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Research Paper

3D-BIOPRINTING

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3D-Printing an innovative approach which has influenced many branches of science spreading its wings from technology, food and medicine and even in to space, There are a lot of materials and processes which are included in additive manufacturing but only a few are to be considered in medicine

Three dimensional (**3D**) **bio printing** is the utilization of 3D printing–like techniques to combine cells, growth factors, and biomaterials to fabricate biomedical parts that maximally imitate natural tissue characteristics. Generally, 3D bio printing utilizes the layer-by-layer method to deposit materials known as bio inks to create tissue-like structures that are later used in medical and tissue engineering fields. Currently, bio printing can be used to print tissues and organs to help research drugs and pills. However, emerging innovations span from bio printing of cells or extracellular matrix deposited into a 3D gel layer by layer to produce the desired tissue or organ.

Materials for Three-Dimensional Bio-Printing

Polymers

Polymer materials are composed of chemical compounds typically formed from carbon, hydrogen, oxygen, and nitrogen. These monomer structures are repeated and bound with itself to create a longer molecular chain. Polymers have become the most popular choice for 3D bio printing in biomedical applications as it is often inexpensive, biocompatible, biodegradable, and can easily be manipulated with regard to its mechanical, chemical, and biological properties. The polymer's manipulative property is particularly important in 3D printing because the printability of a material is dependent on its viscosity. The ink being printed should be stiff enough to support subsequent layers, however if it becomes too viscous, it may lead to blockage of the printing nozzle. Blockage is further avoided by printing the material in its pre-polymerized form. However, a limitation to using synthetic polymers is that the printing process for synthetic polymers induces high temperatures in which cells and growth factors cannot survive or remain active. Thus, cells or biologically active components are incorporated post-extrusion.

Polymeric materials can be printed in various forms as well, including powder, filament, and sheet form. The polymer category encompasses a wide variety of materials that can range from being soft to hard, or synthetic to natural, however, the most commonly used polymers in **craniofacial tissue engineering include PCL, PEEK, PLA, poly(lactic-co-glycolic acid) (PLGA), and chitosan**; the choice of polymer will depend on the goal of the researcher. For example, PLA or PCL are popular choices for drug delivery purposes, while alginates and gelatin are more popular choices for cell encapsulation. The wide variety in polymeric materials makes them highly versatile, and thus may be highly useful in dental tissue regeneration when being combined with the superior spatial resolution provided by 3D printing.

Ceramics

Ceramic materials consist of metals with inorganic calcium or phosphate salts (such as calcium silicate or β-tricalcium phosphate) and are generally osteoconductive and osteoinductive. The composition of these scaffolds also allows them to last longer than hydrogels, permitting more time for structural support and for guided tissue regeneration. Although the properties of ceramic materials allow for cells to quickly proliferate and differentiate on the scaffold, a limitation lies in its inherent brittleness and poor mechanical strength characteristics that may be necessary when dealing with load-bearing defects. Several studies have however aimed to improve the effectiveness of ceramic materials by changing the pore size as well as through the addition of polymers, such as PCL or PLA. These alterations to the ceramic-based materials allow them to resemble better the mechanical properties of natural bone while being able to promote vascularization.

In a review by Jammalamadaka and Tappa, various 3D printing methodologies (such as extrusion, inkjet , and laser sintering) for ceramic-based materials are mentioned, in addition to the use of sintering and freeze-drying methods post-printing to improve the mechanical properties of the scaffolds . The FDM printing of ceramics is briefly outlined in a review by Obregon and colleagues, where scaffold manufacturing consists of three phases that use organic particles to facilitate flowability, which are then burned out with high temperatures leaving behind primarily the inorganic ceramic particles

Composites

Printable composite materials are composed of a minimum of two different materials; mixtures for printable composites being used in dentistry are typically **composed of copolymers, polymer-polymer mixtures, or polymer-ceramic mixtures**its ceramic-based or hydrogel-based and can include the addition of biomolecules, carbon nanotubes, and metals. The mixture will be dependent on the goal of the composite, but it is typically created to manipulate ink properties such as processability, printability, stiffness, and bioactivity. By combining multiple materials, composite materials can harness the benefits of each individual material. For example, the polymer PLA alone has great chemical and physical properties, however, may not be optimally biocompatible as it releases acidic compounds over time. Researchers overcame this issue by creating a composite containing PLA and ceramics such as calcium phosphate, which ultimately lessens the formation of acidic environments formed by PLA.

