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Review

Bio-Inspired Materials: Exhibited Characteristics and Integration Degree in Bio-Printing Operations

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Abstract: In the last decade, additive manufacturing techniques, commonly known under the term "3d printing" have seen constantly increasing use in various scientific fields. The nature of these fabrication techniques that operate under a layer-by-layer material deposition principle features several de facto advantages, compared to traditional manufacturing techniques. These advantages range from the precise attribution of pre-designed complex shapes to the use of a variety of materials as raw materials in the process. However, its major strong point is the ability to fabricate custom shapes with interconnected lattices, and porous interiors that traditional manufacturing techniques cannot properly attribute. This potential is being largely exploited in the biomedical field in sectors like bio-printing, where such structures are being used for direct implantation into the human body. To meet the strict requirements that such procedures dictate, the fabricated items need to be made out of biomaterials exhibiting properties like biocompatibility, bioresorbability, biodegradability, and appropriate mechanical properties. This review aims not only to list the most important biomaterials used in these techniques but also to bring up their pros and cons in meeting the aforementioned characteristics that are vital in their use.

Keywords: 3D Printing, Bio-Printing, Biomaterials, Fused Deposition Modeling (FDM), Stereolithography (SLA) Direct Ink Writing (DIW) Laser-Guided Direct Writing (LGDW)

Introduction

Additive manufacturing, most commonly referred to as "3D printing", can be described as the additive process where a three-dimensional object is fabricated by laying successive material layers at controlled speed and layer thickness. These materials can be biomaterials, metals, ceramics, plastics, resins, concrete, or other materials. Even though printing time, processing speed and printing resolution have been constantly improving over recent years, still, the lack of variety in 3D printable materials persists. Emerging fields like 3D printing of biomaterials, 3D printing of tissues, and high viability cell printing are highly dependent upon printing ink's compatibility and flowability with the current printing techniques available. This study reports the advances in 3D printing materials for emerging biomedical fields and their compatibility with currently available printing techniques.

Bio-printing is the additive process of creating cell patterns by successively depositing cells along with biomaterials that form a substrate thereby fabricating living human constructs with similar biological, chemical and mechanical properties for proper recuperation of tissues, scaffolds and organs. Materials used in this process are known as biomaterials and should exhibit biocompatibility, i.e., being compatible with the human body, bioresorbability, i.e., being able to be naturally absorbed by the human body, biodegradability i.e., the capacity for biological degradation and appropriate mechanical properties dependent on the implantation spot (Bielenstein *et al*., 2022; Kowalewicz *et al*., 2021; Guo *et al*., 2022; Kantaros *et al*., 2016).

In this context, the field where the aforementioned bio-printing technique is growingly being adopted is tissue engineering. Tissue engineering is an innovative, multidisciplinary field that incorporates the fundamental principles of engineering and biology to invent structures with elevated biological functions. Regarding clinical

applications, one of the most decisive targets of tissue engineering is to surpass the numerous barriers imposed by current treatments that are currently based mainly on organ transplants and biomaterial use for implantation (Guillotin and Guillemot, 2011). Organ and tissue malfunctions are a major problem regarding human health and well-being. Especially in cases where the human body fails to self-heal using its mechanisms, targeted medical intervention is crucial.

Even though tissue engineering may be newly introduced, the initial concept of tissue substitution was first expressed in the 16th century. Gasparo Tagliacozzi (1546-1599), Professor of Surgery and Anatomy at the University of Bologna, can be considered as the initial/first documented relevant case when he managed to construct a nose replacement by a forearm flap in “De Custorum Chirurigia per Insitionem” announced in 1597 (O'brien, 2011). The primal clinical application of human cells in this field involved skin tissue development by utilizing fibroblasts, keratinocytes, or a scaffold (that would act as a tissue substrate). In another published work, the regeneration procedure of rabbit articular surfaces by utilizing allograft chondrocytes along with collagen gel is also reported (Wakitani *et al*., 2002). A published literature work by Langer and Vacanti (1993) named "Tissue Engineering" is considered a great contribution towards advancing tissue engineering research on a worldwide scale (Ikada, 2006).

