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Vat polymerization-based bioprinting – process, materials, applications and regulatory challenges

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Abstract:

Over the years, the field of bioprinting has attracted attention for its highly automated fabrication system that enables the precise patterning of living cells and biomaterials at pre-defined positions for enhanced cell-matrix and cell-cell interactions. Notably, vat polymerization (VP)-based bioprinting is an emerging bioprinting technique for various tissue engineering applications due to its high fabrication accuracy. Particularly, different photo-initiators (PIs) are utilized during the bioprinting process to facilitate the crosslinking mechanism for fabrication of high-resolution complex tissue constructs. The advancements in VP-based printing have led to a paradigm shift in fabrication of tissue constructs from cell-seeding of tissue scaffolds (non-biocompatible fabrication process) to direct bioprinting of cell-laden tissue constructs (biocompatible fabrication process). This paper, presenting a first-time comprehensive review of the VP-based bioprinting process, provides an in-depth analysis and comparison of the various biocompatible PIs and highlights the important considerations and bioprinting requirements. This review paper reports a detailed analysis of its printing process and the influence of light-based curing modality and PIs on living cells. Lastly, this review also highlights the significance of VP-based bioprinting, the regulatory challenges and presents future directions to transform the VP-

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3 31 based printing technology into imperative tools in the field of tissue engineering and regenerative
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5 32 medicine. The readers will be informed on the current limitations and achievements of the VP-
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7 33 based bioprinting techniques. Notably, the readers will realize the importance and value of
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9 34 highly-automated platforms for tissue engineering applications and be able to develop objective
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11 35 viewpoints towards this field.

12
13 36 **Keywords: 3D bioprinting; 3D printing; Additive Manufacturing; Vat Polymerization**
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3 37 **1. Introduction**
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6 38 The first three-dimensional (3D) printing method termed as “stereolithography” was
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8 39 demonstrated in 1986 by Charles W. Hull; whereby thin layers of material are crosslinked with
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10 40 ultraviolet light to form complex 3D structures [1]. These solid freeform fabrication techniques
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12 41 were originally developed to replace traditional prototyping methods to accelerate the
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14 42 development of new products using computer design. Since then, the field of 3D printing has
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16 43 evolved and advanced rapidly to eventually facilitate the printing of biocompatible materials,
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18 44 cells and supporting components into highly-complex 3D functional living tissue constructs [2, 3].
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20 45 “3D bioprinting” can be termed as “the use of computer-aided processes for depositing living and
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22 46 non-living materials with a pre-defined 2D or 3D architecture to recapitulate bioengineered
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24 47 structures that are important in tissue engineering and regenerative medicine and fundamental
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26 48 cell biology studies” [4-7]. This technological advancement has led to a paradigm shift in
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28 49 fabrication of tissue constructs from cell-seeding of tissue scaffolds to direct bioprinting of cell-
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30 50 laden tissue constructs in a layer-by-layer fabrication approach [8]. These bioprinting techniques
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32 51 can be classified into three distinct process categories [9]; namely **vat polymerization (VP)**
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34 52 (stereolithography (SLA) [10], digital light processing (DLP) [11, 12] and two-photon
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36 53 polymerization (2PP) [13-15]), **material extrusion** (mechanical/pneumatic [16-20]) and **material**
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38 54 **jetting** (inkjet [21, 22], microvalve [23, 24], laser-assisted [25, 26] and acoustic [27-29]). Despite
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40 55 the significant advances in the field bioprinting, there are still several challenges that need to be
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42 56 addressed. A key challenge in bioprinting is the inability to extrude small volumes of cell-laden
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44 57 bio-inks through a small diameter orifice without experiencing high shear stresses [30].
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46 58 Furthermore, most of the bioprinting techniques are unable to create highly-complex and fine
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48 59 structures that closely mimic the native tissue microenvironment. The VP-based bioprinting
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50 60 process is an emerging biofabrication platform for tissue engineering applications due to its
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52 61 superior resolution and accuracy over other bioprinting technologies. It can achieve printing
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54 62 resolution in the nano-scale region (~100 nm) [31, 32] as compared to the printing resolution of
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56 63 50 – 200 µm in most biofabrication techniques. The high printing resolution is attractive for
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58 64 fabricating biomimetic microenvironment that closely emulates the complex extracellular
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60 65 matrices found in native tissues [33, 34].

2. Vat Polymerization-based bioprinting technique

Although the VP-based printing technique has been commercially available for more than three decades, it is mostly utilized for fabrication of tissue scaffolds which is followed by the traditional cell-seeding approach. As such, extensive research was conducted to enhance the biocompatibility of this printing technique for incorporation of living human cells. Hence, the goal of this review paper is to highlight the significant progresses made in the area of VP-based bioprinting that facilitates the fabrication of cell-laden tissue constructs for various applications. In the following sections, we provide an in-depth evaluation of various VP-based bioprinting techniques (SLA, DLP and 2PP), discuss the important requirements of the VP-based bioprinting technique (system, bio-resin and cellular considerations), present the various applications (tissue engineering, drug development and organ-on-chip) and lastly highlight the outlook of VP-based bioprinting technique.

2.1. Stereolithography (SLA)

Stereolithography (SLA)

Figure 1: Simplified schematic drawing of SLA bioprinting set-up with a scanning beam that scans across the bio-resin surface.

The photo-polymerization process occurs when the photo-curable liquid bio-resin is radiated by its specific curing wavelength. Polymerization is a term used to describe the process of linking

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3 84 small molecules (monomers) into larger molecules (polymers). In the SLA system, photo-curable
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5 85 bio-resin can be cured in two different approaches: (i) **top-down** printing approach and (ii)
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7 86 **bottom-up** printing approach. For the **top-down** printing approach, a scanning laser positioned
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9 87 directly above the vat cures a layer of resin on the build platform and it is lowered into the vat to
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11 88 repeat the curing process. Conversely, the scanning laser is positioned at the bottom of the vat
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13 89 for the **bottom-up** printing approach and the build platform is raised above the bio-resin vat via
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15 90 a “peeling” step between each printed layer. The computer-controlled laser beam scans over the
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17 91 surface of the photo-curable bio-resin along the pre-defined pattern to cure the photo-curable
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19 92 bio-resin to a defined depth which adheres to the previous layer [10]. The build platform is raised
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21 93 to allow the recoating of the liquid photo-curable bio-resin and repeat the curing process to
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23 94 achieve the final 3D complex constructs. The applied radial density should exceed the threshold
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25 95 value to initiate the photo-chemical reactions. It is also important to note that exposure to
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27 96 excessive light can cause bio-resin shrinkage and result in poor accuracy of printed constructs.

28 97 The use of support material is necessary in both bottom-up and top-down SLA systems; both
29
30 98 the support and build structures are of the same material and manual-removal of the support
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32 99 structures is performed after printing. The main role of supporting structures in the top-down
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34 100 SLA system is to facilitate precise printing of complex constructs, whereas the supporting
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36 101 structures in the bottom-up SLA printing process are crucial for the printing of complex
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38 102 overhanging structures. Furthermore, it is critical to ensure good bonding between the printed
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40 103 construct and build platform for a successful print and also reduce the cross-sectional area of
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42 104 each printed layer to minimize the force exerted on the printed constructs during the “peeling”
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44 105 step. The major difference between the top-down and bottom-up SLA printing technique is the
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46 106 printing speed; the bottom-up SLA printing process is generally slower due to the “peeling steps”
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48 107 whereas continuous light exposure can be applied in the top-down SLA printing process.

49 108 The two key parameters to manipulate the voxel size are the average laser power (i.e. the
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51 109 photon flux) and the irradiation time. The typical lateral printing resolution, which is in the range
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53 110 of 20 – 50 μm , is largely influenced by the laser spot size whereas the typical layer height in SLA
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55 111 system ranges between 25 – 100 μm , is largely dependent on the curing depth. The printing
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resolution (voxel diameter $d(N_o, t)$ and length $l(N_o, t)$) of a SLA system can be obtained by integration of the rate equation over the irradiation time t and the condition $R(r, z) \geq R_{TH}$ and results into [35]:

$$d(N_o, t) = w_o \sqrt{\frac{\ln\left(\frac{\sigma_2 \chi t N_o^2}{P_o}\right)}{\ln\left(\frac{P_o}{P_o - R_{TH}}\right)}}$$

$$l(N_o, t) = Z_R \sqrt{\ln\left(\frac{\sigma_2 \chi t N_o^2}{P_o}\right) - 1}$$

where w_o is the beam waist, σ_2 is the two-photon absorption (TPA) cross section, χ is the electric susceptibility, t is the irradiation time, N_o^2 is the squared photon flux, P_o is the initial concentration of initiating molecules, R_{TH} is the threshold radial density and Z_R is the Rayleigh length. The manipulation of these critical parameters shown in the kinetics equations enables the SLA printing system to scan across each individual bio-resin layer to achieve high printing resolution ($< 100 \mu\text{m}$) of fine structures within tissue-engineered constructs to closely mimic the microstructure within the native tissues/organs.

2.2. Digital Light Processing (DLP)

Digital Light Processing (DLP)

Figure 2: Simplified schematic drawing of DLP set-up with a digital micromirror device (DMD) that projects an image onto the surface of the entire bio-resin at pre-defined positions.

DLP is a different approach for crosslinking of photo-curable bio-resins; the use of a digital micro-mirror device (DMD) in DLP facilitates the immediate crosslinking of a layer of photo-curable resin instead of a single dot in SLA [36]. These DMDs are large arrays of micro-mirrors that can be rotated to be in either an 'on' or 'off' positions to facilitate the photo-crosslinking of a bio-resin layer. This results in significant reduction in build time as it is only dependent on the layer thickness and required exposure duration. For each given bio-resin, the cure depth is determined by the energy of the light in the DLP system to which the resin is exposed. This energy can be controlled by adjusting the power of the light source and exposure time.

Although the kinetics of the curing process is highly-complex [37], simplified equations are usually used to describe the polymerization kinetics in the fabrication of structures (projection of an image onto the surface of entire bio-resin to form a solidified layer) during DLP process. A semi-empirical equation is used to describe the exponential decay of light intensity as it passes through a medium in which it is absorbed by correlating the thickness of a solidified layer (cure depth, C_d) to the light irradiation dose, E (mJ/cm²) is used:

$$C_d = D_p \ln \left(\frac{E}{E_c} \right)$$

where C_d is the cured depth, D_p is the "penetration depth" of the laser projection into the bio-resin, E is the light energy on the irradiated surface and E_c is the critical energy required to induce polymerization. The working curve can be obtained [38] by creating a logarithmic-linear plot of C_d vs E which gives the values of E_c that is the intersection of the curve with the x-axis and the value of D_p as the slope of the curve. In DLP, the applied irradiation dose (E) must exceed the critical energy (E_c) in order to form a layer of solidified layer on the bio-resin surface. The E_c is highly dependent on the concentration of the PIs, dissolved oxygen and other inhibiting species [10]. The exposure time required to reach gel point is linearly dependent on the light intensity at that specific location; the cured bio-resin depth (C_d) increases logarithmically with time and

applied irradiation dose (E). Particularly, the macromer conversion at the layer-layer interface should be slightly higher than the gel point to facilitate good chemical and mechanical bonding between the layers. This relationship implies that the printing parameters can be optimized to achieve fine structures with strong interface bonding. The thickness of each individual layer can be controlled by the kinetics equation shown above; the manipulation of these parameters enables the rapid cross-linking of an entire bio-resin layer to fabricate large-volume, complex 3D tissue constructs at a rapid speed.

2.3. Two-photon polymerization (2PP)

Two-photon polymerization (2PP)

Figure 3: Simplified schematic drawing of 2PP set-up that creates a focused beam within the negative/positive photoresist to create complex microstructures.

The 2PP system is a two-photon absorption (TPA) fabrication technique whereby a near-infrared femtosecond laser (typical wavelength is ~ 740 nm from titanium:sapphire femtosecond laser) is used to fabricate precise 3D microstructures with high resolution on the nanoscale [14]. The 2PP process is initiated by three-order non-linear absorption within the focal region; the beam from the femtosecond laser is tightly focused into the photoresist (liquid bio-resin) on a glass cover slip with an oil-immersion objective lens to fabricate high-resolution 3D structures beyond the optical diffraction limit by moving the focused beam within the photoresist [31]. The photoresists consist of negative-tone and positive-tone photoresists [39]; TPA results in

crosslinking of polymer chains through radical polymerization to facilitate direct writing of structures within negative photoresist whereas TPA causes cleavage of polymer chains in the positive photoresist to create the reverse structures. Hence, negative photoresists containing acrylic oligomers/epoxy bio-resins with PIs (for radical generation) and monomers (constitute main skeleton of printed parts) are commonly used for 2PP applications. The polymerization process occurs when a molecule is excited from the ground state to an excited state by absorbing two photons for an extremely short time interval. The 2PP photoresist is transparent to this excitation wavelength and enables the fabrication of highly-complex structures at any spatial position within itself. The working range of the 2PP system is limited by i) the threshold laser power to initiate polymerization process and ii) burning threshold whereby the high power from the laser leads to material degradation and generates bubbles that damage the sample [40]. The scanning speed of 2PP system is typically in the range of millimeters per second as formation of the fine structures occurs at rapid scanning speed. The desired 3D microstructure is achieved by washing out the un-polymerized negative photoresist with ethanol after fabrication process.

