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Davaran, Biodegradable and Biocompatible Polymers for Tissue Engineering Application: A Review, Artif. Cells Nanomed. Biotechnol., 2017, 45(2), p 185–192.Article CAS Google Scholar P. Saini, M. Arora and M.R. Kumar, Poly (Lactic Acid) Blends in Biomedical Applications, Adv. Drug Deliv. Rev., 2016, 107, p 47–59.Article CAS Google Scholar M. Santoro, S.R. Shah, J.L. Walker and A.G. Mikos, Poly (Lactic Acid) Nanofibrous Scaffolds for Tissue Engineering, Adv. Drug Deliv. Rev., 2016, 107, p 206–212.Article CAS Google Scholar As a library, NLM provides access to scientific literature. Inclusion in an NLM database does not imply endorsement of, or agreement with, the contents by NLM or the National Institutes of Health. Learn more: PMC Disclaimer | PMC Copyright Notice 3D printing, an additive manufacturing based technology for precise 3D construction, is currently widely employed to enhance applicability and function of cell laden scaffolds. Research on novel compatible biomaterials for bioprinting exhibiting fast crosslinking properties is an essential prerequisite toward advancing 3D printing applications in tissue engineering. Printability to improve fabrication process and cell encapsulation are two of the main factors to be considered in development of 3D bioprinting. Other important factors include but are not limited to printing fidelity, stability, crosslinking time, biocompatibility, cell encapsulation and proliferation, shear-thinning properties, and mechanical properties such as mechanical strength and elasticity. In this review, we recite recent promising advances in bioink development as well as bioprinting methods. Also, an effort has been made to include studies with diverse types of crosslinking methods such as photo, chemical and ultraviolet (UV). We also propose the challenges and future outlook of 3D bioprinting application in medical sciences and discuss the high performance bioinks. Keywords: Bioprinting, Hydrogel, Extrusion, Inkjet, Stereolithography, Laser-assisted, Review, 3D printing •The most recent promising advances in three-dimensional bioprinting are reviewed. •Extrusion, inkjet, stereolithography, and laser bioprinting studies are cited. •Challenges toward successful employment of bioprinting are discussed. As the main process involved in cell growth and reconstruction of organs, tissue regeneration is currently under extensive study. Organ transplantation, replacement and repair are the options for patients with damaged organs depending on the situation and intensity of the damage. Extensively long waiting lists for organ transplantation exist all around the world. According to U.S. Department of Health & Human Services, as of June 2017, around 120000 patients are in need of lifesaving organ transplant in the United States while only about 5200 donors are available. Also, while the number of transplants performed every year since 2003 has been somehow constant, the number of patients waiting at the year-end has been growing ( ). Under these circumstances, scientists are eager to find alternative ways to compensate for this shortage of organ. Tissue engineering, on the other hand, has been considered as an effective method to help save lives and improve the quality of life. Since proposed in 1993 [1], tissue engineering has been intended to develop practical replacements for damaged tissue by means of applying biology and engineering principles. Scaffolds have found their place in tissue engineering as templates for cell interaction, providing physical support to the afresh developed tissue [2]. Also, scaffolds can function as delivery vehicles to incorporate essential growth factors to control and enhance tissue growth [3]. A combination of cells and biomaterials is often employed as the printing precursor in 3D bioprinting of scaffolds. 3D Bioprinting is an actively studied method in tissue engineering since it shows effective control over scaffold fabrication and cell distribution. Printing resolution of 3D bioprinting techniques is 10–10000 μm which is a wide range showing flexibility of bioprinting compared to other assembly methods such as molding and porous scaffolds [4], [5]. As an additive manufacturing technique, 3D bioprinting is based on deposition of biomaterials, either encapsulating cells or loaded with cells later on, in micrometer scale to form subtle structures comparable to tissue. In most cases, a three-axis mechanical platform controls the movements of extruders printing the bioink in the required algorithm and shape. This platform's movement is governed by coordinates created by the designer and saved in a file format such as g-code that could be easily followed by the printer. Due to vantages such as precise deposition, cost-effectiveness, simplicity, and cell distribution controllability, 3D bioprinting development and application has been increasing constantly over the past few years. As a result, need for new bioinks providing required properties for successful printing, such as printability, printing fidelity, and