The first decade of the current century is linked with the first successful cases of developing the first solely lab-grown organs by utilizing 3D bioprinters. The contribution of Anthony Atala is considered fundamental in this field, where cell therapies, tissue engineering constructs, and organs for many diverse areas of the body have been developed (Anthony, 2022). In addition, he is attributed with the development of 3D bioprinters (Murphy and Atala, 2014) and, in 2006, he and his team developed the first lab-fabricated organ (a human bladder) for implantation to humans (Atala *et al*., 2006). Dr. Paolo de Coppi’s work is also fundamental in this sector, where a tissue-engineered tracheal replacement for a child being developed by him and his team was reported (Elliott *et al*., 2012).

Lattice tissue engineering structures fabricated by bio-printers and compatible biomaterials must exhibit a variety of desired characteristics in the sense of providing a proper substrate for 3D tissue development as the final target. More commonly referred to as "scaffolds", they act as bioresorbable constructs in the spot of the defect with the role of cell-encapsulated tissue constructs containing cells/hydrogels (Dong *et al*., 2017; Nicodemus and Bryant, 2008). They can be categorized as "acellular scaffolds" (scaffolds with no cells, like hip and knee implants, etc.) or "cellular scaffolds" (scaffolds with cells, like skin constructs). Bio-printed scaffolds should exhibit the desired mechanical behavior in terms of providing mechanical integrity in the healing and degradation periods. Controlled porosity is also desired, where an interconnected pore network is considered crucial for blood and nutrient flow. The aforementioned dictates the ability for scaffold fabrication in well-defined geometrical shapes (Kantaros and Piromalis, 2021). Bio-printing techniques exhibit high potential in this field, by being able to provide dimensional stability, reproducibility, and the fabrication of pre-designed interconnected porosity networks at distinct sizes (Moroni *et al*., 2006; Amirkhani *et al*., 2012). Several published works describe cases of bio-printed scaffold structures (Moroni *et al*., 2006; Leong *et al*., 2003; Gauvin *et al*., 2012; Kantaros, 2022). Stages of the bio-printing process are depicted in Fig. 1.

3D Bioprinting Techniques

Stereolithography (SLA)

SLA printing is a 3D printing technique that uses a laser or a DLP projector to cure photopolymer resin layer-by-layer. SLA 3D printers use a UV laser or a DLP projector to cure a specific layer of photosensitive resin in a tank. The light source cures or hardens the resin, forming a very thin sliced solid layer. This slice bonds to the previously formed layer or the build plate. The build plate then moves away from a value that equals the pre-determined layer thickness. This is how the process of object formation is repeated until the complete object is created.

SLA printing produces higher resolution than FDM printing because it uses a light source to solidify the material, resulting in small-sized prints. The horizontal resolution of an SLA scanner depends on the size of the light source spot and can range from 30 to 140 microns. The vertical resolution (or Z-direction resolution) varies from 25 to 200 microns (Jo and Song, 2021). To create a good print, you need to set the layer height and the support placement correctly. Figure 2 depicts an SLA 3D Printer apparatus schematic.

Ink materials compatible with the SLA process should exhibit properties like biocompatibility, stability under exposure to UV light, low viscosity, and optical transparency (Rasheed *et al*., 2021).