The peak irradiance at the focus of the sample (I_o) is represented by an equation [41, 42] along with the following assumptions: (1) only the two-photon processes are considered, (2) the sample thickness is shorter than the Rayleigh range of the focus, (3) the laser is a TEM₀₀ Gaussian beam and (4) it is a perfectly Gaussian beam. A simplified equation in terms of the change in the normalized transmittance during the 2PP process can be expressed as:

$$I_o = \frac{4P_{Avg}}{\pi\omega_o^2 R\tau} \sqrt{\frac{\ln(2)}{\pi}} = \frac{4P_{Avg}}{M^2\lambda z_R R\tau} \sqrt{\frac{\ln(2)}{\pi}}$$

$$T(z) - 1 = \Delta T(z) = \frac{-\beta I_o L_{eff}}{2\sqrt{2} (1 + (z^2/z_R^2))}$$

where P_{Avg} is the average power of the laser source, ω_o is the beam waist, R is the repetition rate, τ is the pulse duration, M^2 is the beam quality, λ is the laser wavelength, z_R is the Rayleigh length, β is the non-linear absorption coefficient and L_{eff} is the effective path length. The simplified equation enables the qualitative comparison between different PI formulations by

measuring the relative change in transmittance at $z = 0$ while accounting for possible differences in the sample path lengths. It is important to maximize the TPA coefficient factor in formulation of new 2PP bio-resin to generate highly efficient absorbers as the TPA (non-linear absorption) is the key limiting factor that initiates the polymerization process.

3. Requirements for VP-based bioprinting

Apart from the concise overview of various VP-based bioprinting techniques in the previous section, we now discuss the important concepts of the VP-based bioprinting techniques in terms of system, bio-resin and cellular considerations in this section.

3.1. System considerations

3.1.1. Laser source

The laser source is the most critical component for VP-based bioprinting technology; the VP process is based on the curing of liquid photo-sensitive polymer by the action of a certain wavelength of light, which supplies the energy to initiate the polymerization reaction to form a 3D polymer network. The selection of suitable laser source (wavelength and intensity) and exposure time are the key factors to induce an appropriate photo-polymerization.

The lasers that are commonly used in SLA and DLP system emit wavelengths in the ultra-violet (UV: 365 – 385 nm) and visible light (405 nm) range, while the 2PP system utilizes near-infrared laser pulses (femtosecond laser) that are tightly focused within the photoresist to fabricate high-resolution 3D structures beyond the optical diffraction limit [43]. The shorter excitation wavelengths of the VP-based bioprinting system (UVA: 320 – 400 nm, UVB: 280 – 320 nm, UVC: 200 – 280 nm) have more harmful effects on the cells [44]. The light can be modeled as packets of energy called photons. The energy carried by a photon, E , can be represented by the Planck's equation,

$$E = h\nu$$

$$E = h \frac{c}{\lambda}$$

where E is the energy carried by a photon, h is Planck's constant (6.63×10^{-34} J.s), ν is the wavelength frequency, c is the speed of light and λ is the excitation wavelength. Thus, this indicates a shorter wavelength possesses greater energy that resulted in more DNA cellular damage. Hence, the choice of a suitable laser source is critical in maintaining high cell viability during the printing process.

3.1.2. Printing parameters

A CAD model is first converted into numerous sliced contours which are sent to the printer and sequentially fabricated in a layer-by-layer printing approach. Some key important printing parameters for VP-based printing include 1) laser scanning speed, 2) layer thickness, 3) Z compensation for material shrinkage and 4) part orientation. These printing parameters are highly inter-dependent; the selection of appropriate printing parameters is critical towards the fabrication of accurate printed parts. The laser scanning speed is critical for the selective curing and solidifying of the photo-polymer bio-resin and also facilitating strong interface bonding between adjacent layers. A rapid laser scanning speed might result in no curing if the applied irradiation dose is lower than that of the critical energy required to reach gel point, whereas a slow laser scanning speed might result in over-curing of surrounding bio-resin in porous structures. Although the macromer conversion at the layer-layer interface should be slightly higher than the gel point to facilitate good chemical and mechanical bonding between the layers, exposure to excessive light would result in bio-resin shrinkage and it is important to factor in Z compensation for material shrinkage. The complex relationship between the various printing parameters implies that the printing parameters should be carefully optimized to achieve high resolution structures with strong interface bonding. Support structure is always required in SLA/DLP printing process; the support and build structures are of the same material and manual-removal of the support structures is performed after printing. Thus, it is important that the build parts are optimally oriented to minimize the amount of support and total number of layers (in top-down approach) and to reduce chances of detachment by minimizing the cross-sectional area of build part as the forces generated during the "peeling" steps are proportional to the cross-sectional area of each layer (in the bottom-up approach). The optimization of the printing

parameters is critical towards achieving high printing resolution and accuracy of the bioprinted tissue constructs.

3.1.3. Vat design

Vat design is an important factor that greatly influences the accuracy of the bioprinted tissue constructs; different important considerations need to be factored in for vat design (SLA and DLP system) in both top-down and bottom-up approaches. In the top-down printing approach, it is challenging to achieve precise control over the uniformity of thickness across each bio-resin layer due to the surface tension. It would require a long time for the photo-sensitive bio-resin to flow over the substrate platform and reach an equilibrium state [45], hence a recoating blade is essential to expedite the spreading of bio-resin and achieve a more homogeneous layer thickness. Furthermore, the exposure of bio-resin to ambient air [46] would lead to oxygen inhibition of the free radical polymerization step [47]. In the bottom-up printing approach, it is necessary to have a transparent base at the bottom of the vat to facilitate the photo-polymerization process. Furthermore, it is critical to reduce the strong adhesive forces between the cured layer and the bottom surface of the vat to minimize the failure rate during the “peeling” steps [48]. A key design strategy is to apply low adhesion materials such as Teflon or silicone as a thin film at the bottom of the vat to minimize the distortion of printed parts during the “peeling” steps [49]. Another approach is to implement a tilting mechanism whereby one side of the vat is slowly tilted to separate the cured layer [50]. Therefore, the force is decreased largely because the structure is peeled off from the bottom surface of the vat instead of being pulled up [51]. Furthermore, this tilting mechanism is also effective for fabrication of highly-porous structures [52].

Although there is an increasing number of bio-resins being processed using VP-based bioprinting techniques, the technique is still mostly limited to the use of single bio-resin at a time. Although the use of complex sequential polymerization and rinsing steps within each printed layer can be implemented to pattern multiple bio-resins in a single construct (or even within a single layer), manual removal/filling of bio-resin and cleaning of the intermediate parts are still required prior to patterning of additional bio-resin [53, 54]. More intensive work can be

conducted to develop new rotating vat carousel system (changing between different bio-resins), rotating platform stage (for washing and drying of multi-material parts) and automatic leveling system (continuation of printing after washing steps).

3.2. Bio-resin considerations

The bio-resins for VP-based bioprinting technique comprise mainly of water as the solvent, monomers/oligomers, and PIs [55]. The PI reacts under light irradiation to produce either radicals or cations that react with monomer/oligomer units. Thereafter, methacrylate- or epoxide-based monomers/oligomers are transformed into solids or cross-linked hydrogels. The stringent requirements for bio-resins used in VP-based bioprinting technique include biocompatibility, suitable degradation kinetics with biocompatible by-products, structural and mechanical properties [56].

3.2.1. Viscosity considerations

Bio-resin properties such as viscosity, functionality, and cure times are important criteria for formulating photo-sensitive bio-resin appropriate for VP-based bioprinting techniques. A bio-resin with low viscosity (0.25 – 10 Pa.s) is preferred for VP-based bioprinting techniques as it improves curing rate, decreases construction time from faster recoating and eases cleaning of excess bio-resin after printing [57]. Particularly, the polymer viscosity affects print accuracy as it influences the recoating process [58]. Although a low viscosity bio-resin is advantageous for VP-based process, it leads to undesirable cell sedimentation due the bioprinting process and results in poor cell homogeneity within the bioprinted constructs. Different strategies that have been implemented to mitigate the cell sedimentation effect will be discussed in the later section. Hence, choosing a suitable range of viscosities for the bio-resins is important to achieve higher printing accuracy and shorter fabrication time.

3.2.2. Laser-material interaction

The laser-material interaction in VP-based bioprinting involves two steps, namely photo-physical and photo-chemical processes [59]. In the photo-physical process, light is absorbed by the chromophores and transits between ground and excited states. Energy of photon from the light source, $E = h\nu$, must be high enough for the excitation from ground to excited state, ΔE . The incident radiation from the light source changes the dipole moment of molecule [59]. In the photo-chemical process, the molecules are transformed through cleavage, electrons transfer reaction, hydrogen abstraction etc.

The light penetration into the bio-resin is directly related to the extinction coefficient in the Beer-Lambert equation and is characterized by D_p (light penetration depth into the polymer) [60].

$$D_p \propto \frac{2}{2.303\varepsilon[PI]}$$

whereby ε is the molar extinction coefficient and $[PI]$ is the molar concentration of PI. The macromer conversion at layer interface should be slightly higher than the gel point to achieve strong interface bonding. However, this over-exposure would result in partial polymerization of surrounding polymer that was intended to remain uncured. A bio-resin with high ε would result in a lower cure depth and facilitate better control of the polymerization process and minimize over-cure. The D_p of light can be decreased by increasing the PI concentration, the use of a dye or photo-absorbers [24] in the bio-resin. This non-reactive dye component or photo-absorber can compete with the PI in light absorption to give better control over the cure depth. This would serve as important information for development of new PIs; an ideal biocompatible PI should have a high molar extinction coefficient to achieve lower cure depth (better control over the layer thickness).

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3.2.3. Photo-curable bio-resin formulation

Some commonly used photo-curable bio-resins include polyethylene glycol acrylate (PEGDA) [61, 62] and gelatin methacrylate (GelMA) [61-64]. Naturally-derived biomaterials such as hyaluronic acid, dextran, and alginate have also been modified with methacrylate groups to form photo-curable bio-resins [65-67]. Furthermore, photo-absorber species can be introduced to improve print resolution by reducing the light penetration depth to achieve better control over the print resolution [68-71]. The photo-absorber absorbs scattered light from the laser source and prevents unwanted curing of the bio-resins to improve print resolution. The understanding of various components within a bio-resin is important for development of new bio-resin with good biocompatibility, printing resolution and cure depth.

3.2.4. PI formulation

Some important design considerations for formulation of new PIs for bioprinting applications include cytotoxic radicals from dissociation of PIs, and any aftermath effect arising from unreacted double bonds [72]. The biocompatibility study of PIs should include evaluation of i) type of PIs, ii) solvent for dissolving PIs, and iii) UV-initiated free radicals; it was shown that the type of free radical species formed during UV photo-dissociation has the most detrimental effect on the cell viability [73].

The choice of PI is crucial in determining the polymerization rate and affecting the resulting properties of printed constructs; the chemical selectivity of PI determines the resolution of fine features as it ensures polymerization of intended volume without triggering cascade of free-radical crosslinking [74]. Another consideration for PI selection is its water solubility and efficiency under hydrophilic condition [75]. The cytotoxicity of PI can be positively correlated to the amount of cellular uptake of the PIs; hydrophobic PIs can easily diffuse through cell membrane, whereas the introduction of more hydrophilic groups to the PIs decreases cellular uptake and reduce cytotoxicity effect [76, 77]. An effective way to produce water-soluble PIs is by introducing water-borne functional groups [78]; some common water-soluble PIs such as 2-hydroxy-1-[4-(2-hydroxyethoxy) phenyl]-2-methyl-1-propanone (Irgacure 2959), riboflavin and

lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) have been used in VP-based bioprinting for printing of cells and tissues (**Table 1**). The PIs used in 2PP require additional considerations such as balancing cross-sectional values (δ), laser intensity, exposure time and wavelength [76]. Particularly, the use of benzylidene cycloketone-based PIs such as G2CK and P2CK for 2PP has shown promising results for the feasibility of 2PP for processing of cell-laden bio-resins [79]. This section highlights the key design considerations for formulation of new PIs; an ideal PI for bioprinting applications should have good biocompatibility, high water solubility and maximum absorption efficiency at longer wavelength.

Table 1: Summary of different PIs used in VP-based bioprinting techniques

	Photo-initiators (PIs)	Maximum Absorption Efficiency Wavelength (nm)	Ref
UV	FMN Riboflavin-based	267	[80]
	Irgacure 2959	275	[81-83]
Vis	LAP	375	[83]
	VA-086	385	[84]
	Eosin Y	514	[81, 82, 85]

Figure 4: Normalized absorption spectrum of different PIs.

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6 366 **4.1. Tissue engineering and regenerative medicine (TERM)**
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11 368 influences on cellular adhesion, proliferation and subsequent downstream cellular behaviors [86]
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13 369 and different types of cells are known to respond distinctively to different extracellular matrices
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15 370 (composition, micro-structures, topography) [87]. Although numerous studies have highlighted
16
17 371 the importance of hierarchical porous structures in various human tissues [88, 89]; it is highly-
18
19 372 challenging to create such biomimetic microenvironment. The advancement of fabrication
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21 373 technologies has allowed us to create more complex microstructures to better mimic native
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23 374 structures [90]. Particularly, the introduction of VP-based bioprinting techniques facilitate the
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25 375 printing of high-resolution structures up to 20 μm in SLA and DLP and up to sub-100 nm region
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27 376 in 2PP. This superior printing resolution of VP-based bioprinting technique as compared to other
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29 377 3D bioprinting techniques had led to an increased interest in fabrication of highly-complex
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31 378 structures to better mimic native microenvironment. The use of VP-based bioprinting technique
32
33 379 can be utilized to create i) surface topography [91], ii) hierarchical structures [92] and iii) high-
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35 380 resolution cell patterning [93, 94] for fabrication of highly-complex 3D tissue constructs. Here,
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37 381 we discuss the use of VP-based techniques for various bioprinting applications in the field of
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39 382 tissue engineering and regenerative medicine (TERM) (**Table 2**).