Composites can also be enhanced with silicate fillers and nanoparticles, which alter its viscosity and stiffness and ability to influence cell morphology. Composite materials are frequently used in craniofacial regeneration due to its unique properties. While hard polymers exist, composite materials are more capable of mirroring complex tissues that withstand higher mechanical stress and loads such as bones and teeth, and thus are more favourable for craniofacial regeneration. For example, in bone regeneration, researchers again have combined PLA with ceramic to take advantage of PLA's mechanical properties while overcoming its brittle nature. Other uses for printable composites include cartilage regeneration and whole-tooth regeneration.

Cell Aggregates

Bio printing of cell aggregates has been used as a scaffold-free methodology of creating tissue engineered constructs. These cell aggregates consist of spheroid structures, which can then be specifically positioned, creating for instance tubular or ring-like structures. Although the constructs are primarily scaffold-free, the cells are usually encapsulated with a hydrogel material that is biocompatible and biodegradable, for cell survival and for mechanical support of the cell construct. The hydrogel also helps to prevent tissue fusion while the cells are maintained in the suspension reservoir of the 3D printer. The use of cross-linking solutions, such as those containing $CaCl_2$ or gelatin, can help to further minimize cell aggregation. As the pH of the bioink is important for cellular survival and scaffold integrity, a study by Lozano and colleagues used the addition of NaOH to stabilize the pH of a modified bio-polymer hydrogel. The advantages of using scaffold-free constructs for tissue engineering include the absence of potentially toxic or immunogenic scaffold materials, as well as the ability to create high cell density constructs. Limitations of the cell aggregate approach include the relatively time-consuming cellular fusion of the spheroids to create larger tissue structures (which may also create non-uniform structures). Certain advances have been made to minimize this limitation such as the development of multicellular cylinders as an alternative structure, which require up to four days to create the appropriate shape. While most 3Dprinting of cell aggregate studies have been performed in vitro, there is a limited understanding of its potential in vivo and further studies must be performed to demonstrate its safety and feasibility as a scaffold-free construct for tissue engineering

Table 2. Summary of 3D printing materials for tissue engineering.

Type	Materials	Applications
Polymers	Compounds typically formed from carbon, hydrogen, oxygen, and nitrogen, such as PCL, PEEK, PLA, PLGA.	Regenerative approach—Uses biodegradable polymers as a guide for tissue regeneration.
Ceramics	Metals with inorganic calcium or phosphate salts (calcium silicate or β-tricalcium phosphate).	Regenerative approach—Longer-lasting ceramic-type scaffolds can permit more time for structural support and for guided tissue regeneration.
Composites	A combination of a minimum of two different materials, for instance copolymers, polymer-polymer mixtures, or polymer-ceramic mixtures.	Regenerative approach—Composites (such as PLA with ceramics) can be created to facilitate the regenerative approach by reducing the formation of acidic environments caused by PLA alone. Replacement approach—Composite hydrogels (such as those containing silica) can be created to facilitate the replacement approach by increasing gene expression of BMPs.
Cell Aggregates	Cell aggregates form spheroid structures, which are then used as a scaffold-free application of tissue regeneration.	Replacement approach—Post-printing fusion of spheroids create structures that can be used as replacements for damaged or missing tissues.

Commonly Used 3D Printing Technologies in Medicine

Among the various types of 3D printing techniques known FDM(Fused deposition modelling), extrusion based bioprinting, inkjet, and polyjet are the most common types of additive manufacturing techniques used in the medical field.

FDM is the most common and in expensive type of additive manufacturing technology. In this technique, a thermoplastic filament is passed through a heated print head and is laid down on to the build platform in layer-by-layer fashion, until the required object is formed. These printers are limited by the variety of the materials being used, and produce lower resolution objects. Expensive FDM printers, which can use wide varieties of materials and can print at higher resolutions are also available,FDM printers can accommodate more than one print head, and thus, can print multiple types of materials at a time.