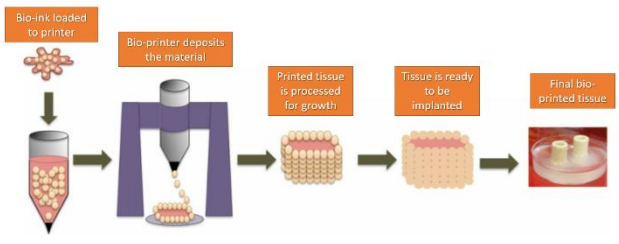

Fig. 1: Distinct stages of the bio-printing process

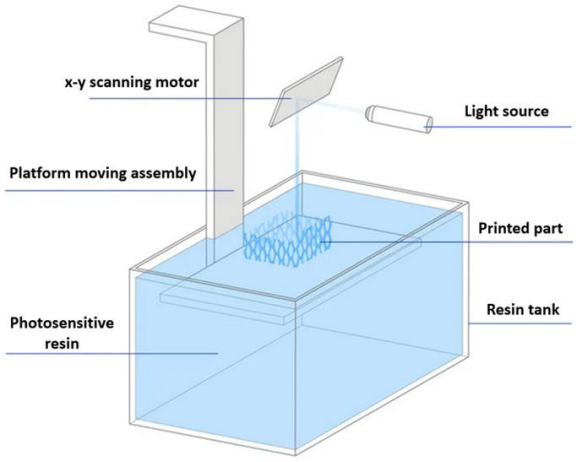

Fig. 2: Stereolithography 3D Printing schematic

Bio-Inks Compatible with SLA Process

Poly (D, L-Lactide) (PDLLA)

PDLLA is a versatile polymer with many applications in the medical field, including as a scaffold material for tissue engineering, as a controlled delivery system for drugs, and as synthetic nerve conduits made out of PDLLA, β-TCP, and collagen for regeneration of peripheral nerves (Hofmann *et al*., 2012; Lin *et al*., 2006; Lin *et al*., 2017).

Literature works describe the fabrication of composite scaffolds from HA biocement embedded in PDLLA oligomers using SLA 3D printing technology using ethyl 2,4,6-trimethylbenzoylphenylphosphinate as a photoinitiator and N-Methyl-2-Pyrrolidone (NMP) as a diluent. With increasing percentages of ceramic in the resin, the viscosity of the resin increases and so a non-reactive diluent, such as NMP, is necessary to help maintain the desired viscosity for SLA. It has been found that as the concentration of HA powders increases, the elasticity of the material increases. (Cai *et al*., 2019).

Poly (Propylene Fumarate) (PPF)

PPF is used in SLA due to exhibiting pho-to-cross-linkability. It is also biodegradable and possesses elevated mechanical properties. In the majority of cases, it is being used in SLA by creating a solution consisting of PPF as the base polymer and Diethyl Fumarate (DEF) as a solvent. The solvents were used in an attempt to avoid premature crosslinking of the polymer. Along with the above-mentioned solution, a photoinitiator is required in SLA. In this case, bisacryl phosphrine oxide is used. A proper balance between PPF and DEF is essential. It has been observed that with a ratio higher than 0.5 of PPF to DEF mechanical strength decreases significantly. On the other hand, adding DEF decreases the viscosity of the solution and hence improves printability. The recent introduction of a ring-opening polymerization method allows the precise determination of PPF molecular mass, viscosity, and molecular mass distribution. Decreased molecular mass distribution assists in the time-certain resorption of the fabricated structures. Newly introduced post-polymerization and post-processing functionalization methods have increased the number of biomedical applications that use PPF material.

Recently, literature works suggest that the influence of PPF molecular mass in scaffolds fabricated via the SLA technique proved critical regarding the degradation rate and bone regeneration *in vivo*. PPF with lower mass exhibited finer behavior in healing rates while no inflammation and host cell acceptability were reported (Kondiah *et al*., 2020; Mamaghani *et al*., 2018).