mechanical properties has been rising leading to extensive work to develop new materials. In the present review, an account of the most recent and functional research studies on bioinks and bioprinting developments is presented. To this end, first outstanding works in major bioprinting methods, including extrusion-based, inkjet, stereolithography-based, and laser-assisted bioprinting methods, are reviewed. Also, a brief review of the above mentioned bioprinting techniques is presented in Table 1 and a short summary of recent outstanding bioprinting studies is tabularized in Table 2. Next, the most fundamental recent studies in bioink development and applications are cited in "High performance bioink" section. Later on, challenges in bioink development and bioprinting, as well as applications and future perspective of bioprinting is discussed. Finally, a short summary of the present article is presented. A brief review of common bioprinting techniques. Extrusion Inkjet Stereolithography Laser-assisted Advantages Simple, capable of printing various biomaterials, ability to print high cell densities Ability to print low viscosity biomaterials, fast fabrication speed, low cost, high resolution Nozzle-free technique, printing time independent of complexity [6], [7], high accuracy and cell viability High resolution, deposition of biomaterials in solid or liquid phase Drawbacks Only applicable for viscous liquids Inherent inability to provide a continuous flow [8], poor functionality for vertical structures, low cell densities UV light source and near-UV blue light's toxicity to cells [9], [10], lack of printing multi-cells, and damage to cells during photo curing [11] High cost, thermal damage due to nanosecond/femtosecond laser irritation [12] Speed Slow [13], [14] Fast [13], [14] Fast [14] Medium [14] Cost Moderate [8], [15] Low [8], [15] Low [8], [15] High [8], [15] Vertical printing ability Good [6] Poor [6] Good [6] Medium [6] Cell viability 89.46 ± 2.51% [16] 80-95% [17], [18] >90% [19], [20] 95% Photoinitiated gelation – Enzymatic degradation at 37°C, controllable degradation 77 , 64 , 78 , 79 Fibrin 74.27% Fibrinogen-thrombin Unique viscoelastic properties among polymers Controlled and adjustable biodegradation 80 Pluronic F127 91.3% Photo-polymerization High elongation at break More than 85% degradation in PBS after 1 week 81 Pluronic F127/alginate 85% Ionic Low mechanical property – 72 , 79 Gelatin methacrylate 75–90% Photo-polymerization Mechanically strong No significant degradation over the 2 months assay 82 Maintaining the structural and mechanical integrity of the printed model is one of the crucial parameters for material selection. The materials should have similar mechanical properties like elasticity and strength to the native biological cellular and tissue components. These properties make the materials better fit than the replica of the original tissues. Mechanical properties vary depending on the different structural requirements from the outer skin to the innermost bone. Nowadays, sacrificial materials are used, which provide required structural and mechanical properties only for a certain period. These materials sacrifice themselves after the task has been over. They are either used at the time of printing for sufficient crosslinking for the complex model or incorporated into the model so that they can function efficiently until the assigned material can efficiently carry out the same function. 43 , 60 , 61 Carbohydrate glass/elastomer was used as a sacrificial material for the 3D printing the soft elastomer. 83 These sacrificial materials also affect the biocompatibility and degradation rate of the bioinks and can affect the host. Mechanical properties like tensile strength and stiffness also play a vital role in reconstructing and 3D printing bones. 84 As described earlier, bio stands for biomimicry's life or natural components, and mimesis means imitation or resemblance. It explains the resemblance of the printed 3D constructs, which resemble biological components present in nature in an analogous or homologous way. It helps us observe and learn from nature to incorporate its patterns to find a solution for our designs. Biomimicry is one of the essential properties of 3D bioprinting. It helps to study the constructed complex natural systems inside and outside our biological system. It helps to fabricate identical cellular and an extracellular component of a tissue or organ. 61 For fabricating a branching pattern of nerve cells, one needs to mimic the branching patterns of the vascular tree with physiologically correct biomaterial types and gradients. 3 A thorough understanding of the micro and macroenvironment and proper composition of the specific material suited for that cause is required to understand the arrangement and position accurately. The precise anatomical and architectural information helps to fabricate a precise 3D printed model. Various bioprinting techniques have been designed and improved for 3D construction. Different bioprinting techniques also affect the tissue and organ design of the construct. Some techniques form continuous structures, whereas discr

