Neeta Raj Sharma
Karupppasamy Subburaj
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Vivek Sharma *Editors*

# Applications of 3D Printing in Biomedical Engineering

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# Preface

The book entitled "*Applications of 3D Printing in Biomedical Engineering*" will be aimed to present various experimental outbreaks on the novel methodologies. The Editorial Team believe that the major reason behind such a scenario is the limited collaborations among the specialized research communities that otherwise can play a vital role to refurbish the current situation of the biomedical industry. Therefore, the primary focus to join or invite different experts in the field of the Biomedical Engineering, Material science, Chemical engineers, Product design and manufacturing, and related fields is to communicate their research ideas. This book will provide a comprehensive knowledge of the innovative Bio-materials, processing routes adopted for treating solid wastes and recycling/reuse of the same as different types of 3D printing feedstock, and different applications for designing, developing of pre-surgical guides, implants like joint replacement, Dental, maxillofacial, artificial organs. This book also covers the market demand for 3D printing Biomedical Products and its future scope. Indeed, this edited book provides a wide variety of literature review, case studies, experiential studies, and technical papers to highlight the scope of 3D printing in Biomedical Engineering. The anticipated audience of this book will mainly consist of researchers, research students, and practitioners in biomedical science and engineering.

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Jalandhar, Punjab, India — Kamalpreet Sandhu
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# Contents

# About the Editors

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**Vivek Sharma** is an Assistant Professor in the Department of Product & Industrial Design at the Lovely Professional University, Punjab. His research interest involves affective computing, biomedical products, and kansei engineering etc. He has also published more than 15 research articles in the peer-reviewed international journals and co-authored 2 books, and 4 book chapters.

# Introduction to Biomaterials

1

Bisola Biobaku-Mutingwende

**Abstract**

Biomaterials are biocompatible materials designed to interface with both living tissue and the environment. They are manufactured from natural sources (living tissue such as silk) or synthetic sources (artificial, such as ceramics, metals and polymers) and can be classified into ceramic, metallic, polymeric, composites (e.g. polymer and metal) and semiconductors (biosensors, implantable microelectrodes). Biomaterials are used in various medical applications to support damaged tissue, replace worn out tissue or enhance biological functions. Biocompatibility is an essential characteristic of a biomaterial but they can also be bioinert, biodegradable or bio-absorbable. They are used in a diverse range of anatomical sites and their applications range from stick-to-skin medical devices, implants, prostheses, transplants to tissue and regenerative engineering.

A wide variety of materials and composites are used due to the broad range of chemical, physical and mechanical properties required. However, biomaterials used in the human body are required to possess certain properties and characteristics so they are not rejected by the patient and the patient does not react to them. These properties and characteristics have to be taken into consideration during the development and manufacturing stages and in the assessment or analysis of their suitability for use. Biomaterials are used in medical devices to save lives and/or improve quality of life. Achieving the right balance is essential and determined by the area of application. Similar to all medical devices, biomaterials must undergo stringent tests to ensure they comply with the legal requirements of the relevant regulating bodies. Advancement in the area of

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biomaterials has progressed from purely interacting with body tissue to influencing biological processes towards the goal of tissue regeneration. Evolving applications include patient-specific 3D printing, drug delivery devices, skin/cartilage, shape memory, tissue engineering-bio printing, wound healing, bioresorbable adhesives and biosensors. 3D printing of patient-specific parts is recently beginning to make important contributions towards improving the safety, biocompatibility and performance of bioresorbable implantable medical devices across a range of application areas.

The field of tissue engineering continues to grow and evolve, heading towards the current goal which is to grow bespoke organs for patients.

**Keywords**

Biomaterials · 3D Printing · Biomedical applications

## 1.1 Introduction to Biomaterials

A biomaterial is a material that is compatible with living tissue and is designed to interface with both living tissue and the environment. Biomaterials are manufactured from natural sources (living tissue such as silk) or synthetic sources (artificial, such as ceramics, metals and polymers) and are used in medical applications to support, enhance or replace damaged tissue or a biological function. Biomaterials are used in medical devices to save a patient's life or improve their quality of life [1, 2]. The history of biomaterials can be traced as far back as the late 1800s when polymers were used in aseptic surgery followed by the 1900s when metal implants were used to support fractures, hip replacements and polymers for use in cornea replacement surgery, artificial hearts, glass lens and synthetic skin [1]. Further development has resulted in the use of polymers surpassing the use of other biomaterials in medical applications. The term biomaterials came into effect in the 1970s and further advancement led to the development of the Society of Biomaterials in 1974 and various academic institutions and companies within the field [3].

The evolution of the multidisciplinary field of biomaterials started with physicians attempting to find solutions to life-threatening medical problems. Followed by researchers and engineers in medicine, material science, biology and chemistry investigating the nature of biocompatibility. The evolution of biomaterials can be said to have gone through three major stages. The first stage focused on the development of the structural properties of the implants, followed by innovations in bioactivity and soft tissue replacement and most recently the regeneration of functional tissue [2]. Recently the focus has been on the function and resorption/degradation of biomaterials. The function of a biomaterial is measured by how well it performs a specific action and how it will be used. The resorption and degradation of a biomaterial consider what happens to the biomaterial as it performs its function and once it has achieved its function. They are also used as tools to facilitate treatment in the area of drug delivery, tissue repair and replacement.

Biomaterials are used in implants, prostheses, transplants, tissue and regenerative medicine. Implants are medical devices inserted or embedded surgically in the body. Prostheses are medical devices used inside the body to replace a diseased or damaged part. Transplants involve the transfer of a tissue or an organ from one body or body part to another. These applications can be grouped under the areas with life sciences known as biotechnology, bioengineering, tissue and regenerative engineering.

## 1.2 Characteristics of Biomaterials

Biomaterials used in the human body are required to possess certain characteristics so they are not rejected by the patient and the patient does not react (i.e. not toxic) to them. When a biomaterial makes contact with a living tissue or system, this living tissue is referred to as the Host. The reaction(s) of the living tissue to the presence of the biomaterial is referred to as tissue/host response and commonly described as the tissue response continuum [5–7].

The host response may vary depending on the location and the nature of the biomaterial. Thus there are two broad phenomenon taking place after every biomaterial interacts with living tissue especially in the case of implants—these are tissue response and biomaterial response [6–8]. The following are the characteristics of biomaterials based on tissue response.

### 1.2.1 Biocompatibility

This is the ability of a material to be compatible with living tissue and not produce a toxic or immunological response when exposed to body tissue or fluids. In essence, the material performs with an appropriate biological response to a specific application [9].

A biomaterial is considered to have good biocompatibility if it does not trigger too strong of an immune response, resists build-up of proteins and other substances on its surface that would hinder its function and is resistant to infection [4]. The dynamic interplay between the host cells/tissue and the biomaterial decides the level of biocompatibility [6, 8].

### 1.2.2 Biotolerant

Biotolerant materials are those that are not necessarily rejected when implanted into the living tissue. They are surrounded by a fibrous layer in the form of a capsule [10].

Examples of biotolerant metals are Co-Cr alloys, stainless steels, gold, zirconium, niobium, tantalum and biotolerant polymers are polyethylene, poly-tetrafluoro-ethylene, polyamide, poly-methylmethacrylate and polyurethane.

### 1.2.3 Bioinert

Bioinert materials have minimal interaction with surrounding tissue and are generally encapsulated by a fibrous layer hence its bio-functionality relies on tissue integration through the implant and direct bone apposition at the implant interface [11]. Examples are stainless steel, alumina, ultra-high molecular weight polyethylene, partially stabilized zirconia-ZrO2 (PSZ), and titanium, Ti alloy, alumina and zirconia.

### 1.2.4 Bioactive

Bioactive refers to a material, which upon being placed within the human body interacts and develops chemical bonds with the surrounding bone and soft tissue. An ion-exchange reaction between the bioactive implant and surrounding body fluids results in the formation of a biologically layer on the implant that is chemically equivalent to the mineral phase in bone. Examples are calcium phosphate cement (CPC), glass ceramics, synthetic hydroxyapatite [12].

### 1.2.5 Bioresorbable

Bioresorbable materials such as calcium oxide, polylactic–polyglycolic acid copolymers and tricalcium phosphate [$Ca_3(PO_4)_2$] start to dissolve (is resorbed) once placed in the body, the by-products are non-toxic and the biomaterial is slowly replaced by advancing tissue (such as bone) [9].

## 1.3 Properties of Biomaterials

The properties of a biomaterial are very important and essential in the development and manufacture of the biomaterial and in the assessment or analysis of its suitability for use. Biomaterials are predominantly used in implants such as artificial joints for the replacement of worn or injured body parts. Thus biomaterials are not only required to exhibit the above characteristics but they are also required to remain stable under high loads and survive/withstand the rigours of everyday living in the form of varying multi-axial and cyclical mechanical loads. These loadings can be considerably high and put the biomaterial under stress and strains. They must also be high wear-resistant, to survive the environment in the body (corrosive saline body fluids) and last the duration of their applications (typically 20 years). These properties can be grouped into bulk and surface properties.

### 1.3.1 Bulk Properties

Stability and longevity of a biomaterial goes hand in hand with its structural and mechanical properties (strength and the mechanics). The structural integrity of a biomaterial takes into consideration the following factors:

#### 1.3.1.1 Modulus of Elasticity (E)

Measure of the change in the dimensions of the biomaterial in response to applied stress. The elastic modulus of an implant should be as close to that of the tissue it is replacing and those around the implant. This is to ensure an even distribution of stress and prevent the relative movement at the implant-bone interface. The elastic modulus of a biomaterial interacting with bone should be similar to that of bone which is 18 GPa.

#### 1.3.1.2 Tensile Strength

This is the ability of a material to resist a pulling force. A biomaterial used in an implant requires high tensile strength.

#### 1.3.1.3 Ultimate Tensile Stress (UTS)

Stress is the force applied on a material per unit area. The UTS is the maximum tensile load a material can withstand per its cross-sectional area, prior to failure [1]. In general, metals possess good tensile strength, whereas in order to compensate for the poor tensile strength of ceramics, they are combined with reinforcement polymeric materials such as glass and Kevlar.

#### 1.3.1.4 Compressive Strength

This is the ability of a material to resist pushing forces and is assessed by the maximum force/load a material can withstand per its cross-sectional area prior to failure. A biomaterial used in an implant requires high compressive strength. Ceramics generally have high compressive strength.

#### 1.3.1.5 Shear Strength

Ability of a material to withstand sliding forces. A biomaterial used in an implant requires high shear strength. Shear strength takes into consideration the maximum shear load a material can withstand per cross-sectional area prior to failure. This property is most relevant in the case of sutures and adhesives.

#### 1.3.1.6 Yield Strength

The stress at which a material exceeds its Yield Point, i.e. no longer returns to its original state due to changes from its region of elastic deformation to its region of plastic deformation as a result of an applied force. Biomaterials used for implants are required to have a high yield strength.

#### 1.3.1.7 Fatigue Strength

When a material is exposed to recurring forces, the stress at which the material fractures is referred to as the fatigue strength. Biomaterials used for implants are required to have a fatigue strength.

#### 1.3.1.8 Young Modulus

A modulus is the value that represents a physical property of a material. The modulus of elasticity represents the easy of stretch or deformation.

#### 1.3.1.9 Ductility

A measure of the degree of plastic deformation prior to fracture when a tensile stress is applied.

#### 1.3.1.10 Hardness

Ability of a material to resist permanent indentation or penetration to its surface. The higher the hardness, the less the wear and tear of the material.

#### 1.3.1.11 Toughness

This is the amount of energy required to fracture a material. The higher the toughness of a material, the less likely it is to fracture.

Stress: force per unit area.

The formula used for calculating stress (σ) is

$$\sigma = P/A,$$

where $\sigma$ stress, $P$ load, $A$ cross section area

#### 1.3.1.12 Strain

The degree of deformation of a material due to an applied force.

The formula used for calculating stress ($\varepsilon$) is

$$\varepsilon = dL/L,$$

where $\varepsilon$ strain, $dL$ extension produced in the rod, $L$ original length

To analyse the strength of a material, a tensile test is performed on the material using a tensile testing machine which applies a tensile load on one end of a specimen of the material and that is fixed at the opposite end. The load and extension graph is then used to calculate and produce a stress–strain curve. Hooke's law states that stress is directly proportional to strain.

#### 1.3.1.13 Surface Properties

The surface properties of a biomaterial is essential as it affects its degree of biocompatibility with its surrounding tissue [13, 14]. The surface properties of a material/implant dictate its level of corrosion resistance as well as the cytotoxicity of the corrosion products. The environment inside the human body can be highly

corrosive to implants. Corrosion is a type of failure in a material. Corrosion Resistance is the ability of a material not to deteriorate as a result of its reaction with its environment. More specifically, corrosion can be defined as the chemical or electrochemical degradation of metals due to their reaction with the environment.

There are different types of corrosion observed in relation to implants:

a. Stress corrosion—This is a crack as a result of increased tensile stress and corrosive environment.
b. Crevice corrosion—Occurs where there is a gap/crack between adjoining surfaces.
c. Pitting corrosion—Similar to crevice corrosion but the holes are produced in the material and blocked by the corrosion product.
d. Fretting corrosion—Failure resulting from corrosion and surface rub.
e. Galvanic corrosion—Occurs as a result of the coupling of two different materials.

#### 1.3.1.14 Cytotoxicity of Corrosion Products

The biocompatibility of a biomaterials improves with an increase in its corrosion resistance and decrease in its toxicity. The toxicity of a biomaterial is determined by the toxicity of its corrosion product. When a biomaterial undergoes corrosion, the residue or corrosion product can be toxic. The degree of toxicity depends on various factors such as the amount of material dissolved by corrosion per unit time, the amount of corroded material removed by metabolic activity in the same unit time and the amount of corrosion particles deposited in the tissue [9]. Surface Characterization: Interaction between host tissue and biomaterial primarily occurs at the implant surface [6, 9]. Thus characterization/preparation of implant surface to suit clinical needs is extremely important to avoid metallosis (metal poisoning), osteolysis (weakening of bone), inflammatory responses and implant loosening, which can be aggravated by metal allergies and sensitivity [13, 15]. Surface characterization can be accomplished by several techniques: Passivation (chemical treatment of a material with a mild oxidant), acid etching (surface treated with nitric or hydrofluoric acid), and sand blasting (sand particles used to get a roughened surface texture which increases attachment at bone implant surface) [9]. Surface coatings involve covering implant surfaces with porous coatings to increase their surface area, roughness, attachment strength at bone implant interface, increase load bearing capacity and biocompatibility [9]. Several coating techniques exist. The plasma sprayed technique is the most commonly used and there are two major types: Plasma sprayed titanium and plasma sprayed hydroxyapatite.

## 1.4 Balancing the Characteristics and Properties of Biomaterials in Medical Devices

Balancing the characteristics, bulk and surface properties of biomaterials are essential. For example, with regard to a heart valve the proper balance can help avoid complications such as tissue degeneration, mechanical failure, post-operative

infection and the induction of blood clots. Vascular grafts also illustrate this principle because they are required to be flexible, porous structures and come in a range of permeabilities. They must also maintain their structural integrity under repeated loads, have a low tendency to clotting, be biostable as well as achieve and maintain homeostasis.

Metal stents commonly used to keep blood vessels open can cause long-term complications, including re-narrowing of the vessel, blood clots and bleeding [4]. Thus recent research has looked into developing a bio-absorbable zinc stent that harmlessly erodes away over time, minimizing the normal chronic risks associated with permanent stents [4]. Metallic biomaterials are predominantly used in orthopaedic implants because of their material and structural properties. Internal fixators such as screws and plates require stability and high bending/pull-out strength hence there are various types of screws to meet the required load bearing needs. Although the artificial hip joint is made out of a combination of biomaterials (Metallic or ceramic femoral head, metallic femoral stem, polyethylene or ceramic insert, metallic acetabular cup and composite bone cement) the implant undergoes high cyclic mechanical stresses that lead to wear.

With regard to tooth filling materials, some general criteria in relation to bulk and surface properties are mechanical strength, wear resistance, minimal dimensional changes on setting and biocompatibility, i.e. non-irritation to pulp, low toxicity, does not dissolve or erode in saliva as well as good aesthetic properties. Artificial skin must prevent the loss of fluids, electrolytes and molecules, it is required to be flexible enough to adapt to the wounded area and movement of the body yet resist storing moisture under the graft. Possible materials used for artificial skin are polymeric or collagen based membranes due to the ability to regulate their properties, however, they are not effective in all burn wounds [16].

### 1.4.1 Bio-adhesives

Tissue adhesives are used to repair fragile, non-suturable tissue in anatomical parts such as livers, kidneys, lungs. The important criteria for tissue adhesives is that they are able to be wet and bond to tissue, they are capable of onsite formation by the rapid polymerization of a liquid monomer without producing excessive heat or toxic by-products [5], they are absorbable, do not interfere with the normal healing process and are easily applied during surgery [5]. They are manufactured from alkyl-o-cyanoacrylates which are low strength and restricted to use in traumatized fragile tissue (e.g. kidney) or fibrin derived from fibrinogen-clotting component of blood and limited mechanical strength. Due to their limited strength wound site leakage sometimes occur and as a result, current research is focused on the use of photothermal therapy, a laser-welding technique for colon repair as an alternative to suturing or stapling [4]. The procedure is minimally invasive and uses photothermal nanocomposites—nano-sized material and gold rods embedded in a matrix that when heated with a laser can fuse with ruptured tissues [4, 17]. Recently a bioadhesive has been developed that is able to bond biological gel to damaged

cartilage in the knee. Cartilage has been very difficult, if not impossible, to repair due to the fact that cartilage lacks a blood supply to promote regeneration [4]. This gel/adhesive combo has been successful in regenerating cartilage tissue following surgery. The biological gel is injected into a cartilage defect and the adhesive helps to keep the gel and newly regrown cartilage in place. In order to avoid adhesive failure, the application of the adhesive must be considered before it is manufactured and the chosen adhesive must be compatible with the manufacturing process intended to mass produce the final product. The adhesive must also be able to withstand the speed and friction of a specific method or the liner materials could break during production, which may compromise integrity. With regard to stick-to-skin products, it is usually the adhesive's main job to keep the device adhered to the user's skin for a specified wear time. Adhesives must also be compatible with the other materials used in the device [18, 19].

### 1.4.2 Materials Property Chart

In order to pick the right material for an application, it is helpful to be able to compare the properties. The wide variety of materials and their varying properties lend itself to the difficulty of comparing them. A materials property chart is a common method of displaying and comparing the properties of various materials [20].

### 1.4.3 Regulations and Standardization

It is a legal requirement that all biomaterials and medical devices must be tested and comply with the requirements of the relevant regulating bodies before they are introduced into the market. The ISO 10993 document highlights the main recommendations for testing a biomaterial or medical device [21].

## 1.5 Classification of Biomaterials and Their Applications

Biomaterials can be classified into ceramic, metallic, polymeric, composites (e.g. polymer and metal) and semiconductors (biosensors, implantable microelectrodes).

Their applications range from stick-to-skin medical devices, implants, prostheses, transplants to tissue and regenerative engineering. Also a wide range of materials are used due to broad range of chemical, physical and mechanical properties required. They are used in a diverse range of anatomical sites.

### 1.5.1 Ceramic Biomaterials

#### 1.5.1.1 Bioceramics

Ceramics are inorganic, non-metallic materials. Bioceramics can be classified as bioinert, bioactive or glass ceramics [22].

#### 1.5.1.2 Bioinert Ceramics

These ceramics show direct bone apposition at implant surface (i.e. close together/side by side) but do not show chemical bonding to bone. They are full oxides, i.e. bulk and surface thus excellent bio compatibility, have good mechanical strength, low ductility which results in brittleness and have similar colour to hard tissue [9]. Examples are aluminium oxide, titanium oxide, zirconium oxide. They are not suitable for load bearing dental implants due to inferior mechanical properties. They are used as surface coatings over metals to enhance their biocompatibility increase the surface area for stronger bone to implant interface [9].

#### 1.5.1.3 Bioactive Ceramics

Bioactive ceramics are not used for load bearing implants due to lack of mechanical strength.

They are used as bone graft material for augmentation of bone and as bioactive surface coating for various implant material to increase biocompatibility and the strength of tissue integration [9]. Examples are calcium phosphate ceramics—(CPC), hydroxyapatite (HA), tricalcium phosphate (TCP), etc. CPC have biochemical composition similar to natural bone. General properties of bioactive ceramics include excellent biocompatibility, lower mechanical tensile and shear strength, lower fatigue strength, lower ductility and brittleness. Though the pores decrease the strength they increase the surface area providing additional regions for tissue ingrowth [9].

#### 1.5.1.4 Glass Ceramics

These ceramics chemically bond to bone due to the formation of a calcium phosphate surface layer. They have high mechanical strength but their low resistance to bending and tensile stresses make them extremely brittle. They are not used as load bearing implants but more often as bone graft material. They also make weak coating bonds between coating and metal substrates. Example is bioglass.

#### 1.5.1.5 Bio Ceramics

With carbon and carbon silicon compounds are biocompatible with a modulus of elasticity similar to that of bone. They are brittle and susceptible to fracture under tensile stress, however, they are used as surface coatings and facilitate osseointegration at bone implant interface.

#### 1.5.1.6 Applications of Bioceramics

Bioceramics are used in bone replacements, heart valves, dental implants (alumina, calcium phosphate), ceramic crowns (glass ceramics + alumina, mica or leucite),

tooth filling materials and hip/knee joint replacement prosthesis (ceramic femoral head, ceramic insert), to name a few.

#### 1.5.1.7 Metallic Biomaterials

Metallic biomaterials are mainly used for load bearing applications such as knee or hip replacement implants, orthopaedic fixation plates and some parts of dental implants. Metallic implants are commonly made from metal alloys comprising of a combination of pure metals. As a result they generally possess a combination of enhanced chemical and mechanical properties. Metallic biomaterials are extracted from other materials such as aluminium from bauxite, however, few materials such as copper and precious metals are naturally found in in their metallic state. Examples of metal and metal alloys are titanium, cobalt, surgical steel (iron, chromium, nickel alloy), molybdenum alloy (vitallium), precious metals (gold, platinum, palladium).

#### 1.5.1.8 Strength of Pure Metals vs. Alloys

Pure metals are tightly packed particles of the same size atoms arranged in an organized pattern. As a result the bonding at the grain boundaries tends to be weaker and susceptible to dislocation when a lateral force is applied. On the other hand, alloys comprise two different sizes of metal elements randomly organized and the difference in size helps prevent the physical dislocation of the lattice structure. The presence of atoms of other metals that are of different sizes disturb the orderly arrangement of atoms in the metal. This reduces the layer of atoms from sliding. Thus, an alloy is stronger and harder than its pure metal [9].

#### 1.5.1.9 Metal Structure and Properties

The area of application in which a metal is used is determined by its physical properties. These physical properties are largely determined by the strength of the metallic bond (degree of attraction) between the closely packed positive metal ions and numerous delocalized electrons. Metallic bonds are naturally strong and the free electrons facilitate the conduction of electricity. Other advantages of metallic biomaterials are that they can be moulded into various forms and complex shapes using a wide range of fabrication techniques, e.g. casting, forging, machining. Their high level of fracture resistance also enables them to bear high loads. The major disadvantages are their susceptibility to corrosion and stress shielding. Stress shielding occurs when metals take the stress off the bone resulting in weakening the bone tissue, hence one of the main aims of metal alloys is to resolve this challenge.

#### 1.5.1.10 Titanium (Ti)

Titanium (Ti) is the highest standard in implant materials. Commercially pure titanium is usually composed of 99.75% Titanium, 0.1% Oxygen, 0.05% Carbon, 0.05% Iron, 0.03% Nitrogen and 0.012% Hydrogen. Titanium Alloy Ti6Al4V consists of titanium, 6% Aluminium—alpha stabilizer, and 4% Vanadium—beta stabilizer. It has excellent corrosion resistance, oxide layer formed is resistant to charge transfer thus contributing to biocompatibility, the modulus of elasticity is 5.6

times that of the bone thus more distribution of stress and the strength of titanium alloy is greater than pure titanium—6 times that of bone hence thinner sections can be made. Titanium Alloys Ti6Al4V also has sufficient ductility and exhibits osseointegration [9].

Titanium Alloy Ti-9Cr-0.2O is used in scoliosis surgery. Its properties include: High stiffness in deformed parts, low stiffness in the non-deformed parts, high strength and good flexibility. These properties make the implant more controllable and surgery easier.