PEGDA and GelMA Inks

Literature works report the use of SLA-based 3D bio-printing for a novel cell-laden cartilage tissue construction. The resin used was comprised of 10% Gelatin Methacrylate (GelMA) as base material, various percentages of Polyethene Glycol Diacrylate (PEGDA), biocompatible photo-initiator and trans-forming growth Factor-Beta 1 (TGF-β1) embedded nanospheres fabricated via a core-shell electrospraying technique. It was found that adding PEGDA to GelMA hydrogel greatly improved printability while compressive modulus also elevated proportionally with PEGDA whilst the swelling ratio decreased. Cells grown on 5/10% (PEGDA/GelMA) hydrogel present the highest cell viability and proliferation rate. The TGF-β1 embedded in nano-spheres can keep a sustained release for up to 21 days and improve the chondrogenic differentiation of encapsulated MSCs. Therefore, such materials feature high potential in cartilage regeneration processes (Martinez-Garcia *et al*., 2022; Jiang *et al*., 2022).

Fused Deposition Modeling (FDM)

In this technique, the material is led to the extrusion nozzle as a liquid of predefined viscosity or it is melted by the heated nozzle to form a layer on the build platform. The required ink is fabricated in the form of a solid filament which is then heated to a semi-molten state in the stage of extrusion. A temperature-controlled nozzle then oozes out the filament material. The forced-out extruded material is deposited onto a platform in a layer-by-layer deposition principle. After completing one layer, the platform gets lowered further and then the next layer gets deposited. The main parameters decisively contributing to the final properties of the material are layer thickness or height, printing speed, infill percentage, nozzle temperature, retraction, shell thickness, and support potential presence (Kantaros and Karalekas, 2013; Antreas and Piromalis, 2021; Kantaros *et al*., 2022; Kantaros and Karalekas 2013; Tsaramirsis *et al*., 2022; Kantaros *et al*., 2013).

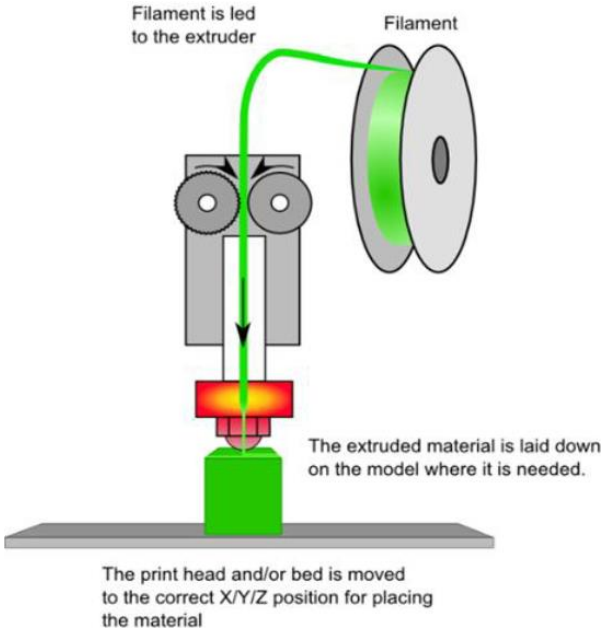

Fig. 3: Fused Deposition Modeling (FDM) schematic

Figure 3 depicts a Fused Deposition Modeling (FDM) 3D Printer apparatus schematic.

The majority of FDM printers are compatible with a wide range of inks. However, the viscosity should be greater than 6×10^7 MPa/s, they should be molten at temperatures between 200 and 250°C and a fast rate of solidification is needed to melt them. In addition, the ratio of elastic modulus to melt viscosity should be less than 5×10^5 s^{-1}.

Bio-Inks Compatible with FDM Process

Poly (Caprolactone)

PCL has properties that make it well-suited for melt-based extrusion processes. The low acquisition cost and shear-thinning properties of this material make it well-suited for use in medical devices and its thermal stability makes it a desirable choice for medical applications. Published literature works suggest that PCL can be used to fabricate a tissue-engineered scaffold to be used as a restoration substrate of breast tissue after a partial mastectomy operation (Jwa *et al*., 2022). Other reported literature cases indicate the use of PCL material combined with sodium Mesoglycan (MSG) which exhibited high rates in targeted wound healing (Liparoti *et al*., 2022) and 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 (Ayran *et al*., 2022).