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41 383 **4.1.1. Skin**
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43 384 The native human skin is a highly-complex organ comprising multiple types of cells with well-
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45 385 defined spatial structures; it consists of hierarchical porous structures with anisotropic
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47 386 distribution of cellular and extracellular matrix components, along with unique undulated feature
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49 387 at the epidermal-dermal junction [95]. At present, none of the bioprinted skin constructs can
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51 388 completely restore all the tissue functionalities [96, 97]. Most of the bioprinted skin constructs
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53 389 are printed using laser-based bioprinting [98, 99] and microvalve-based bioprinting [100-104].
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55 390 These prior research on skin bioprinting mainly focuses on the spatial patterning of skin cells and
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57 391 biomaterials to create biomimetic skin tissue constructs. Overall, the bioprinted skin constructs
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3 392 demonstrated some degree of resemblance to the native skin; as indicated by the presence of
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5 393 stratified keratinocyte region [103], uniform skin pigmentation [104] and presence of vascular
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7 394 networks [105].
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10 395 It is critical to incorporate the skin appendages within the bioprinted skin constructs to fully
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12 396 restore tissue functionalities; it was highlighted that the localized microenvironment plays a
13
14 397 critical role in regulation of the skin stem cell behaviors to achieve regeneration of hair and other
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16 398 skin appendages [106]. Furthermore, it has been demonstrated that the fabrication of
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18 399 physiological-relevant geometrical patterns such as mimicking the mesenchymal and epithelial
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20 400 compartmentalization in native hair follicles and the undulated morphology of the epidermal-
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22 401 dermal region is important for recapitulating important regulatory signaling pathways and
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24 402 physiology of native human skin [107, 108]. The VP-based bioprinting technique holds potential
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26 403 in creating high-resolution features (modulating surface topography of epidermal-dermal
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28 404 junction, creating hierarchical porous structures and patterning of multiple types of cells) to
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30 405 fabricate biomimetic skin tissue constructs.

31 406 **4.1.2. Nerve**

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34 407 Neural defects are commonly caused by trauma or neurological diseases which often leads
35
36 408 to chronic drastic loss of quality of life [109]. It is known that the distal end of the neuron would
37
38 409 gradually degenerate after traumatic injury whilst the proximal end would start to regenerate at
39
40 410 a rate of 1-2 mm/day [110]. However, the nerves would fail to regenerate properly if the damage
41
42 411 is more than 15 mm in length [111]. Intensive research has been conducted to fabricate neural
43
44 412 guidance conduits (NGCs) that can serve as a bridge for nerve regeneration and the VP-based
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46 413 bioprinting approach can facilitate the fabrication of biomimetic 3D *in-vitro* neural tissue
47
48 414 constructs with complex microscale-features [112].
49

50 415 A study has demonstrated the advantages of embedded core-shell nanoparticles with
51
52 416 sustained neurogenic factor delivery system [113]. A SLA system was used to fabricate 3D printed
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54 417 PEGDA neural scaffolds containing encapsulated PLGA nanoparticles with nerve growth factors
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56 418 (NGFs); the sustained release of growth factors enhanced neurite regeneration and the highly-
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3 419 porous structures significantly improve cellular adhesion. Markedly, a recent study has
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5 420 demonstrated the *in-vivo* implantation of 3D printed NGCs with micro-channels by DLP printing
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7 421 into complete sciatic nerve transections of mouse models [114]; optimization of the printing
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9 422 parameters and bio-resin components to match the optimal elastic modulus of native nerves (0.5
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11 423 – 13 MPa [115]) and the effective guidance of regenerating sciatic nerves via branching into the
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13 424 micro-channels and extending toward the distal end of the injury site led to recovery of motor
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15 425 and sensory functions in mouse models 11-weeks post implantation [114]. These results have
16
17 426 highlighted that the key role of NGCs is provide a suitable structural support that enhance the
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19 427 migration and adhesion of neurites across the lesion site.

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21 428 **4.1.3. Liver**

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23 429 The liver is one of the largest glands in the human body and it plays critical roles in
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25 430 production of biochemical for digestion, regulation of metabolism and detoxification of
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27 431 metabolites [116]. Over the years, numerous *in-vitro* liver models have been extensively studied
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29 432 and fabricated for drug screening, metabolism and hepato-toxicity studies. The traditional
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31 433 methods involve culturing human induced pluripotent stem cells (hiPSCs) onto a 2D culture plate
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33 434 and inducing them into hepatic-like cells and prior to evaluating their drug metabolizing activities
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35 435 [117]. However, such induced hepatic-like cells are considered immature as compared to native
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37 436 hepatocytes because they lack many gene expressions and enzyme activities such as cytochrome
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39 437 P450 which is heavily involved in the metabolism of numerous drugs. Furthermore, it is shown
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41 438 that hepatocytes needs to exist in a 3D micro-environment with interactions between other
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43 439 parenchymal cells and ECM in order for them to achieve full maturation with functional
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45 440 properties [118].

46
47 441 A recent study has demonstrated the fabrication of 3D bioprinted hepatic models that
48
49 442 closely mimic the native architecture and cell composition using DLP system [64]; hepatocytes
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51 443 derived from human induced pluripotent stem cells (hiPSCs) along with the supporting
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53 444 endothelial and mesenchymal cells are patterned in a specific manner that resemble anatomical
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55 445 structures of the human liver lobules. The 3D bioprinted tri-culture models showed improved
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57 446 morphological organization, higher liver-specific gene expression, increased metabolic secretion
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and enhanced cytochrome P450 induction as compared to 2D monolayer culture. Another study has also corroborated the importance of biomimetic structures in 3D liver models [119]; the presence of 3D biomimetic hexagonal structures (mimic liver lobules) led to increased albumin and cytochrome P450 expression as compared to monolayer control.

Figure 5: (Top) (a) Morphology of the bioprinted liver tissue model with distinct channel-structure at 10x magnification. (Bottom) (a) Immunohistochemistry staining of the printed liver tissue model after 2 weeks of culture with expression of vimentin (green) co-stained with cytokeratin

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8/18 (red), (b) albumin (Alb), (c) CYP3A4, (d) overview of each staining location within the bioprinted models, (e) negative control and (f) ZO-1 staining. Reproduced with permission [119].

4.1.4. Cartilage

The structural anatomy of cartilage is specifically designed to protect its surrounding tissues; it mainly comprises structural components with limited supply of nerves and blood vessels. The incorporation of glycosaminoglycans (GAGs) within the type II collagen helps to form highly-complex structures that can withstand external compressive forces. The key challenge in bioprinting of cartilage tissue constructs is the limited choice of suitable cell-laden biomaterials with strong mechanical properties [120]. A combination of both natural and synthetic bio-inks can complement each other to achieve a synergistic effect with the hybrid cartilage constructs.

A recent study has demonstrated the use of DLP technique in fabrication of cell-laden biodegradable cartilage constructs [121]; the printed cells within the hybrid bio-resins [poly-D,L-lactic acid/polyethylene glycol/poly-D,L-lactic acid (PDLLA-PEG)]/hyaluronic acid (HA)] are uniformly distributed throughout the constructs with high cell viability. The bioprinted cartilage constructs has a high compressive modulus of 780 kPa and enhanced expression of various chondrogenic genes (Sox9, collagen II and aggrecan) over a period of 4 weeks. Another study performed chemical modification of silk fibroin via a methacrylation process with glycidyl methacrylate (GMA) to yield photo-curable silk-fibroin based (Sil-MA) bio-resin with a high compressive stress of 910 kPa (similar to native cartilages) [122]. The Sil-MA bio-resin is then used to fabricate ring-like trachea cartilage constructs with good structural stability and biocompatibility; *in-vitro* histological evaluation of the chondrocytes-laden Sil-MA cartilage constructs showed good cell organization and extracellular matrix distribution (proteoglycan and collagen).

Figure 6: *In-vitro* histological evaluation of human chondrocytes loaded Sil-MA hydrogel for cartilage tissue engineering showing good cell organization and extracellular matrix distribution within the cartilage-like tissue over time. HE (H&E), MT (Masson's trichrome), SFO (Safranin-O), PKH (PKH26) (red: chondrocytes, green: Sil-MA bio-resin); scale bars represent 1mm (x4) and 500 μ m (x10). Reproduced with permission [122].

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4.1.5. Bone

Current treatment approaches for bone replacement (allografts and autografts) are hindered by limited sites for autologous bone harvesting and the increased risk of infection and rejection from allograft implantations. This has led to the development of tissue-engineered bone implants that can be potentially used for bone implantation. The human bone has a highly-complex hierarchical structure consisting of both organic components (type I, III, IV collagen and fibrillin) and inorganic crystalline mineral (hydroxyapatite) in a highly-specific network [123, 124]. These highly-complex nanoscale structures work in tandem to confer the native bones with high resistance to fracture.

An ideal tissue-engineered bone construct should be osteoconductive, biocompatible, biodegradable and possesses similar mechanical properties (0.22 – 10.44 MPa) to native bones [125]. Although the current bone research is more inclined towards the use of synthetic biomaterials due to its superior mechanical properties, the incorporation of natural biomaterials is important for cell encapsulation within a well-hydrated biomimetic microenvironment. Hence, there has been an interest in development of hybrid tissue constructs (combination of both natural and synthetic biomaterials) for bone tissue engineering applications. Different synthetic bone scaffolds have been fabricated using VP-based printing approaches; most of these synthetic scaffolds demonstrated similar mechanical properties as the native bones [126-129]. Particularly, a recent study has demonstrated the fabrication of cell-encapsulated bone tissue constructs with high compressive modulus (up to 0.2MPa) using a photo-curable nanosilicate-loaded gelatin-based hydrogel (GelMA loaded with Laponite) [130]. The same bio-resin could be potentially applied in VP-based bioprinting to fabricate the highly-complex biomimetic bone structures (highly-dense outer cortical bone with inner porous trabeculae network).

507 **Table 2: Summary of different studies on VP-based bioprinting techniques**

Set-up	λ	Bio-ink Composition			Resolution	Application	Ref
		Materials	PIs, UV absorber	Cells			
SLA	405 nm	PEGDA (10% w/v)	VA-086 (1% w/v)	MCF-7 (2×10^6 cells/ml)	50 μ m	Porous cell-laden tissue constructs	[74]
DLP	Visible light	PEGDA (16% w/v)	LAP (0.2% w/v)	hASCs (1×10^6 cells/ml)	70 μ m	Porous cell-laden tissue constructs	[11]
DLP	405	PEGDA (12% w/v)/ GelMA (8% w/v)	LAP (34 mM) Tartazine (1 – 5 mM)	hMSCs (1×10^6 cells/ml)	50 μ m	Functional multi-vascular networks	[61]
DLP	405	PEGDA (5% w/v)/ GelMA (5% w/v)	Eosin Y (0.01 mM) TEA (0.1% w/v) NVP (37 nM)	NIH3T3 (5×10^6 cells/ml)	50 μ m	Micro-scale cell patterning	[62]
DLP	365 nm	GelMA/HA (2.5%/1% w/v)	LAP (0.15% w/v)	HUVECs (2×10^7 cells/ml), C3H10T1/2 (4×10^5 cells/ml)	-	Vascular networks	[63]
DLP	Visible light	GelMA (15% w/v)	Eosin Y (0.02 mM) TEA (0.2% w/v) NVP (74 nM)	NIH-3T3 (8×10^6 cells/ml)	-	Micro-scale cell patterning	[85]
DLP	Blue light	GelMA (7% w/v)	LAP (0.1% w/v)	HepaRG (9.6×10^7 cells/ml) /human hepatic stellate cells (4×10^6 cells/ml)	-	Hepatic tissue models	[119]
DLP	Visible light	Methacrylated PDLLA-PEG/HA (30%/0.5% w/v)	LAP (0.6% w/v)	hASCs (4×10^6 cells/ml)	-	Cartilage tissue models	[121]
DLP	365 nm	Methacrylated silk fibroin (30% w/v)	LAP (0.2% w/v)	Human chondrocytes (1×10^7 cells/ml)	30 μ m	Cartilage tissue models	[122]
DLP	365 nm	A: GelMA (5% w/v) B: GelMA/GM-HA	A: LAP (0.15% w/v) B: LAP (0.225% w/v)	A: hiPSCs (-) B: HUVEC (2×10^7 cells/ml)	60 μ m	Hepatic tissue models	[131]

		(2.5%/1% w/v)		hASCs (4x10 ⁵ cells/ml)			
DLP	365 nm	PEGDA (15% w/v)	LAP (0.25% w/v)	HUVECs (2x10 ⁶ cells/ml)	-	Cell-laden tissue constructs	[132]
DLP	Visible light	PVA-MA/ GelMA (10%/1% w/v)	Ru/SPS (0.2/2 mM) Ponceau 4R (1% w/v)	hMSCs (5x10 ⁶ cells/ml) ACPCs (2x10 ⁷ cells/ml)	25 µm	Formulation of new bio- resin and photo- initiator	[133]
2PP	800 nm	GelMA (20% w/v)	G2CK (1.827 mM) P2CK (1.827 mM)	MG63 (1x10 ⁷ cells/ml)	-	Formulation of new photo- initiator	[79]
2PP	720 nm	PEGDA (7.5% w/v)	Eosin Y (10 µM) TEA (1.5% w/v) NVP (3.4 µl/ml)	HUVECs (24x10 ⁶ cells/ml)/ C3H10T1/2s (6x10 ⁶ cells/ml)	5 µm	High- resolution matrix patterning	[134]
2PP	720 nm	Gelatin- norbornene (7.5% w/v)	Diazosulfonate -based photo- initiator (0.5 mM)	L929 (1x10 ⁶ cells/ml)	0.5 µm	Fabrication of sub-µm cell-laden tissue constructs	[135]
2PP	800 nm	GelMA (15% w/v)	Hyaluronan- based photo- initiator (HAPI) (1 mM)	MC3T3 (1x10 ⁷ cells/ml)	-	Formulation of new photo- initiator	[136]
2PP	800 nm	GelMA (10% w/v)	Diazosulfonate -based photo- initiator (2 mM)	hASCs (2x10 ⁶ cells/ml)	-	Formulation of new photo- initiator	[137]

5. Regulatory Challenges for Vat Polymerization-based Bioprinting

5.1. Regulatory considerations for customizable tissue-engineered constructs

Although the current regulatory frameworks for tissue-engineered constructs do not consider the manufacturing process (3D bioprinting or conventional manufacturing technique) as an important criterion, the implementation of 3D bioprinting technology will likely increase the regulatory challenges for product quality assurance and testing for fabrication of 3D customizable cell-laden tissue-engineered constructs. Furthermore, it is clearly evident that a lack of harmonization between the regulatory bodies from the U.S. Food and Drug Administration (FDA) and EU European Medicines Agency (EMA) increases the regulatory burden on manufacturers to satisfy different sets of regulatory frameworks and it is important to bridge these existing regulatory gaps. As of now, there are no clear legislation from regulatory bodies on the approval of highly-complex, customizable 3D bioprinted tissue-engineered constructs. We aim to highlight some key regulatory considerations in terms of materials, process and product aspects in the following sections.