#### 1.5.1.11 Cobalt, Chromium and Molybdenum Alloy

Is a composition of 63% Cobalt, 30% Chromium (CrO provides corrosion resistance) and 5% Molybdenum (strength). The properties of cobalt, chromium and molybdenum alloy are high mechanical strength, good corrosion resistance and low ductility (solid material's ability to deform under tensile stress). It is used in the fabrication of custom designs due to ease of castability and low cost [9].

#### 1.5.1.12 Iron, Chromium and Nickel Based Alloy

These are surgical steel alloys. They have a long history of use as orthopaedic and dental implant devices. They are composed of iron, 18% chromium (corrosion resistance) and 8% nickel (stabilize austenitic steel). The properties of iron, chromium and nickel based alloy are high mechanical strength and high ductility. They are used in various applications. They are susceptible to pitting and crevice corrosion and hypersensitivity to nickel has been observed. Bone implant interface has also shown fibrous encapsulation and ongoing foreign body reactions [9].

#### 1.5.1.13 Precious Metals

Precious metals (noble metals) such as gold, palladium and platinum are unaffected by air, moisture, heat and most solvents. They do not depend on surface oxides for their inertness. Their properties include low mechanical strength and very high ductility [9]. However they do not demonstrate osseointegration and cost more per unit weight.

#### 1.5.1.14 Applications of Metallic Biomaterials

Metallic biomaterials are used in dental implants, i.e. pure titanium screws replacing roots for crowns and bridges, orthopaedic screws/fixation, for example, hip/knee joint replacements and spinal implants use titanium, titanium alloys, stainless steel, bone plate are made from stainless steel. Stainless steel is used in heart valves; platinum electrodes are used in cochlear replacements; Staples made of titanium facilitate closure of large surgical incision produced in caesarean procedures.

#### 1.5.1.15 Polymeric Biomaterials

Polymers are organic compounds that consist of chains of molecular units. Examples of polymers: proteins, carbohydrates, plastics, etc. The basic unit of a polymer is a monomer. Examples of monomers: lactic acid, amino acids, glucose, etc. The way monomers are connected/shaped has a very large influence on their properties. They

can be graft, star, multivalent, dendrimer, or dendronized shaped polymers [23, 24]. Polymers are named after the bonds between the monomers. E.g. Polyesters, Polyamides, etc.

There are also various types of polymers, namely Homopolymers (polymers consisting of one type of unit), Copolymers (A polymer consisting of two), Random (units are randomly linked), Alternating (where two units alternate), Block (where blocks comprising of the same units are linked to blocks comprising of different units) and Graft polymers. Polymers can also be referred to as macromolecules since they consist of large molecules. Polymers with low molecular masses and fewer monomers are called Oligomers, for example, peptides [25]. Polymeric Biomaterials have high molecular masses and high melting and boiling points. They are easily modified to various applications and can be polymerized to create synthetic polymers [24]. Although they are biodegradable they are not easily sterilized and they can be subject to surface contamination and leachable compounds. Biopolymers can be grouped into natural or synthetic [25].

#### 1.5.1.16 Natural Polymers (Protein Based)

Natural polymers occur in nature and can be extracted. They are often water-based. They can be divided into functional (DNA, RNA and globular proteins) and structural (Fibres—cellulose, silk, wool or gels and rubbers, e.g. agar and gelatin). Natural polymers can also be classified based on their source, i.e. plants (polysaccharides, e.g. cellulose, starch, alginate), animals (proteins, e.g. gluten (gelatin), albumin and polysaccharides, e.g. chitin (chitosan), hyaluronate) and microbes (polyesters, e.g. poly (3-hydroxylalkonate) derivatives and polysaccharides, e.g. hyaluronate) [9]. Developments in biomaterials have led to additional natural polymers such as reflectins for optical devices, amyloids for biosensors and various plant proteins for tissue regeneration. Together with their genetic variants generated by protein engineering, these natural proteins enable the possibility of creating a combination of properties [26]. Examples of applications of natural polymers are in heart valve replacements (e.g. pig valves), collagen is used in corneal bandage and artificial skin [27]. Absorbable Surgical Sutures made from natural collagen (beef intestine) and fibres (silk) [28]. They are also used in drug delivery [29], prosthetic implants and in tissue engineering for multiple organs.

#### 1.5.1.17 Synthetic Polymers

Synthetic polymers can be grouped into Fibres (polyester, nylon and acrylic), plastic (polyethylene, poly (vinyl chloride), polystyrene, and bakelite) and rubbers (cis-1,4-polyisoprene). They can be degradable or non-degradable. Examples are polyamides (PA), poly (methyl methacrylate) (PMMA), poly (ethylene) (PE)—plastic bags, poly (vinyl chloride) PVC—PVC pipes, polylactic acid (PLA)—plastic bottles, food containers, disposable bags, plastic utensils—polylactides (biodegradable polymer such as those used in brain wafers), polyurethanes (PU)—coatings, adhesives and sealants, automotive building and construction, footwear, appliances [29].

#### 1.5.1.18 Thermoplastic Polymers

These are polymer materials that are consistent in their chemical and mechanical properties regardless of the number of times they are softened when heated and harden when cooled.

#### 1.5.1.19 Thermosetting Plastics

These are polymer materials made of cross-links, they harden when heated and cannot be remoulded once cooled.

#### 1.5.1.20 Elastomers

Elastomers are low crosslink density network polymers (have weak intermolecular forces) that can be stretched easily and recovers upon stress withdrawal. They can be both thermoplastic and thermosets. E.g. rubber

#### 1.5.1.21 Polymer Composite

A polymeric composite is made up of a combination of a polymer and other synthetic biomaterials. It consists of two phases, e.g. glass fibre reinforced plastic. Their main advantage is that their properties can be altered to suit clinical applications.

Bone is an example of a natural polymer composite of collagen (a protein) and apatite (a ceramic). Composites may be isotopic (have the same properties in all directions) or anisotropic (different properties in different directions).

The type of polymer used can be customized to specific applications by considering the type of monomer composition, the molecular weight, the polymer microstructure and architecture and the end group. The substrates used are required to meet the specifications for application and be able to meet the requirements of the scale of production. These substrates come in different forms: granules, powder, filaments, tubes, mono- and multi-filament yarns, sutures, meshes and tapes, foils/membranes/nonwovens, etc. [28]. Within the wound healing and paediatric markets in particular, conventional bioresorbable polymers have lacked the combination of high mechanical strength and the ability to degrade rapidly. As a result, companies seeking to develop bioresorbable wound closure devices such as stomach or ligating clips and vascular closure devices have been forced to either utilize traditional metal-based materials or make compromises in the use of polymeric-based materials that may adversely affect functional performance. For paediatric applications with accelerated bone regeneration, such as for craniomaxillofacial (CMF) implants, imbalanced degradation times could significantly impair the ability of the device to match the natural healing process. Recent innovation has led to the development of PLA-PEG copolymers for use with implantable medical devices. By combining the hydrophobic properties of PLA polymers with the hydrophilic properties of PEG to increase water uptake, the new platform of tri-block (PLA-PEG-PLA) copolymers is able to replicate the mechanical strength of standard, equivalent material grades but degrade up to six times faster [30]. Bioresorbable medical device in orthopaedic applications include: Craniomaxillofacial implants (for skull fractures), sutures anchors (for rotator cuff injury), thorac-lumbar fusion (spinal injury), spinal disk implants (for spinal injury), fixation plates (bone fractures), meniscal darts (for knee injury),

interference screws (for ACL tears), pushlock suture anchor (for anterior knee pain), smart nail (for bone fractures), Achilles implants (for Achilles tendon ruptures), subtalar implants (for flat foot) and hammertoe repair (for hammertoes), biocomposite distal biceps (for tendon rupture), dental membrane (for bone and tissue regeneration), tracheal implant (for airway obstruction), cardiovascular stents (for clogged arteries), breast implants (for breast reconstruction), shoulder balloon (for rotator cuff injury), tissue scaffold (for tissue regeneration), ligating clip (for general surgery) [30]. Evolving applications are patient-specific 3D printing, drug delivery devices, skin/cartilage, shape memory, tissue engineering-bioprinting, wound healing, bioresorbable adhesives and biosensors. 3D printing of patient-specific parts is now beginning to make important contributions towards improving the safety, biocompatibility and performance of bioresorbable implantable medical devices across a range of application areas [31–33]. For example, the orthotic and prosthetic (O&P) field has experienced developments in 3D printing enabling O&P clinicians to seamlessly design and create bespoke devices that are functional, lightweight, affordable, and comfortable for patients, more easily and efficiently than they can with traditional methods [33]. Many of the materials used in the O&P market today, such as carbon fibre sheets, are not the most comfortable. Silicon liners can be used as an alternative to provide a better fit and better comfort, however, this can increase the cost and waiting time for a full prosthesis. 3D printing offers alternatives to carbon fibre and silicon which provides reliable strength and comfort. Currently 3D printing of bioresorbable implants can be done via a bioplotters using granule based gel, inks. Implants can also be 3D printed via SLS using print powder [31].

Recent research is looking into bioplastics as a substitute for single-use plastics. The overall aim with bioplastics is that they are reusable or biodegradable and their mechanical, chemical and physical properties can be tuned to adapt to various applications [1, 34]

#### 1.5.1.22 Semiconductor Biomaterials

Semiconductor biomaterials are used in biosensors and implantable microelectrodes. In the case of the structural design of implantable medical devices and delivery systems, the device should be adequate for handling the electronic data and be the right size to insert in the human body [32]. The delivery methods of the devices are via incision and the use of tools.

Smart wound dressings are a recent development. They combine electronics, wound healing, microfabrication, biomaterials and drug delivery [29]. The dressing integrates sensors and actuators in close contact to skin [4] for the treatment of chronic diabetic ulcers. The smart wound dressing delivers oxygen and blood vessels promoting biochemical factors while monitoring healing and reducing unnecessary dressing replacements and visits to medical facilities [4].

## 1.6 Transplants, Tissue Engineering and Regenerative Medicine

Biomaterials have advanced from purely interacting with tissue to influencing biological processes toward the goal of tissue regeneration [35–38].

Organ transplantation is the process of surgically transferring a donated organ to someone diagnosed with organ failure. Organ transplants performed include kidney, liver, heart, lung and pancreas transplants [16, 27, 32, 39, 40]. The regenerative capacity of tissues can help replicate their biological function in relation to the desired geometry and mechanical properties [21, 32].

Recent research into transplantation proposes bioprinting organs as an alternative to organ donation.

Tissue engineering is a practice within the field of biomaterials that combines scaffolds, cells and biologically active molecules into functional tissues [4].

The goal of tissue engineering is to assemble functional constructs that restore, maintain or improve damaged tissues or whole organs. Artificial skin and cartilage are examples of engineered tissues that have been approved by regulatory organizations; however, currently they have limited use in human patients [4, 17].

Regenerative medicine is a broad field that includes tissue engineering but also incorporates research on self-healing—where the body uses its own systems, sometimes with the help of foreign biological material to recreate cells and rebuild tissues and organs [4, 17].

Tissue Engineering applies engineering principles to either maintain existing tissue structures or enable tissue growth. With regards to the field of engineering materials, tissues can be described as multiple systems of cellular composites each comprising of three main structural components organised into functional units, the extracellular matrix (ECM) [41] (initiates crucial biochemical and biomechanical cues that are required for tissue morphogenesis, differentiation and homeostasis) [42] and scaffolding architecture (highly porous scaffold biomaterials, which act as templates for tissue regeneration, to guide the growth of new tissue), e.g. hydrogels [38, 43]. Currently research is being done to combine silk with tropoelastin, a highly elastic and dynamic structural protein to construct a panel of protein biomaterials. These materials must mimic the elasticity of diverse tissue structures and, consequently, control biological function, particularly the differentiation of stem cells [4, 17].

The main materials for the matrix (scaffold) are synthetic polymers, e.g. polylactic and polyglycolic acid—self-assembling proteins and natural polymers, e.g. fibrin, collagen, collagen-glycosaminoglycan copolymer [44]. Scaffolds and constructs must be biodegradable to enable cells to develop their own extracellular matrix [44, 45]. Ideally, the mechanical properties of the scaffold should be the identical to that of the host tissue and have the strength to withstand the implantation process [38]. In orthopaedic and cardiovascular applications, producing ideal scaffolds is of specific importance and an ongoing challenge because the durability of the implanted scaffold is required to last the

duration of the remodelling process and the rate of healing varies with the age of the patient [38].

Recent developments in 3D bioprinting have combined the processes of tissue and regenerative engineering to address one of the major challenges with transplants by enabling blood vessels to be inserted into new organs thereby maintaining the survival of the organs during transportation from the donor to the receiver [17].

### 1.6.1 Supramolecular Biomaterials

Standardization is another challenge in the field of regenerative medicine as reproducibility is difficult due to physiological differences and changes over time. Therefore, current advancements are in the direction of the development of biomaterials that can be adjusted (tuned) in response to physiological cues or that mimic natural biological signalling [4]. These are called supramolecular biomaterials, they are composed of a combination of molecules engineered to sense and respond, they combine the functionality of the biomaterial and physiological parameters to produce patient-specific applications [17, 46]. The field of Tissue Engineering continues to grow and evolve, heading towards the current goal which is to grow bespoke organs for patients [17, 46].

## 1.7 Conclusion

In conclusion, Biomaterials play an integral role in medicine today—restoring function and facilitating healing for people after injury or disease [4]. The modern field of biomaterials combines medicine, materials science and more recently tissue and regenerative engineering. Biomaterials may be natural or synthetic and can be reengineered into various forms for use in biomedical products and devices. These devices are used in medical applications to support, enhance or replace damaged tissue or a biological function such as heart valves, hip joint replacements, dental implants or contact lenses [4]. They are biocompatible and can be bioinert, biodegradable or bio-absorbable. Doctors, researchers and bioengineers use biomaterials for a broad range of applications [4] and continue to work together towards a common goal.

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# Different Approaches Used for Conversion of Biomaterials to Feedstock

2

Sagarika Bhattacharjee and Harmanpreet Singh

**Abstract**

In this new era of modernization, automation and connectivity are the major players for the progress of today's world. The change from the silicon era to carbon life has brought many ways to simplify our lives by customizing materials based on need and demand. One such technology discussed here is 3D printing. 3D printing has opened up various domains like bioprinting, food printing, and manufacturing of electronics by a bottom-up approach, etc. Bioprinting is one such additive manufacturing process which utilizes biomaterials and/or polymers to form structural and functional bio-component such as cells, membranes, tissues, and organs. This technology allows the manufacturer to tailor the biomaterials as per the requirement such as organ-on-a-chip or some drug trials. Bioprinting has opened up various opportunities in the field of nanotechnology for some state-of-the-art applications like manufacturing of nanorobots, nano-films, and various intricate structures of complex composites, etc. Apart from the medical applications, biocompatible electronics and biodegradable devices can also be manufactured. In that regard, bio-ink is the main component which is responsible for determining the various properties of 3D construct. This chapter talks about the various processing required converting biomaterials to bio-inks, property change observed, and the drawbacks in doing so. As the application of these 3D constructs is in the field of medicine for critical medical issues like end-stage bladder disease, third and fourth-degree burns, and so on, proper

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procedure needs to be followed to allow the 3D construct function properly. Hence, this technology excels in various applications like tissue engineering, bio-sensors, manufacturing of artificial organs as well as skin, etc. This chapter introduces the readers about various printing technologies used for bioprinting along with its applications and limitations. Mainly the current applications of bioprinting along with the touch of the history of the evolution of this technology are the subject of interest here. In the later part of the chapter, the problems arising while acquiring a wide range of applications are also discussed.

**Keywords**

Biomaterials · Conversion method · 3D printing · Biomedical engineering

## Abbreviations

| | |
|---|---|
| 3D | Three-dimensional |
| BMSC | Bone marrow stromal cell |
| DECM | Decellularized extracellular matrix |
| HAMA | Methacrylated hyaluronic acid |
| HepG2 | Human liver cancer cells |
| PEG | Poly(ethylene glycol) |
| PCL | Poly (ε-caprolactone) |
| PLGA | Poly (D,L-lactic-co-glycolic acid) |
| PNIPAAM | Poly(N-isopropylacrylamide) |

## 2.1 Biofabrication

The use of various raw materials including the molecules, living cells, extracellular matrices, and also the biomaterials for producing the complex products (living and non-living) is referred to as the biofabrication [1]. In other words, it is the automated process for generating the biologically functional products which have the structural organization from living cells, various bio-active molecules, few biomaterials, and also the cell aggregates such as micro-tissues, or hybrid cell-material constructs, through bioprinting or bio-assembly and subsequent tissue maturation processes [2].

Organ-on-a-chip is a perfect example of biofabrication. What does this mean? An organ-on-a-chip is a tiny device working on the principles of microfluidics designed in such a way that it mimics any specific organ of our body. With the use of small capillaries and valves (for fluid transport or fluid flow), wells (for injecting external components into the device), and some gates (to regulate the pressure and temperature of the system), this device can be used for various noble purposes such as drug testing, vaccine trials, study various transport phenomenon (device is usually

transparent), and many other. This device is manufactured using 3D bioprinting. Similarly, there are huge applications associated with biofabrication.

There are studies carried out where bio-polymers are printed without cells embedded into them as well as those cells embedded during the post-treatment. Here, the main focus is on biological cells embedded within.

The types of bioprinting method are listed below.

1. The inkjet bioprinting
2. Extrusion-based bioprinting
3. Laser-assisted bioprinting
4. Stereolithography based bioprinting
5. Acoustic droplet ejection bioprinting
6. Magnetic bioprinting

## 2.2 The Inkjet Bioprinting

The inkjet bioprinting was started by Xu et al., in the year of 2005, it was the first bioprinting technique to get the success [3]. In this type of bioprinting method, the setup consists of a storage chamber for the storage of bio-ink (a mixture of living cells and pre-polymer solution) to be used for printing (as shown in Fig. 2.1). Along is the attached printer head where the piezoelectric actuators or heaters are placed. These actuators help to regulate the size of bio-ink droplet formation as well as the speed of deposition during the printing process. Inkjet printing avails 3D printed products at significantly low cost herewith availing with high cell viability. On the other hand, there are various disadvantages including clogging of nozzles, instability in cell concentration, vibration frequencies of piezoelectric actuator causing cell membrane rupture, and inability to print viscous materials. Thus, inkjet printers are still used for printing human fibroblasts with good (>90%) cell viability [4, 5].

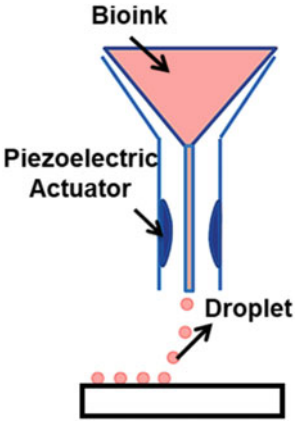


**Fig. 2.1** Schematic diagram of inkjet bioprinting [6]

## 2.3 Extrusion-Based Bioprinting

The technique which is most widely used is the extrusion-based bioprinting. It allows usage of viscous bio-inks which is a very advantageous aspect of this technique. Here, a mechanical force is applied through a screw system that pressurizes the bio-ink filled in the disposable medical-grade plastic syringe to move towards the nozzle (as shown in Fig. 2.2). The tip of the nozzle determines the diameter (usually ~150–350 μm) [7, 8] of the extruded product. Instead of droplet formation, the continuous flow (wire-like) structure is obtained. Mostly cylindrical constructs are obtained on the surface of the stage. Especially, this helps to construct large 3D constructs as per the requirement. But, due to high pressure in the syringe, cell viability is an issue in this case. With controlled parameters and efficient processing, cell viability can be achieved in the range of 80–90% [9]. Pneumatic deposition is preferred for the wide range of bio-ink types and their different viscosities. The main advantage of this process is that it can easily print the viscous bio-inks (nearly $30^{-6} \times 10^{7}$ mPa/s) with very high cell densities, and even cell spheroids, easily into the 3D scaffolds [10, 11].

## 2.4 Laser-Assisted Bioprinting

The laser-assisted bioprinting, also known as The Laser Induced Forward Transfer (LIFT) bioprinting was first introduced in 1999 by Odde [11] especially for the deposition of materials which are inorganic and later was implemented in the field of bioprinting [12]. The setup here consists of energy absorbing layer (Au/Ti) which is present on the ribbon layer of the donor layer [13] as shown in Fig. 2.3. This layer when stimulated using laser pulse on the surface heats a selective region. A layer of bio-ink is suspended under the donor layer. A high-pressure air bubble is generated at the interface of both the layers with the help of this heating up of the donor layer. Thus, stimulates the bio-ink for the formation of a droplet that eventually is received

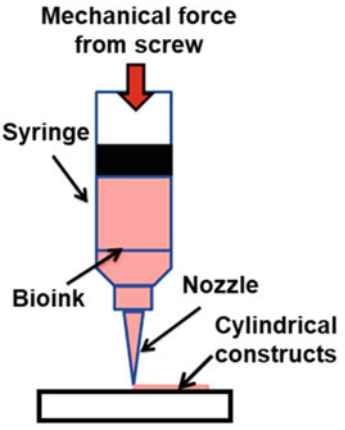


**Fig. 2.2** Schematic diagram of extrusion-based bioprinting [6]

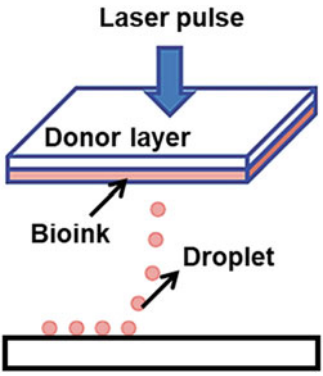


**Fig. 2.3** The Schematic diagram for the laser-assisted bioprinting [6]

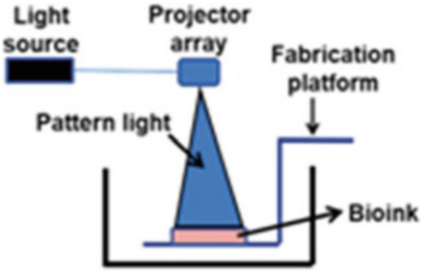


**Fig. 2.4** Schematic diagram of stereolithography bioprinting [6]

by the bottom collecting layer. The formation of a tissue construct is performed by a droplet-by-droplet manner.

The major advantages associated while using laser-assisted bioprinting are compatible with highly viscous materials, non-contact printing, and high cell density. Besides, studies have shown that cells maintain a high cell viability (>95%) because of the short period of laser pulse. On the other hand, the cost-effectiveness is compromised due to the generation of laser pulse and fabrication of the sacrificial donor layer.

## 2.5 Stereolithography Based Bioprinting

As the name suggests, this bioprinting method is a lithography technique that utilizes a light source to crosslink bio-inks in selective manner and deposit layer based onto the fabrication platform [14]. This bioprinting method is only applicable for photo-sensitive resin (bio-inks) like keratin and Decellularized Extracellular Matrix (ECM). These bio-inks consist of photoinitiators as well as reactive monomers. As shown in Fig. 2.4, a projector array is acting as a stimulator to obtain a patterned image on the photo-curable bio-ink layer. This provides a crosslinking of layers on a single printing plane [15]. So, the layer exposed to the high-intensity light gets selectively cured enhancing the structural properties of the tissue construct.

The main advantage of this method is that very complex patterned tissue constructs can be obtained at a faster rate of production as the projection of the total image is obtained at a time. The energy provided by the light source helps in strengthening the structural aspects of the tissue construct. Not only that, among all other techniques stereolithography bioprinting provides one of the highest printing resolutions due to the high resolution of the projection image, i.e. <50 μm [2, 16] as well as high cell viabilities (>90%).

## 2.6 The Acoustic Droplet Ejection Bioprinting

Acoustic droplet ejection bioprinting deals with the formation of droplet due to acoustic waves. Here, the setup consists of an acoustic actuator in the middle surrounded by a reservoir filled with bio-ink as shown in Fig. 2.5. The acoustic ejector makes use of a piezoelectric substrate with interdigitated gold rings which are present on the top to build surface sound waves as per the requirement. Thus, the bio-ink is present in an open pool around the acoustic actuator which gets stimulated when acoustic waves are produced. There is an array of 2D microfluidic channels that take part in the propagation of circular waves generated using acoustic waves. An acoustic focal point is formed towards the exit channel. The formation and ejection of droplet depend upon the force of this focal point, it should exceed the surface tension of the exit channel [17]. The diameter of the ejected droplet is found to be uniform and can be optimized varying the wavelength of the acoustic waves. It also shows good cell viability (>89.8%) [18, 19].