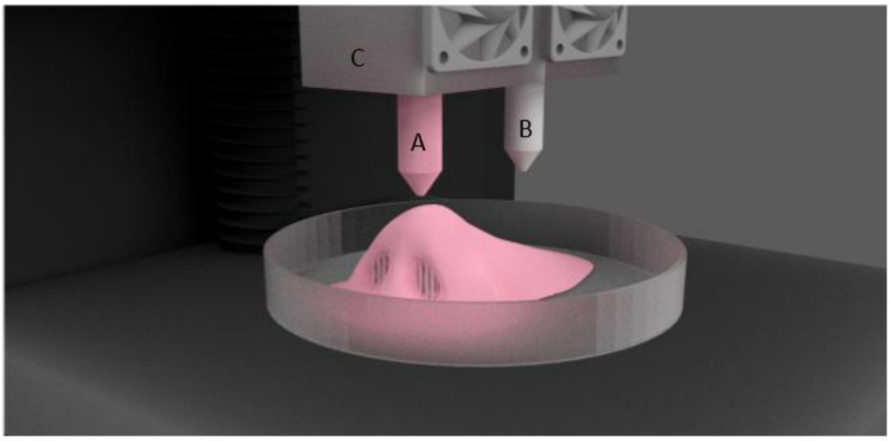


Figure 2. Dual head FDM 3D printer. (A) Building material; (B) Supporting material; (C) Print heads.

ABS(Acrylonitrile butadiene styrene) is the most common thermoplastic polymer used for FDM process. PLA(Polylactic Acid), nylon, polycarbonate (PC), and polyvinyl alcohol (PVA) are some of the other commonly used printing filaments. Lactic acid-based polymers, including PLA and PCL, are well known for their biocompatible and biodegradable properties, and hence, are extensively used for medical and pharmaceutical applications. Additionally, PLA and PCL melt at low temperatures, 175 °C and 65 °C respectively, making it easy to load drugs without losing their bioactivity due to thermal degradation. These polymers undergo hydrolysis in vivo, and are eliminated through excretory pathways comparatively, PCL has lower mechanical strength than PLA, and thus, used for non-load bearing applications. Printing parameters, such as raster angle, raster thickness, and layer height, play a crucial role in fabricating biocompatible scaffolds with required pore size and mechanical strength.FDM has the ability to build constructs quickly, with dimensional accuracy and excellent mechanical properties. Hence it is used widely for prototyping in industry. In medicine, FDM is used for fabricating customized patient-specific medical devices, such as implants, prostheses, anatomical models, and surgical guides. Various thermoplastic polymers are doped with variety of bioactive agents, including antibiotics, chemotherapeutics, hormones, nanoparticles, and other oral dosages for personalized medicine. Using this technology, non-biocompatible materials, such as ABS or thermoplastic polyurethane (TPU), are used for creating medical models for perioperative surgical planning and simulations. These models are also used as a tool to explain the procedures to the patients before they undergo surgery.

MATERIAL USED	INVITRO/INVIVO MODEL	KEYFINDING
PCL + Chitosan	Bone marrow mesenchyme stem cells (BMMSCs)	3D printed scaffolds showed greater cell retention and proliferation of BMMSCs. Stronger osteogenesis and higher bone matrix formation shows their applications in bone tissue engineering
PCL + β-TCP	Alveolar bone defects	The 3D printed PCL/β-TCP membranes showed enhanced bone regeneration capabilities than PCL or collagen membranes alone
PLA + biodegradable calcium phosphate glass	Human monocytes	PLA based scaffolds increased the production of IL-6, IL-12/23 and IL-10
PCL	Estrogen receptor	FDM can be used to fabricate patient specific personalized medicine for drug delivery. The 3D printed hormonal constructs showed biocompatibility and bioactive retention
PLA	Osteosarcoma cells (chemotherapeutics) and E.coli (for antibiotics	3D printed PLA constructs successfully retained the bioactivity. Clear demarcating zones of inhibition was seen for gentamicin constructs and decrease in cell viability of osteosarcoma cells
PVA	DRUG DELIVERY	Novel oral dosage forms were successfully fabricated. Capsules with alternating layers of caffeine and paracetamol were 3D printed.
ABS	CT MODEL SIMULATION	Perioperative surgical simulation of conjoined twin separation surgery The 3D printed models resembled the CT data of the patients and had an overall mean deviation of less than 2 mm
TPU		A 45-year-old man was implanted with this tailor-made fistula implant The 3D printed implant was effective in treating the enterocutaneous fistula