This simultaneous deposition can be time consuming since it is difficult to accurately target cells and materials to their desired location on the printing substrate. To overcome this difficulty “aim and shoot” procedure is developed. This cell-recognition scanning technology is used, which enables the laser beam to select a single cell per pulse specifically, and it ensures that each printed droplet contains a predefined number of cells. 2 Metallic residues are present in the final printed constructs due to the vaporization of the metallic layer from laser radiation. To avoid this phenomenon, a nonmetallic absorbing layer is used nowadays, and the printing process is modified such that it does not need an absorbable layer. 144 , 145 This system's cost is higher than other printing technologies due to monochromatic high-energy laser beams. Owing to such disadvantages, laser-assisted techniques for the printing of 3D complexes are less than 4% share of the total bioprinter available in the market. 114 Bioinks like alginate, collagen, fibrin/fibrinogen, hydroxyapatite, matrigel and blood plasma are the few materials used for laser-assisted bioprinting. 57 This technique was used to fabricate a 3D printed skin model with specific cell densities in a layered tissue construct. 146 Gruene et al. used laser-assisted technology to print the biomaterial incorporated with the mesenchymal stem cells (MSCs). The study showed no effect of the laser pulse on the cells as no change in the gene expression occurred due to heat shock produced by the laser pulse. The system had an enhanced proliferation rate and was similar to the control of the nonprinted cells even after 5 days of cell culture. 147 In another study, laser-assisted technique was used for printing in situ MSCs onto collagen/hydroxyapatite (nHA) disks to favor bone regeneration in a calvaria defect model in mice. The printed disc demonstrated a larger bone volume after 2 months compared with a cellular collagen/nHA disc. 148 Keratinocytes were printed on top of 20 layers of fibroblast placed on the top of a matrix Matriderm. Layers of keratinocytes were printed on the matrix, which provided mechanical stability to the overall printed construct. After the complete development of keratinocytes cells, the in vivo study was conducted for 11 days leading to the conversion of cells into a stratified dense tissue when implanted subcutaneously in mice. 146 The study also demonstrated filling of a 3 mm diameter, 600 μm deep calvarial hole using the composite polymeric biomaterial. Similar polymeric devices is used to fabricate medical components such as a customized, noncellular, bioresorbable tracheal splint, which was used for the patient with localized tracheobronchomalacia. 149 This technique has attracted a lot of attention for its several applications in drug screening for toxicology studies, fundamental cell biology research, tissue models, and wound generation applications. It consists of a 3-axis movable robotic platform and an array of multiple electromechanical microvalve print-heads. Individual gas connection is provided along with the gas regulator to each microvalve print head. It provides a positive pneumatic pressure along with a 0.1 ms valve opening time, which is controlled by the movement of the plunger and the solenoid coil. The magnetic field is induced by the applied voltage, which opens the nozzle by ascending motion of the plunger. The bioink is deposited when the pneumatic pressure overcomes the fluid viscosity and surface tension at the opened orifice. Nozzle diameter, viscosity, and surface tension of the bioink, pneumatic pressure, and valve opening time are vital parameters on which the microvascular process depends 150 ,. 151 The microvascular technique offers several advantages to the printing process, including the synchronized ejection of biomaterial and cells from various print heads and thin material deposition of about 1–2 μm thickness. Precise cellular positioning is also possible, along with higher viability and high throughput printing of about 1000 droplets per second. 150 However, the process is disadvantageous during hydrogel printing within a limited range of viscosities. The cell concentration of up to 106 cells per ml is allowed to print as a small nozzle orifice (100–250 μm) can cause clogging during the printing and disrupt the process. The cells do not always disperse and settle over time, which hampers the overall homogeneity of the cells within the bioinks. 151 , 152 Homogeneous patterning of cells was facilitated on the thin layers of bioprinted ECM in a controlled area. The 3D printed cell displayed more homogeneous cellular layers with ECM 1–2 μm thickness, whereas the manual seeding cells were of 20–30 μm ECM thickness of discrete multilayered cellular clusters. The 3D printed cells induced higher structural and functional resemblance to the native barrier. 