This type of bioprinting is not in much use as the setup is too sophisticated and sensitive to any mechanical disturbances as well as the control over the drop ejection is also difficult. This method also has a limitation on using hydrogels and other

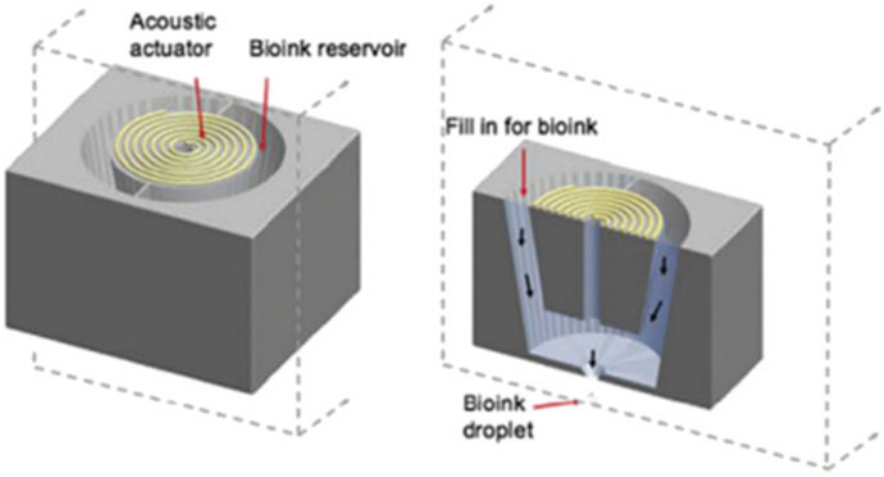


**Fig. 2.5** Schematic diagram of acoustic droplet ejection bioprinting [17]

viscous bio-inks. Moreover, further explorations on this method are carried out to utilize 3D acoustic tweezers using standing surface acoustic wave (SSAW) technology [20].

## 2.7 Magnetic Bioprinting

The contactless technique for assisted assembling of cells into various shapes and structures is known as magnetic bioprinting. This type of bioprinting has two approaches. Firstly, label-free diamagnetophoretic printing deals with mixing the cell-medium with a paramagnetic buffer solution. Followed by exposure to a magnetic field applied externally. Here, the total control of the assisted movement is based on the magnet configuration. While in the other scenario, cells are themselves magnetized before printing as a pre-treatment process using incubation with magnetic nanoparticles for a long time [21, 22, 24]. Cell aggregates are formed through levitation with the help of these cells which are embedded in a low-adherent plate. Then, ring-shaped magnets are used to provide the pattern as required in a separate suspended solution. Variations in the shape of magnetic template provide room for controlling the desired morphology through the spatial patternings of the cell aggregates [23, 24].

The advantages of this technique include direct cell manipulation, scaffold-free as well as a multicellular construct for complex tissue structures. On the other hand, the usage of magnetic nanoparticles also increases the risk of cytotoxicity. Not only can that, but the encapsulation of magnetic nanoparticles also induce internal stresses which can affect the cells and their functioning directly. The similar method has been incorporated for the fabrication of tissues of fat, lung, aortic valve, blood vessels, and tumors of glioblastoma and breast that shows the vivo-like protein expression and ECM [25].

Table 2.1 shows the comparison between various types of bioprinting. Depending upon the required properties, the type of bioprinting technique should be chosen.

**Table 2.1** The Comparison of properties obtained by different methods

| | Inkjet | Extrusion | Laser-assisted | Stereolithography | Acoustic |
|---|---|---|---|---|---|
| Ink viscosity | 3.5–12 mPa/s | Upto $6 \times 10^7$ mPa/s | 1–300 mPa/s | No limitation | 1–18 mPa/s |
| Cell density | Low, $<10^6$ cells/ml | No limitation | Medium, $<10^8$ cells/ml | No limitation | Low, $<16 \times 10^6$ cells/ml |
| Resolution | High | Moderate | High | High | High |
| Print speed | Fast | Slow | Medium | Fast | Fast |
| Cost | Low | Medium | High | Low | Medium |

## 2.8 Bio-ink

Bio-inks are processed biomaterials that can be used for engineering artificial living tissues and organs using 3D printing. They mainly constitute of living cells, materials for encapsulation, and additives to provide the required conditions for the cells to live. The materials used to conjugate with the cells are usually bio-polymers. Bio-polymers are the medium for the living cells to suspend and then get adhered during and after bioprinting. Mainly composite bio-inks consist of bio-polymer, active cells, and bio-active fillers.

The properties which influence the 3D printing capability include viscosity, shear-thinning, viscoelasticity, cytocompatibility and biocompatibility, gelation kinetics, biodegradation, and hydration degree (as shown in Fig. 2.6). Low-viscosity bio-inks that are cyto-compatible can be conjugated with another sacrificial bio-ink for bioprinting applications. The rate of gelation strictly depends on conformational changes or crosslinking of the polymer network. Print fidelity is also affected by the rate of gelation determining rate of crosslinking of the bio-ink post-printing [26].

Bio-inks are also essential for some of the controlling various cell functions including adhesion, migration, proliferation, and also the differentiation. Another

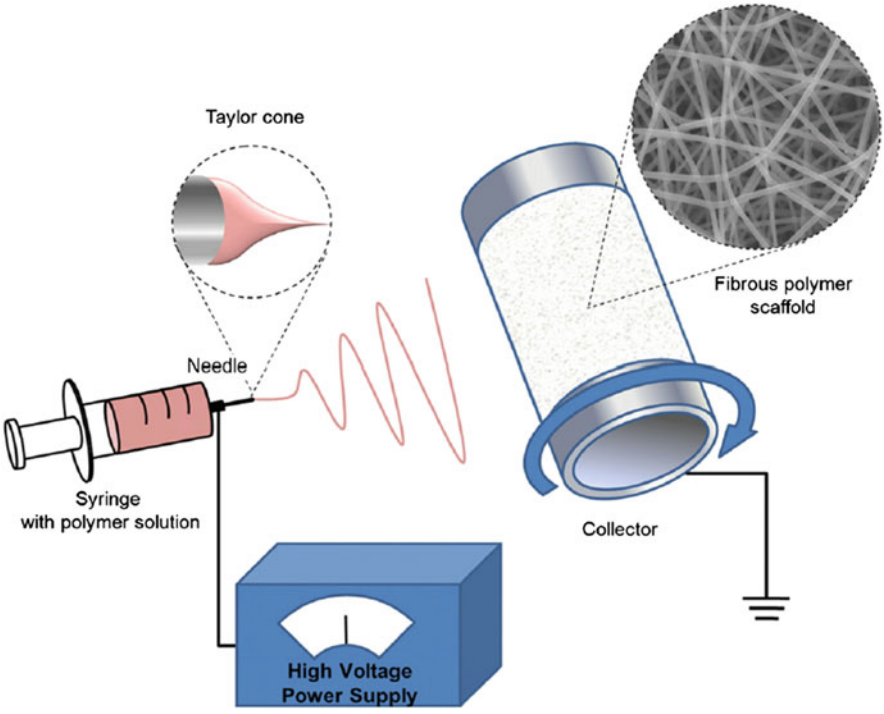


**Fig. 2.6** Schematic representation of electrospinning process [25]

important role of bio-ink is in the development of the capability of the hydrogel network to react to the cell-mediated matrix remodeling. Biodegradation of bio-inks can occur enzymatically (e.g. collagen/gelatin), hydrolytically (e.g. polyester) and through ion exchange (e.g. alginate).

So far, it has been reported that gel-based formulations are efficient for extrusion bioprinting. In the case of fibrous components like fibrinogen/fibrin, electrospinning is suggestible along with 3D printing. Some of these methods of processing biomaterials into bio-inks have been discussed below.

## 2.9 Electrospinning

Electrospinning is a method of fiber formation using electrical energy to withdraw charged fibers of polymer using solution containing polymer or polymer melts, resulting into formation of fibers with diameters in the order of 100 nm. In the electrospinning process, fibers ranging from 50 nm to 1000 nm or greater can be fabricated by providing an electrical potential to a polymeric solution. The polymeric solution is allowed to hang at the tip of a capillary tube, which is possible due to surface tension at the tip. The polymer solution gets charged due to the electrical potential applied. Mutual charge repulsion in the polymer solution induces a force that is directly opposite to the surface tension of the polymer solution. When electrical potential is increased, a cone-like structure is obtained due to the elongation of the hemispherical surface of the solution at the tip of the capillary tube known as Taylor cone.

The electric potential when reaches out to the critical limit, the surface tension forces overcomes to form a jet which gets ejected through the Taylor cone. Due to the elongation and evaporation of the solvent the charges get instable and with time it gets thin in the air. The charged jet eventually forms randomly oriented nanofibers that are collected on a rotating metallic collector.

System parameters like molecular weight of the polymer and distribution determine the degradation rate of the nanofibers. System parameters such as properties of the polymer solution, i.e. its viscosity, surface tension, and also the conductivity, determine the nanofiber diameter and tendency to form the bead gets reduced. There are various process parameters including orifice diameter, the flow rate of the polymer, and electrical potential are responsible for influencing fiber diameter. Some process parameters like capillary and metal collector's distance are responsible for determining the extent of evaporation of solvent from the nanofibers and deposition on the collector (Fig. 2.7).

Electrospun nanofibers are very useful for the tissue scaffold's fabrication due to its versatile as well as tailorable property of the electrospinning process for specific tissue applications. The cell attachment and the orientation within scaffolds such as tendons and ligaments can help in optimizing the orientation of nanofibers.

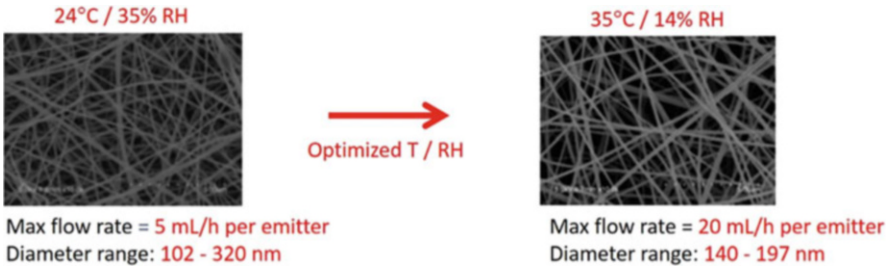


**Fig. 2.7** Effect of process parameters on electrospinning [25]

## 2.10 Ionic Crosslinking

The physical crosslinks through cation solutions are created by the ionic crosslinking. Sodium alginate, a common matrix bio-ink, attaches itself with $Ca^{2+}$ ions. This process is biocompatible and the degree of gelation can be optimized by altering the concentration of $CaCl_2$ in the solution [7, 27]. Reversible interactions offer constant viscosity during printing. But on the other hand, it has some drawbacks like there is the need for post-process of the crosslinking with an additional crosslinking agent along with the mechanically weak constructs [27].

## 2.11 Stereocomplex Crosslinking

Stereocomplex crosslinking involves the coupling of lactic acid oligomers of opposing chirality [7]. Dextran or polyethylene glycol can be used to couple with these oligomers to form solid hydrogels [7, 28]. Additional forms of complexation mechanisms include the formation of inclusion complexes such as cyclodextrins [7]. This mechanism helps us to maintain the viscosity throughout the printing because of reversible interactions which also requires post-process crosslinking and results into constructs which are usually mechanically weak.

## 2.12 Thermal Crosslinking

Thermal crosslinking basically depends on temperature variations. So, this type of crosslinking facilitates only those materials that gel with respect to temperature variations. In case of matrix bio-inks, there is possibility of thermal crosslinking at room temperature (25–37 °C). The main drawback of this crosslinking process is its longer gelation time. But, still thermal crosslinking is more preferred for the simplicity of the gelation mechanism which is highly compatible with biological systems.

## 2.13 Photo-Crosslinking

The process of free radical polymerization helps in the photo-crosslinking. Initially, matrix bio-inks like gelatin, polyethylene glycol, and collagen are modified with acrylate groups. Then, Ultraviolet, Blue, or Visible wavelength light is used to excite the free radicals present in curing bio-ink that serves as the photoinitiator, which leads to the interaction with a matrix bio-ink to form a gel that is usually solid, through covalent bonds. The crosslinking time plays an important role as with increased crosslinking time, the cell viability may get compromised considering the risk of exposure to free radicals and few harmful wavelengths during photopolymerization. This polymerization process occurs after extrusion, helps to avoid any effect on viscosity during extrusion, and leaves room for tuning the mechanical properties through post-process crosslinking.

## 2.14 Enzymatic Gelation

Enzymatic gelation is the process where the enzymatic crosslinking takes place, such as the gelation of fibrin. These materials are converted into gel by the enzymatic reaction, like the interaction of thrombin with fibrinogen to create fibrin gels. Cells are highly compatible with this process, but it requires special extruders or a post-crosslinking process to avoid gelation before extrusion (Fig. 2.8).

These bio-inks are regarded as the most advanced tools for Tissue Engineering and Regenerative Medicine (TERM). TERM mainly deals with the regeneration or replacement of normal biological cells or organs to maintain the basic biological systems in the body. To develop tissues artificially which will be susceptible and accepted by the body, these bio-inks help in obtaining customized organs or tissues to fulfill the biological requirement of a body (Table 2.2).

## 2.15 Hydrogels

A hydrogel is a network of crosslinked natural or synthetic polymer chains which are hydrophilic, at times found as a colloidal gel with water as the dispersion medium. Hydrogels are commonly used as materials for bio-inks as their biocompatibility is good and they are widely used as the cell-laden materials in case of bioprinting. The hydrophilic nature of these polymers absorbs moisture content in the environment. Hydrogels can be highly biocompatible as well as biodegradable, a necessary condition for in vivo applications. Moreover, cells can also be encapsulated in three dimensions when the gelation of hydrogel takes place.

Bio-inks are classified into five subgroups based on their role in the application point of view. They are listed below and discussed in brief as under:

1. Structural bio-inks
2. Sacrificial bio-inks


**Fig. 2.8** Factors affecting properties of bio-ink

3. Functional bio-inks
4. Supportive bio-inks
5. 4-Dimensional bio-inks

## 2.16 Structural Bio-inks

The structural bio-inks can be used to print according to desire using various materials like decellularized ECM, gelatins, alginate, and more. By varying process parameters, the mechanical properties, cell viability, shape, and size can be

**Table 2.2** Summary of some natural, synthetic, and composite materials used as bio-inks for 3D bioprinting

| Type of base biomaterial | Polymer base | Cell type | Bioprinting method | Printed tissue | Ref. |
|---|---|---|---|---|---|
| Natural biomaterials | Alginate | CPCs | Extrusion | Vascular | [29] |
| | | L929 | Extrusion | Vascular | [30] |
| | | HUVSMCs | Extrusion | Vascular | [31] |
| | | HepG2 | Extrusion | Liver | [32] |
| | | NIH 3T3 | Inkjet | Vascular | [33] |
| | Collagen | hMSCs | Extrusion | Bone | [34] |
| | | HFF-1 and HaCaT | Robotic dispensing | Skin | [35] |
| | | NIH 3T3 and HaCaT | Laser-assisted | Skin | [36] |
| | | MC3T3-E1 and hASCs | Extrusion | Liver and bone | [37] |
| | | NIH 3T3 and HaCaT | Laser-assisted | Skin | [38] |
| | Gelatin | BMSCs, ACPCs, and chondrocytes | Extrusion | Cartilage | [39] |
| | | VIC and SMCs | Extrusion | Aortic valve | [40] |
| | | MSCs | Extrusion | Cartilage | [41] |
| | | NIH 3T3 and HepG2 | Extrusion | Vascular | [42] |
| | Fibrin | Rabbit articular chondrocytes | Inkjet | Cartilage | [43] |
| | | hASCs and ECFCs | Laser-assisted | Vascular | [44] |
| | Gellan gum | MSCs | Extrusion | Bone/cartilage | [45] |
| | | Primary neural cells | Extrusion | Brain | [46] |
| | Hyaluronic acid | Chondrocytes and osteoblasts | Extrusion | Osteochondral tissue | [47] |
| | | Chondrocytes | Pneumatic dispensing | Cartilage | [48] |
| | | HAVIC | Extrusion | Heart valve | [49] |
| | Decellularized matrix (DECM) | hASCs | Pneumatic dispensing | Adipose | [50] |
| | | hTMSCs, hASCs and rat myoblasts | Extrusion | Adipose/ cartilage | [51] |

(continued)

**Table 2.2** (continued)

| Type of base biomaterial | Polymer base | Cell type | Bioprinting method | Printed tissue | Ref. |
|---|---|---|---|---|---|
| | | Primary human stellate cells, hepatocytes, and Kupffer cells | Extrusion | Liver | [52] |
| | Matrigel | EPCs | Pneumatic dispensing | Vascular | [53] |
| | | EA.hy926 and A549 | Inkjet | Lung | [54] |
| | | Goat multipotent stromal cells | Pneumatic dispensing | Bone | [55] |
| | Hydroxyapatite | HOP cells | Laser-assisted | Bone | [56] |
| | | hMSCs | Inkjet | Bone | [57, 58] |
| Synthetic biomaterials | PEG | hMSCs | Inkjet | Bone | [56] |
| | | Int-407, HepG2 C3A, and NIH 3T3 | Extrusion | Vascular | [59] |
| | PCL | Rabbit chondrocytes | Inkjet | Cartilage | [43] |
| | | Human nasal septal chondrocytes | Magnetic | Cartilage | [60] |
| | | Chondrocytes and osteoblasts | Extrusion | Osteochondral tissue | [47] |
| Composite materials | Agarose/chitosan | MSCs | Extrusion | Bone | [34] |
| | HAMA-pHPMA-lac/PEG | Primary culture equine chondrocytes | Extrusion | Cartilage | [61] |
| | PG-HA | Human and equine MSCs | Extrusion | Articular cartilage | [62] |
| | Alginate/gelatin | AECs and WJMSCs | Extrusion | Skin | [63] |
| | Fibrinogen/gelatin | HDF-n | Extrusion-based on rotary bioprinting | Vascular | [64] |
| | PLA/PEG/nHAp/dexamethasone | MC3T3-E1 | Extrusion | Bone | [65] |
| | Alginate/CNC | NIH 3T3 and human hepatoma cells | Extrusion | Liver | [66] |
| | SF-PEG4A | NIH/3T3 and keratinocyte | Stereolithography (SLA) | Skin | [67] |
| | Hydroxyapatite/gelatin | MC3T3-E1 | Extrusion | Bone | [68] |
| | LPN-GelMA | HBMSCs | Extrusion | Bone | [69] |

regulated. These factors make structural bio-inks the most basic but same time the most important aspects of a bio-printed design.

## 2.17 Sacrificial Bio-inks

Sacrificial bio-inks are nothing but the support material that are used in the actual hollow structures during printing and are removed after the process to create the channels. The cellular migration along with the nutrients transportation is two important phenomena that take place through these channel or open spaces. Surrounding dependent properties should be present in these materials like water solubility, the degradation under certain temperatures, or natural rapid degradation. Non-crosslinked gelatins and pluronic are examples of potential sacrificial material.

## 2.18 Functional Bio-inks

These are typically the complex forms of inks that are used for the growth, development, also the differentiation of the cells, which is easily done by the integration of the growth factors, biological cues, and also the physical cues including the surface texture and shape. These are the most important materials as they are the biggest factor in developing the various functional tissues as well as the function.

## 2.19 Supportive Bio-inks

Support links are essential for the printed bio-structures as they support the fragile and flimsy overhangs bio-structures so that they can get out of that phase easily. Once the construct is successful in supporting itself these are removed easily.

## 2.20 Dimensional Bio-inks

The concept of 4D bioprinting came in the year 2014. 4D bioprinting deals with printed objects (e.g., biocompatible responsive materials or cells) that have the capability to change their geometries or functionalities with respect to time responding to an external stimulus.

They have a property to react to the stimulus to the introduced energy. Like in future there may be an electricity sensitive bio-ink that based on the different electrical impulses could contract and relax, creating functioning muscle tissue. These kinds of new innovations can revolutionize the goal of printing a viable organ for a patient. It is based on the response of any kind of stimuli like advanced materials as piezoelectric materials or thermo-responsive materials (Table 2.3).

In 2015, Horvaith L and his group mentioned about the engineering of a lung tissue analog closely recapitulating the in-vivo human air–blood barrier architecture

**Table 2.3** Some common bio-inks used in tissue engineering

| Feedstock material | Source | Technique used | Applications |
|---|---|---|---|
| Agarose | Seaweed | Extrusion bioprinting | Cartilage tissue engineering [70] |
| Alginate | Brown algae | Extrusion bioprinting | Biofabrication of artificial human skin [63], Liver tissues [66] |
| Chitosan | The outer skeleton of shellfish/fungal fermentation | Extrusion bioprinting | Drug delivery and tissue engineering [71] |
| Collagen | Skin tissues | Extrusion bioprinting, inkjet bioprinting, laser-assisted bioprinting | Wound healing, skin grafts, cardiovascular tissues, bone, etc. [72] |
| Decellularized extracellular matrix (ECM) | Respective inhabiting native cells | Contact dispensing, inkjet printing, extrusion bioprinting | Biofabrication of in vitro human air-blood tissue [73] |
| Fibrin/ fibrinogen | Blood clot | Extrusion bioprinting, inkjet bioprinting, laser-assisted bioprinting | Wound healing, engineering stem cells, cartilage regeneration [74] |
| Gelatin | Collagen | Partial hydrolysis, inkjet bioprinting, extrusion bioprinting | Bone implants [68] Cartilage [39] |
| Hyaluronic acid (HA) | Connective, epithelial, and neural tissues | Inkjet bioprinting, extrusion bioprinting | Cartilage tissue engineering, heart valve [43, 48, 49] |
| Hydroxyapatite | Teeth/bones | Extrusion bioprinting | Bone tissue engineering [75] |
| PCL/PLA/ PLGA | Polymers | Extrusion bioprinting | Cartilage [43, 60] |

with a highly precise, reproducible biomanufacturing technique. To obtain layer-by-layer fabrication of 3D tissue constructs a valve-based bioprinting process was used. The experimental approach showed that the accuracy of the printer helped to get highly precise and reproducible patterning in a controlled spatial arrangement. Optimization of the printing process was performed to work under cell-friendly, low-pressure conditions to deposit multiple cell types and ECM [73]. Thin layers of ECM were achieved using contact dispensing method compared to the thick layers of ECM which can be obtained manually. In this in vitro model, they employed cell lines which had the advantage of being homogeneous as well as more stable compared to primary human epithelial and endothelial lung cells [76]. This ensures better control and reproducibility at the same time while performing screening tests. This approach had availed the possibility of inter-laboratory comparative studies.

With a background in magnetic 3D bioprinting, Tseng et al. validated spheroid contraction as a biologically relevant cytotoxic endpoint using 3T3 murine embryonic fibroblasts in response to five toxic compounds. This study revealed that the

assay developed, employing M3DB spheroids to determine cytotoxicity in a 3D microenvironment, could overcome limitations of handling, speed, throughput, and imaging of other 3D cell culture platforms [26, 77].

Müller et al., prepared a special bio-ink that was to be used for the cartilage bioprinting, the bio-ink was based on alginate sulfate along with nanocellulose so that they can easily obtain rheological properties that are suitable for printing [78]. Post-printing, the performance majorly depends on the nozzle geometry and dimensions. Low extrusion pressure and shear stress produced migrating cells but at the same time decreased cell proliferation. Conical needles with a wider diameter provided 3D structures with high shape fidelity and cell viability (more than 90%).

## 2.21 Essential Characteristics for a Bio-Ink Material

- Necessarily should be biocompatible. In the case of using magnetic nanoparticles (usually in magnetic bioprinting), cytotoxicity should also be taken care of.
- Mechanical properties should be fulfilled according to the required tissue construct such as structural strength. Mainly the structure of the construct would allow it to withstand some external forces and function properly.
- The various physical properties like viscosity/stiffness, nonlinear viscoelasticity (thixotropy/rheopexy), surface tension, and also the density need to be regulatory. To tune these physical properties, parameters like the polymer/particulate concentration/formulation and/or degree of chemical modification/crosslinking, as well as the salt content, cell density, temperature, and pH need to be manipulated.
- The printability of bio-inks largely depends on rheological properties such as storage and loss moduli, tangent delta, and response to shear stress. Various mathematical models and machine learning can be used to predict and alter the rheological properties.
- Scaffolding material should be such that it holds the living cells and provides them the required living conditions.

