joint	Non-trivial self-evolved structure constructed in “design-fabrication-simulation” workflow where both stretching and bending of 2D grid skeleton were considered. Water-actuation was realized with the use of expanding material, which was composed of hydrophilic acrylated monomers	Raviv <i>et al.</i> ^[47]

	that create linear chains upon polymerization with a small amount of difunctional acrylate molecules.	
Controlled drug delivery	Drug capsule released therapeutics from hydrogel materials in swollen state.	Wang <i>et al.</i> [84]
Smart sensor	Reversible bending movement in a moisture-responsive hydrogel film made of poly(ethylene glycol) diacrylate.	Lv <i>et al.</i> [85]
Soft actuators and solvent-responsive sensors	Reversible deformation demonstrated with a printed multiple-layered poly(glycerol sebacate) strip when exposed to organic solvents vapour.	Lei <i>et al.</i> [86]

Figure 4

3.1.3. Light

Making use of light as a stimulus has attracted much interest in recent research on account of many advantages such as rapid switching, accurate focusing and sustainable properties [87] [88]. Several transitions may be induced upon light irradiation, including size or shape changes, formation of zwitterionic species, charge generation and photodimerizations [89-92]. In numerous efforts to exploit light as the stimulus in AM printed parts, Yang *et al.* incorporated carbon black which exhibited excellent photothermal conversion efficiency into shape-memory polymer polyurethane for FDM printing [93]. In this work, the printed structure recovered from squashed state to original cubic structure upon illumination of either 87 mW cm⁻² of light source or 76 mW cm⁻² of sunshine. Using the same material as the “petal” of “sunflower”, this research group further demonstrated “bud-to-bloom” phenomenon as shown in **Figure 5A**. The underlying mechanism was that the heat generated from absorbed light drives shape recovery. Besides, in Kuksenok’s computational modelling, he designed a composite which consisted of thermo-responsive polymer gel (*i.e.* poly (N-isopropylacrylamide)) and photo-responsive fibers functionalized with spirobenzopyran chromophores [94]. This composite was speculated to be able to undergo repeatable global shrinking and localised bending due to the thermo-responsive gel and light-actuated fibers,

1 respectively. Another approach which used light as the stimulus was demonstrated in Qi's lab,
2 whereby laminated composites exhibited photo-induced bending phenomenon ^[95]. As
3 demonstrated in **Figure 5C**, the composite consisted of two light activated polymer layers
4 (made of pentaerythritol tetra(3-mercaptopropionate), ethylene glycol di(3-
5 mercaptopropionate) and 2-methylene-propane-1,3-di(thioethylvinylether)) with a stretched
6 optical resin NOA65 layer embedded in between. Bending occurred as a result of photo-
7 actuated macroscopic relaxation of the compressive stress applied by this NOA65 layer, and
8 could be useful as a hinge in photo-origami structure (**Figure 5D**). Light has also been used
9 in achieving selectively therapeutic release in Gupta's work ^[96]. He and colleagues printed a
10 capsule with core-shell configuration in which the aqueous core was loaded with delicate
11 therapeutics while the poly(lactic-co-glycolic) acid shell region was loaded with plasmonic
12 gold nanorods (as shown in **Figure 5B**). Selective rupturing of the capsule could be achieved
13 by irradiating laser with specific wavelength which corresponds to the resonance wavelength
14 of the nanorods used. Such rupturing could result in selective therapeutics release in
15 sequential manner, which is extremely useful for cancer therapies and multidrug resistance
16 treatment.

17 The use of light as stimulus has opened up a new avenue in constructing bio-responsive
18 biomedical devices. However, more in-depth research is required to address concerns
19 regarding potential toxicity with the use of photo-activated materials, heat generation during
20 photo-thermal conversion, as well as diminishing shape transformation due to oxygen
21 inhibitory effect in using NOA65 composite ^[97-99]. In addition, when photo polymerization
22 process (*e.g.* SLA or DLP) is used to fabricate light responsive structure, photo-activated
23 materials become limited to those that only respond to specific wavelengths of light. These
24 specific wavelengths should not fall within the range of wavelength used during the UV/laser
25 printing process, in order to avoid unintended conformation or structure transition during

printing. If used as an internal implant, the method to trigger light stimulus on demand should also be considered. For example, if light stimulus is applied externally, the penetration depth of the light should be sufficient and safe at the same time for meaningful transformation to occur.