153 The microvalve technique was used to print organs with improved mimetic nature to the original organs. In one study, this technique was used to print a construct of (6 × 6 × 1.2 mm3) by repeated deposition of the collagen precursor. The printed structure was mechanically stable to retain its shape and dimensions compared with the manually seeded complexes, where profound changes in shapes and dimensions were observed during the culture. 152 It is a 3D printing method that involves photopolymerization to cure the liquid ink placed in a vat into a layered volumetric construct. The technique has been commercially available for more than 30 years, used mainly for the fabrication of tissue scaffolds along with the conventional cell-seeding approach. Stereolithography (SLA) is the primary technique of vat polymerization. It leads to the fast production of volumetric structures with precise internal and external structures in several biomedical applications. This method utilizes a laser beam that helps to sweep around polymerizing single lines of ink in a faster scan mode until each layer has been completed. The SLA system uses a photocurable bioresin to print the construct using top-down and bottom-up printing approaches 154 ,. 155 Another method is used in the Vat polymerization technique, known as digital light-processing (DLP), which has emerged as an improved method in VP printing. It uses a digital micromirror device (DMD) in DLP, which causes immediate crosslinking of a layer of photocurable resin instead of printing at the single dot in SLA. The DMDs provide the rotation to be in either an ON or OFF position, which helps the photocrosslinking of a bioresin layer. The rotation reduces the build time as the process only depends on the thickness of the layer and the necessary time of exposure. 156 , 157 Two-photon polymerization (2PP) is another technique used in vat polymerization to print the material. The process uses a near-infrared femtosecond laser (~740 nm from titanium: sapphire femtosecond laser) to fabricate precise 3D structures with high resolution on the nanoscale. This polymerization process starts with the 3-order nonlinear absorption within the focal region. The laser beam is tightly focused on the photoresist on a glass coverslip with an oil-immersion objective lens. Further, it causes fabrication of high-resolution 3D structures beyond the optical diffraction limit by adjusting the beam within the photoresist. 158 , 159 Bioinks like PEG, PCL, PEG-co-PDP, PEGDA, PCL/HA, HA/PEEK, and titanium are some of the materials used for the vat polymerization for printing a 3D construct. The nature of the 3D construct printed is affected by the fabrication strategy of the different bioprinting techniques. The crosslinking process and the crosslinking agents mainly dominate the fabrication of the 3D constructs. Some hydrogels require support while processing, whereas some are mechanically stable to withstand and to complete the process. These fabricating strategies will be discussed in the next section. 3D bioprinting is a complex process starting with the material selection and ending with the fabrication of a similar biomimetic structure as proposed. Fabrication deals with the crosslinking strategies used for different bioprinting techniques, as discussed earlier. These fabrication techniques are used to accomplish print reliability and resolution. The rheological property of biomaterial plays an important role in preserving the structural integrity of the printed 3D constructs for cell deposition. As the name suggests, this fabricating technique prints the desired complex biomimetic structures. The biomaterials are required to have desired rheological and mechanical properties throughout the printing process to attain a similar mechanical and structural complexity. Viscosity, yield stress, and mechanical reliability are a few necessary factors that need to be controlled during and after printing. 128 , 160 , 161 The viscosity of the biomaterial can be enhanced by pre-exposure of photopolymer to UV light, adjusting temperature and adding enzymes crosslinker. 162 , 163 In a few cases, a thickening semi-crosslinked mixture was used along with the thickening agent to directly print the 3D construct. 164 , 165 , 166 A similar study was performed by synthesizing photocrosslinkable hyaluronan-gelatin hydrogels for printing a complex feasible tubular construct. The synthesized bioink displayed biocompatibility, supporting cell attachment and proliferation of HepG2 C3A, Int-407, and NIH 3T3 cells in vitro. These photopolymers when exposed to UV light displayed improvement in the viscoelastic property and thereby similar values of storage modulus (G′) and loss modulus (G″) of the hydrogel. 163 Zhang et al. directly bioprinted vessels like tubular microfluidic channels. Chitosan and alginate hydrogels were used along with the cartilage progenitor cells and displayed average cell viability of 63% after 12 h of media perfusion postprinting of the complex. The viscosity of the structure was affected by the amount of chitosan used. 167 Only ~3% chitosan was feasible to print microfluidic channels with required fabrication parameters as 2% lacked mechanical integrity, whereas 4% was so viscous that it was not coming out properly from the nozzle. The viscosity of the bioink can be altered using thickening agents like hydroxyapatite, nanocellulose, xanthum, and gum, thereby increasing the printability to form a complex 3D structure. 