Some varied practical applications of the biofabricated assemblies:

1. Biofabrication of human tissues and organ implants: advanced biofabrication technology can assist in designing scalable as well as economical production of organs and organ constructs which are living and can be used appropriately. Recently, it was reported in medical economic data that around $25–30K can be the selling price of the tissue-engineered vascular graft, while exceptional conditions it may be vary up to $50K [79].
2. Biofabrication of in vitro three-dimensional tissue models of human diseases: Using CAD tools, the preparation of bio-ink can be assisted [80]. Moreover, mathematical models help us to have a clear picture of the process and be cautious about the upcoming challenges. Once the required properties are achieved, other alterations can be done via post-treatment.

3. Drug toxicity and drug discovery assays: This avails us with the facility to test new drugs and observe it in vitro condition parameters. This provides enough freedom to experiment with the drugs and their toxicity along with the responsive cell mechanism. For example, lab-on-a-chip is a tiny device mimicking the in vitro condition of a part of a human or any organ without harming any living creatures directly.
4. Bio-sensors and biological reports in space research: Many space programs and challenges are carried out by NASA for 10–12 years. Using 3D printing technology, macro to nanodevices are being manufactured. They are tested to withstand the extremities of parameters like wind velocity, low gravitation, high powered batteries, and many more. Several efforts have resulted fruitful and the outcome has helped us make our life easier. One sophisticated microfluidic bio-reporter has already been developed for National Aeronautics and Space Administration—NASA. It turns out that most challenging task is to create an in vitro tissue analog of human organs which imitate the complexity of human organisms, including the radiation-sensitive immune system along with the circulating lymphocytes and stem cells [80].
5. Biofabrication and bio-art: for a long time, art has been always there in our technological reforms too. For example in recent days, growing semi-living tissue-engineered sculptures is one of them and employed in an art project by provocative bio-artist Oron Catts (http://www.symbiotica.uwa.edu.au/) according to them tissue engineering as a new art medium [81, 82].

So, as per the discussed topics above, 3D printing has already entered our lives on a day-to-day basis. It has already started making our lives easier. Moreover, to continue with the progress made so far, the knowledge of bio-inks is necessary and must in this case. On the other hand, this technology will contribute to maintaining sustainable development altogether. This is why the study of the limitations along with experimentations is need of the time to overcome old limitations and come up as well as explore more advanced materials.

## 2.22 Summary

3D printing is such a technology which provides a lot of scope in terms of biofabrication, tissue engineering, drug testing, etc. The key feature of this technology allows us to obtain complex structural component easily with precision. The availability of various techniques leaves room to explore more biomaterials which can be used for artificial organ fabrication and biocompatible structures. As discussed thoroughly in the chapter, extrusion bioprinting is the most used technique these days due to the distinctive feature which allows viscous hydrogels to be used for bioprinting, good strength at larger scale, i.e. larger tissue constructs and most essentially can form spheroids too. Furthermore, investigations are going on to improve cell viability. Flexible constructs can be obtained using hydrogels and other polymers like PEG and PLC. All of the functionalities of 3D printing have

turned out to be very beneficial for cosmetic surgeries and skin grafts. Not only that, but organ transplantation has also turned out to be a more viable option than accepting a donated organ in terms of blood transfusion and cell acceptability. Apart from this, theoretical and mathematical models have been also proposed to predict the working of various combinations and complex biomaterials under the scaffold. Those studies help us to have a better understanding of the reaction of a drug and another drug-related testing. For all this to happen, few parameters have to be taken care of while converting biomaterials into bio-inks such as temperature exposure, pressure throughout the technique used, scaffold reactivity towards the biomaterials, and most importantly the cytotoxicity (which is often observed in case of magnetic bioprinting). This plays an essential role in cell viability as well as lifespan of these tissue constructs [83]. Based on bio-ink formation, the combination of both, i.e. firstly electrospinning followed by 3D printing would give superior properties, as well as scaffolding, would be benefited [84]. Bio-ink formulation plays a vital role in the property determination of 3D printed products. Thus, there is a lot more scope of further study in optimizing the bio-ink formulation.

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# Bioprinting

3

Tanmay Bharadwaj, Ann Thomas, and Devendra Verma

**Abstract**

Since the discovery of cells in 1665 by Robert Hooke, they are considered nature's building blocks that are primarily responsible for giving us a physical manifestation. These natural building blocks can be utilized to create biological constructs that can serve many applications for the betterment of human health and living. One such application is Bioprinting, which is a rapidly emerging field where additive manufacturing processes are used to fabricate organs and tissue constructs. This technology is one of the most promising technologies introduced to tissue engineering and regenerative medicine. Being a widely accepted and fundamental technology in biofabrication, exploiting various biological components like cells, growth factors, proteins, and biomaterials, this technology can deliver 3D models, replacement organs, and other therapeutic products. Bioprinting has shown incredible growth and is a fast developing technology having the potential to address the currently existing limitations of tissue engineering and regenerative medicine. It also has the potential to develop patient-specific implants establishing itself as the future of organ transplantation and a solution to the organ shortage crisis. 3D-bioprinted tissue models can also serve as a platform for high throughput toxicology screening and drug discovery. This process of additive manufacturing, which has drawn its origin from 3D printing in most ways, has stimulated rapid development in the sector of bioink, which addresses the broad applicability of bioprinting technique in multiple domains of science. The following literature gives more insight on the general background of bioprinting and bioinks and also distinguishes between the types of bioprinting

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processes and discusses 4D bioprinting and the socio-ethical outlook of bioprinting organs. Lastly, it explores open-source bioprinting, and portabilization of bioprinters.

**Keywords**

Bioink · Tissue engineering · Inkjet printing · Biomaterials

## Abbreviations

| | |
|---|---|
| ECM | Extracellular matrix |
| PAB | Pressure-assisted bioprinting |
| CAD-CAM | Computer-aided design and computer-aided manufacturing |
| LAB | Laser-assisted bioprinting |
| TERM | Tissue engineering and regenerative medicine |
| GelMA | Gelatin methacrylamide |
| HAMA | Hyaluronic acid-methacrylate |
| PEG | Polyethylene glycol |
| dECM | Decellularized extracellular matrix |
| iPSCs | Induced pluripotent stem cells |
| EB | Embryoid bodies |
| LIFT | Laser-induced forward transfer |
| PEGDA | Poly (ethylene glycol) diacrylate |
| MSCs | Mesenchymal stem cells |
| hMSCs | Human mesenchymal stem cells |
| PEGDMA | Poly (ethylene glycol) dimethacrylate |
| NSC | Neural stem cell |
| CNS | Central nervous system |
| HTS | High throughput screen |
| BMP-2 | Bone morphogenetic protein |
| hEPCs | Human endothelial progenitor cells |
| VEGF | Vascular endothelial growth factor |
| ALP | Alkaline phosphatase |
| HLA | Hyaluronic acid |
| PVA | Polyvinyl alcohol |
| DFO | Deferoxamine |
| PUA | Poly (urethane acrylate) |
| PLA | Polylactic acid |
| ABS | Acrylonitrile butadiene styrene |
| hESCs | Human embryonic stem cells |
| NEST | New and emerging science and technology |
| HEK | Human embryonic kidney cells |
| YFP | Yellow fluorescent proteins |
| NOSE | Nydus one syringe extruder |

| | |
|---|---|
| HA | Hyaluronic acid |
| BG | Bioactive glass |
| VML | Volumetric muscle loss |
| RGD | Aspartic acid (R)–Glycine (G)–Aspartate (D) |
| TF | Transcription factors |

## 3.1 Introduction

From the invention of the first 3D printer in 1984, which allowed solid 3D objects to be created from digital data to the implantation of the first lab-grown organs in the 2000s, bioprinting is the culmination of technological and biological developments advances that have been decades in the making. Since the development of industrial-grade printing in the fifteenth century, printing has had a groundbreaking effect on society influencing politics, education, language, and religion across the globe. Advancements in printing technology have enabled us to advance from 2D printing to 3D printing, where biomaterials and cells combined in the form of bioink are used to print layers on top of another, creating a height parameter along with already existing parameters of length and breadth. This fabrication technique enables us to create 3D tissue-like structures for medical and tissue engineering use. 3D bioprinting has found application in modeling complex tissue constructs and protein interactions with tailored biological and mechanical properties [1]. In a method called "stereolithography," Charles Hull described 3D printing as a method to create 3D objects by sequentially printing wafer-thin layers of UV curable materials to form a solid, tangible structure [2]. The same principle is applied to create resin molds and form 3D structures using biological materials. The success of solvent-free polymers further helped in the construction of 3D scaffolds, from biological materials, that can be implanted including or excluding the cells. A recent development, enabled by 3D printing, led to medical devices such as stents and splints [3].

The subsequent step was using this technology to engineer tissues for regenerative medicine, and this was facilitated by recent advances in molecular biology and material sciences.

There are different printing techniques that can be used when dealing with a 3D bioprinter and they have been discussed in detail later. These approaches can be used to create living organ constructs with functional biomechanical properties. Despite the significant progress and the research efforts, the goal of fully functional 3D bioprinted organs has yet to be proficient as there is a daunting list of scientific challenges—from cellular density to biomaterial limitations—standing in the way. The life cycle limitations of primary cells mean that stem cells could be used due to their promising self-renewal and pluripotency characteristics. Hence, a fundamental understanding of it needs to be researched. Nevertheless, the primary challenge is to replicate the native structure of the extracellular matrix and cellular complexity of tissue that would suffice biological functions. In this chapter, we shall review about

the different bioprinting approaches and strategies that will be best suited to fabricate the tissue of interest and the challenges that are faced with each type of bioprinter. However, in all these strategies, the bioink formulation is the most essential component. The gelation and crosslinking of this ink determine the infrastructure of the final scaffold. We will discuss the types of bioinks commonly used for tissue engineering applications and the influence of each one on the printing process. A major breakthrough in this field was seen with the integration of stem cells in the bioink which gave the bioengineered tissue cells, ability to renew and self-differentiate. This will be discussed in more detail, with its application in different tissue development like cardiovascular and musculoskeletal tissues. Another field of application where 3D printed tissue models come into play is in the discovery and delivery of various types of drugs. These models have the ability to mimic the spatial and physiochemical characteristics of the microenvironment of the body and they have been established as a better platform for the study and screening of drugs than 2D computational models.

Later segment of this chapter focuses on an upcoming novel approach of bioprinting called as 4D bioprinting where time is taken as the fourth dimension. Scaffolds fabricated in this approach can undergo conformational changes when exposed to specific stimuli even after process of bioprinting. This section also discusses about various approaches of 4D bioprinting, types of stimuli and the mechanism of 4D bioprinting, and finally the applications it can have in the field of biomedical sciences. In addition to this ethical aspects of 3D bioprinting are also discussed as stem cells are fundamental raw materials that are required in this process. Last but not the least concluding this chapter with two important topics, namely portabilization of bioprinters and open-source bioprinting which discuss about the efficacy and portability of bioprinters along with the degree to which a bioprinter can be customized economically that can have wider applications and enhanced interdisciplinary research.

## 3.2 3D Bioprinting Approaches

While there are several approaches to 3D bioprinting, they are mainly based on three fundamental approaches with respect to the guide used for printing the tissues, be it the native tissue or the formation process or both, and they are classified as: (1) biomimicry, (2) autonomous self-assembly, and (3) mini-tissue building blocks.

### 3.2.1 Biomimicry

Nature has inspired several technological innovations such as flight [4], Velcro, injection needles [5], cell culture methods [6], and nanotechnology [6]. It has found application in bioprinting, by reproducing the cellular and extra cellular components present in a tissue or organ [7]. This type of approach is called Biomimicry. This approach's basic principle is to fabricate tissue constructs that mimic native tissue to

a great extent, like branched vasculature or by developing biomaterials similar in composition and structure of the native tissue. This process also requires replicating the microenvironment and framework of the tissues [8]. Understanding the physical nature of the biological forces present, the organization of the supporting cell types, factors involved, and the ECM matrix's composition are important aspects, as the tissues to be replicated need to be carried out on the microscale for this technique to be successful [1]. This method requires more research and understanding in engineering, imaging, biomaterials, and cell biology.

### 3.2.2 Autonomous Self-Assembly

An alternative method of imitating the tissue of interest is to mimic the formation and development of organs as a guide. In their early developmental stages, cells secrete the extracellular matrix building blocks, initiate proper signaling pathways, and self-directed associations and patterns that make up their micro-architecture [9, 10]. A "Scaffold-Free" model that uses spheroids mimics the fusion and cell arrangement of evolving tissues. This approach relies on each cell as the underlying driver of its differentiation and specialization, steering the building of each tissue and its structural and functional properties [10]. It challenges a deeper understanding of embryonic development and organogenesis as well as the microenvironment required to bio-print the tissues [1].

### 3.2.3 Mini Tissue Building Blocks

The principle of this method applies to both the aforementioned approaches. Tissues and organs are made of the basic structural and functional units or building blocks, such as each glia of the brain [11]. Mini-tissues are constructed and accumulated into a considerably larger framework by either mimicking tissue architecture, self-assembly, or combining the two. There are two major approaches to this: One method involves assembling the cell spheres, using designs inspired by the natural world, into a macro-tissue construct [11]. Another method involves replicating the tissue in high resolutions and then allowing its self-assembly into a functioning macro-tissue. Some examples of this can be seen in the self-assembly of cartilage strands to form articular cartilage tissue [12]. This approach of using mini-tissues as building blocks is also used to create "Organs-on-a-chip," which uses 3D bioprinting to replicate functional tissues that are secured together by a microfluidic network. The latter innovation is used to screen drugs or vaccines or to create in vitro models of disease [13] (Table 3.1).

**Table 3.1** Bioprinting strategies Reprinted from Bishop et al. [14], with permission from Elsevier and Copyright Clearance Center

| Strategy | Biomimetic | Self-assembly | Microtissues |
|---|---|---|---|
| Description | Attempts to duplicate environment and growth cues for a target tissue; Relies heavily on bioreactors | Attempts to replicate embryonic environment allowing for autoregulation and self-production of raw elements | Forms smallest possible structural and functional unites that can later be combined to form mature tissue |
| Advantages | Control at each step of tissue development; high degree of precision in cellular positioning | Fast and efficient; scalable for automaton; high cellular density | Fast and efficient; scalable for automation; potential to solve limitations in engineering vascular tissue |
| Disadvantages | Complex given all factors that must be reproduced; slow and often inefficient microtissues are difficult to create | Difficult to change outcome during self-assembly process | Microtissues are difficult to create |
| Scaffold required | Yes | No | No |

## 3.3 Tissue bioprinting techniques

There are several techniques used by bioprinters when it comes to layering and patterning biomaterials to create tissue constructs. These technologies differ when considering the 3D bioprinting factors such as surface resolution, cell viability, and printer cost. The commonly used bioprinters are inkjet based [16], micro extrusion-based, and laser-assisted printing. Each method and their contributing factors in 3D printing will be described in detail:

### 3.3.1 Inkjet Bioprinting

Also termed as drop-on-demand printers are presently the widely accepted technology in both 3D printing as well as bioprinting application (Fig. 3.1b). It uses a non-contact printing method that layers "bioink" in a drop-like manner, in picoliter amounts onto scaffolds made of hydrogels or culture dishes using computer control. The first inkjet printer used was modified from a commercial 2D ink printer [17]. Biomaterials substituted the ink and an elevator, controlled by an electronic database that allowed movement in all three dimensions, replaced the paper. Presently, we can custom design these printers to print biological materials at high resolution, accuracy, and increased speed. Now, based on the mechanism by which the droplets are layered, there are two different types of operating methods:

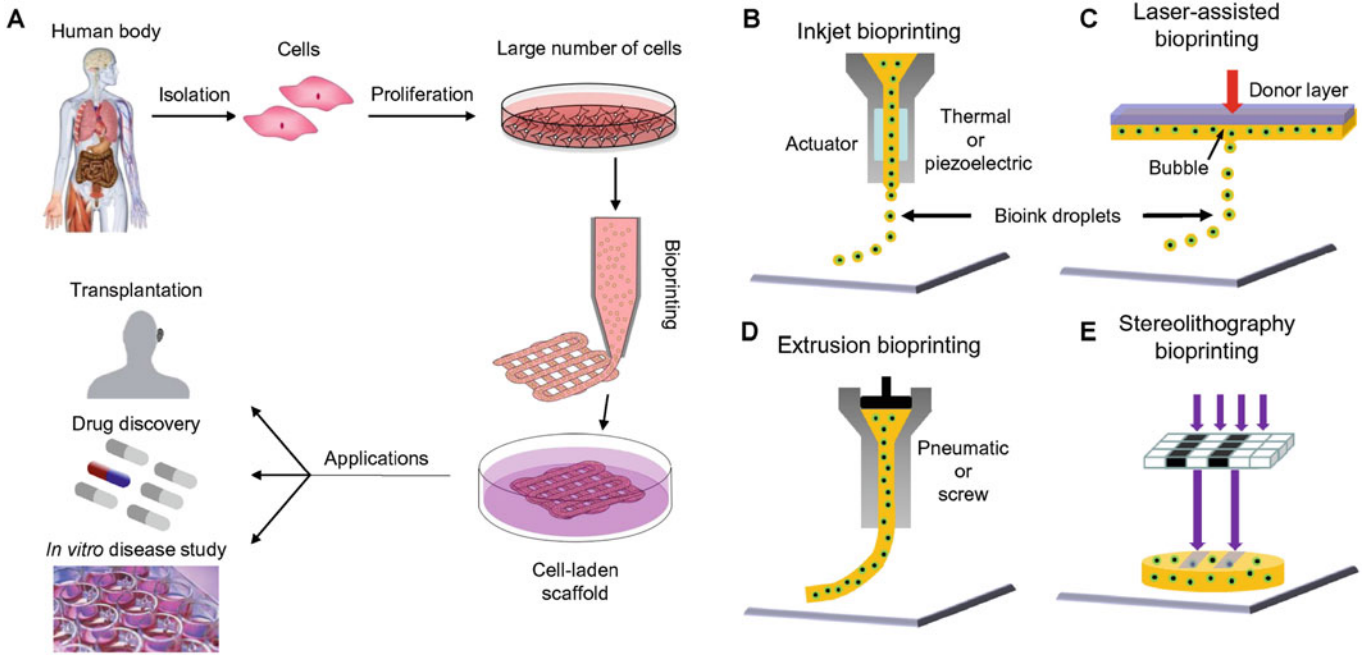


**Fig. 3.1** Bioprinting process and the different techniques. (**a**) The initial step in the bioprinting process includes isolating and culturing the desired cells, followed by integrating them in a bioink to print out cell-laden scaffolds. These scaffolds can then be used for a variety of application such as organ transplant, disease modeling, and in vitro drug study. (**b**) Inkjet printers use a non-contact printing method that layers "bio-ink" in a drop-like manner. (**c**) Laser bioprinters consist of a laser as an energy source to layer biomaterials onto a stationary substrate. (**d**) Extrusion bioprinters rely on extrusion to design and fabricate constructs. (**e**) Stereolithographic printers use a digital light projector to selectively crosslink bioinks plane-by-plane. Reprinted from Mandrycky et al. [15], with permission from Elsevier and Copyright Clearance Center

thermal and piezoelectric. Thermal inkjet printers force the bioink out of the nozzle and onto the substrates. This force is created by inflated bubbles that are formed when the print head is heated electrically and pulses are formed. Research conducted has shown that this print heads can be heated from 200 °C to 300 °C, and was seen to not substantially affect the stability or viability of the biological material, such as DNA [18], or the post-printing functioning of cells like the mammalian cells [19]. The positive side of this method includes more efficient printing speed, inexpensiveness, and broader availability. But, on the other hand, when the biological materials undergo thermal and mechanical stress, the droplets become heterogeneous, unorderly, and unequal in size, and smooth printing becomes difficult as blockages occur. In the second method which involves piezoelectric technology, the droplets are created by a temporary pressure from a piezoelectric actuator that generates acoustic waves that breakdown the continuous liquid in the print head into droplet form at regular intervals [20]. The droplet volume and rate of ejection can be controlled by adjusting ultrasonic parameters, such as pulse, time-duration, and amplitude. Compared to thermal technology, this method does not involve heat and prevents orifice clogging, thus ensuring that the bioink drops remain unidirectional with homogenous size. However, this technology can result in cell membrane damage and cell lysis. This technique reported viability of greater than 90% for mammalian cells layered using piezoelectrical technology such as fibroblasts, human osteoblasts, and bovine chondrocytes [21].

### 3.3.2 Micro-Extrusion Bioprinting

Micro-extrusion bioprinting or pressure-assisted bioprinting (PAB) is one of the more frequently used and affordable 3D printers (Fig. 3.1c). This technique relies on extrusion to design and fabricate constructs using biomaterials which usually comprise solutions, pastes, or dispersions. This instrument uses a temperature-monitored, material handling and dispensing system which can move in all three dimensions, a fiber-optic light source that helps in the visualization of the deposition area and also plays a role in photoinitiator activation, a camera with a three-dimensional control base, and a humidifier [11]. The printer functions by a robotic extrusion of the biomaterial, controlled by the movement of pneumatic pressure or plunger in the form of a continuous strand through a microscale orifice or a micro-needle onto an immobilized substrate. This technique yields continuous beads of material, as controlled by the computer-aided design and computer-aided manufacturing (CAD-CAM) software, as compared to the liquid droplets in the other method [22]. There are numerous materials like hydrogels, polymers with good biocompatibility, and cell spheroids that have displayed great compatibility with micro-extrusion devices.

This method of printing became popular mainly due to its ability to layer cells at very high densities. It also includes room temperature handling, direct assimilation of the cells, and even spreading of the cells. This technique is used for the fabrication of different tissue types, such as aortic valves [23], vasculatures [24], and in vitro

pharmacokinetics [25] and tumor models [26]. Although it is a slow process for high-resolution structures, the 3D structures that can be created vary from large to microtissues in microfluidic chambers. The bioprinted grafts obtained from this technology were seen to retain their functionality in vivo [27].

### 3.3.3 Laser-Assisted Bioprinting (LAB)

Laser-induced forward transfer forms the basis for the working of LAB (Fig. 3.1d) [28]. Although comparatively less in use than the other bioprinting technologies, this technique is more commonly used in tissue- and organ-engineering fields. It consists of a laser as an energy source to layer biomaterials onto a stationary substrate. This technique was previously used for the metal transfer, but now, by substituting with biomaterials, we can print materials such as nucleic acids, proteins, and some cells. It has three main components: a pulsed laser source, a focusing system or "ribbon" made of glass, usually coated with liquid biological materials (e.g., cells and/or hydrogel) that are deposited on the metal film (e.g., gold or titanium), and a receiving substrate. LAB functions by irradiating the lasers onto the absorbing layer of the ribbon, causing the evaporation of the biological materials towards the collector substrate. The receiving layer is usually a cell culture medium that ensures the cellular adhesion and unaffected cell growth after biomaterial is transferred to the ribbon. This method uses lasers in the UV or near-UV wavelength to print the hydrogels, cellular components, and ceramic materials. Factors—such as the thickness of the material on the film, the rheological properties, wettability of the substrate, the wavelength of the laser, and the speed and precision of the printing as well as the organization of the structure—affect the resolution of the printed material and this usually ranges from pico- to micro-scale [29].

There are several challenges to this technology, such as the preparation of each ribbon, which is a gradual, drawn-out, and strenuous process, especially if different types of cells and biomaterials have to be co-integrated and layered together to form tissues. Another problem is that as the ribbon is coated with different liquid biological materials, it poses a challenge to precisely target and layer the cells on the substrate. This can be solved by using a cell-recognition scanning technology that can detect the properties of a cell within each pulse. Also, metallic residues present on the metallic laser absorbing layer vaporize and form on the bioprinted construct. This can be avoided by using non-metallic absorbing substrates and choosing a different strategy such that it does not require such a layer. Current research has shown that this technology can be used for the printing of cells, such as human dermal fibroblasts, mouse myoblasts, breast cancer cells, and rat neural cells. In 2013, scientists were successfully able to create Grafts as substitutes for skin by this technology, a turning point in the field of LAB [30]. This technology has an edge to the other methods due to the absence of a nozzle and the fabrication of cells in high resolution. It also provides the user with increased control of the bioink drops and its positioning characteristics (Table 3.2).