Figure 5

3.1.4. Magnetic field

The use of magneto-responsive materials for biomedical uses can be dated back to around 2600 years ago ^[100]. In current biomedical applications, magnetic fields offer effective and safe manipulation via non-contact remote mode, in areas ranging from magnetic resonance imaging, targeted drug delivery to artificial muscle applications ^[101, 102]. To create magneto-responsive constructs using AM technology, Wei *et al.* formulated an ink composed of poly(lactic acid) polymer infused with magnetic iron oxide nanoparticles and utilized direct-write printing to fabricate tubular structures (**Figure 6A**) which possess magnetically guidable and shape recovery properties ^[103]. The shape recovery was actuated as a result of induction heating of iron oxide in the presence of alternating magnetic field, thus generating sufficient energy and allows temporary shape to transform back into the initial configuration. In another interesting work, Zhu *et al.* created magnetically responsive structures with a fast response time by using poly(dimethylsiloxane)/Fe (PDMS/Fe) composite ink ^[104]. The quick response could be achieved due to the presence of Fe particles, which obtained or lost their high magnetization immediately by turning on or off the external magnetic field. In an effort of constructing origami robots, Miyashita and colleagues printed a device which could travel at a speed of 3.8 body-length/s remotely under external magnetic field ^[105]. As a side remark, this miniature untethered robotic device possessed additional advantages such as good bio-

resorbability and the ability to self-fold via heat stimulus for drug delivery applications. Self-folding could also be realized using magnetic forces. For instance, Boncheva *et al.* created PDMS elastomeric sheets patterned with magnetic dipoles and self-folding was achieved as an interplay between the material's elastic bending energy and the externally applied magnetic energy ^[106]. Besides, magnetic field applied during the printing process can be useful to control the orientation of magneto-sensitive particles to achieve anisotropic heterogeneous composites. By varying the particles orientation and density, this kind of composites possess different mechanical properties at different regions, mimicking muscles-like properties where shape distortion occurs very quickly and disappears abruptly when external magnetic field is applied and removed, respectively ^[107, 108]. Using this concept where magnetic field was applied during printing process, Kim *et al.* embedded neodymium–iron–boron (NdFeB) alloy in silicone rubber matrix and created complex 3D structures with programmed ferromagnetic domains as shown in **Figure 6B**. These structures were capable of undergoing fast shape changes via magnetic actuation ^[109].

In spite of the benefits of these magneto-responsive materials in biomedical applications, their highly reactive nature and aggregation affinity remain a concern ^[110, 111]. In addition, the frequency of the magnetic field should be in the clinically safety range (*i.e.* 50 – 100 kHz) to avoid potential elevated temperature which might traumatize body tissue ^[112]. Future efforts should be placed on researching safer magneto-induced materials to be put into actual medical practices.

Figure 6

3.1.5. Electric field

Electric fields have the capability of manipulating cells to predefined positions or orientating cells in particular directions, these phenomena are known as galvanotaxis and electrotropism respectively [113-115]. There are various research which focus on the search and fabrication of electroactive materials for tissue regeneration [116-118]. In developing electrically conductive scaffolds to promote muscle tissue regeneration, Sayyar *et al.* printed 3wt% graphene-incorporated multilayer methacrylated-poly(trimethylene carbonate) scaffold as shown in **Figure 7A**, that exhibited enhanced electrical conductivity values of $\sim 0.001 \text{ S cm}^{-1}$ [119] which is comparable to that of muscle tissues ($\sim 0.004 \text{ S cm}^{-1}$) [120]. The scaffolds displayed good cellular viability and promoted osteogenesis of mesenchymal stem cells upon application of electrical stimulation. Recent work by Jakus *et al.* demonstrated that higher graphene content scaffolds (60 vol% graphene in polylactide-co-glycolide (85:15) copolymer) displayed greater electrical conductivity of 0.08 S cm^{-1} [121]. These scaffolds serve as potential platform for nerve regeneration as they supported neurogenic differentiation of human mesenchymal stem cells, possessed exceptional handling characteristics and could be intraoperatively manipulated. Another work by Dong *et al.* showed that by adding aniline tetramers conjugated polyethyleneimine into the Pluronic F127 formulations, the printed scaffolds had sufficiently high conductivity values of more than 0.002 S cm^{-1} for tissue electrical stimulation [122]. Besides using electroactive materials, another approach is to use cell laden structures which could be actuated under electrical stimulation to achieve locomotion. This concept is demonstrated from Cvetkovic and colleagues' work as shown in **Figure 7B** [123], which used cell laden skeletal muscle strip cultured on stereolithographic 3D printed poly(ethylene glycol) diacrylate hydrogel. This work has advanced one step forward in realizing functional integrated cellular systems, which can have a myriad array of

1 applications especially in programmable muscle tissue engineering and biomimetic machine
2 design.