5.2. Materials aspects

Although numerous 3D printing resins have been cleared by FDA for dental printing (Class I devices – surgical guides, retainers, aligners and Class II devices – denture base) [138], there are currently no approved bio-resins for applications such as tissue-engineered human constructs. To date, no single bio-resin has had a history of clinical (human) use and the short-term clinical translation perspective of bio-resins seems poor. Nevertheless, more intensive works are necessary at current stage to further validate the safety and effectiveness of bio-resins against current gold-standards biomaterial such as collagen used in most FDA-approved tissue-engineered living skin substitutes [139].

It is imperative to understand the effect of vat polymerization printing on the photo-curable bio-resins as small deviations in the physical, mechanical and chemical properties can influence the performance, structural integrity and biocompatibility of the 3D bioprinted constructs. Furthermore, it is necessary to evaluate the effects of impurities such as unreacted photo-

initiators, free radicals or even degradation by-products on encapsulated living cells during manufacturing, post-processing and even after implantation. The manufacturer would need to assess and determine if the toxicity level is at an acceptable level using either conventional testing approaches as described in ISO10993-1 [140] or long-term biocompatibility studies to detect the effect of printing process and bio-resins on cellular behavior and proliferation and assess the potential occurrence of an adverse biological reaction upon contact during implantation.

5.3. Process aspects

An interesting question would be how to apply existing regulatory frameworks to on-demand fabrication of autologous patient-specific tissue-engineered constructs using an industrialized manufacturing process? The manufacturing complexities arise from the customization of patient-specific tissue-engineered constructs; from the computer-aided design – computer-aided manufacturing (CAD – CAM) model compilation to code generation steps for bioprinting [141]. The identification of clinically-relevant design parameters, threshold limit range (maximum/minimum) for the parameters, and tunable parameters to achieve customizable patient-specific tissue-engineered constructs. The patient’s anatomy is prone to errors in file conversion due to the difficulties in challenging conversions of complex anatomic curves, hence it is important to maintain data integrity throughout the file conversions. The implementation of automated designs via controlled mechanisms in customizable manufacturing processes can help to ensure predictable outcomes. Advanced 3D imaging and modelling software are integral to creating automated computational processes with well-defined design parameters that can be applied to generate patient-specific 3D architectures [142]. It would help to map pre-determined hatching geometry, biomaterials and different types of cells onto anatomically-relevant regions of the bioprinted construct.

The process validation requires a control strategy that implements a multi-parametric, risk-based approach to analyze the potential errors and the important relationships between process and build parameters on the critical quality attributes (CQAs) of the 3D bioprinted tissue-engineered constructs. The establishment of threshold process parameter limits and well-

defined processing steps would provide assurance and validation of the product's design and build specification. A detailed production flow diagram and a high-level summary of each critical manufacturing step would be important for determining the root cause of failures from manufacturing defects with a clear understanding of each step. Furthermore, it is also critical to demonstrate the process reproducibility by documenting the manufacturing tolerances of the individual machine and validating the software workflow to ensure file compatibility during format conversions by identifying critical file attributes and verifying performance criteria.

Furthermore, the 3D bioprinted tissue constructs should be manufactured in a reproducible manner that is sterile and contamination-free throughout the entire process. The sterility assurance processes encompass the raw materials for preparation of bio-resins, cleaning processes for 3D bioprinters and final 3D bioprinted tissue constructs during printing and post-printing. A recent study has evaluated different sterilization techniques for photo-curable bio-resins [143]; the use of ethylene oxide (EtO) was the least destruction sterilization methods with no damaging effect on the photo-crosslinkable methacrylate/methacrylamide groups and the EtO treatment also eliminated a bacterial challenge of *E. coli* without causing a significant decrease in viability and proliferation of encapsulated hMSCs.

5.4. Product aspects

The regulatory routes for tissue-engineered constructs are documented under Title 21 of the Code of Federal Regulations (CFR) Part 1271 [144]. Although the manufacturing process (conventional or 3D bioprinting) is not taken into account, it is critical to provide the regulators with well-defined regulatory frameworks on how to apply existing laws and regulations on 3D-bioprinted personalized tissue-engineered constructs. It is also important to achieve standardization and documentation of best practices for design and fabrication of 3D bioprinted patient-specific tissue constructs.

There are huge regulatory challenges in the implantation of customizable 3D bioprinted tissues and organs; 1) the combination of living cells and bioactive materials in 3D bioprinted tissue constructs gives rise to complex mechanisms with little information on its long-term

influence in human hosts, 2) it is difficult to meet a regulatory definition of potency as the mechanisms of action in 3D bioprinted tissue constructs is highly-complex due to its multiple components and 3) highly-complex manufacturing and product characterization as minor manufacturing changes for customizability might have significant, unexpected effects on the product features. Hence, it is imperative to implement a well-defined and integrated regulatory pathway to avoid redundant and overburdened regulatory pathways.

6. Future Outlook

6.1. Developing new biocompatible PIs

Most of the commercially available PIs are limited in terms of the extinction coefficient and water-solubility. A recent study has demonstrated the development of new water-dispersible nanoparticles of 2, 4, 6-trimethylbenzoyl-diphenylphosphine oxide (TPO) with high extinction coefficient that enables rapid printing of complex 3D hydrogel structures [145]. On the other hand, development of water-soluble PIs for 2PP requires synthesizing photo-sensitizers with two-photon absorption capability and the benzylidene cyclanone dyes (large cross section values of 400 – 3300 GM at ~800 nm) have the potential for making hydrophilic PIs [75, 76, 146]. The use of visible light sensitive PIs of high molar absorptivity enables printing with high resolution features; tris-bipyridyl ruthenium hexahydrate (Ru) with sodium persulfate (SPS) has a significantly higher molar absorptivity of 300 times as compared to lithium phenyl-2, 4, 6-trimethylbenzoylphosphinate (LAP) [68]. The use of Ru/SPS PIs is also compatible with different natural biomaterials such as fibrin and collagen without any further chemical modification [147]. Carboxylation of conventionally insoluble camphorquinone resulted in formulation of PIs with higher photo-reactivity, increased water solubility and better biocompatibility [148].

6.2. Improving cell homogeneity in VP-based bioprinting

An existing challenge that is typically overlooked in most bioprinting systems is the cell sedimentation effect, which occurs as a result of gravitational forces and leads to poor cell homogeneity within the bio-inks over time [149, 150]. One of the key design considerations in 3D bioprinting is to achieve uniform cell distribution within the bioprinted tissue constructs and

it is of paramount importance to ensure good cell homogeneity within the bio-resin. A common strategy to mitigate the effect of gravitational forces is to achieve a state of neutral buoyancy [151] between the encapsulated cells and bio-resin. A study has demonstrated the incorporation of Percoll at 37.5% v/v, nano-level colloidal silica particles coated with polyvinylpyrrolidone (PVP), helps to increase the density of bio-resin to achieve good homogeneity of the encapsulated cells [11]. However, the use of Percoll led to some unexpected extraneous photo-crosslinking especially during the fabrication of porous structures as indicated by the partial pore occlusion. Hence, more work is still required to test the feasibility of other agents in improving the cell homogeneity within the bio-resin.

6.3. High speed VP process

Recently, a novel and rapid VP-based printing approach known as continuous liquid interface production (CLIP) has been proposed by eliminating the recoating process using an oxygen-containing “dead zone” between fabrication part and the vat of polymer [152]. Oxygen inhibition of free radical polymerization is an undesirable scenario during the VP-based printing process; the oxygen can either quench the photo-excited PIs or create peroxides by combining with the free radicals from the photo-cleaved PIs. An oxygen-permeable, UV-transparent window (amorphous fluoropolymer window, Teflon AF 2400) is placed below a liquid resin bath to create a “dead zone” and the curing part is continuously drawn out of the resin above the “dead zone” to create suction forces that constantly renew the reactive liquid resin. This continuous printing process is fundamentally different from the traditional bottom-up SLA systems, whereby the UV exposure, resin renewal and part movement must be conducted in separate and discrete steps.

The “dead zone” thickness and the polymerization of the cured thickness above the “dead zone” can be represented by:

$$\text{Dead zone thickness} = C \left(\frac{\Phi_0 \alpha_{PI}}{D_{c0}} \right)^{-0.5}$$

$$\text{Cured thickness} = \frac{1}{\alpha} \ln \left(\frac{\Phi_0 \alpha_{PI} t}{D_{c0}} \right)$$

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3 643 whereby the C is a proportionality constant, ϕ_0 is the number of incident photons at the image
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5 644 plane per area per time, α_{PI} is the product of PI concentration and the wavelength-dependent
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7 645 absorptivity, D_{c0} quantifies the resin reactivity of a monomer-PI combination, α is the resin
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9 646 absorption coefficient and t is the exposure time.

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11 647 A dead zone thickness in the order of tens of micrometers can be maintained by careful
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13 648 selection of control parameters such as photon flux, resin optical and curing properties. A printing
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15 649 resolution of 50 μm can be achieved and complex 3D structures can be fabricated at the rates of
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17 650 hundreds of millimeters per hour. This high printing speed would potentially enable the rapid
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19 651 fabrication of parts within minutes instead of hours [152].

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21 652 A recent study has demonstrated the ability to fabricate geometrically complex, centimeter-
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23 653 scale cell-laden tissue constructs at a rapid printing speed via volumetric bioprinting (VBP)
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25 654 technique [153]. The mechanism behind this new concept is to achieve a minimum absorption of
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27 655 light dose to overcome the gelation threshold; the resin container is set into rotation and
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29 656 synchronously irradiated with a sequence of 2D light patterns. The light dose from each single
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31 657 exposure is insufficient to crosslink the bio-resin and it only solidifies in specific regions where
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33 658 the accumulation of multiple angular exposures results in an absorbed dose exceeding the
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35 659 gelation threshold. The bioprinted tissue constructs showed high volume accuracy and
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37 660 homogeneous cell distribution (avoid cell sedimentation due to its rapid fabrication speed).

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39 661 **6.4. 4D bioprinting using VP-based printing technique**
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42 663 Most native tissues undergo dynamic changes during tissue conformations to form complex
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44 664 3D structures, microarchitectures, extracellular matrix [154]. The potential use of 4D bioprinting
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46 665 technology based on 3D bioprinting with the embedded ability of shape transformation may
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48 666 more accurately mimic the dynamics of the native tissues which is not achievable in conventional
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50 667 3D structures with static properties and behavior [155]. 4D bioprinting can be defined as “groups
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52 668 of programmable self-assembly, self-folding or self-accommodating technologies which include
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54 669 three main defining or essential components: (i) man-made programmable design, (ii) 2D or 3D
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bioprinting process, (iii) post-printing programmable evolving of bioprinted constructs which could be driven by cells or biomaterials and triggered by external signals” [156].

The use of VP-based printing technique is common for 4D printing applications; various photo-curable stimuli-responsive materials have been used [157-160]. A recent study has reported a ultrafast fabrication approach that relies on generated stresses during swelling of responsive polymer to evolve from printed 2D structures into highly-complex 3D structures [161]. Polymer networks with variable degrees of monomer conversion and crosslinking density are produced using DLP technique, which led to differential swelling within the network, creating stresses that transformed printed 2D structures into complex 3D structures. Another recent work has demonstrated the 4D printing of smart biomedical scaffolds using a novel soybean oil epoxidized acrylate [162]; the fabricated scaffold is able to change to a temporary shape at -18°C and exhibit its shape memory effect and recover to its original shape at physiological temperature of 37°C . Similarly, a temperature-responsive mixture of tert-Butyl acrylate with di(ethylene glycol) diacrylate (tBA-co-DEGDA) polymer network based on a dual-component phase switching mechanism was fabricated using SLA technique to form shape-memory 4D printed structures [163]. However, most of these materials used in 4D printing applications are not designed for biomedical applications.

4D bioprinting is an exciting field that enables the transformation of patterned structures via exploiting the shape-changing features of various stimuli-responsive biomaterials. Some of the key challenges that remain to be solved include (i) development of biocompatible bio-resins suitable for cell encapsulation, (ii) interplay between the cellular viability and function/dynamic modulation of the bioprinted constructs (influence of cells on shape-changing properties and also influence of shape-changing stimuli on cell viability), (iii) design mechanics of the stimuli-responsive bio-inks for repeated responsiveness. To advance the innovation in 4D bioprinting, it is necessary to develop more advanced smart biomaterials and also incorporate rational computer designs of sophisticated multiple stimuli-responsive procedures to facilitate its widespread use in building highly-complex self-morphing 3D tissue constructs.