**Table 3.2** Comparison of different bioprinters based on key parameters

| Bioink | Inkjet | Micro-extrusion | Laser-assisted |
|---|---|---|---|
| Cell viability | >85% | 40–80% | >95% |
| Print speed | Fast | Slow | Medium |
| Viscosity | 3.5–12 mPa/s | 30 mPa/s to $>6 \times 10^7$ mPa/s | 1–300 mPa/s |
| Resolution | <1 pl to >300 pl droplets, 50 μm wide | 5 μm to millimeters wide | Microscale resolution |
| Cost | Low | Medium | High |
| Bioink material | Fibrin, alginate, hap, GF based, PCL, PEG, PVP, dermamatrix | Alginate, gelatin, collagen, gellan gum, hyaluronic acid, agarose, dECM, PEG, pluronic, novogel, cryo-ink | Alginate, collagen, fibrin, hap, matrigel, blood plasma |
| Applications | Vascular tissue, bone, cartilage, neurons, lung tissue | Bone, cartilage, neurons, muscle, tumor Organ-on-a-chip | Vessels, bone, cartilage, adipose, skin |

## 3.4 Bioink

Bioprinting is based on the manufacturing of organs or tissues using biomaterials and cells. These biomaterials called "Bioinks" should have high cytocompatibility. Bioink often composed of cells that are being used but at times, contain additional materials that encompass the cells. They must often meet a specific criterion such as rheological characteristics, physicochemical properties, bio-functionality, and biocompatibility [31]. These properties before and after gelation are important factors for printing and ensuring feasible structural resolution, shape fidelity, and cell viability. These bioinks are among the most advanced tools employed in tissue engineering and regenerative medicine (TERM) [32].

Some ways in which bioinks differ from traditional 3D printer ink, which are:

- Provide growth and function to cells.
- Functions at a comparatively lower temperature (37 °C or below).
- Does not compromise cell viability.
- Bioactive.

Traditional bioprinting required the deposition of the material in a layered pattern, but recently, volumetric bioprinting, a new technology that enables the creation of entire constructs at once, was introduced. In this technique, the bioink present in the liquid cell is selectively irradiated through optical 3D dose distribution, which triggers polymerization, which in turn comprises the final structure [33]. This was a turning point in material science, that led to the quick construction of customizable biomaterials. But before this technique can be used in any significant bioprinting applications, further studies need to be conducted on its working and bio-mechanisms.

### 3.4.1 Classifications of Bioink

Different types of bioinks can be used depending on the construct being fabricated as well as the material used. Certain bioinks can form the structural backbone on which the entire scaffold is built and also provide stimulations for the growth of cells, whereas some are detached after the tissue is formed, leaving behind a cavity for the transport of nutrients. Each organ or tissue being formed requires a certain type of ink to suit its functioning and development and these types are discussed in detail:

#### 3.4.1.1 Structural

These types of bioinks mainly find applications when dealing with the skeleton of the desired construct. And it uses materials that can gelatinize or crystallize such as alginate or gelatine which also allows in the modification of the cell properties such as shape and size. These factors are the reason that, though structural bioinks are more of a basic type, they still form a fundamental feature in bioprinted designs.

#### 3.4.1.2 Sacrificial

Sacrificial types of bioinks provide a support system during the fabrication of the tissues or organ and then are dissolved or detached, which leaves behind conduits or void regions on the structure surface. This assists in the transport of nutrients or cells throughout the tissue and might be the keypoint for the fabrication of vascular systems. These materials exhibit certain properties such as water solubility under certain temperatures or rapid degradation which allows its removal from the construct. Some examples of potential sacrificial materials are non-crosslinked gelatine and pluronic.

#### 3.4.1.3 Functional

Functional bioinks can be classified under a more specialized form of ink which is responsible for imparting functionality to the construct. They are necessary for guiding the growth of the cells to the proper formation of the desired tissue construct. These bioinks may contain certain growth factors that can stimulate the differentiation of cells to recapitulate the native ECM. These components are some of the biggest factors of bioinks as they play a huge role in developing functional tissue from a bioprinted construct.

#### 3.4.1.4 Support

Depending on the tissue, most constructs lack the necessary biomechanical properties required to support themselves until development occurs. So, until then, supportive bioinks are used to provide the necessary support system, especially if they are to be planted in vivo. The printed tissue can be quite delicate due to the patterns and overhangs, and these bioinks provide them with the rigidity they require until they can support themselves.

#### 3.4.1.5 4-Dimensional

4-dimensional bioinks are the next step in the development of bioinks. They allow the fabrication of high functioning tissue systems that can respond to stimuli. For example, they can change the properties of the tissue such as shape and function, depending on an external altercation. An electric sensitive bioink that responds to repeated electric stimulations causing it to contract, could be the basis of developing fully functional muscle tissue [34]. This can also help in the construction of a functional nerve cell. The endless possibilities posed by this field of bioink development can change the way we look at tissue engineering and it can also bring us one step closer to printing a fully functional organ for a patient.

## 3.5 Types of Bioink

### 3.5.1 Polysaccharide Based Bioink

#### 3.5.1.1 Alginate

Alginate is a naturally occurring polysaccharide made of mannuronate and guluronate residues obtained from the cell wall of brown algae. It gelatinizes on the addition of calcium ions, to form a tough hydrogel, due to the sodium-calcium crosslinking. It has been widely used due to its bio-inertness and compatibility and also due to its inexpensiveness [35]. This bioink can be integrated with other types of biomaterials that help in the fabrication of tissue, such as the use of nanocellulose for printing cartilage (Fig. 3.2a, b) [36]. Even though alginate is a widely used bioink, especially in vivo studies, it poses several challenges such as slow crosslinking and comparatively softer gels which lead to the formation of low-resolution constructs.

#### 3.5.1.2 Gellan Gum

Gellan gum is an exo-polysaccharide secreted by *Sphingomonas elodea*. It is usually used as a plant-based alternative to gelatine.It has the ability to gel at low temperatures around 20 °C where the random coil conformation converts into a double helix structure. Gellan Gum has been approved by the FDA to be used as an additive that can stabilize or binding processed foods [31]. But due to its low viscosity at room temperatures, gellan gum is usually integrated with gelatin-methacryloyl/gellan gum (GelMA/gellan) hydrogels with and without methacrylated hyaluronic acid (HAMA). The incorporation of hyaluronic acid (HLA) in gellan gum increased filament stability due to the interaction of the negative charge of glucuronic acid of the gellan gum and the positive charge of lysines of gelatin methacrylamide [37].

#### 3.5.1.3 Agarose

Agarose is a polysaccharide, comprised of D-galactose and L-galactose, obtained from certain types of rhodophytes. Its most common application is in agarose and now has found application in the bioink industry owing to its gelling properties. An

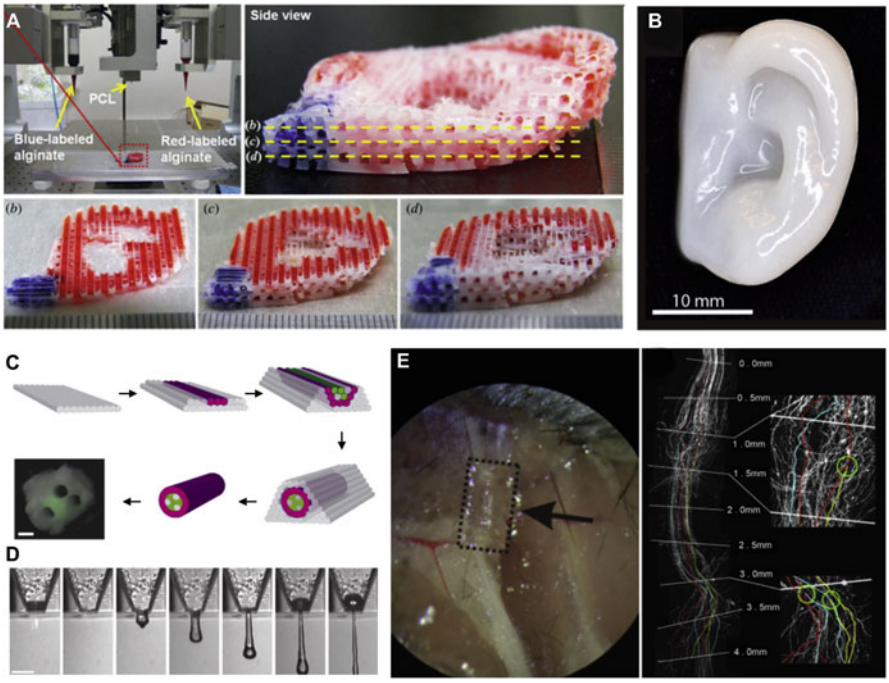


**Fig. 3.2** Types of bioprinted tissues and organs. (**a**) Bioprinted ear using polycaprolactone and alginate. (**b**) Cartilaginous ear scaffolds fabricated by integrating chondrocytes in an alginate bioink. (**c**) Schwann cells and BSMC used in creating synthetic neural tissues. (**d**) Bioprinting of ganglion and glial cells. (**e**) PEG based constructs used in the repair of neural cell. Reprinted Mandrycky et al. [15], with permission from Elsevier and Copyright Clearance Center

important aspect that improves its use in printing is the adaptability of the melting and gelling temperatures depending on the function posed.

## 3.5.2 Protein Based Bioink

### 3.5.2.1 Gelatin

Gelatin is a collagen material, usually from pork or calfskin, and has found wide application for use in engineering tissues. The physical chain entanglement of the material which enables it to form a gel at low temperatures is a dictating factor in the formation of gelatin scaffolds. However, when operated at room temperatures, the viscosity of the pure gelatin drops making it unsuitable for bioprinting applications. One approach to tackle this is by integrating gelatin with other more viscous polymers such as methylacrylamide. Gelatin Methacrylamide (GelMA) with gellan gum is a common solution for this challenge and the resulting scaffolds could be fabricated at 37 °C [38].

#### 3.5.2.2 Collagen

Collagen is one of the main proteins present in the ECM of mammalian cells and contains several RGD (Aspartic acid [R]–Glycine [G]–Aspartate [D]) domains which are essential for binding onto cells. There are several types of collagen and the most commonly used is Type 1 which can gelatinize over room temperatures. Moreover, collagen has already found applications for use in biomedical applications due to its ability to protect cells while being printed. Present research indicates collagen as a prime component in the engineering of soft tissues like skin and muscle, as well as hard tissues like bone [31].

### 3.5.3 Synthetic Polymers

#### 3.5.3.1 Pluronics

Pluronics are widely used temperature-sensitive hydrogel having a Sol–Gel transition property. This hydrogel attains a solid conformation at room temperature and converts into the liquid below room temperature [31]. Physical interactions dominate the solid phase of the gel. A long-term pluronic-based network, with increased mechanical integrity, can be formed by modifying the pluronic chain to chemically crosslink with acrylate groups [39]. This was also seen to reduce the toxicity of pluronic acid on cells.

#### 3.5.3.2 Polyethylene Glycol (PEG)

Polyethylene glycol is a hydrophilic polymer generated by ethylene oxide polymerization. It is a widely used polymer for bioinks due to its versatility of properties and has found wide application in engineering soft tissues, especially neural cell repair and tissue synthesis (Fig. 3.2e) [31]. It is not very applicable in micro-extrusion bioprinting due to its low viscosity but this can be countered by using derivates of PEG with other polymers which result in increased mechanical strength. Another added advantage of PEG is its low toxicity and immunogenicity when working with biological components.

### 3.5.4 Other Types

#### 3.5.4.1 Decellularized ECM (dECM)

Decellularized ECM based bioinks are the answer to recreating the natural extracellular matrix and can be derived from almost any mammalian tissue. Due to this, they contain several growth factors that help in the differentiation of tissues. Tissues like heart and muscle can be decellularized and pulverized to form a solid that can then be made into a gel. One disadvantage of using dECM is its low shape fidelity due to its low viscosity. But this can be countered by crosslinking with riboflavin which helps increase its mechanical integrity [40] (Table 3.3).

**Table 3.3** Types of bioinks

| Bioink | Polysaccharide based bioink | Protein based bioink | Synthetic Bioink |
|---|---|---|---|
| Description | Forms hydrogel by crosslinking<br>Mimic natural environment of cells | Tissue matching physiochemical properties<br>Derived from ECM, bones, or tendons of animals | Chemically modified with functional groups<br>Can attach domains responsive to RGD domains or to mechanical stimulus |
| Advantages | Biocompatibility, ease of gelation | Gelation occurs at low temperatures<br>Low antigenicity and some polymers contain RGD domains for binding (e.g., Gelatin) | Adjustable mechanical strength and chemical manipulation<br>Decreased immunogenicity |
| Disadvantages | Softer gels unless integrated with another polymer, lower print resolution | Gelation is a time-consuming process | No cell attachment sites |
| Applications | Vascular tissues, osteogenic and adipogenic differentiation | Aortic valves, cartilage, skin, bone | Bone, cartilage, vascular tissue |

### 3.5.5 3D Bioprinting Using Stem Cells

Since 3D bioprinting received its first-ever U.S. patent in 2006 [41], there have been quite a few developments in the field of 3D bioprinting, creating an arena that holds immense potential for artificial organ printing and regenerative medicine [1]. When discussing the cell sources to carry out this technology, it has been understood that, along with ensuring that the cells maintain their capacity to restore the biological function of the native tissue, the bioprinted cells must enlarge, but not to the limit where it can cause hyperplasia or cell lysis. In most instances, primary cell lines were seen to be comparatively more strenuous to identify and separate and they have restrictive life spans [42].

A breakthrough in the field of tissue regeneration and disease modeling was seen with the integration of stem cells which had the innate potential to self-renew and differentiate. However, obtaining autologous stem cells is a cumbersome process, which involves either bone marrow regeneration, tissue extraction, or apheresis. The next advancement in this field was seen with the integration of iPSCs [43] which gave rise to a more patient-specific cell line from just mature cells by biopsies. This breakthrough to reprogram cells to become patient-specific has paved the way for the possible treatment of genetic diseases, by bioprinting and studying them [44]. This can lead to a better understanding of how the diseases are caused and the genotypic variances and at the same time, reducing chances of graft rejection when implants are transferred into hosts for tissue regeneration.

## 3.6 Current Advances

### 3.6.1 3D Bioprinted Cardiovascular Tissue

Various bioprinting methods have been tested in fabricating cardiovascular tissue structures, using different types of proliferative cells. These tissue structures exhibit a vessel like a channel when it is grown along with endothelial cells [45]. Cardio genesis can be directed by controlling the formation and size of embryoid bodies (EBs). This can be done by using Laser direct-write bioprinting technology that can synthesize EBs from mouse ESCs [46]. On the other hand, angiogenesis can be directed by using laser-induced-forward-transfer (LIFT) cell bioprinting method, to print MSCs onto a cardiac patch and improve cardiac function [47]. Another method applicable in creating tissues with cardiogenic potential is the Extrusion bioprinter that prints human cardiac-derived cardiomyocyte progenitor cells onto a polysaccharide based scaffold [48]. These cells were seen to have high viability and commitment to the cardiac lineage as well as an improved expression of transcription factors. A recent study focused on creating a bioink using decellularized ECM taken from heart tissue and incorporating it in extrusion bioprinters. In another experiment, it was seen that a similar decellularized ECM bioink synthesized using the heart tissue, loaded with muscle cells, was used to 3D print heart tissue using micro-extrusion bioprinting [49]. These organs printed on decellularized ECM showed an increased life span and improved cardiac-specific genes than those constructs that were bioprinted using collagen bioinks.

#### 3.6.1.1 Applications

In most heart failure cases; a transplantation procedure is the only viable treatment. So an approach based on bioprinted tissues using stem cells will be a huge improvement for high throughput toxicology screening and more effective research, drug discovery, and regenerative therapies for cardiac diseases [50, 51]. As of now, pre-clinical studies are carried out for bioprinted myocardium grafts in the hope that they might, someday, provide economically feasible remedies for myocardial infarctions [45, 52]. This field of research can offer several possibilities when it comes to treating valvular diseases, as bioprinting can help in accurately recreating heart valves and this has a huge clinical implication when it comes to surgical processes as well as in regenerative therapies [53].

#### 3.6.1.2 Limitations

When it comes to the heart, it is a complex and dense tissue that requires a detailed vascular system and biocompatibility. Thus, a more intense study is required to generate a vascularized heart tissue with an appropriate density that can accurately respond to electrical stimuli and keep up with the necessary beating pattern. This is most essential since the heart is a metabolically active tissue [52]. Heart valve engineering poses several design issues and a deeper study in printing this is necessary to print biologically acceptable valves. Also, isolating and obtaining these cells is not an easy task, and hence, a further study into using stem cells to

construct heart valves is essential. Also, studies should be carried out to look into the printing of next-gen constructs that help in the synchronous heartbeats of the bioengineered tissue by integrating aspects to it that can increase its ability to sense and respond to stimuli, which will enhance the imitation, for example, the integration of carbon nanotubules in hydrogels [54].

### 3.6.2 3D Printed Musculoskeletal Tissue

Various bioprinting techniques are used for the construction of musculoskeletal tissue using stem cells. Precisely patterned 3D skeletal muscle has been constructed using C2C12 myoblastic cells which has unlimited growth and differentiation capabilities into multinucleated myotubes [55]. These bioprinted cells showed increased viability and synchronous response to electric pulse [56]. Inkjet bioprinting is used to incorporate GFs onto micro-porous polymer fibers that were synthesized using electrospinning [57]. This helps EFs to specialize in different cell lines that can help in the fabrication of muscle-tendon-bone tissue. Based on growth factor patterning of BMP-2 onto fibrin-coated glass slides, inkjet bioprinters are employed in enabling the specialization of primary myoblasts into osteogenic and myogenic cell subpopulations [58].

#### 3.6.2.1 Applications

Bioengineered muscle cells that can be activated by actin–myosin motors, to generate force, have found widespread applications to create microelectromechanical biological devices, for example, bio-motors, actuators, heart pumps, and biosensors. These novel bioengineered microdevices can be created by conjugating the devices with bioprinted skeletal muscle tissue and regulating the action by excitation–contraction coupling [55]. Skeletal muscle cells constructed using C2C12 cells have shown to have a high imitation of the musculoskeletal properties seen in the body [59]. And though these cells have found a wide application when incorporated with bio-microdevices, it is necessary to ensure that these devices are constantly conjugated to produce reliable results. These applications have paved the way for a deeper study of the principles of cellular development and mechanisms that enable a more innovative bio-inspired approach to improve the treatment design for mesenchymal stem cells (MS) diseases and trauma as well as for regenerative therapy [57].

#### 3.6.2.2 Limitations

Proper alignment of bioprinted tissue is essential to maintain functionality and recreate biological functions such as contractile force generation by myocytes [57]. Though current studies conducted, to evaluate the conjugation of microelectromechanical system devices with bioprinted skeletal muscles and the response of the cells to electrical impulses have proven to be successful, more research has to be done to study the biomechanical functioning of fabricated organs. Another aspect is the vascularization of the skeletal tissue, and though they have not proven to be successful yet, studies are being conducted to perfuse the fabricated

scaffolds and enable the development of neuromuscular junctions. Recently, a group of scientists used the stereolithographic printer to create poly (ethylene glycol) diacrylate (PEGDA) hydrogel-based biological robots, or bio-bots, that could create neuromuscular junctions in vitro when integrated with neurons and showed impulsive movement when they were grown with rat cardiomyocytes [60].

### 3.6.3 3D Printed Bone

Bone is one of the few tissues known for its self-healing capabilities, yet, beyond a critical size, it cannot heal itself completely without external intervention. One of the major causes for bone repair and implantation is high impact trauma and tumor resections. But these implants fail due to several reasons such as repetitive loading. This brings in the need for an engineered tissue that can be modeled into a bone, at the same time, maintain its functionality.

A breakthrough in this field occurred when hydrogel-based bioinks were used in inkjet bioprinters to print tissues at high resolution. The use of hydrogels, like fibrin or alginate, increased the chances of biocompatibility of the newly engineered tissue constructs. But a drawback of this technique was the compressive modulus of the scaffolds was less than 5 kPa and this was not ideal. Hence a photopolymerization technique using synthetic polymeric hydrogel was developed and this resulted in constructs with a compressive modulus of >500 kPa and is of the same order of magnitude as human musculoskeletal tissue [61]. PEG hydrogel has an added advantage of promoting cell viability and ECM production. Isolated human mesenchymal stem cells (hMSCs) were seen to promote osteogenic growth during monolayer growth and hence are preferred in skeletal tissue reconstruction in orthopedic engineering.

Other materials that promoted osteogenic growth are bioactive glass and hydroxyapatite. And it is observed that the integration of hMSCs and poly (ethylene glycol) dimethacrylate (PEGDMA) along with the above materials is used to develop homogenous bone constructs [62]. An added biochemical test of these constructs showed a higher total collagen production and alkaline phosphatase activity than those constructed with hyaluronic acid (HA) or bioactive glass (BG) (Fig. 3.3a).

HA is seen to stimulate hMSCs osteogenic differentiation ECM production whereas decreasing cell toxicity at the same time. In conjunction with cartilage bioprinting, these techniques can hold a huge potential for the fabrication of osteochondral interface tissues, which is a pivotal point in bone tissue engineering.

#### 3.6.3.1 Applications

Bone defects and tissue loss due to infection, trauma, and tumor resection lead to a huge demand for bone substitutes for repair and replacement in the field of orthopedics. Bone tissue engineering involves the use of binders, i.e. bone scaffolds with required osteoconductivity and necessary mechanical strength to withstand the growth of the tissue. CT and MRI scans can be used to image the bone defects in

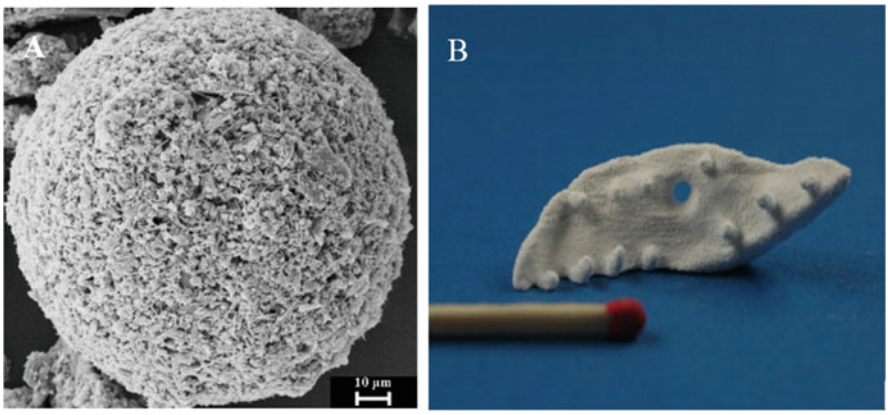


**Fig. 3.3** (**a**) SEM micrograph of scaffold made by integrating bioactive glass with β-tricalcium phosphate in a homogeneous manner. (**b**) 3D bio-printed implant fabricated using CT data. Reprinted from Bergmann et al. [63] with permission from Elsevier and Copyright Clearance Center

the body which can then be used as a template for 3D bioprinting [64]. Present research on bone bioprinting involves integrating hydroxyapatite and methacrylated gelatin (GelMA) in the bioink which results in increased bioactivity of gelatin. The use of hydroxyapatite, which is a major component of bone mass, leads to a higher cytocompatibility of the ink and supported osteogenic differentiation of the cells, due to the collagenous origin [65]. Another study integrated hMSCs in different types of collagen–agarose matrices to observe the type of bioink required for osteogenic differentiation. It was seen that the collagen matrix was able achieve this as osteogenesis requires soft collagen rich substrates [66].

#### 3.6.3.2 Limitations

One of the major challenges faced in the field of 3D bioprinting is the ability of the construct or implant to mimic the in vivo microenvironment. Scaffolds need to be constructed such that they can hold up, from the time it is implanted into the body till the bone tissue can remodel itself. The materials used also play a huge role in determining the efficacy of the construct. A material with increased mechanical strength results in decreased porosity. Another major issue is the declined rate of repair in an older person as compared to the fast healing process in a younger individual. Some materials that have shown promising in vitro potential give out in the body microenvironment due to lack of vascularization. This is one major issue that needs extensive research to be curbed [67].

## 3.7 3D Printed Neural Tissue

Several bioprinting methods have been able to bio-print neurons with success in developing the voltage-gated potassium and sodium channels [68]. Neural stem cells (NSC) are an essential part of the development of neurons and have found application in fabrication of 3D tissues with precise spatial patterning, as differentiation of these cells is administered by active macromolecules and transcription factors (TFs). Glioma stem cells are seen to be underlying reasons for high-grade glioma, and now they are being used to create glioma tumor models [69]. It has been observed that mesenchymal stem cells (MSCs) grown on softer matrices gave rise to neurogenic cells, whereas when cultured on stiffer mediums, it promoted the growth of myogenic and osteogenic cell differentiation [45].