3 Overall, tissues such as muscles or nerves could benefit from electrical stimulation <sup>[117, 118, 124-
4 ^{126]}</sup>. Nonetheless, there may be issues such as localized heating, membrane disruption or cell
5 death when the electric current applied is too high ^[127]. Hence, precautionary measures and
6 precise actuation should be taken while using electric field as the stimulus.

7 8 **Figure 7**

9 10 **3.2. Chemical Stimuli**

11 Variations in physiological conditions such as change in pH or ionic concentration are often
12 critical hallmarks for distinct types of diseases, such as cancer, degenerative diseases,
13 infections and cardiovascular diseases, rendering them important targets of consideration
14 when designing bio-responsive materials.

15 16 *3.2.1. pH*

17 Local acidification near the cancerous or inflammation sites as well as the difference in pH
18 along gastrointestinal tract has prompted the use of pH-responsive materials to achieve
19 controlled anticancer drug delivery and organ-specific release of orally administered drugs,
20 respectively ^[128-131]. pH- responsive materials are capable of swelling, shrinking, dissociation
21 or degradation upon change in environmental pH, attributed to either protonation of ionizable
22 groups or degradation of acid-cleavable bonds. When there is a change in pH, pH-responsive
23 materials display globule-to-coil transition: polymer chains stretch to coil form due to
24 electrostatic repulsion of charged functional groups or forming globule structure when charge
25 of the functional groups is neutralised ^[132, 133].

1 There are several natural proteins displaying pH-responsiveness, such as collagen, gelatin and
 2 keratin. Collagen is the main structural protein existing in various connective tissues in the
 3 body ^[134]. Its pH-responsiveness has been demonstrated by Bear *et al.*, whereby minimum
 4 swelling occurred at neutral pH range (*i.e.* pH 4 to 8) while maximal swelling occurred in
 5 mild acid and alkali environments (*i.e.* pH 2 to 4 and pH 8 to 12), followed by decline in
 6 swelling at extremely acidic and alkaline environments (*i.e.* < pH 2 and > pH 12) ^[135].
 7 According to Donnan theory, as the pH drifts away from collagen's isoelectric point, the
 8 increase of charge bounded at protein surface caused the formation of excess ions with
 9 opposite charges inside the collagen gel. These opposite charged ions in turn initiate action of
 10 osmotic forces and hence swelling occurs ^[136, 137]. Keratin is another pH-responsive protein
 11 that is naturally derived from human and can be found excessively in hair and nails ^[138].
 12 Compared to collagen, keratin adopts a different swelling profile when responding to
 13 different pH environments, as shown in **Figure 8C**. According to Ramos *et al.*, keratin shrank
 14 into disordered and collapsed network in acidic environment (< pH 6), while swelling
 15 increases significantly above pH 6 with maximal swelling being observed above pH 8 ^[139].
 16 This pH responsive behaviour of collagen and keratin has been employed in research studies
 17 for smart release of functional compounds such as doxorubicin ^[140, 141] or antimicrobial ZnO
 18 nanoplates ^[142] for anticancer therapy and prevention of wound infection respectively.
 19 Generally, natural proteins has gain attention as potential bioactive materials which can
 20 enhance cell-material interaction and proliferation due to the presence of cell adhesion motifs
 21 ^[143-145]. Despite numerous studies done in printing these proteins using AM technology, focus
 22 were placed on achieving bioactive printed constructs for improving cell interaction ^[146-148],
 23 while their unique property of pH responsiveness has yet been explored.
 24 Besides natural polymers, wide varieties of synthetic polymers, which are pH-responsive,
 25 have been used for drug release applications. Printing technology was utilized to fabricate the