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6.5. Drug development

There has been a growing interest in 3D printing of drugs since the first approval of 3D printed drug by the Food and Drug Administration (FDA) in 2015 [164-166]. The use of VP-based printing technique for drug printing has several advantages such as rapid fabrication speed and high printing resolution. Nevertheless, the use of photo-polymerizable resin is relatively uncommon in pharmaceutical manufacturing and the remnants of uncured resin may potentially pose some adverse effects. A recent study has demonstrated the manufacturing of drug-loaded tablets with specific extended release profiles using SLA system [167]; different drugs such as paracetamol and 4-aminosalicylic acid (4-ASA) are loaded into the photo-polymerizable resin consisting of polyethylene glycol diacrylate (PEGDA), polyethylene glycol 300 (PEG 300) and diphenyl(2, 4, 6-trimethylbenzoyl)phosphine oxide to manufacturing drug-loaded tablets of varying drug concentrations. There is a high retention of drug content (> 90%) within printed tablets and the drug release profile can be manipulated via selection of suitable polymer combination (PEGDA/PEG 300 ratio) within the resin. Another study has demonstrated the influence of water content on release rates of SLA-printed drug-loaded hydrogels using biocompatible, non-toxic riboflavin (vitamin B2) PI [168]. The main mechanism for drug release is via drug diffusion through the swollen matrix of the hydrogels; a formulation with higher initial water content can swell to its equilibrium state faster and hence resulting in faster drug release.

The key attributes that favor 3D drug printing over traditional manufacturing practices include personalized medicine (dose flexibility), increased product complexity (controlled drug release, precise dosing) and lastly on-demand manufacturing. Numerous researches have highlighted the importance of personalizing drug release based on patient anatomy and population-level variability in drug metabolism [169-171]; the use of 3D printing enables the fabrication of multiple small, individualized batches of personalized drugs more economically feasible and it also allows for tailoring of drug dosing in accordance to the patient's mass and metabolism. The use of advanced 3D printing technique not only enables the manipulation of drug release profile via geometric design and architectural complexity of the printed drugs, but also facilitates more accurate control over the precise dosing to achieve higher batch-to-batch

consistency. Lastly, the ability to print on-demand would potentially improve medicine access, reduce wastage of drugs with limited shelf-life.

6.6. 3D printing of micro-fluidics devices

Micro-fluidics is widely utilized for precise manipulation of fluids in channels with dimensions of tens of micrometers [172]. Over the years, numerous research groups are thriving to use 3D printing techniques for fabrication of improved and highly-complex micro-fluidics designs within a single step [173]. Due to its low operating costs, VP-based printing process (SLA/DLP) is a potential manufacturing tool for micro-fluidics and organ-on-a-chip studies [174]. A re-usable template PDMS micro-fluidic device is fabricated using VP-based printing technique at a low cost of ~ USD \$0.48 at high printing resolution of 50 μm [175]. The integrity test of the printed device is performed using fluorescein solution; there is no observation of leakage or blockages from the microscopy images. Another similar study has developed a one-step direct molding technique for fabricating 3D truly micro-fluidic channels; an optimized procedure is applied to remove the residual PDMS via heat treatment, followed by oxygen plasma treatment and lastly coating of the inner channels with fluorinated silane molecules [176]. Another study has demonstrated the use of VP-based printing technique for fabrication of mixer design with through holes for micro-fluidics purposes; it facilitates the rapid fabrication of highly-sophisticated features at low development cost (USD \$0.57) [177].

Some other interesting application of VP-based printing technique for fabrication of micro-fluidics devices include the fabrication of integrated valves and functional modules. Up till now, most valves in micro-fluidics devices are made up of PDMS. However, the integration of PDMS valves require significantly high engineering expertise and costly equipment. A recent study has demonstrated the fabrication and testing of 3D printed micro-fluidics devices with integrated membrane-based valves [178]; a customized resin formulation is used to fabricate the micro-fluidics devices with integrated valves that can last up to 800 actuations. Furthermore, another study has also demonstrated a built-in valve fabrication inside micro-channels using SLA which can be further modified to form larger operational units such as multi-way switches and pumps [179]. An advanced fabrication and assembly method for modulated micro-fluidics devices is

demonstrated using SLA technique [180]; it enables easy integration of different functional modules onto the block chip assembly for various bio-sensing applications.

Despite the various advantages of VP-based printing techniques for printing for micro-fluidics devices, the key challenge with printing enclosed micro-fluidics structures is the removal of uncured resin [181]. It is highly challenging to remove the liquid resin due to the fabrication of small structures within the micro-fluidics devices. The use of low viscosity resin and the ability to exclusively constrain light within the pre-defined region (to prevent over-exposure) are critical towards the fabrication of improved micro-fluidics devices with easy removal of any uncured resin. The continued development of VP-based printing technique with improved printing resolution will facilitate the fabrication of micro-fluidics devices at a low-cost and rapid manner.

Figure 7: Applications of vat polymerization-based bioprinting. i) tissue engineering and regenerative medicine, ii) drug development and iii) 3D printing of microfluidics devices.

7. Concluding Remarks

Although the VP-based printing technique is a well-established printing method, the limited choice of biocompatible materials and the use of single bio-resin has hindered its widespread use in the field of tissue engineering. The understanding of various important requirements of VP-

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3 771 based bioprinting techniques equips potential new users with the critical knowledge on material
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5 772 formulation and machine development. As the technology continues to grow, it can be
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7 773 anticipated that the regulatory bodies will implement a more centralized and defined regulatory
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9 774 pathway. The development of advanced bio-resins and the incorporation of rational computer
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11 775 designs for sophisticated printing procedures would facilitate its widespread use in building
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13 776 highly-complex multi-material 3D tissue constructs. The VP-based bioprinting is an emerging
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15 777 bioprinting technique due to its superior printing resolution which currently cannot be matched
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17 778 by other bioprinting techniques. It is advantageous to leverage its superior printing resolution for
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19 779 creating highly-complex surface topography, hierarchical structures and high-resolution cell
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21 780 patterning that are critical for various applications such as tissue engineering and regenerative
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23 781 medicine (TERM), drug manufacturing and fabrication of complex microfluidics devices.

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References

[1] C.W. Hull, Apparatus for production of three-dimensional objects by stereolithography, Google Patents, 1986.

[2] W.L. Ng, C.K. Chua, Y.-F. Shen, Print Me An Organ! Why We Are Not There Yet, *Prog. Polym. Sci.* 97 (2019) 101145.

[3] T.A. Mir, S. Iwanaga, T. Kurooka, H. Toda, S. Sakai, M. Nakamura, Biofabrication offers future hope for tackling various obstacles and challenges in tissue engineering and regenerative medicine: A Perspective, *Int J Bioprint* 5(1) (2019) 153-163.

[4] J. Groll, T. Boland, T. Blunk, J.A. Burdick, D.-W. Cho, P.D. Dalton, B. Derby, G. Forgacs, Q. Li, V.A. Mironov, Biofabrication: reappraising the definition of an evolving field, *Biofabrication* 8(1) (2016) 013001.

[5] F. Guillemot, V. Mironov, M. Nakamura, Bioprinting is coming of age: report from the International Conference on Bioprinting and Biofabrication in Bordeaux (3B'09), *Biofabrication* 2(1) (2010) 010201, 1-7.

[6] T.-S. Jang, H.-D. Jung, M.H. Pan, W.T. Han, S. Chen, J. Song, 3D printing of hydrogel composite systems: Recent advances in technology for tissue engineering, *Int J Bioprint* 4(1) (2018) 126 - 153.

[7] J.M. Lee, W.L. Ng, W.Y. Yeong, Resolution and shape in bioprinting: Strategizing towards complex tissue and organ printing, *Appl. Phys. Rev* 6(1) (2019) 011307, 1-16.

[8] K.-f. Ren, M. Hu, H. Zhang, B.-c. Li, W.-x. Lei, J.-y. Chen, H. Chang, L.-m. Wang, J. Ji, Layer-by-layer assembly as a robust method to construct extracellular matrix mimic surfaces to modulate cell behavior, *Prog. Polym. Sci.* 92 (2019) 1 - 34.

[9] J.M. Lee, S.L. Sing, M. Zhou, W.Y. Yeong, 3D bioprinting processes: A perspective on classification and terminology, *Int J Bioprint* 4(2) (2018) 151.

[10] F.P. Melchels, J. Feijen, D.W. Grijpma, A review on stereolithography and its applications in biomedical engineering, *Biomaterials* 31(24) (2010) 6121-6130.

[11] H. Lin, D. Zhang, P.G. Alexander, G. Yang, J. Tan, A.W.-M. Cheng, R.S. Tuan, Application of visible light-based projection stereolithography for live cell-scaffold fabrication with designed architecture, *Biomaterials* 34(2) (2013) 331-339.

[12] R. Gauvin, Y.-C. Chen, J.W. Lee, P. Soman, P. Zorlutuna, J.W. Nichol, H. Bae, S. Chen, A. Khademhosseini, Microfabrication of complex porous tissue engineering scaffolds using 3D projection stereolithography, *Biomaterials* 33(15) (2012) 3824-3834.

[13] E.D. Lemma, B. Spagnolo, M. De Vittorio, F. Pisanello, Studying Cell Mechanobiology in 3D: The Two-Photon Lithography Approach, *Trends Biotechnol.* 37(4) (2018) 358-372.

[14] J.-F. Xing, M.-L. Zheng, X.-M. Duan, Two-photon polymerization microfabrication of hydrogels: an advanced 3D printing technology for tissue engineering and drug delivery, *Chem. Soc. Rev.* 44(15) (2015) 5031-5039.

[15] D. Mandt, P. Gruber, M. Markovic, M. Tromayer, M. Rothbauer, S.R.A. Kratz, S.F. Ali, J. Van Hoorick, W. Holnthoner, S. Muehleder, Fabrication of biomimetic placental barrier structures within a microfluidic device utilizing two-photon polymerization, *Int J Bioprint* 4(2) (2018) 144 - 155.

[16] I.T. Ozbolat, M. Hospodiuk, Current advances and future perspectives in extrusion-based bioprinting, *Biomaterials* 76 (2016) 321-343.

[17] W.L. Ng, W.Y. Yeong, M.W. Naing, Development of Polyelectrolyte Chitosan-gelatin Hydrogels for Skin Bioprinting, *Procedia CIRP* 49 (2016) 105-112.

- [18] W.L. Ng, W.Y. Yeong, M.W. Naing, Polyelectrolyte gelatin-chitosan hydrogel optimized for 3D bioprinting in skin tissue engineering, *Int J Bioprint* 2 (1) (2016) 53-62.
- [19] P. Zhuang, W.L. Ng, J. An, C.K. Chua, L.P. Tan, Layer-by-layer ultraviolet assisted extrusion-based (UAE) bioprinting of hydrogel constructs with high aspect ratio for soft tissue engineering applications, *PLoS One* 14(6) (2019) e0216776, 1-21.
- [20] W.L. Ng, Yeong, W. Y. & Naing, M. W., Potential of Bioprinted Films for Skin Tissue Engineering, *Proceedings of the 1st International Conference on Progress in Additive Manufacturing* (2014) 441-446.
- [21] R.E. Saunders, B. Derby, Inkjet printing biomaterials for tissue engineering: bioprinting, *Int. Mater. Rev.* 59(8) (2014) 430-448.
- [22] H. Gudupati, M. Dey, I. Ozbolat, A Comprehensive Review on Droplet-based Bioprinting: Past, Present and Future, *Biomaterials* 102 (2016) 20-42.
- [23] W.L. Ng, J.M. Lee, W.Y. Yeong, M. Win Naing, Microvalve-based bioprinting – process, bio-inks and applications, *Biomater. Sci.* 5(4) (2017) 632-647.
- [24] W.L. Ng, W.Y. Yeong, M.W. Naing, Microvalve bioprinting of cellular droplets with high resolution and consistency, *Proceedings of the International Conference on Progress in Additive Manufacturing* (2016) 397-402.
- [25] L. Koch, M. Gruene, C. Unger, B. Chichkov, Laser assisted cell printing, *Curr. Pharm. Biotechnol.* 14(1) (2013) 91-97.
- [26] L. Koch, O. Brandt, A. Deiwick, B. Chichkov, Laser assisted bioprinting at different wavelengths and pulse durations with a metal dynamic release layer: Aa parametric study, *Int J Bioprint* 3(1) (2017) 42-53.
- [27] F. Guo, P. Li, J.B. French, Z. Mao, H. Zhao, S. Li, N. Nama, J.R. Fick, S.J. Benkovic, T.J. Huang, Controlling cell–cell interactions using surface acoustic waves, *Proc. Natl. Acad. Sci. U.S.A.* 112(1) (2015) 43-48.
- [28] S.M. Naseer, A. Manbachi, M. Samandari, P. Walch, Y. Gao, Y.S. Zhang, F. Davoudi, W. Wang, K. Abrinia, J.M. Cooper, Surface acoustic waves induced micropatterning of cells in gelatin methacryloyl (GelMA) hydrogels, *Biofabrication* 9(1) (2017) 015020, 1-11.
- [29] Y. Sriphutkiat, S. Kasetsirikul, Y. Zhou, Formation of cell spheroids using Standing Surface Acoustic Wave (SSAW), *Int J Bioprint* 4(1) (2018) 130-141.
- [30] J.M. Townsend, E.C. Beck, S.H. Gehrke, C.J. Berkland, M.S. Detamore, Flow behavior prior to crosslinking: the need for precursor rheology for placement of hydrogels in medical applications and for 3D bioprinting, *Prog. Polym. Sci.* 91 (2019) 126 - 140.
- [31] S. Kawata, H.-B. Sun, T. Tanaka, K. Takada, Finer features for functional microdevices, *Nature* 412(6848) (2001) 697.
- [32] V.F. Paz, M. Emons, K. Obata, A. Ovsianikov, S. Peterhänsel, K. Frenner, C. Reinhardt, B. Chichkov, U. Morgner, W. Osten, Development of functional sub-100 nm structures with 3D two-photon polymerization technique and optical methods for characterization, *Journal of Laser Applications* 24(4) (2012) 042004.
- [33] B. Walters, J. Stegemann, Strategies for directing the structure and function of three-dimensional collagen biomaterials across length scales, *Acta Biomater.* 10(4) (2014) 1488-1501.
- [34] X.-Y. Yang, L.-H. Chen, Y. Li, J.C. Rooke, C. Sanchez, B.-L. Su, Hierarchically porous materials: synthesis strategies and structure design, *Chem. Soc. Rev.* 46(2) (2017) 481-558.
- [35] S. Engelhardt, Direct laser writing, In: Schmidt V, M. R. Beleggratis MR, editors. *Laser Technology in Biomimetics.*, Heidelberg, Springer-Verlag, 2013, pp. 13-65.