Several researchers have illustrated the functional application of patterned scaffolds in directing the phenotypic growth of the tissue. For instance, imprinting poly(methyl methacrylate) substrate on linear patterns using nano-lithography causes astrocytes to grow in radial glia like manner, in the absence of biochemical factors [70]. It is thought that perhaps the patterned scaffolds mimic the embryonic neural stem cell niche, causing the cells to favor a regenerative response. In one certain experiment, a bioink comprised of natural polymers was used to bio-print NSCs through micro-extrusion. This bioink led to the formation of a porous scaffold which enabled specialization of the stem cells, in situ, into a functioning neural cell [71].

### 3.7.1 Applications

Bioprinted neurons can help ease the way for the study of how neural cells work, their bio-mechanisms, and disease progression. It can also enable translational drug screening in vitro. In cases of a chronic degenerative disease or acute traumatic injury, this method finds application for central nervous system (CNS) tissue replacement [72]. As mentioned, patterned printing can be used to research more in detail on the brain and neural tissue cancer. This tackles the challenges placed by chemotherapy drug resistance, and thus using disease models that mimic the tumor microenvironment can be used to improve present therapies and develop more effective regenerative treatment methods [69]. Bioprinted tumor models can be used to assist in personalized therapies for patients and developing individualized drug resistance and susceptibility tests.

### 3.7.2 Limitations

When fabricating the tissue constructs for neural tissue synthesis or for cell repair, the bioink plays a crucial role in ensuring the success of the 3D print. Optimization of the bioink must be carried out to develop a more complex neural tissue structure. The thickness of the scaffolds means a higher gel modulus and this can secure the

stability of the structure even after multiple layering. But a study conducted showed that a thicker scaffold leads to a decreased cell viability which can affect the neural tissue development [72].

## 3.8 3D Bioprinting for Drug Discovery and Delivery

The successful invention and manufacture of any commercial drug require a huge investment of time and financial resources which can prove to be catastrophic if it leads to late-stage failures. This creates a space to infuse the constantly changing innovations in technology which can lead to a prediction of the efficacy of the drug as well as study the toxicology of the compound well before the clinical trials. Due to the ability of 3D tissue models to mimic the spatial and chemical characteristics of native tissues, they have proven to provide a better platform for drug screening than 2D models.

### 3.8.1 Drug Discovery

Cell-based drug assays, at present, utilize cells attached to a smooth stratum made of either glass or polystyrene, i.e. the 2D monolayer culture method. This time and tested method have proved to be valuable in several cell-based studies; however, it fails to replicate the 3D environment in vivo, with the surrounding cells and ECM. Therefore, two 3D culture systems were developed, that includes scaffold and scaffold-free systems [73]. The former type of system can mimic the characteristics of the cells in the body and is made of biocompatible substances like the ECM or polymers, that can exhibit mechanical and toxicity properties [74]. Different techniques are used to construct in vitro tissue models, like hydrogel culture, hanging drop method, and microwell based method [75]. To date, 3D constructs of disease models like pulmonary edema and cancerous growth have been fabricated for target identification and drug discovery and optimization of assays and high throughput screen (HTS) for use in various fields [76]. Looking at the intricacies involved in vivo, creating a biomimetic structure with necessary stimulations for its proper functioning is still a hurdle. Right now, only some 3D structures are ready for industrial drug optimization [77, 78]. One major limitation is the integration of different types of cellular materials resulting in statistical discrepancy than two-dimensional models. Another problem is that ECM matrices derived from natural polymers exhibit batch-to-batch variations which result in inconsistent experimental results [79]. However, the most prominent limitation is the lack of vascularization, which determines the cell behavior as it controls the transport of the oxygen, nutrients, drugs, and intercellular factors throughout the 3D structure [80].

#### 3.8.1.1 Genetic Engineering Applications

The thermal inkjet bioprinter was seen to disrupt the cell membranes for a brief moment, creating temporary pores that allow the movement of nucleic acids. The pores then closed after some time, maintaining cell viability. This micro-disruption

of the cells creates a passage for the genetic material in the plasmid to cross over in the orifice of the print head. This allows the genetic material contained inside the droplets to be spatially delivered within the target sites of the matrix [81]. Alginate based constructs loaded with MSCs and calcium phosphate were extruded in porous or solid shape. And then, the non-viral plasmid DNA that encodes for a bone morphogenetic protein (BMP-2) was genetically transplanted into cells. Two weeks post culture, the bioprinted structures with BMP-2 plasmids displayed a higher osteogenic differentiation percentage than non-transfected cells, as seen when comparing alkaline phosphatase (ALP) activity and osteocalcin [82]. Another group of scientists developed an inkjet based method of immobilizing the "solid-phase" pattern of growth factors (GF) on biomaterial substrates [57]. Controlled drug delivery is an important aspect of generating physiologically-relevant tissue models. For example, only when microscopic collagenous material, encapsulated with vascular endothelial growth factor (VEGF) was formed and integrated to 3D constructs of human endothelial progenitor cells (hEPCs), the possible ways to extend the vascular action of the VEGF at the specific niche was recognized [83]. Multiple biomolecules were delivered with different release profiles by using compounded constructs as a combined arrangement, such as sodium alginate by the extrusion-based printer and gentamicin sulfate through electrospun polyvinyl alcohol (PVA) nanofibers and deferoxamine was integrated into an electrospun core/shell [84]. It was also seen that vertically graded porous structures enabled the delivery of deferoxamine (DFO) in a sustained manner over a longer time frame. This showed that composite scaffolds could be used for achieving different release profiles independent of each drug, through manipulation of the struts and nanofibers [84].

## 3.9 4D Bioprinting

### 3.9.1 Introduction

The technique of 3D printing has done immense contributions in the areas of bio fabrication like tissue engineering and regenerative medicine [1–3]. This technique is quite old and was first proposed by Hull and co-workers in the year of 1986 [85]. Presently, 3D printing is a widely used technology that covers almost all aspects of applications in science and engineering fields. 3D printing opened a gateway to many novel materials, techniques, and devices which got instant commercialization and wide recognition all over the globe. In today's world, 3D printing technology has a strong grasp in the majority of advanced manufacturing labs and research communities all over [5–10]. 3D printing has been categorized into different types based on the types of extrusion process approaches, namely inkjet, micro-extrusion, and laser-assisted printing techniques. The technique functions using a special type of resins and plastics to make 3D structures until more recently cell embedded bioink with bioactive agents in the form of hydrogels came into the picture and established the foundation of bioprinting. Overall this technique can deposit cells at high density and can function with a wide range of viscosities of

bioink [1]. 3D bioprinting strategies have also been combined with microfluidic platforms for achieving precision for regulation bioink flowrate and optimal resolution [86, 87], thus making it possible to print multi-material heterogenous and biomimetic tissue constructs like blood vessels, liver, heart, and tumors [12, 13, 16]. The native tissues have the property of responding to the dynamic microenvironment that exists three-dimensionally surrounding the tissue construct. The response generated due to these stimuli can be a secretory response from the tissue or contraction–relaxation response. For example, the brain emits electrical signals that initiate musculoskeletal movement, sequential peristalsis in case of esophagus and gut, relaxation and contraction movement of smooth muscle walls for transmission of food bolus along [17–19]. These tissues have built-in dynamic actuators that help in conformational changes as a response for a particular stimulus. This is where the conventional 3D structures fail to deliver dynamic properties similar to that of native tissues and 3D bioprinting is not having the essential technology to deliver a more appropriate biomimetic tissue construct for the use in tissue engineering and regenerative medicine. This limitation marks the development of a new technology called 4D bioprinting, which has its foundation from 3D bioprinting but also comes with imparting the ability of conformational flexibility in a controlled manner. This strategy of generating stimuli-responsive 3D scaffolds takes 4D bioprinting one step ahead of 3D bioprinting when it comes to mimic not only the natural structure of native tissues but also their response dynamics. 4D bioprinting has evolved from 3D printing limitations and other conventional fabrication limitations. Scaffolds with encapsulated cells generated using 4D bioprinting have the potential to grow, develop, and functionalize under applied physical, chemical, and biological stimuli as shown in Fig. 3.4 [88]. So far 4D bioprinting has shown great progress in numerous applications in the field of biomedical engineering, tissue engineering, regenerative medicine, and drug screening. Fabrication of blood vessels is one of the most persisting limitations when it comes to bioprinting and 4D bioprinting has shown a promising approach to develop one. It can be done by printing a flat structure and then giving it a suitable stimulus to enable it to roll it into a cylindrical structure similar to that of blood vessels. Apart from this, another important application of 4D bioprinting is generating tissue constructs required for studying drug effects. To date, this technology has drawn the attention of many scientific communities all over leading to many new advances in this field. Meanwhile, with recent progress in material science, printing technology and biofabrication have immensely boosted the advancement of 4D bioprinting and its applications.

#### 3.9.1.1 Challenges Associated with 3D Bioprinting

3D printing is a highly multipurpose technique that has extensive usage in many areas of fabrication and biomedical engineering. But it also comes with some limitations particularly in the field of biomedicine. The successful development of a three-dimensional construct using 3D bioprinting technology requires a proper understanding of the material (bioink) properties. Certain important parameters like cell viability and print resolution define the aptness of a considered bioink as these materials contain cells that can lose their viability and functionality under shear

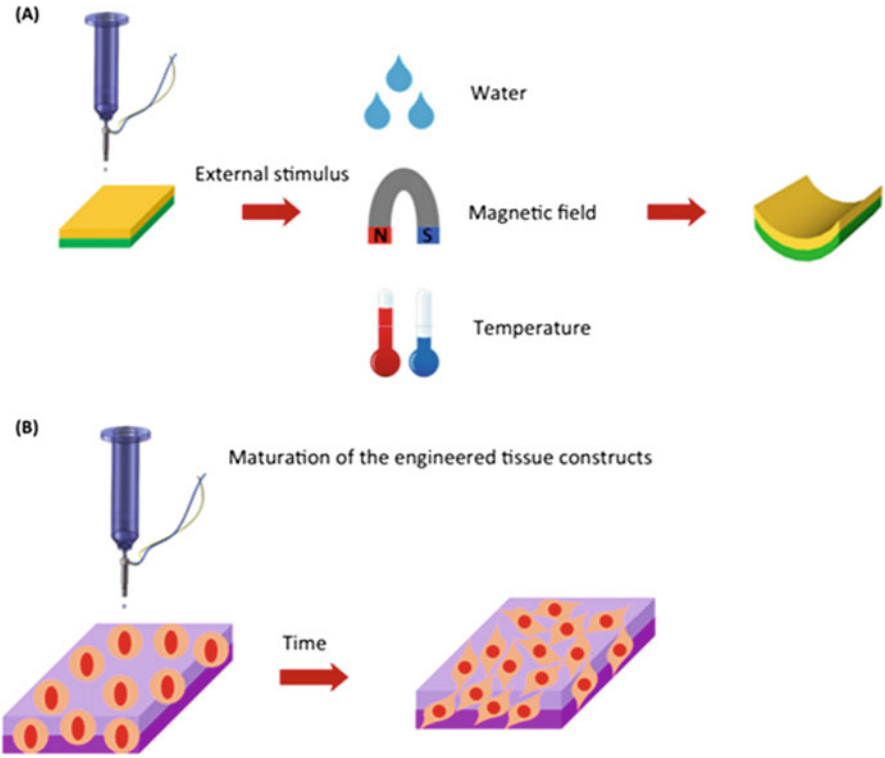


**Fig. 3.4** Diagrammatic representation of 4D bioprinting technique (**a**) Deformation of stimuli-responsive hydrogel under the presence of different types of stimuli. (**b**) Development of 4D bioprinted tissue constructs. Reprinted from Gao et al. [89], with permission from Elsevier and Copyright Clearance Center

stress during the printing process [90]. Bioinks with low viscosity often have heterogeneous cell densities [91], whereas the one with high viscosity offers high flow resistance and shear stress that can cause a reduction in the number of viable cells [92]. The development of a three-dimensional scaffold with high positional complexity is still the most persisting limitation in this technique. Most of the 3D bioprinting platforms presently available perform bioprinting using a single variety of bioink during each fabrication process. This limitation requires the blending of bioinks in required proportions to achieve swift concentration gradients present between two types of tissues or addressing the complexity of a tissue. The two above limitations are still open for development and upgrades but the following limitation can be effectively addressed using the novel 4D bioprinting. To achieve the closest proximity of mimicking native tissues the fabricated scaffolds must be stimuli-responsive in dynamic proportions. This desired trait is not possible to achieve using the conventional 3D bioprinting as its output constructs are static once they have been printed. If these scaffolds are deposited inside the body, create

positional contrast in comparison to surrounding tissue which is responsive and shows conformational flexibility [24, 25]. Considering an example of heart that has a four chambered design which on receiving an electrical stimuli undergoes rhythmic contraction enabling it to pump blood throughout the body similarly brain can stimulate musculoskeletal system movements using electrical signals. Besides electrical stimuli there are other biochemical signals that generate dynamic changes in tissues. The conventional 3D bioprinting does not suffice these dynamic modulation to tissues [34]. In 4D bioprinting which is considered as an upgrade to 3D bioprinting where "time" is added as the fourth dimension should not be confused with "duration of bioprinting process" but rather the fabricated scaffolds with embedded cells continue to proliferate and evolve gradually even after the completion of bioprinting process [89]. Thus to fill in the void of mimicking tissues to an extent of generating conformational changes in bioprinted scaffolds 4D bioprinting is the required option.

## 3.10 Transition to 4D Printing and Bioprinting

The fourth dimension that comes with 4D printing is the shape alteration ability similar to that of protein folding and origami that employ directed transformation. These complex mechanisms are an interesting phenomenon and are expected to develop into 4D bioprinting in the later future [24–30]. Simple design that comprises the 4D printing and can be adopted in bioprinting is the deposition of heterogenous materials having differential properties like swelling, for example, can determine the folding and unfolding conformational dynamics. Tibbits and the team explored both design and engineering aspects of 4D printing and inferred that material programmability, multi-material printability, and scrupulous designs ensuring accurate transformations are some essential requirements [93]. Recently Lewis and team developed a bioink that is printable hydrogel with the differential swelling property. The bioink consists of hectorite clay, cellulose nanofibers, and monomers of N-isopropyl acrylamide and these ingredients contribute to shape morphing ability which when stimulated, displays a conformational change in structure [94]. Besides swelling, a team lead by Studart has demonstrated conformational flexibility using magnetic materials. The designed bioink consists of aluminum platelets doped poly (urethane acrylate) (PUA) which are responsive to externally applied low magnetic fields [95]. The aluminum platelets embedded inside the printed architecture can undergo directed pattern transformation once stimulated with an external magnetic field. Innovative examples similar to the ones stated above have paved a promising approach demonstrating the incorporation of the time factor in 3D printing. Because 4D printing is still a novel technique in the world of biofabrication there is a void space in understanding and implementing the processes that can help in generating a three-dimensional tissue construct having the potential to that of native tissues present in heart muscles involved in pumping activity, tissues in esophagus and gut involved in the peristaltic activity, and so on. Biomaterial research communities

still have to deliver a library of stimuli-responsive cytocompatible materials specifically for 4D bioprinting to meet the biomedical goals for treatment.

## 3.11 Approaches and Definition

4D bioprinting has three approaches as suggested by Mironov and co-workers in 2014 and is completely different from each other as shown in Fig. 3.5. In the first approach, a smart material or responsive biomaterial on stimulation changes its structural conformation seeded by the user. This approach was developed in MIT and cells embedded in the smart material simply utilize the framework and proliferate to generate a tissue construct. The second approach is the type of "in vivo 4D bioprinting" method where a 3D printed construct is first implanted inside the body which gradually houses a growing tissue or organ after surgery. As the tissue or organ gains strength simultaneously the 3D printed construct loses its strength but maintains its shape. The medical device in due course keeps degrading until it finally absorbed. The third category of 4D bioprinting undergoes self-assembly or self-organization on demand by their microenvironment. In this approach microdroplets containing cells are arranged in a particular order which in due course changes their structure by undergoing self-assembly. The exact triggering mechanism is yet to be understood at this current stage. Thus, considering the three above mentioned categories or approaches of 4D bioprinting it is difficult to reach on a consensus in determining the exact form of this technology. Based on these observations Mironov and team concluded defining this technique as "groups of programmable self-assembly, self-folding, or self-accommodating technologies which include three main defining or essential components:

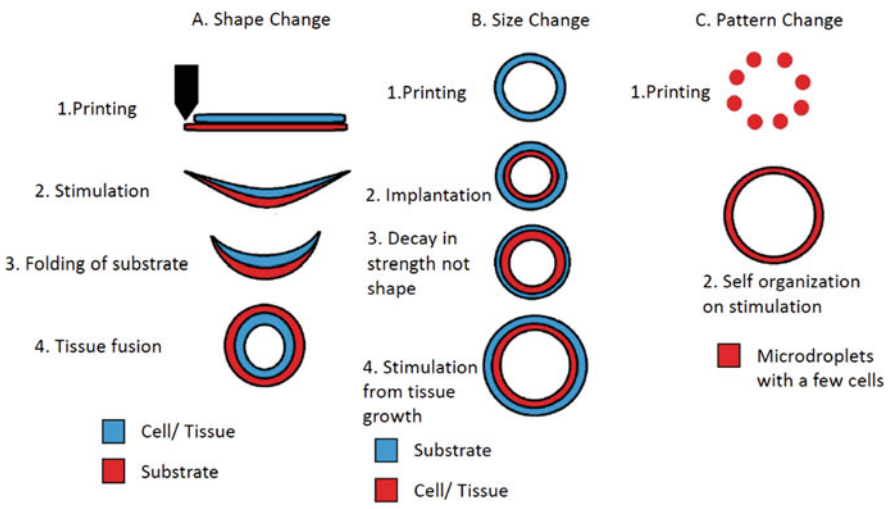


**Fig. 3.5** Three approaches in 4D bioprinting

1. Man-made and not nature-made programmable design,
2. 2D or 3D bioprinting process,
3. post-printing programmable evolving of bioprinted constructs that could be driven by cells or biomaterials and triggered by external signals" [88].

There are distinct differences between 3D Bioprinting and 4D Bioprinting techniques of biofabrication most important being predefined conformation change on being stimulated. 4D bioprinting can also be considered as the combination of 3D Bioprinting and bioreactor provided the transformation in tissue construct or cells is predetermined. This technology is rapidly evolving and with this, the differentiation between 3D bioprinting and 4D bioprinting is also increasing. Presently 4D bioprinting is limited to changes in shape, size, and pattern but it can be extrapolated to a future where apart from undergoing macrostructural changes 4D bioprinting will deal with parameters like microstructure, property and even functionality of the bioprinted construct.

### 3.11.1 Mechanism of 4D Bioprinting

The most defining feature of 4D Bioprinting is the ability to alter size, shape, and to some extent functionality. The variation in size of 4D printed constructs is given by swelling and shrinking parameters, whereas in case of a change in shape the constructs can deform into geometries differing from its basic shape. Similarly, in case of a change of functionality, the cells process the property to initiate various cellular functions like cell fusion, cell assembly, and other biological activity. All these changes require an external stimulus to dictate the necessary actions.

### 3.11.2 Types of Stimuli

4D bioprinting is conducted using stimulus-responsive material which is also called smart material which can detect external stimulus which can be physical (water, temperature, light, electric and magnetic field), chemical (pH value and ion concentration), or biological (glucose and enzymes) in nature.

Humidity or water is an element that was first used in 4D bioprinting to recreate a stimulus. This requires a water-sensitive material on the other end to give the necessary response. An example of water based stimulus can be seen in Fig. 3.6. On exposing these materials to a stimulus inducing surrounding they can intake and expel water as a response. Due to which subsequent response is generated in the form of swelling, twisting, bending, and other deformations. A similar working model was designed by Zhang and co-workers on the modification of cellulose with stearoyl moieties [96]. This composition was used for developing a film that had a non-uniform moisture absorption coefficient. This differential moisture absorption potential was able to create a response in the form of bending of the film when

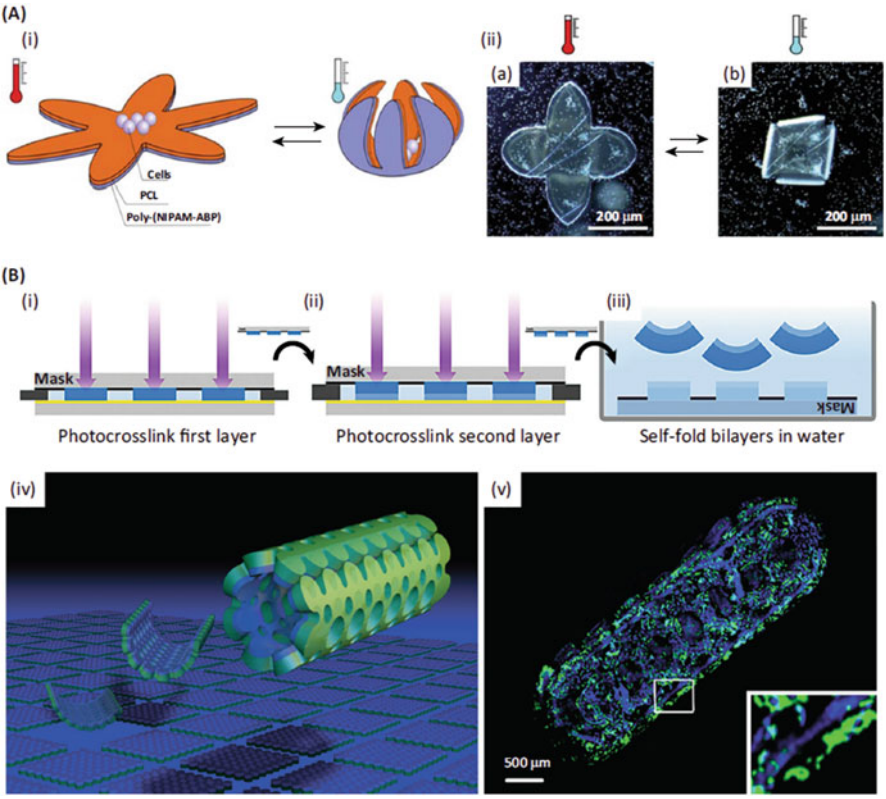


**Fig. 3.6** Mechanism of conformational changes shown by the materials as a response to stimuli (**a**) Temperature based deformation (i) A flower shaped printed polymer bilayer containing cells undergoes conformational change on decreasing the temperature. (ii) On increasing the temperature, the petals unfold and open up releasing the cells inside. (**b**) Water based deformation (i–iii) Development process of PEG bilayer. (iv) Folding process of PEG bilayer in response to water. (v) Fluorescent image having fibroblasts stained with Hoechst stain [blue] present on the inner side of the printed construct and the outer side contains fibroblasts that are stained with calcein AM [green]. Reprinted from Gao et al. [14], with permission from Elsevier and Copyright Clearance Center

exposed to a surrounding having a varying percentage of moisture. Lewis and co-workers used cellulose fibrils along with acrylamide together to form a mixture. This mixture was exploited to 4D print a flat object that can undergo deformations into various geometries like flower petals and so on when immersed in water [94]. Apart from water, other materials have been used for 4D printing like photocrosslinkable material soybean oil epoxidized acrylate that is responsive when immersed in ethanol. The differential crosslinking of the material at different depths leads to non-uniform deformation throughout the structure. Temperature is a desired stimulus in the case of thermo-responsive materials. Materials that display

shape memory property are some of the notable examples. In this category, the deformation is caused due to a variable internal thermal stress within the fabricated model. Constructs designed using these materials deform and regain the original shape on exposing them to specific transition temperatures. Reversible deformation is common in the case of thermo-responsive material and these materials have acquired a wide range of usage in 4D bioprinting. An example of temperature based stimulus can be seen in Fig. 3.6. Polyelectrolytes that come under the category of electric field responsive materials possess the ability to modulate their swelling property to create swelling, shrinking, and bending conformational changes depending on initial structure and the electric field applied [97]. Poly(acrylic) acid is a typical example of this technique that shows fast and precise shrinkage in the electric field when exposed to electrophoresis and electroosmosis. Light is also a convenient and widely used stimulus as it can be regulated both spatially and temporally with high precision. The underlying mechanism is termed as an optothermal effect that leads to a change in temperature on the absorption of light [98]. Magnetic field responsive materials like composite magnetic nanoparticles can be used to regulate material properties within which they are embedded in the microscopic level. Kokkinis and co-workers embedded stiff magnetic platelets in inks and modulated particle orientation on applying a low magnetic field [95]. Deformations can also be induced directly by utilizing external forces like cell tension force and surface tension. Takeuchi and the team used NIH-3T3 cells to deform an initially flat parylene C microplate using cell traction force. The previously flat microplates folded into cylindrical tubes within a few days [99]. Similar to this, surface tension can also initiate deformation in 4D bioprinted models. Li and the team took a triangular soft film and used a bioprinter to deposit droplets on it. Gradually the liquid evaporated that resulted in inducing surface tension at the edge of the film leading the folding of the film into a pyramid structure [100]. Apart from using physical stimuli, chemical and biological stimuli also play a significant role in developing a required response. Some polymers like keratin, gelatin, and collagen are pH-sensitive. The polymers undergo a conformational change in their chain structure from global to coil form causing swelling, stretching, and bending. Huang and the team printed various models using potassium-3-sulfopropylmethcrylate that showed reversible conformational change by regulating the ionic concentration [101]. Some materials that are used to generate biological stimuli are responsive to glucose or enzymes and are thus used to detect physiological parameters in vivo after fabricated in various structures.