Extrusion Based Bio printing

This method is a Modification of FDM materials are extruded through a print head either by pneumatic pressure or mechanical force. Similar to FDM, materials are continuously laid in layer-by-layer fashion until the required shape is formed, as shown in Figure 3. Since this process does not involve any heating procedures, it is most commonly used for fabricating tissue engineering constructs with cells and growth hormones laden. Bio inks are the biomaterials laden with cells and other biological materials, and used for 3D printing. This 3D printing process allows for the deposition of small units of cells accurately, with minimal process-induced cell damage. Advantages such as precise deposition of cells, control over the rate of cell distribution and process speed have greatly increased the applications of this technology in fabricating living scaffolds. A wide range of materials with varied viscosities and high cell density aggregates can be 3D printed using this technique. A large variety of polymers are under research for the use in bio printing technology. Natural polymers, including collagen, gelatin, alginate, and hyaluronic acid (HA), and synthetic polymers, such as PVA(Polyvinyl Alcohol) and PEG(polyethylene glycol), are commonly used in bioinks for 3D printing. Often these bioinks are post-processed either by chemical or UV crosslinking to enhance the constructs mechanical properties. Depending on the type of polymer used in the bioink, biological tissues and scaffolds of varied complexity can be fabricated. Multiple print heads carrying different types of cell lines for printing a complex multicellular construct can be possible with this technique. Lee et al., have used six extrusion headed 3D printer with six different bio inks, including PEG as a sacrificial ink to fabricate a living human ear. Laronda et al., has used this extrusion bio printing to fabricate gelatin based ovarian implants which can accommodate ovarian follicles. These implants restored the ovarian functions of the sterilized mice, and they even bore offspring

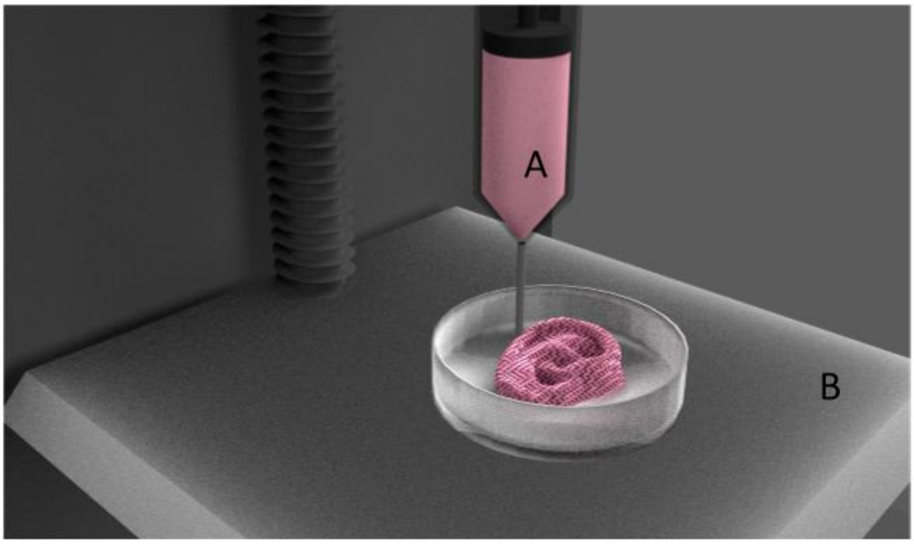


Figure 3. Extrusion based bioprinting. (**A**) Bioink; (**B**) Build platform.

Extrusion bio printing has been used for fabricating scaffolds for regeneration of bone, cartilage, aortic valve, skeletal muscle, neuronal, and other tissues. In spite of all this success, material selection and mechanical strength still remains a major concern for bio printing. **Fabricating vascularization within a complex tissue is still an unanswered problem faced by this technology.** To address this issue, researchers have focused on using sacrificial materials, which are incorporated within the construct while 3D printing, and are removed in post-processing, leaving the void spaces to act as vascularization channels, Now we shall see some of the biomaterials currently used by researchers, and their applications.