- [36] W. Zhu, X. Ma, M. Gou, D. Mei, K. Zhang, S. Chen, 3D printing of functional biomaterials for tissue engineering, *Curr. Opin. Biotechnol.* 40 (2016) 103-112.
- [37] E. Andrzejewska, Photopolymerization kinetics of multifunctional monomers, *Prog. Polym. Sci.* 26(4) (2001) 605-665.
- [38] P.F. Jacobs, *Rapid prototyping & manufacturing: fundamentals of stereolithography*, Society of Manufacturing Engineers 1992.
- [39] Y.-L. Zhang, Q.-D. Chen, H. Xia, H.-B. Sun, Designable 3D nanofabrication by femtosecond laser direct writing, *Nano Today* 5(5) (2010) 435-448.
- [40] A.K. Nguyen, R.J. Narayan, Two-photon polymerization for biological applications, *Mater. Today* 20(6) (2017) 314-322.
- [41] M. Sheik-Bahae, A.A. Said, T.-H. Wei, D.J. Hagan, E.W. Van Stryland, Sensitive measurement of optical nonlinearities using a single beam, *IEEE J. Quantum Electron.* 26(4) (1990) 760-769.
- [42] A. Ajami, W. Husinsky, R. Liska, N. Pucher, Two-photon absorption cross section measurements of various two-photon initiators for ultrashort laser radiation applying the Z-scan technique, *JOSA B* 27(11) (2010) 2290-2297.
- [43] B.H. Cumpston, S.P. Ananthavel, S. Barlow, D.L. Dyer, J.E. Ehrlich, L.L. Erskine, A.A. Heikal, S.M. Kuebler, I.Y.S. Lee, D. McCord-Maughon, J. Qin, H. Röckel, M. Rumi, X.-L. Wu, S.R. Marder, J.W. Perry, Two-photon polymerization initiators for three-dimensional optical data storage and microfabrication, *Nature* 398 (1999) 51.
- [44] R. Masuma, S. Kashima, M. Kurasaki, T. Okuno, Effects of UV wavelength on cell damages caused by UV irradiation in PC12 cells, *J. Photochem. Photobiol. B: Biol.* 125 (2013) 202-208.
- [45] J.P. Kruth, K. Renap, Recoating issues in stereolithography, *Rapid Prototyping Journal* 1(3) (1995) 4-16.
- [46] A. Boddapati, C.L. Henderson, F. Ding, D.W. Rosen, A.S. Jariwala, V. Breedveld, M.A. Grover, Modeling effects of oxygen inhibition in mask - based stereolithography, *Rapid Prototyping Journal* 17(3) (2011) 168-175.
- [47] M. Shirai, K. Mitsukura, H. Okamura, Chain Propagation in UV Curing of Di(meth)acrylates, *Chemistry of Materials* 20(5) (2008) 1971-1976.
- [48] Q. Lian, F. Yang, H. Xin, D. Li, Oxygen-controlled bottom-up mask-projection stereolithography for ceramic 3D printing, *Ceramics International* 43(17) (2017) 14956-14961.
- [49] Y.-M. Huang, C.-P. Jiang, On-line force monitoring of platform ascending rapid prototyping system, *Journal of Materials Processing Technology* 159(2) (2005) 257-264.
- [50] X. Wu, Q. Lian, D. Li, Z. Jin, Tilting separation analysis of bottom-up mask projection stereolithography based on cohesive zone model, *Journal of Materials Processing Technology* 243 (2017) 184-196.
- [51] F. Liravi, S. Das, C. Zhou, Separation force analysis and prediction based on cohesive element model for constrained-surface Stereolithography processes, *Computer-Aided Design* 69 (2015) 134-142.
- [52] R. Felzmann, S. Gruber, G. Mitteramskogler, P. Tesavibul, A.R. Boccaccini, R. Liska, J. Stampfl, Lithography-Based Additive Manufacturing of Cellular Ceramic Structures, *Advanced Engineering Materials* 14(12) (2012) 1052-1058.
- [53] K. Arcaute, B. Mann, R. Wicker, Stereolithography of spatially controlled multi-material bioactive poly (ethylene glycol) scaffolds, *Acta Biomater.* 6(3) (2010) 1047-1054.
- [54] J.-W. Choi, H.-C. Kim, R. Wicker, Multi-material stereolithography, *J. Mater. Process. Technol.* 211(3) (2011) 318-328.

- [55] J. Zhang, P. Xiao, 3D printing of photopolymers, *Polym Chem-Uk* 9(13) (2018) 1530-1540.
- [56] S.V. Murphy, A. Atala, 3D bioprinting of tissues and organs, *Nature biotechnology* 32(8) (2014) 773-785.
- [57] R.J. Mondschein, A. Kanitkar, C.B. Williams, S.S. Verbridge, T.E. Long, Polymer structure-property requirements for stereolithographic 3D printing of soft tissue engineering scaffolds, *Biomaterials* 140 (2017) 170-188.
- [58] S.C. Ligon, R. Liska, J. Stampfl, M. Gurr, R. Mulhaupt, *Polymers for 3D Printing and Customized Additive Manufacturing*, *Chemical Reviews* 117(15) (2017) 10212-10290.
- [59] P.J. Bártolo, *Stereolithographic processes*, *Stereolithography*, Springer 2011, pp. 1-36.
- [60] J.H. Lee, R.K. Prud'Homme, I.A. Aksay, Cure depth in photopolymerization: Experiments and theory, *J. Mater. Res.* 16(12) (2001) 3536-3544.
- [61] B. Grigoryan, S.J. Paulsen, D.C. Corbett, D.W. Sazer, C.L. Fortin, A.J. Zaita, P.T. Greenfield, N.J. Calafat, J.P. Gounley, A.H. Ta, F. Johansson, A. Randles, J.E. Rosenkrantz, J.D. Louis-Rosenberg, P.A. Galie, K.R. Stevens, J.S. Miller, Multivascular networks and functional intravascular topologies within biocompatible hydrogels, *Science* 364(6439) (2019) 458-464.
- [62] W. Zongjie, A. Raafa, P. Benjamin, S. Roy, G. Sanjoy, K. Keekyoung, A simple and high-resolution stereolithography-based 3D bioprinting system using visible light crosslinkable bioinks, *Biofabrication* 7(4) (2015) 045009.
- [63] W. Zhu, X. Qu, J. Zhu, X. Ma, S. Patel, J. Liu, P. Wang, C.S.E. Lai, M. Gou, Y. Xu, K. Zhang, S. Chen, Direct 3D bioprinting of prevascularized tissue constructs with complex microarchitecture, *Biomaterials* 124 (2017) 106-115.
- [64] X. Ma, X. Qu, W. Zhu, Y.-S. Li, S. Yuan, H. Zhang, J. Liu, P. Wang, C.S.E. Lai, F. Zanella, Deterministically patterned biomimetic human iPSC-derived hepatic model via rapid 3D bioprinting, *Proc. Natl. Acad. Sci. U.S.A.* 113(8) (2016) 2206-2211.
- [65] M.T. Poldervaart, B. Goversen, M. De Ruijter, A. Abbadesse, F.P. Melchels, F.C. Öner, W.J. Dhert, T. Vermonden, J. Alblas, 3D bioprinting of methacrylated hyaluronic acid (MeHA) hydrogel with intrinsic osteogenicity, *PloS one* 12(6) (2017) e0177628.
- [66] K. Szafulera, R.A. Wach, A.K. Olejnik, J.M. Rosiak, P. Ulański, Radiation synthesis of biocompatible hydrogels of dextran methacrylate, *Radiat. Phys. Chem.* 142 (2018) 115-120.
- [67] H. Mishbak, G. Cooper, P. Bartolo, Development and Characterization of a Photocurable Alginate Bioink for Three Dimensional Bioprinting, *Int J Bioprint* 5(2) (2019) 189.
- [68] K.S. Lim, R. Levato, P.F. Costa, M.D. Castilho, C.R. Alcalá-Orozco, K.M.A. van Dorenmalen, F.P.W. Melchels, D. Gawlitta, G.J. Hooper, J. Malda, T.B.F. Woodfield, Bio-resin for high resolution lithography-based biofabrication of complex cell-laden constructs, *Biofabrication* 10(3) (2018) 034101.
- [69] M.P. Lee, G.J.T. Cooper, T. Hinkley, G.M. Gibson, M.J. Padgett, L. Cronin, Development of a 3D printer using scanning projection stereolithography, *Scientific Reports* 5 (2015) 9875.
- [70] D. Han, Z. Lu, S.A. Chester, H. Lee, Micro 3D Printing of a Temperature-Responsive Hydrogel Using Projection Micro-Stereolithography, *Scientific Reports* 8(1) (2018) 1963.
- [71] S. Sakai, H. Kamei, T. Mori, T. Hotta, H. Ohi, M. Nakahata, M. Taya, Visible Light-Induced Hydrogelation of an Alginate Derivative and Application to Stereolithographic Bioprinting Using a Visible Light Projector and Acid Red, *Biomacromolecules* 19(2) (2018) 672-679.
- [72] R.F. Pereira, P.J. Bartolo, 3D bioprinting of photocrosslinkable hydrogel constructs, *J Appl Polym Sci* 132(48) (2015).

1
2
3 966 [73] A.D. Rouillard, C.M. Berglund, J.Y. Lee, W.J. Polacheck, Y. Tsui, L.J. Bonassar, B.J. Kirby,
4 967 Methods for Photocrosslinking Alginate Hydrogel Scaffolds with High Cell Viability, *Tissue Eng*
5 968 Part C Methods 17(2) (2011) 173-179.
6
7 969 [74] Z.J. Wang, X. Jin, Z.L. Tian, F. Menard, J.F. Holzman, K. Kim, A Novel, Well-Resolved
8 970 Direct Laser Bioprinting System for Rapid Cell Encapsulation and Microwell Fabrication, *Adv.*
9 971 *Healthcare Mater.* 7(9) (2018).
10 972 [75] L. Brigo, A. Urciuolo, S. Giullitti, G. Della Giustina, M. Tromayer, R. Liska, N. Elvassore, G.
11 973 Brusatin, 3D high-resolution two-photon crosslinked hydrogel structures for biological studies,
12 974 *Acta Biomaterialia* 55 (2017) 373-384.
13
14 975 [76] X. Huang, X. Wang, Y. Zhao, Study on a series of water-soluble photoinitiators for fabrication
15 976 of 3D hydrogels by two-photon polymerization, *Dyes and Pigments* 141 (2017) 413-419.
16 977 [77] I. Mironi-Harpaz, D.Y. Wang, S. Venkatraman, D. Seliktar, Photopolymerization of cell-
17 978 encapsulating hydrogels: Crosslinking efficiency versus cytotoxicity, *Acta Biomaterialia* 8(5)
18 979 (2012) 1838-1848.
19 980 [78] J. Torgersen, X.-H. Qin, Z. Li, A. Ovsianikov, R. Liska, J. Stampfl, Hydrogels for Two-
20 981 Photon Polymerization: A Toolbox for Mimicking the Extracellular Matrix, *Adv. Funct. Mater.*
21 982 23(36) (2013) 4542-4554.
22
23 983 [79] A. Ovsianikov, S. Mühleder, J. Torgersen, Z. Li, X.-H. Qin, S. Van Vlierberghe, P. Dubruel,
24 984 W. Holthöner, H. Redl, R. Liska, Laser photofabrication of cell-containing hydrogel constructs,
25 985 *Langmuir* 30(13) (2013) 3787-3794.
26 986 [80] S. Turunen, E. Käpylä, K. Terzaki, J. Viitanen, C. Fotakis, M. Kellomäki, M. Farsari, Pico-
27 987 and femtosecond laser-induced crosslinking of protein microstructures: evaluation of
28 988 processability and bioactivity, *Biofabrication* 3(4) (2011) 045002.
29
30 989 [81] K. Kastrup, H. Sikes, Using photo-initiated polymerization reactions to detect molecular
31 990 recognition, *Chem. Soc. Rev.* 45(3) (2016) 532-545.
32 991 [82] C. Bahney, T. Lujan, C. Hsu, M. Bottlang, J. West, B. Johnstone, Visible light photoinitiation
33 992 of mesenchymal stem cell-laden bioresponsive hydrogels, *European cells & materials* 22 (2011)
34 993 43.
35 994 [83] R. Holmes, X.-B. Yang, A. Dunne, L. Florea, D. Wood, G. Tronci, Thiol-ene photo-click
36 995 collagen-PEG hydrogels: impact of water-soluble photoinitiators on cell viability, gelation kinetics
37 996 and rheological properties, *Polymers* 9(6) (2017) 226.
38
39 997 [84] M. Ayer, S. Schrettl, S. Balog, Y. Simon, C. Weder, Light-responsive azo-containing
40 998 organogels, *Soft matter* 13(22) (2017) 4017-4023.
41 999 [85] Z. Wang, H. Kumar, Z. Tian, X. Jin, J.F. Holzman, F. Menard, K. Kim, Visible light
42 1000 photoinitiation of cell-adhesive gelatin methacryloyl hydrogels for stereolithography 3D
43 1001 bioprinting, *ACS Appl. Mater. Interfaces* 10(32) (2018) 26859-26869.
44
45 1002 [86] Y.-H. Lin, Y.-C. Chiu, Y.-F. Shen, Y.-H.A. Wu, M.-Y. Shie, Bioactive calcium silicate/poly-
46 1003 ϵ -caprolactone composite scaffolds 3D printed under mild conditions for bone tissue engineering,
47 1004 *J. Mater. Sci. Mater. Med.* 29(1) (2018) 11.
48 1005 [87] F. Liu, C. Liu, Q. Chen, Q. Ao, X. Tian, J. Fan, H. Tong, X. Wang, Progress in organ 3D
49 1006 bioprinting, *Int J Bioprint* 4(1) (2018) 128 -142.
50 1007 [88] H. Wang, X.Y. Liu, Y.J. Chuah, J.C. Goh, J.L. Li, H. Xu, Design and engineering of silk
51 1008 fibroin scaffolds with biomimetic hierarchical structures, *Chem. Commun.* 49(14) (2013) 1431-
52 1009 1433.
53
54
55
56
57
58
59
60