### 3.11.3 Applications of 4D Bioprinting

4D bioprinting has started a wave of innovation all around the world. Because 4D bioprinting is still in its infancy, a lot of fascinating ideas are pouring in to take 4D bioprinting to a stage where it can be applied for biomedical applications [102]. Recently 4D bioprinting has shown immense potential in solving some prevailing challenges such as microscale vascular models, drug delivery systems,

wound healing, and so on. 4D bioprinting can mimic dynamic changes similar to that of native tissue and due to this property, it has convinced many tissue engineering and biomedical research communities all around the world that it can be a better replacement to sync with body physiological parameters. Few successful examples have been discussed in the following literature that has been designed recently.

#### 3.11.3.1 Stents

Stents can be defined as small hollow tube-like constructs similar to that of artery or veins that help in the transfer of components like blood or urine from one place to another. Previously stents were fabricated using bioprinting and had to be surgically implanted at required sites but after the advent of 4D bioprinting, there are many strategies in development with the potential of minimally invasive surgery. The 4D printed stents are comparatively smaller in size and when implanted at the site of requirement on receiving the necessary stimulus they undergo self-deformation to attain proper size and shape thus minimizing the invasiveness of the surgery.

Ionov and team developed 4D bioprinted stent from a shape morphing biopolymer hydrogel having a minimal diameter of 20 μm. The deformation mechanism is based on calcium ion ($Ca^{2+}$) concentration. On changing the concentration of $Ca^{2+}$ ions, the polymer shows reversible conformational changes. The hydrogel shows high cell viability for 7 days [103]. Ge and co-workers designed a stent that had high conformational flexibility. At the initial stage, the designed stent had a small diameter and after transplanted into a vessel, the thermo-responsive material of stent changed its conformation to its original shape having a bigger diameter [104].

Stents have been used widely for vascular stenosis but recently stents have also been applied in other endoluminal structures in the body. The trachea is also a type of endoluminal component in the body and under certain disease conditions, it also develops stenosis. Cohn et al. designed a smart stent that is thermo-responsive and has shape memory ability. The base model of the stent is small in size due to which its implantation is comparatively easy and with a customized design, the stent has the potential to reduce migration of stents that causes frequent stent deployment failure [105]. Shape memory polymers are responsible for the development of smart materials and this ability is immensely essential for a broad range of biomedical requirements. Bioprinted stents have shown promising features in many aspects of surgical treatment techniques but better biocompatibility is still a demanding area that requires significant scientific attention.

## 3.12 Drug Delivery

As 4D bioprinted scaffolds can be transformed into multiple conformations with the required stimulus, this ability gives it the advantage to be a potential drug delivery medium. Biological systems that are utilized for controlled drug delivery can be categorized into three categories, namely directly activated, progressively activated, and self-regulated [106]. Ph responsive bioprinted dermal patches containing drugs were designed by Akbari and co-workers which on detecting a change at wound site

released the drugs to prevent infection [107]. 4D bioprinting has great potential to be used for designing a replacement for heart valves and these replacements can be embedded with drugs to be released when appropriately triggered. Apart from these examples, many other notable works have already entered clinical trials and have tremendous potential to impact the quality of life. At present 4D bioprinting is at the nascent stage and it requires more research to validate its potential for robust clinical safety and efficacy along with scaling up of the manufacturing process.

## 3.13 Wound Healing

4D bioprinting has shown promising innovations to target wound healing ranging from fracture and damage of bones, muscles, and nerves. Recently a bioprintable scaffold was designed for treating bone aberrations having good cytocompatibility and polymorphic properties. The scaffold was embedded with human adipose-derived stem cells, and when exposed to physiological body temperature, the scaffold promoted bone growth by activating protein production, expression of necessary genes, and depositing vital minerals at the site of the bone defect [108]. Apart from treating bone defects, 4D bioprinting is also used to print cartilage tissue especially articular cartilage that plays a vital role in joint movements. Betsch and team designed cartilage tissue by using magnetically aligned collagen fibers. It was the first time when multi-layer bioprinting was conducted using human knee articular chondrocytes [109].

Severe injuries lead to nerve damage that stalls the wound healing procedure. 4D bioprinting has good potential in treating such damaged nerves reinstating good electrical conductivity and nerve regeneration. Similar work has been done by Zhang et al. fabricated a closed conduit scaffold that can be temporarily opened and fixed, facilitating the implantation process of the conduit. The printed material was made from graphene and soyabean oil mixture epoxidized with acrylate. The conduit was embedded with human mesenchymal stem cells, which can differentiate into neural cells [110]. Wound repair is a new domain in the area of 4D bioprinting and it requires more test run to ensure biocompatibility and in vivo efficacy of the approach. 4D bioprinting has the potential to develop a novel approach in the field of biofabrication and also to revolutionize tissue engineering and regenerative medicine. Tissues in the human body are dynamic, plastic, and possess a specific function. Conventional 3D printed scaffolds do not possess the structural flexibility and responsive property similar to that of tissues. On the other hand, 4D bioprinting flawlessly meets this requirement by mimicking tissues to a great extent and also derives responses from physiological stimuli generated by the body. 4D bioprinting can also be considered for bioactuation, soft tissue robotics, and biosensing. Nevertheless, this technique is still in the proof of concept stage and requires more research to understand the clinical significance of this approach. To date, apart from the mathematical model, there is no computer model to help us understand the deformation kinetics. Models printed require heat and trial approach to understand the folding kinetics and to modulate it as per the requirement. Another challenging

part is the development of biological stimulus-responsive material in the form of bioink. Native tissues are exposed to a variety of multiple stimuli simultaneously and in comparison to this functional complexity, 4D bioprinted models have a long way to go as they are responsive to a very limited stimulus. 4D bioprinting requires more understanding and contributions from material engineering, printing technology, and computer-based numerical modeling. The era of 4D bioprinting has already arrived and awaiting to unfold more innovations that can revolutionize biomedical sciences to a great extent.

## 3.14 The Socio-Ethical Outlook of 3D Bioprinting

The future belongs to an era where scientists can create or biofabricate personalized organs for individuals using a process called as three-dimensional (3D) bioprinting [111, 112]. 3D bioprinting is the biological version of the famous rapid prototyping technique 3D printing, which is used widely for small scale manufacturing and do-it-yourself (DIY). The development of this printing stems way back to the twentieth century with the development of photocopier and inkjet printers where the hardware component was optimized. While 3D printing is used widely for constructing models with inorganic components like polylactic acid (PLA) or acrylonitrile butadiene styrene (ABS) plastic, 3D bioprinting is used to design biological scaffolds that can replace native tissues. These bioprinters use specialized inks called bioink, which contain living cells like differentiated, human embryonic, or induced pluripotent stem cells for fabricating replacement biological scaffolds. Similar to an era when printing technology was newfound, the biological constructs will also impart same innovative and democratizing effect as that of book printing in its applicability for tissue engineering and regenerative medicine, hinting to a future where artificially designed personalized biological body components will be available as text in this modern society.

Bioprinting in long term can be a revolutionary technique, generating artificially designed biological organs without the need for organ donation from active or deceased individuals. Considering the research point of view of 3D bioprinting, it has been well studied and established in my countries showing good advancement. But considering the commercialization sector and medical applicability of this technology, it still requires more promising and long-lasting results. Drug screening has achieved good progress utilizing this technology with embedded lab-on-chip, as it helps to create organ components affected by the drug. Fabrication of human heart valves for the younger generation of a population experiencing ailment from mechanical heart valves or other bioprosthetics can satisfy the mid-term gain of this technology. As the required components will be developed from the patient's cells with a patient-specific organ geometry, this technique has the potential to eliminate the need for mechanical components shortly. The fabricated biological structures once implanted at the required site can grow normally with the patient without requiring any further surgical intervention for component replacement. Bioprinting can cope with ethical dilemmas related to organ donations,

xenotransplantation, and clinical organ transplantation but it has its challenges related to practical, clinical, and ethical issues that have to be further articulated. For example, the cost of developing a customized biological organ can generate a waiting list similar to that of allograft or human transplantation. 3D bioprinting undoubtedly a cutting-edge technology in today's generation but it requires established clinical guidelines, ethical oversight, and regulations.

### 3.14.1 Design Parameters to Consider

Scientific communities involved in bioprinting research need to be mindful of the fact that there is no single regulatory board that governs and overlooks the entire bioprinting process and also of the fact that there are different regulatory methods in different jurisdictions [113]. Nevertheless, there are regulations and guidelines relevant to the technique and type of research and this ascends from the techniques used in 3D bioprinting as well as union of materials from various synthetic, natural, and biological sources in novel ways. With the mercuric advancement in the field of materials science and technology, regulatory frameworks have failed to keep up the pace with novel 3D bioprinting applications [113]. Considering the current scenario, researchers need to focus on regulations of individual components like materials to be used and its applicability in research instead of waiting for an overall bioprinting regulatory guideline. The researchers also need to be mindful of potential regulatory voids and predict the ethical questions their work can raise as there are no clear guidelines. Thus, before conducting any research in this area, there is a list of

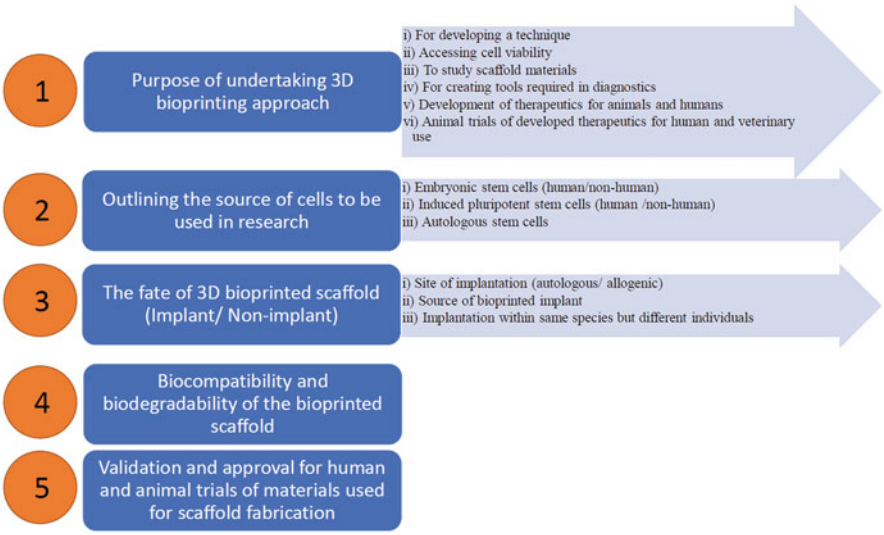


**Fig. 3.7** Design parameters to consider

parameters for consideration and consultation with governance units for providing updates related to their research (Fig. 3.7).

### 3.14.2 Social Aspects

The chance of success of a novel technology can only be determined by considering the acceptance percentage of the general public. There are innovative technologies that get an underrated outlook in society due to some ethical concerns. Considering reproductive cloning as an example, which is an innovative technique one of its kind, gets simply dismissed by a majority of society due to a persisting notion of "playing God." Bioprinting can meet a similar fate if certain social aspects are not taken into consideration. The soft impacts which can be considered as the human psyche, cultural and religious outlook of people, play an important role in successfully translating technology from lab to society.

#### 3.14.2.1 Soft Impacts of Society

Assessment of new technology not only depends upon the scientific output, which can be considered as direct output but also on the indirect output of the technology. These indirect outputs are also termed as soft impacts or societal relevance of the technology [114]. Soft impacts or societal relevance can be defined as the impact of a novel technology on the quality of human life. More precisely, it is mainly concerned with the effect of the technology on psychological parameters like human emotions, habits, experience, and so on. Any novel technology that is invented imparts a tremendous effect on these parameters of a human being. A mobile phone can be one of the best examples where technology has greatly affected human emotions with an altered version of love and friendship [115, 116]. If this existing emotional quotient of society will not be considered while designing new technology, then the framework of that technology can be considered as short-sighted and this mistake has enough potential to jeopardize the technology in the near future. Tough bioprinted constructs have successfully mimicked native tissues in many aspects and are undoubtedly more natural than the synthetic implants used but the constructs will still be regarded as non-self or foreign implants and this will generate a range of human emotions towards the technology which is important to understand.

There is a series of parameters that one has to consider before coming up with any novel technique

- People's perception of bioprinted products.
- Comparison between bioprinted products and organ transplantation.
- Preferability of bioprinted organ over organ donation.
- Necessary changes to be considered to improve social acceptance of bioprinted products.

Focusing on these parameters can improve the implementation of technology by society, along with clearing basic doubts.

## 3.15 Cultural and Religious Perspective

Cultural and religious values are an important pillar in society and are an inseparable factor of the community, and any technology that repels these notions will be immediately dismissed as it will be against people's value and interest. Human embryonic stem cells (hESCs) have promising applicability in many areas of science especially in tissue engineering but catholic and orthodox views are against the use of hESCs in research for therapeutic and reproductive cell cloning as the belief lies as life begins at conception. Different religions have different beliefs and concepts about life and its existence which do not sync with the rationality of science. On the other hand, it has to be clearly understood that reproductive cell cloning is the only approach of genetic modification and considering the stage of technology, the percentage of successful modulation of genes is very low, around 95–98% of mammalian cloning have failed via miscarriages or leading to complications in the long run [117]. Similarly, in the case of bioprinting, considering the stage of technology, it can be a debatable subject similar to other technologies that clash with religious beliefs. There is a high probability that some religions might accept a fabricated tissue construct while others will not or some might approve the use of bioprinted tissue while refusing a bioprinted organ as it concurs with the concept of "playing God".

Acceptance is not only limited to religion rather it varies from country to country. Some countries are in favor of reproductive cell cloning like the United Kingdom, Denmark, Japan, the Netherlands, and Korea, while there is a list of countries that do not support the technique like France, Germany Italy, India, Ireland, and so on. Certain countries also discriminate based on each technology. For example, stem cell engineering and reproductive cell cloning have a lot of overlapping areas between them but the previous technique is authorized in many countries like Israel, Belgium, Sweden, and India and later is prohibited in some countries. Human perspectives and beliefs are dynamic and vary with time so it should be clearly understood that evaluation of technology should be done from time to time and without restricting technological advancement. Because it is possible that a technology that is not accepted at a specific time scale can be acceptable later in the future. This outcome can be due to lack of proper understanding related to a technology which if later on gets clarified can welcome the adoption of the concept. The hesitance in acknowledging a technology mainly lies in fear and inherent psychological resistance to change. Bioprinting is also a novel technology waiting for its clinical and social approval and with time may also face controversies, thus it requires a critical study of this technology on society, religion, and culture.

## 3.16 Ethical Aspects

Ethical aspects stem from the word "Ethics" which itself is a broad field and is a discipline of philosophy. Ethics are of different types, namely applied, normative, descriptive, and meta [118]. Scientific innovations primarily deal with applied ethics which can be defined as ethics related to morality undertaken in different aspects of scientific approaches and practices like engineering, medical, bioethics, and environmental ethics. There are certain ethical principles already existing related to stem cells, animals, and utilization of cells but these are few aspects of bioprinting thus there is a requirement of proper regulatory guidelines covering the entire bioprinting process.

NEST-Ethics is a promising model that can be utilized for establishing ethical guidelines for bioprinting. NEST-Ethics can be defined as the ethics of the New and Emerging Science and Technology model and is based on two important parameters, namely recurring tropes and argumentative patterns. A trope can be defined as a recurring motif that can lead to a specific impact, whereas argumentative pattern means a set of ethical arguments that counter exist each other.

NEST-Ethics can be divided into two levels, namely meta-ethical issues and techno-ethical issues. Meta-ethical issues include—

- Collective unity (consequentialism or utilitarianism).
- Deontology.
- Theories of justice.
- Conceptions of a good life.

Deontology deals with the basic principles, rights, and duties; similarly theories of justice consist of appropriate distribution of finance and benefits, and finally, conceptions of a good life are about virtue ethics. The second level deals with the study of the relationship between technology and moral change [119, 120].

Meta-ethical issues deal with two different viewpoints, namely technological determinism and social determinism. Technological determinism defined as the benefits of a particular technology to humankind and social determinism consists of people's perspective of the novel technology. Bioprinting will eventually evolve and has the potential to deliver promising clinical results replacing damaged organs and tissues with new ones thus saving lives of many people but, on the other hand, it is also possible where an individual with a bioprinted prosthetic will be face discrimination on certain grounds in comparison to a person with a normal heart transplant. Though the reaction to such technological novelty is normal the main problem stays with the resistance to change, cultural, and moral perspective.

Techno-ethical issues can be understood using two factors, namely hard impacts and soft impacts. Hard impacts can be defined as quantifiable consequences of novel technology on the well-being of humankind and soft impacts deal with non-quantifiable consequences like habits experience and perception as mentioned in the above literature under soft impacts. Advanced technologies have a great impact on society based on moral standards. With bioprinting in the picture, a

process that generates organs similar to original ones may provoke a changing awareness of unhealthy habits like alcoholism and smoke from bad to good. Considering smoking as an example, cigarette packets come with a warning label to highlight the causality of smoking but with the advancement of bioprinting there might be organ shops who can promote their products as "Smoking and Alcoholism are no longer injurious as you can enjoy new sets of liver and lungs." This may sound farfetched and shocking for now, but a possibility of moral change will always exist.

### 3.16.1 Ethics Related to Source and Donation of Cells

The incorporation of cells is an important aspect of bioprinting and the type of cells used to determine the property of the tissue or organ generated using bioprinting. As the incorporation of the stem is the ultimate goal of this technology, thus ethics regarding utilization of cells especially stem cells are important to consider. As reported by De Vries [121] in his review on ethical aspects of tissue engineering, nearly 70% of articles have referred to the moral problems of using human embryonic stem cells. Similarly 20% of selected articles have shown the moral problems of using therapeutic cloning in research. By doing so, four important factors raising ethical concerns come into the picture—

a. Source of cells.
b. Donation of cells.
c. Using animals for research.
d. Morally problematic research technique.

One has to clearly understand that bioprinting will be an incomplete process without the utilization of stem cells at certain stages of the process. Use of fetal cells and embryonic germ cells procured by conducting induced abortion raises a lot of ethical and moral controversies as in some ways it promotes abortion. Thus, many articles have reported alternate sources for research—

a. Bone marrow-derived stem cells.
b. Amniotic fluid-derived stem cells.
c. Placenta.
d. Stem cells from umbilical cord.

Collectively these can be referred to as mesenchymal stem cells. Apart from these induced pluripotent stem cells are also used.

There have been studies for evaluating risks of using xenogeneic cells, cells from other species of animals. Disadvantages mainly remain with disease transmission from animals to humans as there is a high probability of transmission of pathogens like bacteria, viruses, prions, and other infectious agents that can lead to immunological complications in the receiving host. The use of allogenic cells is

comparatively safer but the probability of a certain amount of disease transmission also remains [122, 123].

Donors play a significant role in stem cell research and there are four important parameters to be considered.

- Donor's privacy.
- Donor's informed consent.
- Possible invasiveness from source.
- Ownership of donated tissues or organs.

Confidentiality of donor information should be dealt with great priority and it can be established by anonymizing the samples to be used in research. Informed consent of the donor is the next important parameter in the picture where the donor must be informed of the possible utilization of its sample in research. There are certain donor reservations about research practices to be conducted and it is important to ethically respect it.

Directed donation and anonymous donation are two important aspects of altruistic donations which are widely debated topics in organ/tissue donation. The primary argument that persists is if the donor gets the right to choose the receiving host or not. In a directed donation, the donor chooses the receiving host who might be related to the donor in some ways. This type of donation is often labeled for partiality as it favors specific recipients leading to skipping certain names on the waiting list. Bioprinting undoubtedly has an advantage when it comes to altruistic donations as the organs will be bioprinted. Tough the debate regarding cell source still exists but are expected to subside when proper regulatory and ethical guidelines will be established.

### 3.16.2 Ethics Related to Clinical Trials

Bioprinting, as previously referred, is an umbrella term that covers many aspects like cells, biomaterials, devices, and drugs, which is a more advanced and simultaneously more complicated process than designing therapeutic drugs and biologics [124]. Thus translation of this technology into clinics is also difficult. The ethics related to clinical trials can be categorized into two groups:

a. Pre-clinical trials.
b. Clinical testing.

#### 3.16.2.1 Pre-clinical Trials

Pre-clinical trials are required to study the effect of specific elements of a final bioprinted product on an individual. This is a significant step to access the reaction of non-self-components of bioprinted product on the receiving host. The implant may initiate an irreversible or partially irreversible change within an individual once implanted and the host has to accept the lifetime consequence of the transplantation

thus pre-clinical studies should be dealt with the highest priority. If an implant creates any type of side-effect or reaction, it is equally difficult to detect which specific component of bioprinted construct is provoking a discomfort or a reaction. For evaluating the effects of in vivo studies, animal models are utilized that vary from small animals like rats, nude mice, rabbits, and so on to large animals like primates. Large animal models like primates are required as the data generated from small animal models for biochemical and physiological interactions are not in sync with that of humans making the research inconsequential. Animal models are important as they mimic human physiology and disease manifestation in certain ways. Apart from this, certain animal models have a small life span that helps to study an effect during the whole life span. Nevertheless, the use of animals for research has always raised ethical issues even if there are established regulatory guidelines. Many animal welfare organizations compel the government to ban the use of animals for research and press charges of animal cruelty.

### 3.16.2.2 Clinical Testing

One of the biggest challenges faced in clinical trials is the selection of a human candidate for conducting tests. There are two important factors to be considered for selection purpose—

1. Patients should be healthiest or sickest among the considered group.
2. The patient should give proper informed consent.

Healthy individuals who volunteer for money without being concerned about the process and the consequence it can have on their health and mind should not be encouraged to participate. Complete information on the process should be presented to the patient and their family members. It is also important to keep in mind to access the individual on how much has the person understood as the language used in the consent form may be complicated scientific terms which are not understood by the general public, thus it is an important priority of medical professionals to explain the entire process in its simplest form before obtaining the official signature. The cost of clinical trials is also a valid limitation and the government should take complete responsibility to ensure proper funding of these trials. Identifying 100% true negative individuals, i.e. a person without any disease is an ideal condition and screening should be conducted to reach the closest proximity to an idle scenario. Considering the stage of bioprinting, similar clinical trial tests should be conducted and every minute details of the experimental observation should be recorded without any sort of favoritism and cover-up.

3D bioprinting is evolving as its applications are increasing gradually in number and due to this evolution of bioprinting technology, process complexity is also increasing. Apart from the fact that this technology needs proper regulatory guidelines, it also seeks faith and willingness of society to understand the positive aspects of bioprinting rather than focusing on prejudices and superstitions that have been greatly corroding many aspects of the scientific community and our society. This technology has brought more questions than answers when it comes to ethical,

social, and legal issues of bioprinting and it is obvious from the fact that the speed of development of research and technological advancement occurring has surpassed the rate of understanding. But given time 3D bioprinting can solve some of the major persisting problems like organ donation and transplantations and with the establishment of proper regulations, clinical approval of this process will be apparent. The government should make sure of creating committees to govern the technological advancements and access their clinical feasibility. Last but not least, the commercialization of bioprinted organs which even if not discussed in this chapter but is a highly essential parameter that has to be regulated legally to subjugate any illegal trafficking of bioprinted organs. In the end, bioprinting is a revolutionary technology and if given proper opportunity, it can create wonders in the history of clinical sciences.