required drug delivery systems with special designs and precise dimensions, which otherwise are not possible through conventional manufacturing methods. This precision is crucial to achieve unique drug release profiles. In Larush's work, acrylic acid-based hydrogel has been designed and printed to obtain tablets capable of faster drug release at high pH environment [149]. Similarly, Okwuosa *et al.* printed tablets with core-shell configuration using polyvinylpyrrolidone and methacrylic acid co-polymer as core and shell materials respectively [150] (as shown in **Figure 8A**). The tablets fabricated in these two studies displayed gastric resistant properties and pH responsive drug release pattern, making them a promising system for enteric drug delivery purposes. In an effort of printing hypromellose acetate succinate-based tablets for paracetamol delivery, Goyanes and colleagues demonstrated delayed and selective release at different pH through employing enteric polymers with different grades. This is especially useful in realizing the development of personalised dose medicines which cater for different patients with different medical needs [151]. Other than drug delivery, pH-responsivity has been useful in achieving multifunctional dressing, for example GelDerm as shown in **Figure 8B**. GelDerm is a smart dressing which can indicate bacterial infection using pH-dependent colorimetric measurement and release antibiotic agents at the wound site [152]. The colorimetric feature was achieved through printing of color-changing alginate fibers that were loaded with mesoporous resin beads, doped with pH-responsive dye. In addition, pH-responsive synthetic polymers are also printed to obtain flow-regulating valves. Nadgorny *et al.* demonstrated that reversible pH-dependent swelling/shrinking of the poly(2-vinylpyridine) hydrogel, which was reinforced by acrylonitrile-butadiene-styrene owing to the superior plasticizing and strengthening properties, resulted in dynamic flow control [153].

Overall, the use of natural pH-responsive polymers in practical applications is limited by their intrinsic weaker mechanical properties [79, 154, 155]. Existing solution to this limitation are

blending natural polymers with other materials to create hybrid constructs with adequate structural integrity. As for synthetic polymers, despite the established abundant experimental in exploring their potential use for biomedical purposes, future work is necessary to achieve facile, low-cost and controlled synthesis of novel materials with well-defined chemical compositions, biodegradability and long term structural stability, prior to commercializing them for clinical applications.

Figure 8

3.2.2. Ionic concentration

There are relatively few studies investigating the potential use of ionic strength as stimulus in 4D printing technologies. These studies generally showed that shape morphing could be realized through changing salt concentration. For instance, Huang *et al.* printed hydroxyethyl acrylate-based hydrogel with reversible shape changing characteristics in response to ionic strength as shown in **Figure 9B** ^[156]. Potassium 3-sulfopropylmethacrylate used in the ink during printing process accounted for the shape changing properties due to its intrinsic swelling nature in response to ionic level / salt concentration. Another example from Kang and colleagues' work demonstrated the potential use of photonic lamellar film, which consists of polystyrene-b-quaternized poly(2-vinyl pyridine), as colorimetric sensors for skin monitoring applications ^[157]. In their study, the film's swelling / de-swelling behaviour (as shown in **Figure 9A**) which was actuated by ionic concentration, could effectively modulate both the domain spacing and refractive-index. In a study by Thérien-Aubin *et al.*, composite gel composed of poly(acrylamide-co-butylmethacrylate), poly(methacrylic acid) and poly(N-isopropylacrylamide) was fabricated at specific regions of the gels. The gel changed from cylindrical shape to drum shape upon changing of ionic concentration to 1.5 M. The shape-

transformation arised from internal stresses from swelling of different gels, and thus could be very useful in applications such as soft robotics, actuation and sensing ^[64].

Despite relatively little research on material's response to ionic concentration, it is important to note that physiological electrolytes level can be a critical indication for various diseases. For instance, excessive level of calcium in blood, known as hypercalcemia condition, is caused by certain types of cancer like lung and breasts cancer ^[158-160]. Also, Alzheimer's disease patients was found to have increased intracellular [Na⁺] and [K⁺] levels in their brain regions ^[161]. Therefore, integration of these biological observations with the latest advancement of material technology would certainly bring in more efficient therapeutic options in the future through studies on materials response behaviour in changing ions concentration.

Figure 9

3.3. Biological Stimuli

In the human body, biological processes often rely on feedback-controlled communication involving biological small molecules or bio-macromolecules such as glucose, enzymes, nucleic acids, polypeptides, and proteins. A typical example is blood glucose regulation through insulin secretion by islet cells, in response to blood sugar level ^[162]. Recent studies have been devoted to endowing materials with specific functionality that allows responsive behaviour upon being exposed to these stimuli ^[163].

3.3.1. Glucose

With the rising trend in diabetes mellitus worldwide, considerable attentions has been given on the development of glucose responsive materials and their potential application in glucose

monitoring and insulin delivery^[164-166]. Generally, management of type I and type II diabetes involves regular blood sugar monitoring. In view of this, glucose responsive materials are being printed into usable glucose detection. For instance, using amperometric principle, Adams *et al.* printed glucose sensor using graphene polylactic acid filament, which was preconditioned with glucose dehydrogenase flavin adenine dinucleotide^[167]. The detection level of glucose concentrations was found to be between 0 and 400 mg/dL and can be useful for glycemic management. Similarly, as shown in **Figure 10**, Song and colleagues^[168] fabricated glucose sensor by placing glucose oxidase (GOx) with platinum nanoparticles (PtNP) in between carbon-nanotube (CNT) conductive electrodes. A polyaniline nanowire network was then inkjet-printed over CNT/GOx/Pt-NP-deposited area to form resistive bridge between the CNT electrodes. In the presence of glucose, the sensor will be catalysed by GOx to produce hydrogen peroxide (H₂O₂) as the by-product. The H₂O₂ will be further catalysed by PtNP to induce local acidification. The pH-responsive conductivity of polyaniline hence serves as chemiresistive sensor to quantitatively determine the glucose concentration for diabetes management, with detection limit of 2 mM.