Poly (Lactic Acid)

Polylactic Acid (PLA) is one of the most commonly used polymers for FDM due to advantages such as biocompatibility, biodegradability, and low cost. The melting point temperature of this material makes it suitable for forming filaments and it can be extruded at a temperature of 180-250°C (Kantaros *et al*., 2021). One of the challenges with PLA is the release of acidic by-products when it degrades. There was a significant decrease in the physiological acidity level due to the release of lactic acid. To reduce the likelihood of acidic release, PLA and ceramics are combined to create a composite material. The composite material also tends to increase the strength of compressive forces, making it a good candidate for tissue engineering processes.

Polyether Ether Ketone (PEEK)

Peek is a semi-crystalline thermoplastic polymer that has a melting temperature between 330-340°C and a service temperature of 260°C (Ikada, 2006). Due to its high melting point, it was initially excluded from its use in FDM processes. Recent advances in FDM printer technology have allowed the use of PEEK in FDM printers. Published literature suggests that the main factors affecting the 3D printing of PEEK in FDM processes are the melting point temperature, the extrusion speed, and the extrusion force. (Dorovskikh *et al*., 2022).

Poly-Vinyl Alcohol (PVA)

Poly-Vinyl Alcohol (PVA) is a synthetic polymer created by vinyl alcohol and acetate monomers. The presence of the latter provides biocompatibility, biodegradability, and bio-inertia. PVA is soluble in lukewarm water and can be used in FDM techniques in filament form. The tensile properties of this material are highly comparable to those of human articular cartilage, thus, providing a suitable substrate for bone cell ingrowth (Chua *et al*., 2004). Its hydrophilicity and chemical stability allow extreme pH and temperature exposure and its semi-crystalline form ensure proper oxygen and nutrients flow to the cell. PVA is extensively used in various load-bearing implant cases like cranio-facial defects and bone tissue regeneration treatments (Oka *et al*., 2000).

Direct Ink Writing (DIW)

DIW is an extrusion-based 3D printing exhibiting similarities with the FDM method that uses a nozzle to extrude materials onto a build platform in a layer-by-layer manner. By utilizing this technique, controlled deposition of raw materials in a highly viscous liquid state is achievable which allows them to retain their shape upon the deposition stage. DIW can be considered more versatile than FDM as it can use a large variety of materials ranging from ceramics, hydrogels, plastic, food, and even living cells. The prime parameters which decide the final properties of the product are nozzle size,

viscosity and density of the material, printing speed, and thickness kept between the layers. In DIW, similarly to FDM, the use of support structures is vital in cases of complex geometrical shapes featuring overhangs and steep deposition angles. However, the use of dissolvable materials as supports helps towards overcoming this issue due to their ability to be easily removed upon the completion of the printing procedure. The post-fabrication processing stages also assist in the elevation of the printed item's mechanical properties (such as elastic modulus) by using UV-curing apparatus (Shuai *et al*., 2013a-b). Figure 4 depicts a Direct ink writing schematic

Ink materials using the DIW method should exhibit a fast rate of gelation and maintain proper structural integrity upon the completion of the printing process.

Laser-Guided Direct Writing (LGDW)

LGDW is a laser-assisted direct writing technique that is capable of depositing cells with micrometer accuracy. The technique of cell deposition using a weakly focused laser beam can be used on a variety of surfaces and matrices. Laser-guided bio-printing is a process in which a laser beam is used to direct cells onto a receiving substrate (Bunea *et al*., 2021).