MATERIALS	INVITRO/INVIVO MODEL	KEYFEATURES
Gelatin (partially cross-linked)	CD-1 strain (Harlan) female mice	3D printed implant restored ovarian function in the sterilized mice. Additionally, these mice successfully bore offspring
Nano-fibrillated cellulose (NFC) + alginate	Human nasoseptal chondrocytes	Successfully 3D printed constructs resembling human organs (ear). The cytotoxicity and cell viability analysis proved the biocompatibility of this novel hydrogel (bioink) formulation
NFC + alginate; NFC + HA	Human derived induced pluripotent stem cells (iPSCs)	Cells are pluripotent for at least 5weeks,and then formed in to hyaline cartilage type-ii collagen
Methacrylated hyaluronic acid (MeHA	Mesenchymal stromal cells	Bio printed scaffolds maintained good cell viability for more than 3 weeks. Increased concentrations of MeHA promoted osteogenic differentiation
PVA and phytagel (1:1)	Human dermal fibroblast cells	PVA/phytagel hydrogel was successfully 3D printed cryogenically and have mechanical properties similar to soft tissue. Additionally, coating with natural polymers (chitosan or gelatin) increased the cell attachment of the fibroblasts

Material Sintering

In material sintering type of 3D printing technique, the powdered form of printing material in a reservoir is fused into a solid object, either by using physical (UV/laser/electron beam) or chemical (binding liquid) sources. SLA(Stereolithography) type is the oldest and widely used technology among metal sintering 3D printers. Unlike extrusion based printers, there is no contact between the print head and printing object. The objects can be 3Dprinted with high accuracy and resolution with this technique. The major limitation of this technology includes limited availability of photocurable polymer resins. Majority of the SLA resins currently available are based on low molecular weight polyacrylate or epoxy resins. For biomedical applications, polymer ceramic composite resins, made up of hydroxyapatite based calcium phosphate salts, are commonly used

Inkjet or Binder Jet Printing

This process is similar to SLS(Selective laser sintering); instead of fusing the powder bed with laser or electron beam, binding liquid is selectively dropped on to the powdered bed to bind the materials in a layer-by-layer fashion as shown in Figure 4. This process is continued until the final object is formed.

1. Thermal
2. piezoelectric

Are two types of printing heads used in this technique. In thermal print head systems, an electric heating unit is present inside the deposition head, which vaporizes the binding material to form a vapour bubble. This vapour bubble expands due to pressure, and comes out of the print head as a droplet. Whereas in the piezoelectric print head system, the voltage pulse in the print head induces a volumetric change (changes in pressure and velocity) in the binder liquid, resulting in the formation of a droplet. These printers are known for their precise deposition of the binder liquid with speed and accuracy

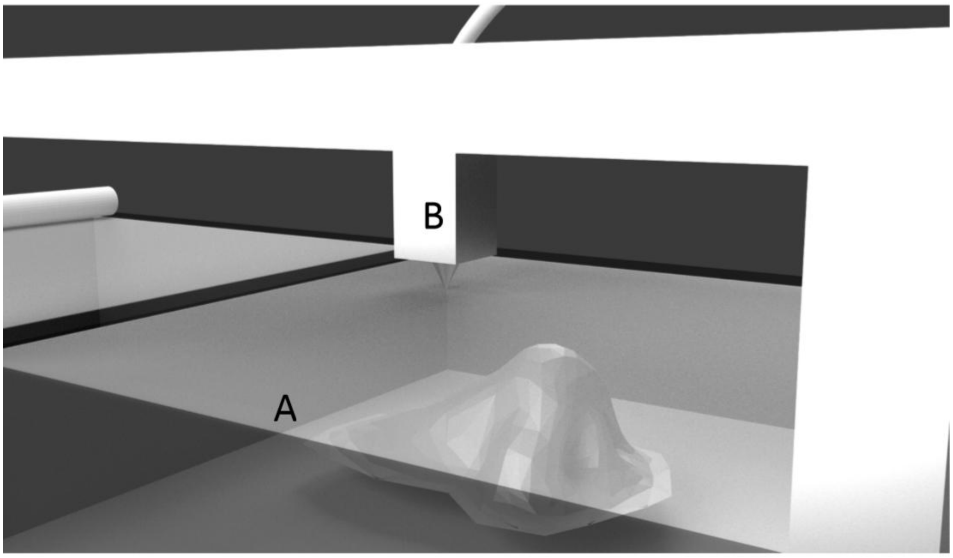


Figure 4. Inkjet 3D printing. (**A**) Powdered bed; (**B**) Binding liquid spraying nozzle.