Besides blood glucose monitoring, diabetes management also include subcutaneously administration of insulin several times a day. However, consistent patient vigilance and compliance is difficult to manage leading to high risk of diabetes complications. This has thus necessitated the desire for self-regulated glucose-responsive medications through controlled insulin delivery. Current approach in developing glucose-responsive insulin delivery is primarily based on two mechanisms in controlling blood glucose level. These two mechanisms are: 1) directly triggered model in which insulin is released through competitive binding of glucose with glycopolymer–lectin complexes^[169, 170]; and 2) progressively activated model whereby insulin is released due to swelling caused by localized acidification from glucose oxidase-based enzymatic reaction^[171]. Although these mechanisms are well

established, there are currently no studies involved in printing glucose responsive device based on such mechanism for insulin delivery. Alternatively, Song *et al.* demonstrated a new approach in which insulin is delivered through the secretion of β cells in high glucose environment. This is achieved by printing a graft composed of polylactic acid and growing stem cell-derived β cells clusters on the graft to introduce glucose responsivity into the β -cells laden constructs ^[172]. It was found that this cell laden printed graft could induce higher insulin secretion in response to the presence of glucose.

The major concern in developing glucose responsive materials is the use of enzymes in the fabrication process. Hence, benign processing conditions are critical to ensure preservation of enzymes bioactivity after fabrication. Additionally, glucose responsive materials used for insulin delivery should be limited to biocompatible materials for practical usage.

Figure 10

3.3.2. Enzymes

Enzymes are highly specific and selective molecules which modulate many biological processes, including protein expression, formation of cellular adhesions and muscle contraction ^[173-176]. The use of enzyme-responsive materials has caught tremendous attention due to the fact that a large variety of enzymes is already present in the body.

Enzyme stimulation can be intrinsically triggered by the biological environment itself and the level of stimulation is naturally modulated by the state of the body. For example, expression of certain enzymes such as matrix metalloproteinases (MMP) has been shown to have association with tumour invasion and metastasis at specific regions in the body. The expression of MMP thus served as site-specific biological trigger for targeted release of anti-inflammatory drug from negatively charged hydrogel ^[177, 178]. The mechanism behind this

trigger is the enzymatic degradation of the drug-loaded hydrogel, resulting on-demand
 delivery from the hydrogel at the specific regions in the body. Wang *et al.* and Ceylan *et al.*
 have also employed the proteolytic degradative property of gelatin methacryloyl to fabricate
 micro-robots for targeted drug delivery purpose. These magnetically driven micro-robotic
 devices (as shown in **Figure 11** A and B) were shown to be completely removed in the
 presence of collagenase ^[179] and MMP2 enzymes ^[180], with no detectable toxic residues left.
 With this enzymatic degradability, their studies have addressed the general issues in AM-
 printed micro-robots such as non-degradability or cytotoxic by-product.
 Other than drug delivery, enzymatic degradable materials are also of significant interest in the
 field of tissue regeneration because implants made of these materials are able to be broken
 down and removed naturally from the body after they have fulfilled their functions. With this
 intention, Song *et al.* printed micro-channels embedded in a protease-degradable peptides
 support structure for vascularization purposes. The endothelial cell-seeded micro-channels
 were shown to have “angiogenic sprouting” as a result of enzymatic degradation of the
 support structure ^[181].
 Besides material degradation, there are many other responses that could be triggered by
 enzymes. For examples, supramolecular architectures changes, swelling/collapse of structures,
 and transformation of surface properties ^[182]. However, current use of printed enzyme-
 responsive materials is limited to their enzymatic-triggered degradability. The challenge in
 using enzyme-responsive materials lies in controlling their response behaviour due to
 possibilities of unwanted cross-reactivity with other enzymes in the body. This specificity
 issues is of particular concern especially when DNA sequences or longer peptides are used
^[182].