88 , 164 , 166 , 168 Wouter et al. used gelatin methyacrylamide hydrogels to print tissue-engineered cartilage constructs. The printed hydrogel was tested with chondrocyte cells; efficient viability and differentiation were seen in cells, and enhancement in mechanical properties was also seen in the hydrogel. Gelatin methacrylate solution has low viscosity at normal body temperature, making it incompatible with biofabrication processes. However, the addition of HA or codeposition with thermoplastic enhanced the viscosity and, thus, favored as a compatible bioink material. 169 During the fabrication process, the biological hydrogels, composed of polysaccharides and proteins, are difficult to print as the in situ gelation is a critical stage as they need to be provided with support so that they do not collapse or deform under their own weight. 170 The support bath is used widely to fabricate complex hydrogel-based overhanging structures. The support bath materials should have a rigid matrix, which yields by a passing nozzle and rapidly recovers itself after the motion of the nozzle. The support bath provides the necessary gelation to the hydrogel before integrating the subsequent layers without clogging the nozzle. Several materials have been used for providing support in the form of a support bath. Pluronic is one of the biocompatible materials playing the dual role as fugitive bioink and support bath due to its thermoreversible sol–gel phase transition property. The mechanical weakness and rapid dissolution are the main concerns with this material as they limit the duration up to which the hydrogel can be supported by the PF baths. 170 , 171 , 172 Wu and his research group modified PF with triblock copolymer to increase its efficiency as a support bath. They synthesized a fluorescent dyed fugitive ink, which comprises an aqueous solution of pluronic F127, a triblock copolymer with a hydrophobic poly(propylene oxide) (PPO) segment and two hydrophilic poly(ethylene oxide) (PEO) segments in a PEO–PPO–PEO fashion was used as a fluid filler. The diacrylate functionalized pluronic F127 solution was used as a physical gel reservoir. The 3D biomimetic microvascular networks with a 3-generation hierarchical branching topology of 200–600 μm microchannel diameter were constructed using this system. Fumed silica nanoparticles-based suspension was used as a hydrophobic support bath for the 3D extrusion printing. Hydrophobic silica-mineral oil suspension was used to deposit hydrophobic inks like PDMS, SU-8 resin, and epoxy-based conductive ink. The structural integrity was maintained during printing as well as curing was done up to 90°C. The bath was further able to provide feasibility, versatility, and a much better resolution such as 30 micron for PDMS filaments. 173 , 174 , 175 Crosslinking is a vital step in 3D bioprinting that influences the printed construct's mechanical, physiochemical, and cellular properties. 88 Hydrogels with a rapid gelation mechanism are primarily used through the in-process crosslinking method. This crosslinking is accomplished either by changing the extrusion head for coaxial extrusion of the precursor and crosslinker. This can be obtained by depositing precursor into a crosslinker bath. 176 , 177 The crosslinking can be modified by changing the material or by varying the printer's tool path design. 178 , 179 Recently, extrusion bioprinters are customized for specific applications, including core-shell printing, combined extrusion and ES, and UV curing during and postprinting. 113 Photoinitiators are the critical components of photocurable polymerization systems. Various UV –light photoinitiators are utilized for photocrosslinking. Several limitations like low water solubility, inhibition with oxygen, high-energy UV light exposure requirement and cell damages have also been reported in this crosslinking process. 180 Layer-by-layer UV curing of bioprinted photocurable GelMA-based hydrogels was prepared using a rapid extrusion-based bioprinting technique with an in-built ultraviolet (UV) curing system. The GelMA improved the bioink printability and shaped fidelity before crosslinking and led to the fabrication of soft tissue constructs with a high aspect ratio (length to diameter) of ≥5. The cell viability of the printed layers was above 80% in all cases. 125 In another study, water-soluble photoinitiator lithium phenyl-2,4,6-trimethyl-benzoyl phosphinate (LAP) was emulsified in pentaerythritol triacrylate monomers. Extrusion-based printing technique was used to print the 3D construct at the optimum concentration of 10 mM and 1.62 M for LAP and triethanolamine, respectively. 180 Billiet and his coworkers used and optimized VA-086 as a photoinitiator to fabricate highly viable 3D printed macroporous gelatin methacrylamide constructs. 