- [89] W.L. Ng, M.H. Goh, W.Y. Yeong, M.W. Naing, Applying Macromolecular Crowding to 3D Bioprinting: Fabrication of 3D Hierarchical Porous Collagen-based Hydrogel Constructs, *Biomater. Sci.* 6(3) (2018) 562-574.
- [90] S.J. Hollister, Porous scaffold design for tissue engineering, *Nat Mater.* 4(7) (2005) 518-524.
- [91] A.P. Zhang, X. Qu, P. Soman, K.C. Hribar, J.W. Lee, S. Chen, S. He, Rapid fabrication of complex 3D extracellular microenvironments by dynamic optical projection stereolithography, *Adv. Mater.* 24(31) (2012) 4266-4270.
- [92] F.P. Melchels, K. Bertoldi, R. Gabbrielli, A.H. Velders, J. Feijen, D.W. Grijpma, Mathematically defined tissue engineering scaffold architectures prepared by stereolithography, *Biomaterials* 31(27) (2010) 6909-6916.
- [93] P. Zorlutuna, J.H. Jeong, H. Kong, R. Bashir, Stereolithography - based hydrogel microenvironments to examine cellular interactions, *Adv. Funct. Mater.* 21(19) (2011) 3642-3651.
- [94] K. Arcaute, B.K. Mann, R.B. Wicker, Stereolithography of three-dimensional bioactive poly (ethylene glycol) constructs with encapsulated cells, *Ann. Biomed. Eng.* 34(9) (2006) 1429-1441.
- [95] D.J. Tobin, Biochemistry of human skin—our brain on the outside, *Chem. Soc. Rev.* 35(1) (2006) 52-67.
- [96] S. Vijayavenkataraman, W. Lu, J. Fuh, 3D bioprinting of skin: a state-of-the-art review on modelling, materials, and processes, *Biofabrication* 8(3) (2016) 032001, 1-31.
- [97] W.L. Ng, S. Wang, W.Y. Yeong, M.W. Naing, Skin Bioprinting: Impending Reality or Fantasy?, *Trends Biotechnol.* 34 (9) (2016) 689 - 699.
- [98] S. Michael, H. Sorg, C.-T. Peck, L. Koch, A. Deiwick, B. Chichkov, P.M. Vogt, K. Reimers, Tissue Engineered Skin Substitutes Created by Laser-Assisted Bioprinting Form Skin-Like Structures in the Dorsal Skin Fold Chamber in Mice, *PloS one* 8(3) (2013) e57741, 1-12.
- [99] L. Koch, A. Deiwick, S. Schlie, S. Michael, M. Gruene, V. Coger, D. Zychlinski, A. Schambach, K. Reimers, P.M. Vogt, Skin tissue generation by laser cell printing, *Biotechnol. Bioeng.* 109(7) (2012) 1855-1863.
- [100] W. Lee, J.C. Debasitis, V.K. Lee, J.-H. Lee, K. Fischer, K. Edminster, J.-K. Park, S.-S. Yoo, Multi-layered culture of human skin fibroblasts and keratinocytes through three-dimensional freeform fabrication, *Biomaterials* 30(8) (2009) 1587-1595.
- [101] W. Lee, V. Lee, S. Polio, P. Keegan, J.H. Lee, K. Fischer, J.K. Park, S.S. Yoo, On-demand three-dimensional freeform fabrication of multi-layered hydrogel scaffold with fluidic channels, *Biotechnol. Bioeng.* 105(6) (2010) 1178-86.
- [102] V. Lee, G. Singh, J.P. Trasatti, C. Bjornsson, X. Xu, T.N. Tran, S.-S. Yoo, G. Dai, P. Karande, Design and Fabrication of Human Skin by Three-Dimensional Bioprinting, *Tissue Eng Part C: Methods* 20(6) (2013) 473-484.
- [103] N. Cubo, M. Garcia, J.F. del Cañizo, D. Velasco, J.L. Jorcano, 3D bioprinting of functional human skin: production and in vivo analysis, *Biofabrication* 9(1) (2016) 015006.
- [104] W.L. Ng, Z.Q. Tan, W.Y. Yeong, M.W. Naing, Proof-of-concept: 3D bioprinting of pigmented human skin constructs, *Biofabrication* 10(2) (2018) 025005, 1-13.
- [105] X. Han, J. Courseaus, J. Khamassi, N. Nottrodt, S. Engelhardt, F. Jacobsen, C. Bierwisch, W. Meyer, T. Walter, J. Weisser, Optimized vascular network by stereolithography for tissue engineered skin, *Int J Bioprint* 4(2) (2018) 134-150.
- [106] C. Huang, Y. Du, C.S. Nabzdyk, R. Ogawa, T. Koyama, D.P. Orgill, X. Fu, Regeneration of hair and other skin appendages: A microenvironment - centric view, *Wound Repair and Regeneration* 24(5) (2016) 759-766.

1
2
3 1055 [107] J. Pan, S. Yung Chan, J.E. Common, S. Amini, A. Miserez, E. Birgitte Lane, L. Kang,
4 1056 Fabrication of a 3D hair follicle - like hydrogel by soft lithography, *J. Biomed. Mater. Res. A*
5 1057 101(11) (2013) 3159-3169.
6 1058 [108] P. Admane, A.C. Gupta, P. Jois, S. Roy, C.C. Lakshmanan, G. Kalsi, B. Bandyopadhyay, S.
7 1059 Ghosh, Direct 3D bioprinted full-thickness skin constructs recapitulate regulatory signaling
8 1060 pathways and physiology of human skin, *Bioprinting* 15 (2019) e00051.
9 1061 [109] Q. Valeria, C. Tonda-turo, Trends in the design of nerve guidance channels in peripheral
10 1062 nerve tissue engineering, *Progress in Neurobiology* (2015) 1-18.
11 1063 [110] C.-C. Chen, J. Yu, H.-Y. Ng, A. Lee, C.-C. Chen, Y.-S. Chen, M.-Y. Shie, The
12 1064 physicochemical properties of decellularized extracellular matrix-coated 3D printed poly (ϵ -
13 1065 caprolactone) nerve conduits for promoting Schwann cells proliferation and differentiation,
14 1066 *Materials* 11(9) (2018) 1665.
15 1067 [111] B.N. Johnson, K.Z. Lancaster, G. Zhen, J. He, M.K. Gupta, Y.L. Kong, E.A. Engel, K.D.
16 1068 Krick, A. Ju, F. Meng, L.W. Enquist, X. Jia, M.C. Mcalpine, 3D Printed Anatomical Nerve
17 1069 Regeneration Pathways, *Materials Views* (2015) 6205-6217.
18 1070 [112] P. Zhuang, A.X. Sun, J. An, C.K. Chua, S.Y. Chew, 3D neural tissue models: From spheroids
19 1071 to bioprinting, *Biomaterials* 154 (2018) 113-133.
20 1072 [113] S.-J. Lee, W. Zhu, L. Heyburn, M. Nowicki, B. Harris, L.G. Zhang, Development of novel
21 1073 3-D printed scaffolds with core-shell nanoparticles for nerve regeneration, *IEEE Trans. Biomed.*
22 1074 *Eng.* 64(2) (2016) 408-418.
23 1075 [114] W. Zhu, K.R. Tringale, S.A. Woller, S. You, S. Johnson, H. Shen, J. Schimelman, M.
24 1076 Whitney, J. Steinauer, W. Xu, Rapid continuous 3D printing of customizable peripheral nerve
25 1077 guidance conduits, *Mater. Today* 21(9) (2018) 951-959.
26 1078 [115] M.K. Kwan, E.J. Wall, J. Massie, S.R. Garfin, Strain, stress and stretch of peripheral nerve,
27 1079 *Acta Orthop Scand* 63 (1992) 267-272.
28 1080 [116] K.-H. Lee, J. Lee, S.-H. Lee, 3D liver models on a microplatform: well-defined culture,
29 1081 engineering of liver tissue and liver-on-a-chip, *Lab Chip* 15(19) (2015) 3822-3837.
30 1082 [117] Y. Kondo, T. Iwao, K. Nakamura, T. Sasaki, S. Takahashi, N. Kamada, T. Matsubara, F.J.
31 1083 Gonzalez, H. Akutsu, Y. Miyagawa, An efficient method for differentiation of human induced
32 1084 pluripotent stem cells into hepatocyte-like cells retaining drug metabolizing activity, *Drug*
33 1085 *metabolism and pharmacokinetics* (2013) DMPK-13-RG-104.
34 1086 [118] K. Kim, K. Ohashi, R. Utoh, K. Kano, T. Okano, Preserved liver-specific functions of
35 1087 hepatocytes in 3D co-culture with endothelial cell sheets, *Biomaterials* 33 (2012) 1406-1413.
36 1088 [119] T. Grix, A. Ruppelt, A. Thomas, A.-K. Amler, B. Noichl, R. Lauster, L. Kloeke, Bioprinting
37 1089 perfusion-enabled liver equivalents for advanced organ-on-a-chip applications, *Genes* 9(4) (2018)
38 1090 176.
39 1091 [120] Z. Izadifar, X. Chen, W. Kulyk, Strategic design and fabrication of engineered scaffolds for
40 1092 articular cartilage repair, *J Funct Biomater* 3(4) (2012) 799-838.
41 1093 [121] A.X. Sun, H. Lin, A.M. Beck, E.J. Kilroy, R.S. Tuan, Projection stereolithographic
42 1094 fabrication of human adipose stem cell-incorporated biodegradable scaffolds for cartilage tissue
43 1095 engineering, *Frontiers in bioengineering and biotechnology* 3 (2015) 115.
44 1096 [122] S.H. Kim, Y.K. Yeon, J.M. Lee, J.R. Chao, Y.J. Lee, Y.B. Seo, M.T. Sultan, O.J. Lee, J.S.
45 1097 Lee, S.-i. Yoon, Precisely printable and biocompatible silk fibroin bioink for digital light
46 1098 processing 3D printing, *Nat. Commun.* 9(1) (2018) 1620.
47 1099 [123] J.D. Currey, *Bones: structure and mechanics*, Princeton: Princeton university press. 2013;
48 1100 436 pp.