Figure 11

4. 4D printing for biomedical application and challenges ahead

In the last few decades, maturation of AM technology, together with advances in material sciences and better understanding in biological systems, have opened up and prompted the exploration of stimuli-responsive materials in 4D printing. Particularly for biomedical applications, 4D printing can potentially benefit many different areas such as tissue regeneration, medical device fabrication, drug delivery, and medical diagnosis ^[183]. In the case of tissue regeneration, the ultimate goal is to engineer functional scaffolds to replace diseased or injured tissues. Ideally, the engineered materials should possess bio-mimicking features and bio-responsiveness to assist tissue remodelling. 4D printing technology provides a good platform for fabrications of scaffolds with intricate designs and controlled change in properties over time. In addition, 4D printing also facilitates the fabrication of medical devices which can initially be compact and small in size before implantation to minimize surgical wound and expand itself into precise geometry which fit nicely upon implantation into the body ^[63]. In targeted drug delivery systems, 4D printing makes multi-drugs sequential delivery feasible through allowing precise deposition of therapeutics into separated compartments in a tablet and accounting for the timely release profile triggered by stimuli such as pH, temperature, liquid and enzymes ^[184, 185]. As for medical diagnosis, 4D printing becomes useful in fabricating smart materials and depositing them at precise locations in circuit boards for sensing applications ^[152], for example GelDerm for wound management and chemiresistive glucose sensors as mentioned.

The tremendous efforts invested have propelled the rise of 3D printing for biomedical applications and 4D printing seems to be in the next inevitable trajectory of AM. Though research in the latter has progressed significantly, translation of the technology is still in its infancy. Firstly, fundamental understanding of the complexity and dynamism of biological

1 systems needs to be well integrated into engineering solutions. Engineering solutions that are
2 isolated or overly simplifying biological systems would not be predictable nor programmable.
3 Consequently, the outcome would be just as unpredictable and riddled with uncertainties.
4 Secondly, even given due consideration of the above, high-precision controlled responses
5 remain a challenge. Just as in all smart stimuli-triggered devices, the trigger is key in the
6 success of such devices. There is a large variation in availability and stability of
7 stimuli/trigger at differing parts of the body and not to mention from patient to patient. This
8 adds to the ambiguity of the effectiveness of the outcome. Despite the challenges, the outlook
9 of 4D printing remains optimistic. Just like all breakthrough research and technology, they all
10 start with the simplest condition/situation and in the case of 4D printing for biomedical
11 applications, choosing the right tissue/organ with simpler environment with limited
12 interaction with blood for example may be a good starting point. For specialised technology,
13 instead of targeting a generic patient base, perhaps personalisation e.g. personalised drug
14 release stimuli may also help to mitigate the challenge. With the emerging complementary
15 technology such as big data and artificial intelligence, better prediction and simulation could
16 probably be achieved sooner. This would significantly help in the predictability of
17 environment (stimuli) and responses alike. Lastly, the close collaboration of scientists and
18 engineers of different disciplines encompassing areas like biology, materials science,
19 modelling and engineering is imperative to deliver the potential of 4D printing for biomedical
20 applications.

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List of Figure Captions

Figure 1: Self-folding box: A) Different materials printed at different hinges, where materials with low T_g experiencing shape recovery first, vice versa; B) Images showing sequential folding of the printed structure into box upon heating, demonstrating the importance of assigning smart-responsive materials at specific locations for desired shape transformation. Reproduced with permission from ^[26].

Figure 2: Possible material distribution in multi-material 4D printing: A) Uniform distribution; B) Gradient distribution; C) Special distribution. Black and white box represents different materials in 4D structure.

Figure 3: Thermo-responsive materials and their use in biomedical applications: A) Recovery of polylactide/hydroxyapatite scaffolds for self-fitting small bone defect implant. Reproduced with permission from ^[61]; B) Schematic showing grippers with rigid poly(propylene fumarate) panels and flexible thermo-responsive poly(N-isopropylacrylamide-co-acrylic acid) hinges open and close reversibly. Reproduced with permission from ^[67]; C) Thermally-actuated tracheal stent expand and fit well upon being deployed into the body, preventing potential injury during the insertion process. Scale bar = 1 cm. Reproduced with permission from ^[62].

Figure 4: 4D printing of liquid-responsive materials: A) Macroscopic image of network containing different osmolarities, folding spontaneously into a hollow sphere (i to iv) over a period of 8 hours. Scale bar = 200 mm. Reproduced with permission from ^[78]; B) Schematic illustrating self-folding of poly(ethylene glycol)-based bi-layered hydrogel, and forming cylinders with (v) small and (vi) big radii, scale bar = 200 μm . Reproduced with permission

from ^[79]; C) Liquid-actuated reversible responsibility could be achieved in 4D printed cellulosic composites. Scale bar = 10 mm. Reproduced with permission from ^[80].

