3D Bioprinting Techniques

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Additive manufacturing, more often referred to as “3D printing,” is the method of fabricating three-dimensional objects by adding successive layers of materials at a regulated rate and thickness. These materials could be made of concrete, metals, ceramics, polymers, resins, biomaterials, or other substances. The dearth of variety in 3D-printable materials continues even though printing time, processing speed, and printing resolution have all increased. The compatibility and flowability of printing ink with the current printing procedures are crucial for developing fields such as the 3D printing of biomaterials, tissues, and high-viability cells.

Keywords: 3D printing ; bio-printing ; fused deposition modeling (FDM) ; stereolithography (SLA) ; direct ink writing (DIW)

1. Fused Deposition Modeling (FDM)

The continuous layer-by-layer extrusion of a thermoplastic polymer filament characterizes fused deposition modeling (FDM), a revolutionary scaffold-building technology. The absence of an organic solvent, the quick solidification of the extruded polymer, and the structural integrity of the resulting three-dimensional matrix are all advantages of FDM. The FDM bio-printer’s goal is to fabricate configurable, reproducible scaffolds that facilitate homogeneous cell distribution. Due to its potential to generate regenerative tissues and organs, this technique has paved the way for the development of artificial multicellular tissues and organs. Three-dimensional bioprinting now uses a wide range of biomaterials and different methods developed by researchers.

In this method, the material is either melted by the heated nozzle to form a layer on the build platform or it is fed into the extrusion nozzle as a liquid with a predetermined viscosity. The necessary ink is created as a solid filament, which is subsequently heated throughout the extrusion process to a semi-molten condition. The filament material is then oozed out through a temperature-controlled nozzle. Layer by layer, the extruded material that was forced out is deposited onto a platform. The platform is further lowered after one layer is finished, and the subsequent layer is then deposited. Layer thickness or height, printing speed, infill rate, nozzle temperature, retraction, shell thickness, and the potential inclusion of supports (structures that assist deposited materials in being correctly printed in cases of steep angles) are major factors that significantly affect the material’s final qualities [1][2][3][4][5][6][7]

Materials Compatible with the FDM Process

Poly(caprolactone): PCL is an excellent choice for melt-based extrusion operations because of its characteristics. This material is an attractive option for medical applications because of its thermal stability, low acquisition cost, and shear-thinning capabilities. It is also well-suited for usage in medical devices. According to published literature, PCL may be employed to create a tissue-engineered scaffold that can be used as a substrate for restoring breast tissue after a partial mastectomy procedure [8]. Other cases mentioned in the literature include the design and bio-printing of a novel wound-dressing material by incorporating Juglone (5-hydroxy-1,4-naphthoquinone) to a 25% Polycaprolactone (PCL) scaffold, which demonstrated high rates of targeted wound-healing [9], and the use of a PCL material combined with sodium mesoglycan (MSG), which exhibited high rates of healing [10].

Poly (lactic acid): One of the most often-used polymers for FDM is polylactic acid (PLA), which features benefits such as biocompatibility, biodegradability, and affordability. This material’s melting point allows for the formation of filaments, and it can be extruded at temperatures between 180 and 250 °C [11]. When PLA breaks down, acidic byproducts are released, which is one of its problems. The release of lactic acid results in a considerable reduction in physiological acidity. A composite material made of PLA and ceramics is used to lessen the possibility of acidic leakage. The composite material is a promising choice for tissue-engineering operations due to the fact that it also has a tendency to improve the strength of compressive pressures.

Polyether ether ketone (PEEK): Peek is a semi-crystalline thermoplastic polymer with a service temperature of 260 °C and a melting point between 330 and 340 °C [12]. It was previously not used in FDM procedures because of its high melting point. PEEK can now be used in FDM printers thanks to recent improvements in printer technology. According to the research that has been published, the melting point temperature, the speed of the extrusion, and the amount of force are the three most important factors that affect the 3D printing of PEEK using FDM procedures [13].

Poly-vinyl alcohol (PVA): Vinyl alcohol and acetate monomers combine to form the synthetic polymer known as poly-vinyl alcohol (PVA). These latter qualities—biocompatibility, biodegradability, and bio-inertia—are provided by their presence. PVA can be utilized in filament form in FDM processes, and is soluble in lukewarm water. This substance's tensile characteristics are quite similar to those of human articular cartilage, making it an ideal substrate for the ingrowth of bone cells [14]. Its semi-crystalline shape permits optimal oxygen and nutrient delivery to the cell, and its hydrophilicity and chemical stability allow for exposure to high pH and temperature conditions. PVA is widely employed in a variety of load-bearing implant applications, including bone-tissue-regeneration procedures and cranio-facial deformities [15][16].