Water, phosphoric acid, citric acid, PVA, poly-DL-lactide (PDLLA) are some of the commonly used binding materials for inkjet 3D printing. A wide range of powdered substances, including polymers and composites, are used for medical and tissue engineering applications. Finished 3D printed objects are often post-processed to enhance the mechanical properties. Wang et al., have used phosphoric acid and PVA as binding liquids to bind HA/β-TCP powders for bone tissue regeneration applications. The accuracy and mechanical strength of constructs printed using phosphoric acid were higher than constructs printed using PVA. Sandler et al., have fabricated precise and personalized dosage forms using concentrated solutions of paracetamol, theophylline, and caffeine. Uddin et al., have surface coated metallic transdermal needles with chemotherapeutic agents using **Soluplus,** a copolymer of PVC–PVA–PEG, for transdermal drug delivery.

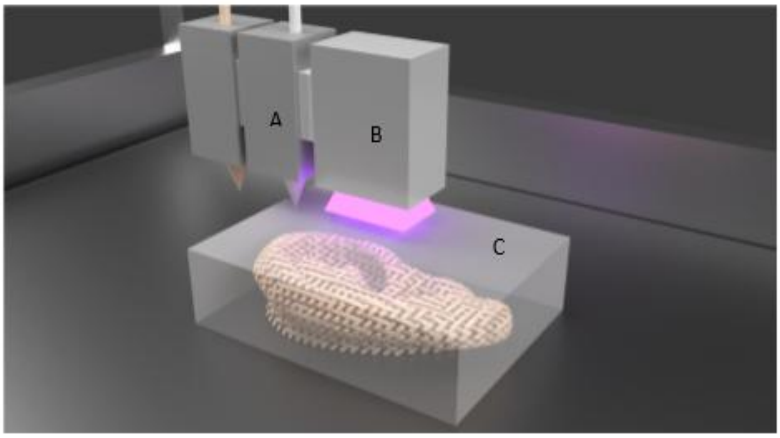
Table here shows the types of binding liquids and respective powder materials.

MATERIALS	INVIVO/INVITRO METHOD	KEYFEATURES
Powders: hydroxyapatite + β-TCP)	Rabbit bone marrow stromal stem cells(BMsc)	Constructs printed with phosphoric acid showed better fabrication accuracy and mechanical properties than constructs printed with PVA, Both binding liquids showed good constructs printing
Substrate: paper and polyethylene terephthalate (PET); Binding liquid: concentrated solution of paracetamol, theophylline, and caffeine	Drug Molecules	Active pharmaceutical ingredients were successfully 3D printed using inkjet technology. The accurate deposition and crystallization of the drugs can be highly controlled. Precise and personalized dosing of the drug substances is possible with this technology
Powders: β-TCP+hydroxyapatite + dextrin; Binding liquid: water + glycerol	Male Rats	3D printed constructs with BMP-2 and osteoblast cells showed enhanced ectopic bone formations
Powder: α-TCP; Binding liquid: 8.75 wt % phosphoric acid	Female mice	Unlike PMMA, co-delivery of drugs vancomycin and rifampin was possible with 3D printed constructs. Thus, significantly improving implant-

		associated osteomyelitis. Additional PLGA coating further prolonged the antibiotic release.
Binding liquid: Miconazole;	Candida albicans	Antifungal agents were successfully incorporated using inkjet printing technology and clear zone of inhibition was demonstrated. Fabricated constructs can be effectively used for transdermal treatment of cutaneous fungal infections
Binding liquid: 2-pyrolidinone; Substrate: calcium sulfate hemihydrate	Osteoblast like sarcoma cells	Binder solution toxicity was assayed by sintering specimens at temperature ranging from 300–1100◦C. High temperature sintered samples were compatible

Polyjet Printing

Similar to inkjet printing, layers of photopolymer resin are jetted on to the build platform and are simultaneously cured using UV light source, as shown in Figure 5. Unlike inkjet process, multiple types of materials can be jetted simultaneously and cured. This gives us the ability to fabricate a complex multi-material object. Due to these capabilities, polyjet is widely used in the medical field to fabricate an atomical models for surgical planning and pre-operative simulations. High resolution objects with varied modular strengths can be 3D printed with high dimensional accuracy using polyjet technique. Since the UV source is right next to the jetting nozzle and cures the resin instantaneously, post-processing of the construct will not be necessitate, This technology is relatively new to the additive manufacturing field. Many types of photopolymers, such as ABS like, Veroclear, Verodent, and Fullcure are commercially available for use in polyjet printing. Table below shows some of the photopolymers used in medical applications.