Bio-Inks Suitable for DIW and LGDW

Hydrogel Inks

Hydrogels are complex three-dimensional networks of hydrophilic polymers that can absorb a large amount of water. The key advantage of those materials is their high biocompatibility and biodegradability rates because of their ability to supply the proper conditions that favor the encapsulation of viable cells further protecting the cells without hindering cell-cell interaction. Hydrogel inks should be able to flow fluently under working pressure conditions by exhibiting controlled viscosity. Also, they ought to provide sufficient structural integrity upon the completion of the printing, furthermore as a fast rate of gelation which will be controlled by exploiting shear thinning (Tamo *et al*., 2022). The literature desired approach to design a hydrogel ink is to fabricate a polymer solution that forms a network upon the completion of the printing process. The network thus formed could be physically or chemically crossed-linked using external stimuli like temperature, light, or ion concentration (Rioux *et al*., 2022). The majority of natural polymers such as gelatin, cellulose, collagen, fibrinogen, alginate, and agar and synthetic polymers such as polyacrylamide, polyurethane, Polyethene Glycol (PEG) are being utilized in hydrogel fabrication in 3D bio-printing (Ramezani *et al*., 2022; Teixeira *et al*., 2022).

The rate of proliferation towards the targeted tissue decreases as the polymer concentration and cross-linking density increase. However, due to their increased viscosity, higher polymer concentrations are ideal for extrusion-based DIW. Mechanical properties increase as polymer concentration increases. Shear stress rises with viscous inks, high pressure, and small diameter nozzles and can cause cell death during extrusion. Shear stresses greater than 60 MPa have been found to cause 35% or more cell death (Duan, 2017). Thus, when using the DIW technique, shear stress is the most important parameter influencing resolution in hydrogel bioprinting.

Gelatin-Methacryloyl (GelMA)

GelMA is a semi-synthetic hydrogel consisting of gelatin derivatized with methacrylamide and methacrylate groups. Sauty *et al*. (2022). experimented that GelMA hydrogels can be synthesized with a specific Degree of Functionalization (DoF) and adjusted to the intended application as a three-Dimensional (3D) cell culture platform and GelMa was also shown to support cartilage tissue formation using chondrocytes and MSCs (Sauty *et al*., 2022). Piao *et al*. (2021) found out that while dispensing cell-laden GelMa from a glass capillary significantly higher pressure was required; however, cell vitality and proliferation weren't significantly affected.

Bio-printing neural tissues via DIW dictate some specific ink material requirements. More specifically, the elastic modulus (i.e., stiffness of the matrix of composite ink used) affects neural cell growth, vitality, and cell signaling. It has been found that brain tissues are compatible with a stiffness of 0.5 MPa approximately which is much less as compared to bone or cartilage tissues. Therefore, soft hydrogels need to exhibit low interfacial tension which allows cells to move across the tissue implant line. This can be achieved by a combination of two or more printing inks where one will possess the required biological property and the other with the task to regulate stiffness percentage (Mohd *et al*., 2022).

Recent research suggests several approaches to combining nucleic acid delivery and bio-printing. One of them is developing gene-activated bioink. In this case, the nucleic acid of interest and its delivery mechanism could be incorporated in a single step by encapsulating it in a bio-printable material, resulting in a gene-activated bio-ink (Wu *et al*., 2019).

Inkjet Bioprinting

Inkjet printing is a non-contact, controlled 3D printing process that allows for the dispersion of droplets with volumes ranging from 1-100 picoliters that include cell viability. Droplets are extruded from a nozzle in one of two ways: Drop by Drop (DOD) or Continuous Inkjet (CI) (CIJ). DOD is the more suited of the two for tissue engineering. Furthermore, DOD can be classified into three types according to the depositing techniques.