Figure 5: Photo-sensitive materials in 4D printing: A) Deformation of photo-sensitive printed “sunflower” from “bud to bloom” upon light illumination. Scale bar = 2 cm. Reproduced with permission from ^[89]; B) Schematics showing fabrication of programmed rupture drug capsule: printing of therapeutics core followed by printing of PLGA containing AuNRs. The latter was then selectively triggered, resulting in therapeutics release. Reproduced with permission from ^[92]; C) Schematics showing stretched NOA65 being embedded in between LAP layer forming laminated structure, and (D) its bending behaviour upon light irradiation. Scale bar = 5 mm. Reproduced with permission from ^[91].

Figure 6: Magneto-sensitive materials in 4D printing: A) Images showing tubular nanocomposites recovered from temporary shape under alternating magnetic field. Scale bar = 5mm. Reproduced with permission from ^[99]; B) i) Ferromagnetic particles in the composite ink reoriented according to magnetic field applied during printing process; and ii) under magnetic fields, structures experienced shape changing and the experimental results matched with finite-element simulations. Scale bar = 10 mm. Reproduced with permission from ^[104].

Figure 7: Electro-sensitive materials in 4D printing: A) Conductive graphene composites scaffold was printed and supported mesenchymal stem cell growth and osteogenic differentiation upon electrical stimulation. Scale bar = 200 μ m. Reproduced with permission from ^[114]; B) (i) “Bio-bots”, a solid muscle strip were formed through cells and matrix compacted around the pillars (scale bar = 1 mm) and (ii) time lapse images showing movements of “bio-bots” upon electrical stimulation. (iii) Increasing stimulation frequency

resulted in increasing number of contractions, velocity and displacement. Reproduced with permission from ^[118].

Figure 8: pH-sensitive materials in 4D printing: A) (i) Stereolithographic design file showing core-shell configuration; (ii) Macroscopic image showing 30% completed core-shell tablet, where the inner core contains API while outer layer is an enteric shell for gastric protection. Reproduced with permission from ^[144]; B) Schematic showing GelDerm, a multifunctional dressing for monitoring and management of wounds, containing pH sensitive indicator and drug-eluting components. Reproduced with permission from ^[145]; C) Keratin hydrogel demonstrated reversible swelling/de-swelling upon change of pH. Reproduced with permission from ^[134].

Figure 9: Ion-responsive materials in 4D printing. A) Schematic showing structure of photogenic gel and tuning mechanism. Reversible swelling / de-swelling phenomenon observed due to the change of ions concentration, modulating domain spacing and thus the associated refractive index. Reproduced with permission from ^[150]; B) Shape change of hydroxyethyl acrylate-based hydrogel in response to ionic concentration. Scale bar = 1cm. Reproduced with permission from ^[149].

Figure 10: Glucose-responsive devices in 4D printing: (i) Configuration of chemiresistive glucose sensor. A current meter is connected with the sensor to measure current flow through the polyaniline nanowires. (ii) Catalytic reaction mechanism used in the chemiresistive sensor: the presence of glucose will be catalysed with H_2O_2 as by product, H_2O_2 is subsequently catalysed by the PtNP and result in local acidification. This change can be detected by measuring polyaniline nanowires resistance. Reproduced with permission from ^[159].

Figure 11: Enzyme-responsive devices in 4D printing: A) (i) Image showing biodegradable micro-robot and (ii) energy dispersive spectroscopic analysis confirmed the presence of iron oxide nanoparticles which are used to direct device movement. Scale bar = 4 μm . Reproduced with permission from ^[171]; B) Series of images showing enzymatic degradation of the device in a collagenase solution. Scale bar = 15 μm . Reproduced with permission from ^[170].

List of Table Captions

Table 1: Comparison of common printing techniques for biomedical applications: material extrusion, vat polymerisation, powder fusion and material jetting. Reproduced with permission from ^[18, 19] .

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Table 1 (4)

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Table 1: Comparison of common printing techniques for biomedical applications: material extrusion, vat polymerisation, powder fusion and material jetting. Reproduced with permission from [18, 19] .