2. Stereolithography (SLA)

A popular bioprinting technique for meeting the needs of fabricating complex tissues is stereolithography (SLA) 3D bioprinting. The potential of SLA 3D bioprinting in a variety of subsectors, including bone and neural tissue engineering and the creation of controlled microenvironments to study cell behavior, is increased by the development of novel photocrosslinkable biomaterials with improved physical and chemical properties. SLA bioprinting is an innovative bioprinting technology with a wide range of potential and clinical applications thanks to its adaptable design and versatility. Light-based bioprinting, which is an adaptation of stereolithography (SLA) procedures, creates constructions by starting chemical reactions that firm or cure bioinks only where they have been illuminated. Such bioprinters are often faster, using many types of light-based technologies, such as digital light processing (DLP), because they cure full layers concurrently. Light-based bioprinters can also recreate more delicate details at considerably greater resolutions due to millions of microscopic points of light.

Materials Compatible with SLA Process

Poly(D,L-lactide) (PDLLA): PDLLA is a flexible polymer with numerous medicinal uses, such as scaffolding for tissue engineering, controlled drug delivery, and synthetic nerve conduits constructed of PDLLA, -TCP, and collagen for the regeneration of peripheral nerves [17][18][19]. The fabrication of composite scaffolds from HA biocement embedded in PDLLA oligomers using SLA 3D printing technology is described in the literature. N-methyl-2-pyrrolidone (NMP) is used as a diluent and ethyl 2,4,6-trimethylbenzoylphenylphosphinate acts as a photoinitiator. A non-reactive diluent, such as NMP, is required to help maintain the desired viscosity for SLA, because the viscosity of the resin increases as the percentage of ceramic in the resin increases. It has been discovered that the elasticity of the material rises as the concentration of HA particles increases [20].

Poly (propylene fumarate) (PPF): PPF is used in SLA due to it exhibiting pho-to-cross-linkability. It also has superior mechanical properties, and it is biodegradable. In the majority of cases, it is used in SLA by combining diethyl fumarate (DEF) as a solvent with PPF as the base polymer. A photo-initiator is needed in SLA in addition to the previously mentioned fix. Bisacryl phosphrine oxide is utilized herein. The right amounts of PPF and DEF must be present. Mechanical strength has been seen to significantly decrease at PPF to DEF ratios greater than 0.5 [21]. On the other hand, adding DEF reduces the solution's viscosity and enhances its printability. PPF molecular mass, viscosity, and molecular mass distribution can now be precisely determined thanks to the recent development of a ring-opening polymerization process. Reduced molecular mass distribution helps the constructed structures to dissolve over time. PPF material is now used in more biomedical applications thanks to newly developed post-polymerization and post-processing functionalization techniques. According to the recent literature, the influence of PPF molecular mass in scaffolds made using the SLA approach was crucial for controlling the degradation pace and bone regeneration in vivo. While there was no documented inflammation or host cell acceptance, PPF with a lesser mass demonstrated finer behavior in healing rates [21].

PEGDA & GelMA inks: The application of SLA-based 3D bio-printing for a unique cell-laden cartilage-tissue assembly has been documented in the literature. The resin utilized was made up of transforming growth factor-beta 1 (TGF-1)-embedded nanospheres created using a core-shell electrospraying process, 10% gelatin methacrylate (GelMA) as a base material, and varying percentages of polyethene glycol diacrylate (PEGDA). It was discovered that adding PEGDA to GelMA hydrogel significantly enhanced printability, and that PEGDA also increased compressive modulus correspondingly while decreasing the swelling ratio. The maximum cell viability and proliferation rates were observed in cells cultured on

5%/10% (PEGDA/GelMA) hydrogel. The TGF-1 implanted in nano-spheres can maintain a sustained release for up to 21 days and enhance the encapsulated MSCs' chondrogenic development. As a result, such materials exhibit high potentials in cartilage-regeneration applications [22][23].

3. Direct Ink Writing (DIW)

The extrusion-based 3D-printing technique known as DIW uses a nozzle to extrude materials onto a build platform layer-by-layer, similarly to the FDM method. By using this method, it is possible to control the deposition of raw materials in a highly viscous liquid condition, allowing them to keep their shape during the deposition stage. Due to the fact that it can use a wide range of materials, including ceramics, hydrogels, plastic, food, and even living cells, DIW can be seen as being more versatile than FDM. Nozzle size, material viscosity and density, printing speed, and thickness maintained between layers are the main factors that determine the final properties of the fabricated item. Similarly to FDM and SLA, support structures must be used in DIW when complicated geometric shapes with overhangs and steep deposition angles are present. However, the use of dissolvable materials as supports helps to overcome this problem because they can be quickly removed after the printing process is complete. By using UV-curing equipment, the post-fabrication processing phases further aid in improving the printed item's mechanical characteristics (such as elastic modulus) [24].