- 1101 [124] J.H. Cole, M.C. van der Meulen, Whole bone mechanics and bone quality, *Clin. Orthop.*
- 1102 *Relat. Res.* 469(8) (2011) 2139-2149.
- 1103 [125] Y.-c. Chen, M.-y. Shie, Y.-h.A. Wu, K.-x.A. Lee, L.-j. Wei, Y.-f. Shen, Anti-inflammation
- 1104 performance of curcumin-loaded mesoporous calcium silicate cement, *Journal of the Formosan*
- 1105 *Medical Association* 116 (2017) 679-688.
- 1106 [126] Y.-j. Seol, D.Y. Park, J.Y. Park, S.W. Kim, S.J. Park, A New Method of Fabricating Robust
- 1107 Freeform 3D Ceramic Scaffolds for Bone Tissue Regeneration, *Biotechnology and Bioengineering*
- 1108 110 (2013) 1444-1455.
- 1109 [127] L. Elomaa, S. Teixeira, R. Hakala, H. Korhonen, D.W. Grijpma, J.V. Seppälä, Preparation
- 1110 of poly (ε-caprolactone) -based tissue engineering scaffolds by stereolithography, *Acta*
- 1111 *Biomaterialia* 7 (2011) 3850-3856.
- 1112 [128] A. Hoffmann, H. Leonards, N. Tobies, L. Pongratz, K. Kreuels, F. Kreimendahl, C. Apel,
- 1113 M. Wehner, N. Nottrodt, New stereolithographic resin providing functional surfaces for
- 1114 biocompatible three-dimensional printing, *Journal of Tissue Engineering* 8 (2017) 1-9.
- 1115 [129] S. Tanodekaew, S. Channasanon, P. Uppanan, Preparation and degradation study of
- 1116 photocurable oligolactide - HA composite: A potential resin for stereolithography application, *J.*
- 1117 *Biomed. Mater. Res. B Appl. Biomater.* 102(3) (2014) 604-611.
- 1118 [130] J.R. Xavier, T. Thakur, P. Desai, M.K. Jaiswal, N. Sears, E. Cosgriff-Hernandez, R. Kaunas,
- 1119 A.K. Gaharwar, Bioactive nanoengineered hydrogels for bone tissue engineering: a growth-factor-
- 1120 free approach, *ACS nano* 9(3) (2015) 3109-3118.
- 1121 [131] X. Ma, X. Qu, W. Zhu, Y.-S. Li, S. Yuan, H. Zhang, J. Liu, P. Wang, C.S.E. Lai, F. Zanella,
- 1122 G.-S. Feng, F. Sheikh, S. Chien, S. Chen, Deterministically patterned biomimetic human iPSC-
- 1123 derived hepatic model via rapid 3D bioprinting, *Proceedings of the National Academy of Sciences*
- 1124 113(8) (2016) 2206-2211.
- 1125 [132] Y. Shanjani, C.C. Pan, L. Elomaa, Y. Yang, A novel bioprinting method and system for
- 1126 forming hybrid tissue engineering constructs, *Biofabrication* 7(4) (2015) 045008.
- 1127 [133] K.S. Lim, R. Levato, P.F. Costa, M.D. Castilho, C.R. Alcala-Orozco, K.M. van Dorenmalen,
- 1128 F.P. Melchels, D. Gawlitta, G.J. Hooper, J. Malda, Bio-resin for high resolution lithography-based
- 1129 biofabrication of complex cell-laden constructs, *Biofabrication* 10(3) (2018) 034101.
- 1130 [134] J.C. Culver, J.C. Hoffmann, R.A. Poché, J.H. Slater, J.L. West, M.E. Dickinson, Three -
- 1131 dimensional biomimetic patterning in hydrogels to guide cellular organization, *Adv. Mater.* 24(17)
- 1132 (2012) 2344-2348.
- 1133 [135] A. Dobos, J. Van Hoorick, W. Steiger, P. Gruber, M. Markovic, O.G. Andriotis, A.
- 1134 Rohatschek, P. Dubruel, P.J. Thurner, S. Van Vlierberghe, Thiol-Gelatin - Norbornene Bioink for
- 1135 Laser - Based High - Definition Bioprinting, *Adv Healthc Mater.* (2019) 1900752.
- 1136 [136] M. Tromayer, P. Gruber, M. Markovic, A. Rosspeintner, E. Vauthey, H. Redl, A.
- 1137 Ovsianikov, R. Liska, A biocompatible macromolecular two-photon initiator based on hyaluronan,
- 1138 *Polym. Chem.* 8(2) (2017) 451-460.
- 1139 [137] M. Tromayer, A. Dobos, P. Gruber, A. Ajami, R. Dedic, A. Ovsianikov, R. Liska, A
- 1140 biocompatible diazosulfonate initiator for direct encapsulation of human stem cells via two-photon
- 1141 polymerization, *Polym. Chem.* 9(22) (2018) 3108-3117.
- 1142 [138] MicronDental, FDA Approved 3D Printer Resins, 2017. (Accessed 23 Nov 2019).
- 1143 [139] M.H. Kathawala, W.L. Ng, D. Liu, M.W. Naing, W.Y. Yeong, K. Spiller, M. Van Dyke,
- 1144 K.W. Ng, Healing of Chronic Wounds—An Update of Recent Developments and Future
- 1145 Possibilities, *Tissue Eng Part B: Reviews* 25(5) (2019) 429 - 444 DOI: 10.1089/ten.teb.2019.0019

1
2
3 1146 [140] U.S. FDA, Use of International Standard ISO 10993-1, "Biological evaluation of medical
4 1147 devices - Part 1: Evaluation and testing within a risk management process", 2016. (Accessed 23
5 1148 Nov 2019).
6
7 1149 [141] P. Hourd, N. Medcalf, J. Segal, D.J. Williams, A 3D bioprinting exemplar of the
8 1150 consequences of the regulatory requirements on customized processes, *Regenerative medicine*
9 1151 10(7) (2015) 863-883.
10 1152 [142] C. Kengla, E. Renteria, C. Wivell, A. Atala, J.J. Yoo, S.J. Lee, Clinically Relevant
11 1153 Bioprinting Workflow and Imaging Process for Tissue Construct Design and Validation, *3D*
12 1154 *Printing and Additive Manufacturing* 4(4) (2017) 239-247.
13 1155 [143] C. D O'Connell, C. Onofrillo, S. Duchi, X. Li, Y. Zhang, P. Tian, L. Lu, A. Trengove, A.
14 1156 Quigley, S. Gambhir, Evaluation of sterilisation methods for bio-ink components: gelatin, gelatin
15 1157 methacryloyl, hyaluronic acid and hyaluronic acid methacryloyl, *Biofabrication* 11(3) (2019)
16 1158 035003.
17 1159 [144] Food, D. Administration, Regulatory considerations for human cells, tissues, and cellular
18 1160 and tissue - based products: Minimal manipulation and homologous use; guidance for industry
19 1161 and food and drug administration staff; availability, *Federal Register* 82(221) (2017) 54290-54292.
20 1162 [145] A.A. Pawar, G. Saada, I. Cooperstein, L. Larush, J.A. Jackman, S.R. Tabaei, N.-J. Cho, S.
21 1163 Magdassi, High-performance 3D printing of hydrogels by water-dispersible photoinitiator
22 1164 nanoparticles, *Science Advances* 2(4) (2016) e1501381.
23 1165 [146] J. Van Hoorick, P. Gruber, M. Markovic, M. Tromayer, J. Van Erps, H. Thienpont, R. Liska,
24 1166 A. Ovsianikov, P. Dubruel, S. Van Vlierberghe, Cross-Linkable Gelatins with Superior
25 1167 Mechanical Properties Through Carboxylic Acid Modification: Increasing the Two-Photon
26 1168 Polymerization Potential, *Biomacromolecules* 18(10) (2017) 3260-3272.
27 1169 [147] J.W. Bjork, S.L. Johnson, R.T. Tranquillo, Ruthenium-catalyzed photo cross-linking of
28 1170 fibrin-based engineered tissue, *Biomaterials* 32(10) (2011) 2479-2488.
29 1171 [148] E.A. Kamoun, A. Winkel, M. Eisenburger, H. Menzel, Carboxylated camphorquinone as
30 1172 visible-light photoinitiator for biomedical application: Synthesis, characterization, and application,
31 1173 *Arabian Journal of Chemistry* 9(5) (2016) 745-754.
32 1174 [149] M.E. Pepper, V. Seshadri, T.C. Burg, K.J. Burg, R.E. Groff, Characterizing the effects of
33 1175 cell settling on bioprinter output, *Biofabrication* 4(1) (2012) 011001.
34 1176 [150] W.L. Ng, W.Y. Yeong, M.W. Naing, Polyvinylpyrrolidone-Based Bio-Ink Improves Cell
35 1177 Viability and Homogeneity during Drop-On-Demand Printing, *Materials* 10(2) (2017) 190, 1-12.
36 1178 [151] D. Chahal, A. Ahmadi, K.C. Cheung, Improving piezoelectric cell printing accuracy and
37 1179 reliability through neutral buoyancy of suspensions, *Biotechnol. Bioeng.* 109(11) (2012) 2932-
38 1180 2940.
39 1181 [152] J.R. Tumbleston, D. Shirvanyants, N. Ermoshkin, R. Januszewicz, A.R. Johnson, D. Kelly,
40 1182 K. Chen, R. Pinschmidt, J.P. Rolland, A. Ermoshkin, E.T. Samulski, J.M. DeSimone, Continuous
41 1183 liquid interface production of 3D objects, *Science* 347(6228) (2015) 1349-1352.
42 1184 [153] P.N. Bernal, P. Delrot, D. Loterie, Y. Li, J. Malda, C. Moser, R. Levato, Volumetric
43 1185 Bioprinting of Complex Living - Tissue Constructs within Seconds, *Adv. Mater.* (2019) 1904209.
44 1186 [154] Y.-C. Li, Y.S. Zhang, A. Akpek, S.R. Shin, A. Khademhosseini, 4D bioprinting: the next-
45 1187 generation technology for biofabrication enabled by stimuli-responsive materials, *Biofabrication*
46 1188 9(1) (2016) 012001.
47 1189 [155] C.M. González-Henríquez, M.A. Sarabia-Vallejos, J. Rodríguez-Hernandez, Polymers for
48 1190 additive manufacturing and 4D-printing: Materials, methodologies, and biomedical applications,
49 1191 *Prog. Polym. Sci.* 94 (2019) 57 - 116.

- [156] J. An, C.K. Chua, V. Mironov, A perspective on 4D bioprinting, *Int J Bioprint* 2(1) (2016) 3-5.
- [157] H. Xie, K.-K. Yang, Y.-Z. Wang, Photo-cross-linking: a powerful and versatile strategy to develop shape-memory polymers, *Prog. Polym. Sci.* 95 (2019) 32 - 64.
- [158] R.T. Shafraneck, S.C. Millik, P.T. Smith, C.-U. Lee, A.J. Boydston, A. Nelson, Stimuli-responsive materials in additive manufacturing, *Prog. Polym. Sci.* 93 (2019) 36 - 67.
- [159] H. Sun, C.P. Kabb, M.B. Sims, B.S. Sumerlin, Architecture-transformable polymers: Reshaping the future of stimuli-responsive polymers, *Prog. Polym. Sci.* 89 (2019) 61-75.
- [160] Y.Y.C. Choong, S. Maleksaeedi, H. Eng, P.-C. Su, J. Wei, Curing characteristics of shape memory polymers in 3D projection and laser stereolithography, *Virtual Phys Prototyp* 12(1) (2017) 77-84.
- [161] L. Huang, R. Jiang, J. Wu, J. Song, H. Bai, B. Li, Q. Zhao, T. Xie, Ultrafast Digital Printing toward 4D Shape Changing Materials, *Advanced Materials* 29(7) (2017) 1605390.
- [162] S. Miao, W. Zhu, N.J. Castro, M. Nowicki, X. Zhou, H. Cui, J.P. Fisher, L.G. Zhang, 4D printing smart biomedical scaffolds with novel soybean oil epoxidized acrylate, *Scientific Reports* 6 (2016) 27226.
- [163] Y.Y.C. Choong, S. Maleksaeedi, H. Eng, J. Wei, P.-C. Su, 4D printing of high performance shape memory polymer using stereolithography, *Materials & Design* 126 (2017) 219-225.
- [164] J. Norman, R.D. Madurawe, C.M. Moore, M.A. Khan, A. Khairuzzaman, A new chapter in pharmaceutical manufacturing: 3D-printed drug products, *Adv. Drug Del. Rev.* 108 (2017) 39-50.
- [165] E. Lepowsky, S. Tasoglu, 3D printing for drug manufacturing: A perspective on the future of pharmaceuticals, *Int J Bioprint* 4(1) (2018) 119.
- [166] S.J. Trenfield, A. Awad, A. Goyanes, S. Gaisford, A.W. Basit, 3D printing pharmaceuticals: drug development to frontline care, *Trends Pharmacol. Sci.* 39(5) (2018) 440-451.
- [167] J. Wang, A. Goyanes, S. Gaisford, A.W. Basit, Stereolithographic (SLA) 3D printing of oral modified-release dosage forms, *Int. J. Pharm.* 503(1-2) (2016) 207-212.
- [168] P.R. Martinez, A. Goyanes, A.W. Basit, S. Gaisford, Fabrication of drug-loaded hydrogels with stereolithographic 3D printing, *Int. J. Pharm.* 532(1) (2017) 313-317.
- [169] G.S. Ginsburg, J.J. McCarthy, Personalized medicine: revolutionizing drug discovery and patient care, *Trends Biotechnol.* 19(12) (2001) 491-496.
- [170] M.A. Hamburg, F.S. Collins, The path to personalized medicine, *New Engl. J. Med.* 363(4) (2010) 301-304.
- [171] N.J. Schork, Personalized medicine: time for one-person trials, *Nature News* 520(7549) (2015) 609.
- [172] G.M. Whitesides, The origins and the future of microfluidics, *Nature* 442 (2006) 368.
- [173] C.M.B. Ho, S.H. Ng, K.H.H. Li, Y.-J. Yoon, 3D printed microfluidics for biological applications, *Lab Chip* 15(18) (2015) 3627-3637.
- [174] W.L. Ng, W.Y. Yeong, The Future of Skin Toxicology Testing - 3D Bioprinting Meets Microfluidics, *Int J Bioprint* 5(2.1) (2019) 237.
- [175] G. Comina, A. Suska, D. Filippini, PDMS lab-on-a-chip fabrication using 3D printed templates, *Lab Chip* 14(2) (2014) 424-430.
- [176] H.N. Chan, Y. Chen, Y. Shu, Y. Chen, Q. Tian, H. Wu, Direct, one-step molding of 3D-printed structures for convenient fabrication of truly 3D PDMS microfluidic chips, *Microfluid. Nanofluid.* 19(1) (2015) 9-18.
- [177] G. Comina, A. Suska, D. Filippini, Low cost lab-on-a-chip prototyping with a consumer grade 3D printer, *Lab Chip* 14(16) (2014) 2978-2982.

1
2
3 1238 [178] C.I. Rogers, K. Qaderi, A.T. Woolley, G.P. Nordin, 3D printed microfluidic devices with
4 1239 integrated valves, *Biomicrofluidics* 9(1) (2015) 016501.
5 1240 [179] A.K. Au, N. Bhattacharjee, L.F. Horowitz, T.C. Chang, 3D-printed microfluidic automation,
6 1241 *Lab on a Chip* 15 (2015) 1934-1941.
7 1242 [180] K.G. Lee, K.J. Park, S. Seok, S. Shin, D.H. Kim, J.Y. Park, Y.S. Heo, S.J. Lee, T.J. Lee, 3D
8 1243 printed modules for integrated microfluidic devices, *RSC Advances* 4(62) (2014) 32876-32880.
9 1244 [181] S. Waheed, J.M. Cabot, N.P. Macdonald, T. Lewis, R.M. Guijt, B. Paull, M.C. Breadmore,
10 1245 3D printed microfluidic devices: enablers and barriers, *Lab Chip* 16(11) (2016) 1993-2013.
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