Polyjet 3D printer. **(A)** Nozzle spraying photopolymer; **(B)** UV source; **(C)** Supporting material.

MATERIAL USED	INVIVO/INVITRO	KEY FINDING
Elastic photopolymer (FullCure 930)	Mock Surgical procedure was performed under live fluoroscope using the 3D printed phantom	Pre-surgical planning & simulation was possible with patient-specific abdominal aortic aneurysm phantom. Simulation was effective in planning surgical challenges & complications than standard procedures
Rigid acrylic resin	3D printed patient-specific intrahepatic vessel models,Preoperative planning in hepatocellular carcinoma resection procedure	The use of 3D printed intrahepatic vessel models from patient's data (CT files) has greatly improved the surgical quality of the hepatocellular carcinoma procedure
Photopolymer resin	Genioplasty performed on 88 patients with dentofacial deformities	3D printed genioplasty templates provided greater accuracy in the surgical procedures than traditional intraoperative measurements
Multiple photopolymer resins printed using Connex 350	Used as preoperative surgical guidance model liver transplant	6 patient specific liver models were 3D printed (3 living donor and 3 recipients). Significantly improved surgery and minimized intraoperative complications
Multiple photopolymer resins printed using Objet 500 Connex	Printed anatomical model of head with different materials for skin, bone and tissues,Used these models as a training tool for neuro surgery	Significantly improved the training experience of surgeons by improving navigation and planning
Photopolymer RGD525 and Connex 500	Printed with polymers that are visible under MRI scanners	Anatomically accurate phantoms that can be imaged under CT and MRI were developed.

	Spine model containing C6–C8 vertebrae including tumors in them	Improving preoperative planning for MR guided minimally invasive surgeries
Multiple photopolymers and Objet 350 Connex	Materials with different rigidity were used to mimic native tissue's mechanical properties. Different models such as hollow aneurysm, craniocerebral aneurysm, and craniocerebraltumors	Aneurysm clippings and tumor resection planning were efficiently planned with these models
Multiple photopolymers and Objet studio	Materials with different flexibilities were used 50 patients were randomly chosen to explain medical procedure using 3D printed model	3D printed model of nasal sinus anatomy was used as educational tool to enable patients to make informed decision. Results suggest improved patient comfort levels and outcomes
Projet 3512 HD	Rigid material was used to create molds for nephrology sectioning. 5 patient specific slicing guides were 3D printed for partial nephrectomy	Enabled accurate sectioning of tumors for colocalization analysis for radiomic and radiogenomic analyses

Limitations

Although 3D printing has the ability to fabricate on-demand, highly personalized complex designs at low costs, this technology's medical applications are limited due to lack of diversity in biomaterials. Even with the availability of variety of biomaterials including metals,ceramics,polymers, and composites,medical 3D printing is still confined by factors such as biomaterial printability,suitable mechanical strength, biodegradation, and biocompatible properties,Usually, in extrusion based bio printing, higher concentrations of polymers are used in fabricating bioinks to obtain structural integrity of the end product. This dense hydrogel environment limits the cellular network and functional integration of the scaffold. For any moderate sized biological scaffold to be functional, vascularization is of utmost importance, and is not possible with the current 3D printing technology. Small scale scaffolds currently printed in the laboratories of researchers can easily survive through diffusion, but a life-size functional organ must have a profuse vascularization. To address this problem, incorporation of sacrificial materials during the scaffold fabrication has been usedbymanyresearchers. These materials fill up the void spaces, providing mechanical support to the printing materials, and once constructs are fabricated, they are removed by post-processing methods. Many sacrificial/fugitive materials including carbohydrate glass , pluronic glass and gelatinmicroparticles are currently under investigation, Additionally, design induced limitations cause material discontinuity, due to poor transformation of complex CAD design into machine instructions. Process induced limitations include differences in porosities of CAD object and finished 3D printed product .

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