4. Laser-Guided Direct Writing (LGDW)

LGDW, a laser-assisted direct writing method, can deposit cells with micrometer-level precision. On a number of surfaces and matrices, cell deposition with a focused laser beam is possible. In the process of "laser-guided bio-printing," cells are guided by a laser beam onto a receiving substrate [25].

Materials Suitable for DIW & LGDW

Hydrogel Inks: Complex three-dimensional networks of hydrophilic polymers that have a high water-absorption capacity are called hydrogels. Due to their capacity to provide the ideal conditions that favor the encapsulation of viable cells, as well as protecting the cells without obstructing cell–cell communication, these materials have a number of advantages, but their greatest benefit is their high biocompatibility and biodegradability rates. The regulated viscosities of hydrogel inks should enable fluid flow under working pressure conditions. Additionally, they must offer sufficient structural integrity when printing is finished, as well as a quick rate of gelation that can be managed by utilizing shear thinning [26]. According to the literature, creating a polymer solution that solidifies into a network after printing is the preferred method for creating hydrogel ink. Utilizing external stimuli such as temperature, light, or ion concentration, the network thus created could be physically or chemically crossed-linked [27]. The majority of synthetic and natural polymers, including polyacrylamide, polyurethane, and polyethene glycol (PEG), are used in the fabrication of hydrogels in 3D bioprinting [28][29]. Examples of natural polymers used in this process include gelatin, cellulose, collagen, fibrinogen, alginate, and agar.

As the polymer concentration and cross-linking density rise, the rate of proliferation toward the targeted tissue declines. Higher polymer concentrations, however, have been discovered to be ideal for extrusion-based DIW due to their increased viscosity. As the polymer concentration rises, the mechanical properties increase. Shear stress can result in cell death during extrusion because it increases with viscous inks, high pressures, and small diameter nozzles. According to research, shear stresses greater than 60 MPa can result in 35% or more cell death [30]. Shear stress is the parameter that has the greatest impact on resolution in hydrogel bioprinting when using the DIW technique.

Gelatin-methacryloyl (GelMA): Using gelatin that has been derivatized with methacrylamide and methacrylate groups, GelMA is a semi-synthetic hydrogel. GelMA hydrogels were demonstrated to support the formation of cartilage tissue using chondrocytes and MSCs in experiments conducted by Sauty et al. [30]. GelMA hydrogels can be synthesized with a specific degree of functionalization (DoF) and tailored to the intended application as a three-dimensional (3D) cell-culture platform. Piao et al. discovered that significantly higher pressure was needed to dispense cell-laden GelMa from glass capillary, but that cell vitality and proliferates weren't significantly impacted [31][32].

5. Inkjet Bioprinting

A non-contact, controlled 3D-printing technique called inkjet printing enables the dispersion of droplets with volumes ranging from 1 to 100 picoliters that still contain cell viability. One of two methods—continuous inkjet (CI) or drop-by-drop (DOD)—is used to extrude droplets from a nozzle (CIJ). Of the two, DOD is better suited for tissue engineering. In addition, DOD can be divided into three categories based on the depositing method. Some examples include mechanical, electromagnetic, and thermal (which use heat to expand and deposit the material before the nozzle). In DOD inkjet 3D

bio-printing, individual drops with diameters ranging from 25 to 50 μm are produced in accordance with predetermined specifications. A continuous stream of individual droplets with a volume of 100 μm in diameter are expelled during CIJ 3D bioprinting. Due to the fact that these techniques have a tendency to influence the cell wall and its vitality after being sonicated at 15–25 Hz, it is discovered that electromagnetic and thermal inkjet printing have not been widely accepted as far as tissue engineering is concerned. Due to this, thermal inkjet is used more frequently to promote better cell vitality [33]. The process is accelerated by the use of many inkjet print heads, each of which has a number of separate nozzles. When using a thermal inkjet printer, the ink is heated to a high temperature, which causes bubbles to form. The bubbles continue to grow until the ink is discharged from the nozzle. The survivability of biologically printed DNA, cells, tissues, and other organs is not affected by the heating temperature, which can reach up to 300 °C for a brief period of time. In this instance, cell viability has been determined to be about 85% [34].

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