Material Extrusion: Fused-deposition modelling (FDM), Bioprinting	<p>The diagram illustrates the material extrusion process. A material spool feeds filament into an extrusion head. Inside the head, a heater element melts the filament, which is then pushed through a nozzle onto a build platform. The extruded material forms an object or model, with support material being deposited alongside it. The build platform moves downwards as the object is built layer by layer.</p>		<ol style="list-style-type: none">1. Melted materials are fed through a extrusion head onto print bed;2. Subsequent layers are deposited, fused with previous layers and solidified;3. Process 1-2 repeat until finish;4. Remove printed part.
	Available Printers	MakerBot Replicator+ 3D printer; Ultimaker 2+; 3D Bioscaffolder	
	Materials	Thermoplastics (e.g. ABS, PLA); photocrosslinkable hydrogels	
	Pros	Inexpensive process; wide choice of materials	
	Cons	Average accuracy, low speed	
Vat polymerization: Stereolithography (SLA); Two-Photon Polymerization (2PP); Digital light processing (DLP)	<p>The diagram shows the vat polymerization process. A laser beam is directed through a transparent window into a vat filled with photo resin. The laser cures a layer of resin, forming an object. A Y platform moves vertically within the vat, lowering the cured layer and exposing a new surface for the next layer of resin to be cured. The process repeats until the object is complete, after which the vat is drained and the part is removed.</p>		<ol style="list-style-type: none">1. A light source (<i>e.g.</i> laser or ultra-violet light) emitted and cured resin layer by layer;2. Cured resin moves either further into the vat or away from the vat, leaving cured portion just under the surface;3. Process 1-2 repeat until finish;4. Drain vat and remove part.
	Available Printers	Formlabs Form 2; 3D Systems ProJet 1200; EnvisionTec Aria	
	Materials	Ultra-violet photopolymer resin	
	Pros	High accuracy; quick process; large build areas	
	Cons	Limited material use; toxic; expensive	

Powder Bed Fusion:

Selective laser sintering (SLS);
Electron beam melting (EBM);
Selective laser melting (SLM)

1. Powders dispersed over build platform;
2. Laser melts and fused powders;
3. Build platform moves down and feeder bed rises, spreading new layer of powders;
4. Process 2-3 repeat until finish;
5. Part is left to cool before being removed; loose materials brushed away.

Available Printers	Sinterit Lisa; EOS P 500; ARCAM A2X; 3D Systems ProX300
Materials	Powder based metal (e.g. titanium, steel) or polymers (e.g. nylon)
Pros	Isotropic mechanical properties; complex geometries
Cons	High power usage; grainy surface finish

Material Jetting:

Drop on Demand (DOD) approach

1. Droplets of liquid resin are jetted to the desired locations while the printhead travels over the build platform;
2. Deposited materials solidified either by laser or blower. Build platform moves downwards.
3. Process 1-2 repeat until finish;
4. Completed part is left to cool or laser cured and removed.

Available Printers	Mimaki 3DUJ-553 UV LED
Materials	Polymers (e.g. PS, polypropylene, PMMA, HDPE)
Pros	Smooth surface finish; high accuracy; homogeneous mechanical and thermal properties
Cons	High cost; support materials required; limited material choice

Table 2: Water-actuated smart materials and applications.

Application	Description	Reference
Cell-resembling structures for tissue engineering and targeted drug delivery	Picoliter aqueous flower-shaped network spontaneously self-folded into hollow sphere, arising as a result of different swelling from two layers of lipid droplets (1,2-diphytanoyl-sn-glycero-3-phosphocholine) with different osmolarities (Figure 4A).	Villar et al. ^[79]
Cell encapsulation for insulin production	Self-folding cylindrical scaffolds made of poly(ethylene glycol)-based bi-layered hydrogel used to encapsulate cells in benign environment (Figure 4B).	Jamal et al. ^[80]
Tissue regeneration or smart valve application	Water-actuated reversible natural hydrogel construct composed of carboxymethyl cellulose hydrocolloid (Figure 4C).	Mulakkal et al. ^[81]
Smart joint	Non-trivial self-evolved structure constructed in “design-fabrication-simulation” workflow where both stretching and bending of 2D grid skeleton were considered. Water-actuation was realized with the use of expanding material, which was composed of hydrophilic acrylated monomers that create linear chains upon polymerization with a small amount of difunctional acrylate molecules.	Raviv et al. ^[47]
Controlled drug delivery	Drug capsule released therapeutics from hydrogel materials in swollen state.	Wang et al. ^[82]
Smart sensor	Reversible bending movement in a moisture-responsive hydrogel film made of poly(ethylene glycol) diacrylate.	Lv et al. ^[83]
Soft actuators and solvent-responsive sensors	Reversible deformation demonstrated with a printed multiple-layered poly(glycerol sebacate) strip when exposed to organic solvents vapour.	Lei et al. ^[84]