181 Alginate and fibrin are among the most common used materials for this type of printing due to their capacity to sustain structural stability and are versatile to be incorporated with any cellular component. 182 Nishiyama constructed the gel structure using state-of-the-art inkjet technology. Sodium alginate solution was ejected from the inkjet nozzle and mixed with calcium chloride. The nozzle could move in three directions, thereby leading to a new fabrication method for the detailed fixation of 3D gel structures using living biological components. 176 Crosslinking is essential to improve the biomechanical features of the polymers through network bonding. It establishes a connection between two functional groups of a polymer chain through covalent or noncovalent bonding. 183 However, in a few cases, extrusion technology was used for crosslinking. Still, it cannot be completed before and during the printing as the bioinks may consist of different types of polymer with different crosslinking patterns and mechanisms. In such cases, the printed construct is exposed to crosslinkers after printing. This bioink mixture used in this process is made of two types of materials: primary and secondary. Primary material forms the basic framework and helps to enhance printability and shape accuracy during the complete printing process. At the same time, secondary material undergoes crosslinking after printing to provide structural accuracy. 88 Seung et al. fabricated alginate scaffolds in a two-step process; a modified dispensing process and an aerosol spraying method using calcium chloride as a crosslinking agent. A 3D pore-structured, cell encapsulated alginate scaffold of 20 × 20 × 46 mm3 was constructed with varying crosslinker concentrations of the aerosol and dispensing process. The printed construct displayed 84% cell viability to prepsteoblast (MC3T3-E1) cells. 184 In another gelatin-methacrylamide (GelMA)/hyaluronic acid (HA) polymeric system, GelMA alone could not provide the desired structural property and integrity as well as printability. The acid improved the viscosity and therefore enhanced the printed structural 3D construct. The HA also improved the cell viability to 82% compared with the pristine GelMA system toward chondrocyte cells. 169 Postprocess crosslinking was used to construct heterogeneous aortic valve conduits using alginate/gelatin polymeric mixtures. The constructed aortic valve had viability over 80% for aortic root sinus smooth muscle cells (SMC) and aortic valve leaflet interstitial cells (VIC) (Figure 2B). 76 This process involves using a sacrificial agent followed by inducing a crosslinking agent, which gets removed upon final printing of the desired 3D construct. This type of printing commonly uses build/support configuration for printing constructs or models with high structural reliability. Build materials are generally engineered tissue components (cells and/or hydrogels), which support or help base materials to provide mechanical strength to hold the structure with a strong grip 84 tightly. In most cases, the support material may be deposited with the build material but is removed through postprocessing procedures. This process has allowed the use of particular material, which was not favorable earlier due to its characteristics and can be used in indirect printing. 185 The 3D filament networks printed using carbohydrate glass displayed a rigid behavior. The printed networks were used for the biocompatible sacrificial template in several engineered tissues. These templates were further used to create cylindrical networks. The cylindrical networks were lined with endothelial cells also permeated with blood under pulsatile flow of high pressure. This approach allowed independent control of endothelialization, network geometry, and extravascular tissue. 161 Alginate and gelatin precursors are mixed with different concentrations of hydroxyapatite to print a 3D complex. It is a two-step mechanism that combines gelatin's thermosensitive and alginate's chemical crosslinking properties to form the structure. 186 Materials like gelatin, pluronic F127, and agarose show reversible crosslinking mechanisms, making them suitable for support material. 88 Alginate was used to construct a scaffold for nerve tissue applications through indirect printing. It involved printing a sacrificial framework from gelatin, impregnating the framework with a low concentration of alginate, and finally, the gelatin framework was removed using an incubation process. The printed lower alginate scaffold has better cell viability and functionality as compared with the higher alginate scaffold. 