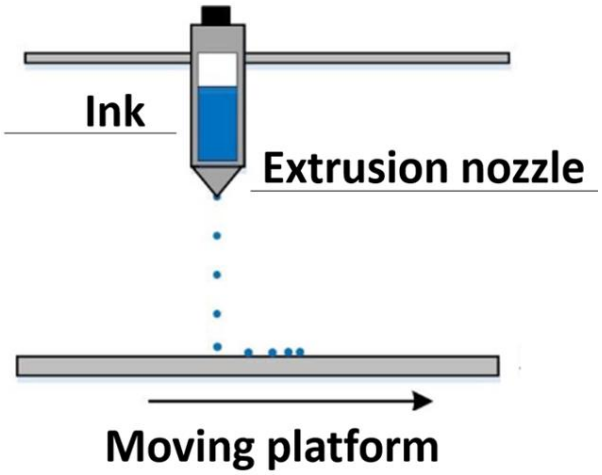

Fig. 4: Direct ink writing schematic

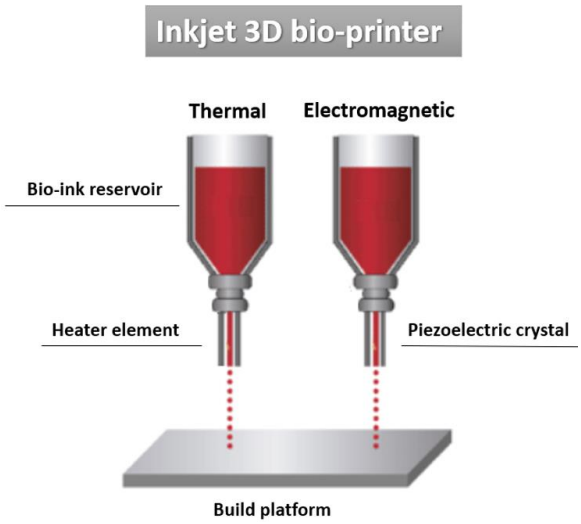

Fig. 5: Different Inkjet 3D bio-printer schematic

Thermal (using heat to expand and deposit the material before the nozzle), electromagnetic and mechanical are considered some examples. Individual drops with diameters ranging from 25 to 50 μm are generated according to predetermined requirements in DOD inkjet 3D bio-printing. In CIJ 3D bio-printing continuous streams of individual droplets possessing a volume of 100 μm in diameter are ejected. It is found that as far as tissue engineering is concerned, electromagnetic and thermal inkjet printing has not been broadly adopted since these processes tend to affect the cell wall and its vitality after sonication at 15-25 Hz. That is the reason why thermal inkjet is more widely used for better cell vitality (Xiao *et al*., 2022; Yang *et al*., 2022; Aversa *et al*., 2021a-b; Aversa *et al*., 2022; Yang *et al*., 2022). Multiple inkjets print heads consisting of several individual nozzles are being utilized to fasten the process. In the case of Thermal Inkjet Printing, ink is super-heated and bubbles are created which expand further until the ink is released through the nozzle. The heating temperature can reach up to 300˚C for a few microseconds, thus, not affecting the viability of biologically printed DNA, cells, tissues, and other organs. Cell viability, in this case, has been found out approximately 85% (Tofan *et al*., 2022). Figure 5 depicts different inkjet3D bio-printer schematics.

In this technique, ink requirements dictate properties like desired viscosity and surface tension as viscosity affects clogging, and surface tension has an immense contribution to the shape of the drop not only after emerging from the nozzle but also on the substrate. The viscosity of the ink should be below 10 centipoises while surface tension should ideally range between 28-350 mn m^{-1} (Yang *et al*., 2022).

Conclusion

3D Printing technology has greatly evolved in the past decade, with several different techniques being introduced in various fields and sectors. Available equipment now features reduced fabrication times as well as newly introduced materials, exhibiting a variety of properties. The introduction of 3D bio-printers utilizing bio-materials compatible with the human body offers an unprecedented ability to fabricate highly controlled porous interconnected structures that act as biological substrates for human cells to proliferate and lead to grown tissues. These structures must exhibit a variety of properties such as biocompatibility, bioresorbability as well as appropriate mechanical behavior. In this context, advanced biomaterials, like bio-inks acting as raw materials for 3D bio-printers, now offer the ability to produce high viability cell, tissue, and even direct DNA fabrication. The careful tuning of process parameters in the 3D bio-printer settings as well as the continuous introduction of new biomaterials possesses an unrivaled way to realize the full potential of this technology.

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Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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