187 Hybrid printing involves using computer-aided technology and several other fabrication processes. Multiscale parts have been constructed due to the integration of bioprinting techniques with melt-plotting and ES apparatus. Kang et al. used a hybrid printing method to fabricate the human-scale tissue construct with enhanced structural properties. The tissue was constructed by representing clinical imaging data as a computer model of the anatomical defect and then translating the model into a program that can control the motion associated with the printer's nozzle. Additionally, it can help distribute the cells to discrete locations. The microchannel was fabricated inside the tissue constructs, facilitating the diffusion of nutrients to printed cells. 20 Printing ECM with accurate structural integrity and exact components is critical for several technologies. A large amount of materials has been used for printing the matrix, but the complexity of the natural ECM leads to complications in printing them with intrinsic cellular morphologies and functions. A hybrid method was developed to bioprint the cell-laden constructs with a novel decellularized ECM (dECM) bioink, which can provide an optimized microenvironment favorable for the 3D growth of tissues. 32 PCL and PLGA are commonly used as support for hybrid printing. These polymers are generally processed at high temperatures, making it difficult for cells to deposit them directly. In order to overcome this factor, cells are deposited onto them using hydrogel as a carrier. 88 PCL/alginate hybrid scaffolds were fabricated using the aerosol crosslinking process for even cell distribution (Table 3). Scaffolds can provide repeated and appropriate pore structure but low biological activity, low cell-seeding efficiency, and nonuniform cell density. PCL scaffold coated with alginate provides enhanced cell viability, cell seeding efficiency, and cell distribution for prosteoblast cells (MC3T3-E1). 188 , 189 Bioprinting techniques and their crosslinking strategy Bioprinting technique Crosslinking method/agent Bioink Target tissue Reference Inkjet Ionic-CaCl2 Alginate Bone tissue 190 Ionic-CaCl2 Alginate Not specified 191 Thermal Pre-gelation + ionic-CACL2 Alginate/gel Not specified 192 Ionic-SrCl2 Alginate/Guar Gum Human cartilage 166 Electrostatic interaction/hydrophobic Peptide/keratin Not specified 193 Hydrogen binding + π–π stacking Supramolecular-PCL Not specified 194 UV crosslinking/(Irgacure 2959) PEGDA Cartilage tissue 195 Extrusion Ionic-CaCl2 Alginate Cardiac tissue 196 Thermal Pre-gelation + ionic-CaCl2 Alginate/gel Lung 113 Ionic-CaCl2 Alginate/guar gum Not specified 197 Ionic-CaCl2 Alginate/PCL Human cartilage 198 Electrostatic interaction PEG/Clay Not specified 199 Electrostatic interaction/ UV crosslinking GelMA/κCA /Clay Not specified 200 Electrostatic interaction Gel/Ch Skin 27 Hydrogen bonding Gly/Clay Bone regeneration 201 DNA hybridization Polypeptide/DNA Not specified 202 UV crosslinking/(Irgacure 2959) GelMA/Silicate Bone tissues 203 UV crosslinking/(Irgacure 2959) GelMA/CNT Myocardial tissue 204 Ionic-CaSO4 Ch-HAp Bone tissue 205 Electrostatic interaction Alg/MC/Clay Bone tissue 206 Laser-assisted Ionic-CaCl2 Alginate Skin tissue 207 Thermal pre-gelation + ionic-CaCl2 Alginate/gel Not specified 208 Electrostatic interaction HA Corneal structures 209 Visible-light crosslinking/rose bengal Tyramine-HA Not specified 210 Stereolithography Hydrogen binding + UV crosslinking PCL-Pyrimidinone Not specified 211 UV crosslinking/(LAP) GelMa Vascular tissue 212 UV crosslinking(Irgacure 2959) GelMA/PEGDA Cartilage tissue 213 Photo/Lap GelMA/PEGDA Spinal cord 214 UV crosslinking/(Irgacure 2959) GelMA/M-HA Adipose tissue 215 Visible-light crosslinking/(VA-086) PEGDA Not specified 192 In the 3D printing design of organs or tissue, 3D models allow the researchers to create a functional prototype of the model faster than any other prototyping process. This also enhances the research efficiency as less time is consumed by these prototype models used for different works. Several factors affect the design factors and capabilities of bioprinting, which can be listed and explained in the next section. Bioprinting technology uses computer-aided technology to create engineered tissues. The software-based technology has allowed greater precision and control while printing as it has allowed variation in terms of cell-material shape and deposition along with the modification in single or multiple depositions of the cells on a single substrate. This digital technology has allowed the introduction of digital analysis to understand the printing process at a super refined and cellular level. This understanding has helped fabricate even a complex structure like skin with different cell densities and cell types printed on the same substrate. 88 3D printing is one of the advanced, evolving technology known for creating or printing novel or biomimetic 3D complex constructs. It helps to produce a replica of a structure similar to the original one with great resolution and precision. It helps to create unique 3D constructs with the help of biomaterials and cells, thereby replacing the orthodox approach of using metals and certain plastics to create 3D models. The shape of the printed 3D constructs must be anatomically precise in order to replace the required organ, cellular or tissue model. 88 Several factors like printing technology, biomaterial, and fabrication strategy affect the shape of the 3D construct to be printed. Printer resolution, accuracy, and platform size for printing also affect the shape and features of the desired printed 3D constructs (Figure 2C). 76 , 181 3D bioprinting needs a computer and computer-aided design to fabricate the assigned 3D design perfectly. SL plays a vital part in this 3D printing for describing the surface geometry of a 3D model or object by creating a series of linked triangles. Triangles are linked in a serial order for better resolution. It plays a significant part in accurately printing the desired 3D constructs. 216 Mannor et al. constructed a 3D printed bionic ears with its electrical components, which increased the printed ear's efficiency and broadened human hearing capability. 217 Duan et al. attempted to create a 3D printed heart valve using an extrusion-based printing method, and the reverse engineering approach has been exploited for this method. A porcine aortic valve was harvested, and the data were imported into an open-source bioprinter and anatomically correct the aortic valve using this approach. 76 In another work, heart valves were drawn into solid work and were printed afterward. 3D bioprinting helps print an anatomically correct virtual shape with reasonable accuracy and precision. Resolution is one of the properties while considering good image. In 3D printing, resolution refers to the smallest movement of the nozzle, which can deposit a material on the substrate with better precision. The 3D printers have two types of resolution XY resolution and Y resolution. The resolution of the horizontal movements of the printer can be controlled by XY resolution, and the Y resolution governs the resolution of the vertical movements associated with the printer. 218 The smallest material unit constructed affects the resolution and accuracy of the printed 3D constructs. The layer function and thickness affect the resolution in the Z direction. Printing further layers in this direction will affect the overall structure. Sometimes the printing path height, path space, and nozzle diameters also cause a change in the resolution of the printed 3D constructs. 219 , 220 The bioprinting phenomenon involves the basic approach of using biomaterials, cells and growth factors to print a desired 3D complex construct of specific cellular, tissue or organ model for the treatment of specific infections or diseases. However, these components can vary based on the necessity and specificity of the biomedical applications, which can lead to heterogeneity in the cells, materials and growth factors required to print a desired 3D construct. 3D printed materials consist of two vital components, biomaterial, which will form the skeletal framework of the printed construct and live cells, which needs to be incorporated within the framework to create a replica of the proposed structure, organ or model. Single cells, cell aggregates and multiple cell types can be varied while utilizing the cells. Cells that are young and immature take on individual characteristics and become mature to form specialized cellular forms and function. 221 The specialized human cells that have the ability to differentiate into various cell types are known as stem cells. They have been used in regenerating many organs and tissues in the human body for their unique properties. Pluripotent and multipotent stem cells have been widely used in 3D bioprinting. Pluripotent stem cells are the specialized cells having the ability to undergo self-renewal. All the cells of the tissue of the body can be generated by these stem cells. In contrast, multipotent stem cells have the capacity to self-renew. This renewal is done by dividing and can develop into multiple specialized cell types present in a specific tissue or organ. 222 Schuurman et al. worked on printing PCL fibers with alginate constructs using extrusion-based printing, and the mechanical property was varied by changing the fiber spacing, orientation and thickness. Cell viability was calculated using C20A4 cells, and cell viability became high immediately after printing and was close to 75% even after three days of in vitro culture. 189 Zhu et al. constructed gelatin methacrylamide hydrogel with graphene nanoplatelets using SL printing. This model was used to provide a biocompatible microenvironment for survival and growth, leading to the successful construction of neural stem cells. Well differentiation, architecture and homogeneous cell distribution were observed in the printed cells. 223 Seol et al. fabricated a 3D printed biomask for facial skin wounds and reconstruction. The layers of skin were constructed using an extrusion-based bioprinting technique. A porous PU layer, a keratinocyte-laden hydrogel layer and a fibroblast-laden hydrogel layer were used to create different layers of skin. Human epidermal keratinocytes and human fibroblasts were used to study the biological activities. In the animal study, the subcutaneous implants reduced the wound area by

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