## 3.17 Open-Source Bioprinting and Portabilization of Bioprinters

3D bioprinting can be defined as a type of additive manufacturing where cells, biopolymers, and growth factors combine to develop bioink, which can be further utilized for synthesizing a three-dimensional scaffold utilizing a bioprinter. 3D bioprinting is a versatile and revolutionary technology that has good potential to address some of the major persisting problems in the field of tissue engineering and regenerative medicine. In biofabrication, the top priority is given for tissue-mimicking and till now, it is a persisting challenge as native tissues are immensely complex and dynamic. 3D bioprinting is a well-equipped technique that can be utilized to address the limitations as it provides high throughput, reproducible outputs, and high precision. Bioprinting is a complex process and does require proper infrastructure and skilled manpower for its functioning and troubleshoot issues. These requirements can act as a circumventing factor for many research labs and educational institutes that function with low setups. Apart from this, the requirements also discourage translation of bioprinting into other fields of science like drug screening or cancer biology and so on. Versatility is an important technological advantage in case of bioprinting gets severely affected when it comes to accessibility, cost, and expenses of a commercial bioprinter. Bioprinting despite being a cutting-edge technology with a wide range of applications, is still inaccessible to a broad range of scientific communities and educational demonstrations. Currently available commercial bioprinters come with a trade-off where one has to compromise resolution for throughput or vice versa, along with an expensive price range of 10,000 to $200,000. Most of the commercially available bioprinters are bulky and huge and most importantly non-customizable when it comes to hardware and closed source in software. Thus, bioprinting despite having promising applications is still under adapted and much less exploited. There are two aspects of bioprinting that are still underdeveloped and not yet exploited, which can address the accessibility issue of bioprinting. They are:

a. Open-source bioprinting.
b. Portabilization of bioprinter.

These two parameters are important in defining the degree of accessibility and convenience of bioprinting to a great extent. Currently, there is a very small segment of products that are available that respect these two aspects of bioprinting. This area is not that highlighted and thus requires more research and study to access the applications and other potential it can have. In countries that are new to bioprinting have a basic problem that has to be addressed to increase the technological awareness expertise of this novel technology. The problem lies in the academic career of researchers who are involved in bioprinting research. Many research scholars never had bioprinting exposure during their academic years as educational institutes simply lack the infrastructure, skilled personnel, and finance to acquire a commercial bioprinter. Though bioprinting can be taught as a theoretical chapter but without practical knowledge and proper demonstration, the conceptual clarity cannot be achieved. Thus, for developing the foundation of bioprinting, the technology needs to have good accessibility to educational institutes.

### 3.17.1 Open-Source Bioprinting

The main problem that arises with closed source bioprinting is that the software does not allow accessibility into the source code that could have been modified for several experiments rather than on something specific. Hardware modification opportunities are also limited and even if available, have to be purchased and are expensive. Apart from that, any customization implemented on the device can revoke the warranty of the instrument. These limitations make proprietary available bioprinter useful for a very limited purpose. Comparing the finance invested in these instruments, the amount of output is not satisfactory. Open-source bioprinting, on the other hand, has the potential to remove these above limitations as the technology utilized in making the device functional has copyright, thus allowing user-specific customizability. This method apart from reducing the overall cost of the project, also encourages easy innovation at the ground level. It must be clearly understood that open-source bioprinting is only limited to technology invested into the devices only but bioink and the technology utilized in making them can have copyright.

There are also examples where research groups have designed their open-source bioprinter and have shown a significant amount of work on bioprinting. Goldstein and team [125] designed a bioprinter from a Makerbot Replicator 3D printer, using PLA as the fabrication element and syringe injection unit for the extruder. The modified bioprinter was then organized by calibrating and optimizing the process parameters to support cell incorporated bioink. Post-printing it was observed that the cells were stable, viable, and were gradually proliferating. The cells utilized for bioprinting were of chondrogenic origin harvested from rat knees and successfully retained the cartilaginous phenotype. Similarly, another group led by Jaehoo Lee [126] designed both open-source bioprinter and software where the source code can

be modulated to match the functionality. The bioprinter consists of a multichannel rotating extruder that enables it to dispense multiple biomaterials maintaining an isolated and stable environment for each biomaterial used. This device bioprinter costs about 1/10 the price of commercial bioprinters like RegenHU, EnvisionTEC, and so on. This device can be further modified based on hardware and software due to its open-source background. Another work is done by Reid [127] and the team where they designed a bioprinter using an inexpensive off-the-shelf 3D printer to study tumorigenesis and microenvironmental redirection of breast cancers. They developed the bioprinter by modifying a Felix 3.0 3D printer. The bioprinted scaffold showed good cell viability as they reduced shear stress on cells using a pulled glass capillary pipette which also gave good positional control. Using the setup, the team was able to print human induced pluripotent stem cells (iPSCs) into Geltrex while maintaining its pluripotency. The important milestone they were able to achieve was single-cell resolution print within 50 μm range which is first of its kind and simultaneously were able to minimize mechanical stress on cells. The bioprinter used can be fabricated on any 3D printer. Melanie Kahl and team [128] worked on a similar project where they developed an ultra-low-cost bioprinter which is very simple in design and takes very little time to assemble. They modified Anet A8 Desktop 3D printer with Prusa i3 DIY kit thus most of the components used in this work were not purchased rather they were 3D printed. This modified bioprinted was approximately €150 in price furthermore it was lightweight, small, portable, and easier to functionalize with laminar airflow. The bioprinter was used to print 2D and 3D scaffolds using recombinant human embryonic kidney cells (HEK) that expressed yellow fluorescent protein (YFP). The bioprinted scaffold showed good cell proliferation and cell viability post-print. The most impressive mechanism of this bioprinter is that it can be easily reverted into a 3D printer that can be used for many other purposes as well as to print replacement parts for the bioprinter as well. This work is completely open-source and well documented using a time-lapse video. Nils Bessler and co-workers [129] designed an impressive bioprinter using a Prusa i3 3D printer. This modification of the 3D printer was done by modifying the firmware of MERLIN and replacing the plastic extruder with Nydus One Syringe Extruder (NOSE). This design has the flexibility of using syringes with multiple nozzle diameter and volumes. Experimental verification of the device was conducted using HEK293 cells and embryonic stem cells. The results obtained post-print showed good cell viability around 81% and 85% respectively.

These aforementioned projects are some of the examples of open-source bioprinters that have been successful in experimental validation using stem cells similar to that of commercial bioprinters. As these are open-source derivatives, the finance invested in these projects is minimal, which is a very important requirement for academic institutions or research groups that function with low finance setup. All the models developed are available on the Internet in the form of .stl files which can be used directly to print the components of bioprinter using any 3D printer. Open-source bioprinters apart from drastically reducing the overall cost, also encourage multiple scientific disciplines that have an interest in connecting with bioprinting.

### 3.17.2 Portabilization of Bioprinters

Portabilization of bioprinters can be defined as the degree of mobility offered by a bioprinter to its user. Portabilization became an important aspect after the development of a concept called "onsite bioprinting." This novel concept of bioprinting in the current scenario is limited to tissue-specific aberrations. Handheld bioprinter is a revolutionary bioprinter that has its dimension reduced to an extent where it fits in a palm. This device presents the major extent of mobility to its user, unlike other bioprinters that are localized to a desk or laminar hood installments.

A working prototype was first developed by O'Connell and team [130] with a UV light that helps in photocuring. The prototype was specifically designed for targeting chondral defects where the process of surgery requires an initial stage of debridement step for removing the excess amount of fibrous tissue from the site of aberration. This surgical step leads to a change in the size and shape of the defect before surgery. Pre-fabrication will not be an idle approach for such conditions as the site dimension has changed, and to address this limitation bioprinting has to be conducted onsite of the defect. This technique of fabricating replacement tissues onsite of defect is a novel approach and can be termed as onsite bioprinting. Currently available bioprinters are not flexible enough to address this surgical requirement of onsite bioink delivery. To address this limitation, a handheld bioprinter was designed that has the potential to deposit bioink in a direct-write manner on site. Considering the dimensions of the bioprinter, the name "Biopen" was given to this invention. It serves several advantages like—

a. Being a handheld device, a surgeon can manually sculpt any replacement tissue construct in the desired order.
b. With proper skill, this device can be used to fill crevices and make a complex and precise deposition.
c. Being a handy tool, it is ideal for in-situ biofabrication.
d. The small dimension of this device helps in the easy sterilization process.
e. The devices itself is fabricated using a 3D printer; this supports low finance investment.

At the current stage, this prototype of bio pen requires a bioink with UV crosslinking property, but it can be further modulated to be compatible with other categories of bioink. To evaluate the applicability of this prototype, a bioink was designed consisting of gelatin–methacrylamide/hyaluronic acid–methacrylate (GelMa/HAMa) with photoinitiator VA-086. The bioink was embedded with human infrapatellar fat pad derived stem cells to check the cell viability. Post-printing results on day 7 showed 97% cell viability indicating minimal shear stress on cells. This prototype comes with certain limitations like—

a. The outer pore diameter of the extruder is approximately 1 mm, which can be a limitation in treating defects less than 1 mm dimensions.

b. The heat generated from the user's hands can disturb the thermal equilibrium of bioink with thermo-gelling property, disturbing the flow rate of the bioprinter.
c. As this working prototype is the first of its kind, a compatible bioink has to be developed to match with this method of delivery.

This model was further perfected by O'Connell and the team [131] by focusing on osteochondral aberration on joints rather than focusing individually on cartilage and bone. The Biopen was modified to house two different bioinks and a novel coaxial extrusion system for Core-Shell extrusion architecture as shown in Fig 3.8a. The extrusion from each chamber can be individually regulated making it ideal for targeting cyto-metrical variations found commonly in cartilages. The handheld bioprinter was given a more ergonomic design similar to that of handheld surgical tools to enhance the surgeon's dexterity. This device was used to conduct the world's first live in-situ 3D bioprinting surgery using a sheep as an animal model.

A similar innovation by Hakimi and team [132] came into the picture that supports the in-situ biofabrication of printing sheets of biomaterial on site of the defect. This handheld bioprinter can print tissue sheets from a microfluidic cartridge and the rate of deposition of biomaterials is in sync with the movement of a pair of

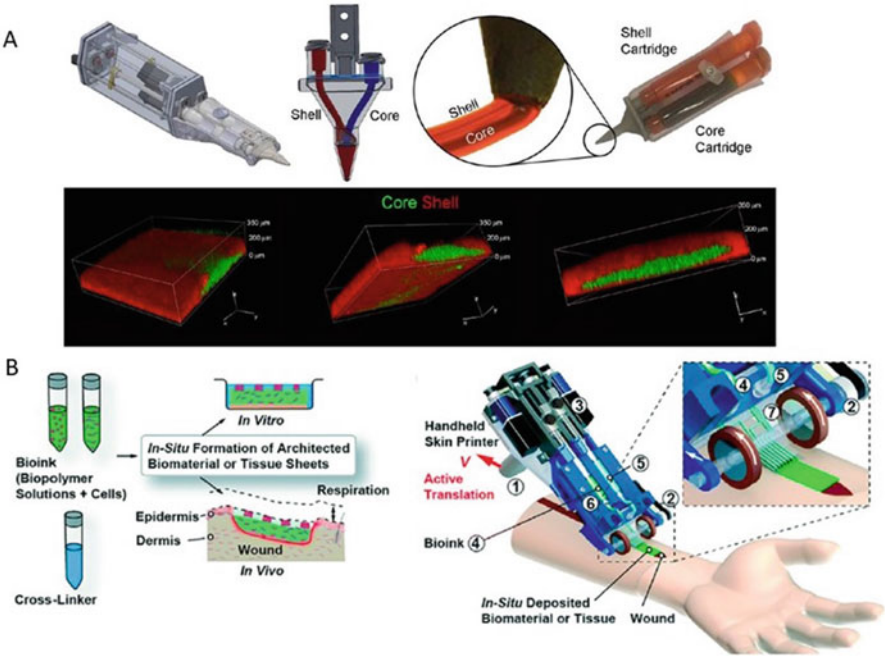


**Fig. 3.8** Types of handheld bioprinter (**a**) For printing bones, cartilage, and also the bone-cartilage gradient found in joints as mixing of two lineages of bioink is possible [127]. (**b**) For printing replacement skin. Reprinted from Singh et al. [136], Copyright (2020), with permission from Elsevier and Copyright Clearance Center

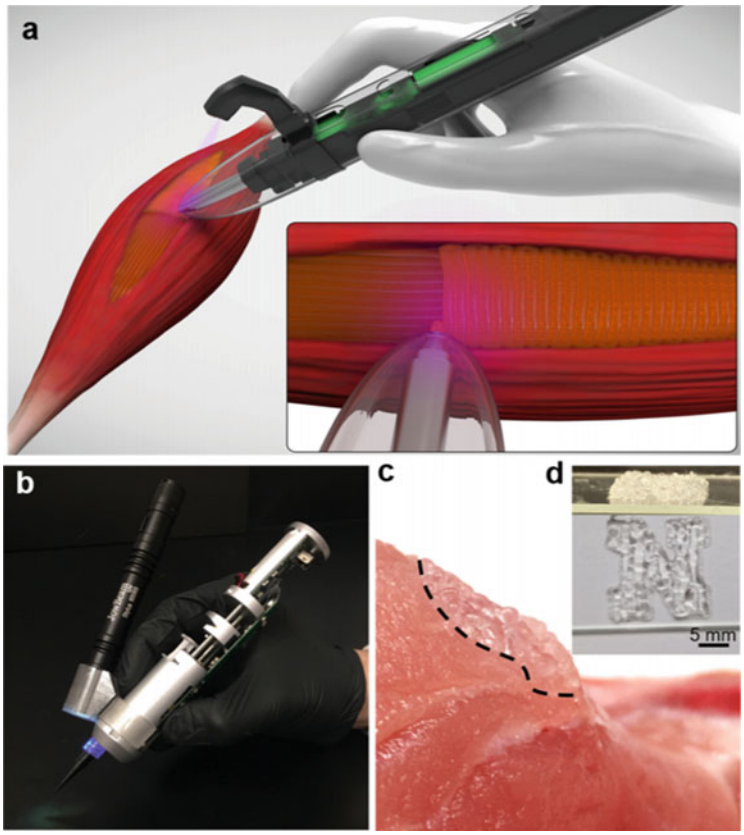


**Fig. 3.9** Usability of a handheld bioprinter (**a**) Representation of the device treating a muscle segment affected by VML. (**b**) Image showing the ease of usability of the device. (**c**, **d**) Section of muscle treated with a bioprinted scaffold using the handheld bioprinter along with its dimensions. Reprinted (adapted) with permission from Russell et al. [134]. Copyright (2020) American Chemical Society

rollers that can print along the defect as shown in Fig 3.8b. This device primarily addresses the complications of acute and full-thickness wounds where dermis, hypodermis, and epidermis all three layers are damaged and the normal healing procedure of skin is compromised leading to no healing or delayed healing [133]. This current model can house up to 3 ml of bioink solution and has a coverage area of approximately 100 $cm^2$ within a 0.8–2.1 min. The handheld bioprinter requires three different compositions of bioink for generating the replacement skin. First being an alginate and collagen type one mixture followed by bioink for dermis which consists of fibrinogen and hyaluronic acid (HA) mixture to which collagen type 1 was added and lastly for epidermis fibrin and HA were used. This bioink is highly biocompatible and biodegradable providing a conducive environment for incorporated keratinocytes to proliferate and attach at the site of deposition.

Recently a handheld bioprinter was developed by Russell and the team [134] to address the treatment of volumetric muscle loss (VML), which is common in the majority of skeletal muscle injuries. VML requires immediate surgical intervention as it leads to altogether skeletal muscle loss with corollary muscle impairment. VML injuries are often associated with damage to soft tissue, extensive muscle fibrosis, and incomplete tissue regeneration [135]. To target these challenges, the handheld bioprinter was designed with a UV source as shown in Fig 3.9. The basic bioink composition was GelMA, which is a collagen-derived product mimicking the natural extracellular matrix. The bioink was embedded with C2C12 myoblasts to act on the muscle degenerated sites. The myoblast cells formed multinucleated myotubes after 24 days. Its applicability was tested in the murine VML model. Histological samples validated strong interaction of printed scaffold with surrounding tissues.

These are some examples of developed handheld bioprinters which have shown great potential in the treatment of the various category of tissue aberration, which is complex and requires precision. The mobility and ease of usability offered by these bioprinters, along with enhanced surgeon dexterity, can be a blessing for challenging surgical scenarios that cannot be addressed by any other currently available techniques.

## 3.18 Conclusion

Biofabrication, in a nutshell, aims to design and construct scaffolds that mimic the complex microenvironment experienced by each cell and tissue. However, the next step in bioprinting is to generate scaffolds that can monitor the growth and status of the cells in real-time, rather than remain passive. It should be able to monitor the bioactivity of the cells and respond to it as necessary. Another aspect of being looked at is developing technologies that fabricate constructs that enable the differentiation of stem cells within the microenvironment and allow their maturation into adult tissues. This would further speed up the process of developing organs in vitro, and can thereby lower or even eliminate the need for organ donations. Most of the present challenges posed by this field relate to the materials that are used in the process. Currently, the selection is based on either the extent of biocompatibility or the ease of extrusion and crosslinking parameters. An ideal biomaterial should be compatible and manipulatable as well as maintain cell survival and function. 3D bioprinting has further evolved into a concept of bioprinting that includes time as its fourth dimension, called 4D bioprinting, taking biomimetics to the next level. As aforementioned, this technique helps to fabricate dynamic tissue that can respond to stimuli by undergoing conformational change, very similar to that of native tissues. This technology is still in an incipient stage and requires further research to evaluate its potential and to address the existing limitations. Socio-ethical issues are one of the most significant sectors of any novel technology and need to be addressed with proper fundamental ethical guidelines. Bioprinting is one such technology that will be utilized to generate tissues and organs that can be utilized by individuals using stem cells. Thus, there is an urgent need for an ethical committee to govern the entire

process. Open-source bioprinting as mentioned, can help in making this technology accessible to a broader sector of science other than tissue engineering and regenerative medicine further enhancing the interdisciplinary research. It also makes the technology inexpensive thus increasing its availability to academic institutes for demonstration purposes, encouraging the development of practical concepts along with theory and research groups that operate on a low budget setup. Another promising clinical application can be seen in the development of handheld bioprinters or print heads with digital controls that can allow direct tissue repair. By creating 3D constructions of scanned lesions, the print heads can directly deliver the necessary growth factors and scaffolds that can repair the lesion, irrespective of their size or thickness. This field can give rise to several potential applications, and this technology of bio-fabricating tissues and organs can revolutionize medicine and health care as we know it.

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# 3D Printed Implants for Joint Replacement

4

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**Abstract**

The 3D printing technology started and developed at the early stages of the eighties, where it is based on constructing the object layer by layer by using the appropriate material and technology and where it helps in producing a physically real object through a digital transformation process. Using 3D printing technology has many benefits, especially in the medical sector, where it could be used for speeding up the surgical operation, replacing human organs, to manufacture customized prosthetic limbs, a wide range of the material that can be used, customized designs that can be implemented since the anatomy of any person is unique and this means the geometry and dimensions of anyone bone are different from anyone else, producing surgical tools and instrumentation like jigs that can be used by the surgeon to position the implant correctly. In this chapter, the innovation of using 3D printing technology is addressed and elaborated to tackle the rapid progress and development in this sector.

**Keywords**

3D printing · Bio-medical engineering · Joint replacement

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## 4.1 Introduction

3D printing (3DP), also known as additive manufacturing (AM) technology has significantly evolved over the last decade and continues to open new routes to the production of high-performance and complex structures with enhanced properties and intricate shapes that are unattainable by conventional fabrication methods. Due to its innovation-driven approach [1], 3DP has been expanding in several fields such as material science providing increased optical and mechanical performance, in membrane technology for increased adsorption of chemicals, as well as being incorporated in complex systems such as space technology to develop tools on-site [2–9]. The ease of modeling and experimentation with the 3DP technology helps in progressive growth and expansion of knowledge in the fields, which can be of significant value in several applications.

Over the past decade, 3DP has recently gained interest in the bio-medical field, especially in biomodeling, fabrication of scaffolds and implants such as for dental restorations and tissue engineering, and even for drug delivery as it is highly flexible and faster than the current methods [10, 11]. Moreover, the positive impacts on the biological and mechanical properties of the designed medical implants and devices manufactured by industrial 3DP technology promote the use of 3D printed items as viable candidates for further research in clinical applications [2, 3, 11–13]. 3DP can also be a highly cost-effective approach, thereby cutting down costs and improving economic efficiency. The ease of development and lower costs has led to the adoption of 3DP to develop complex parts such as joints in the medical field which can easily be tailored to the patient's need [2–9]. In this chapter, the innovation of using 3DP technology to develop joints and prostheses is addressed, which is one of the leading outcomes of the rapid progress and development in this sector.

The breakdown of existing metal-based implants into the surrounding biological fluid leads to a decreased mechanical performance and an unsuccessful execution of the healing of the orthopedic damages (i.e. bone fractures in joints). Moreover, metal-based implants still portray a high risk of cytotoxicity and inflammation which still needs to be addressed. With the use of biodegradable materials, the mechanical integrity of the medical implants can be prolonged [14]. In addition to this, a high permeability is preferred in the transportation of nutrition and waste to and from the cells which promote bone re-growth resulting in prolonged implantation. As 3DP can have an upper hand in accurately determining the porosity and pore arrangement in the implants, the incorporation of such a technique compared to conventional ones (such as machining) can be considered as the better alternative [15]. Additionally, easier surface modification of implants using the 3DP technology through techniques such as adding a layer of a coating as the final layer is a simpler way to enhance the joint implants as the manufacturing technique itself can impact the properties of the material and therefore impact the functionality of the implant.

## 4.2 3D Printed Implants/Prostheses

### 4.2.1 Knee Joints

The knee is a complex joint comprising of patellofemoral and the medial and lateral tibiofemoral articulations. Any of these areas can be the source of pain and leading to reduced functions during preoperative or post-operative procedures. Replacing articulations particularly the patellofemoral during implantation is often not given that attention it needs, and hence can lead to unsuccessful replacements. Often at times, malalignment of lines of pull through at the patellofemoral articulations ends up causing further dislocation as the knee extends. Moreover, the movement at the tibiofemoral articulations is also complex. The shape and surface contour of the medial side of the tibiofemoral articulation are very different than its lateral side. Currently, describing these contours still is considered a major challenge in designing prostheses or implants. Failure due to exact fit and alignment can cause a major drawback in the successful bio-functionality of the implants and may lead to infections and in some cases tumors. In addition to this, the limitations that come with the manufacturing techniques for both metal-based and plastic-based prostheses result in a limited range of motion and are in capable of mimicking the exact movement of the original knee. Due to the inevitable friction introduced to the knee joints with the addition of implants, the currently available knee-prostheses are suggested to be beneficial for patients that require it for low daily activity [15].

As a result to provide improved knee-reconstructions, the introduction of knee joint-preserving intercalary tumor resection has been carried out to provide better positioning and functioning of the joint after reconstruction, and at the same time combat the occurrence of first grade and metastatic bone tumors around the joint area.

In a study conducted by Liu et al. [16], joint-preserving intercalary resections were introduced to a set of 12 patients with malignant bone tumors around the knee joint. The osteotomy guide plates and reconstructions used in the intercalary procedure were 3D printed. Observations of the cross-sections at the resection plane with a 3D printed surface of the prosthesis were performed. The patients were observed for their results, complications, and remarks for 7–32 months, with a follow up at around 22 months. Results revealed that the resections performed were highly accurate in the similarity between the prosthesis and residual bone. Analysis using the mean Musculoskeletal Tumor Society (MSTS) score revealed that an average score of 28 ($26 > \text{MSTS score} > 30$) was reported without the need for any type of support. However, the presence of superficial infection was reported in two cases, a local recurrence was observed in one case, and a pulmonary metastasis was observed in one case. Nevertheless, through this research, it can be concluded that the use of 3D printing in manufacturing the tailored osteotomy guide plates aided in the joint-preserving tumor resection and reconstruction. However, further clinical studies are a must on a larger sample group to ensure the efficacy of such prostheses.

Regular wear and tear of the knee joint can lead to a constant loss in the articular cartilage of the knee joint and over a prolonged period can result in a total

meniscectomy. This degenerative joint condition is commonly known as knee osteoarthritis. One of the most extensively used materials used in meniscal tissue regeneration is poly (ε-caprolactone). However, using scaffolds that are cell-free can lower the tissue regeneration process and instead promote the degeneration of the joint. In this research presented by Zhang et al. [17], mesenchymal stem cells (MSCs)derived from bone marrow were seeded in a novel 3D printed PCL scaffold which aimed to provide enhanced meniscal regeneration and improved protection of the cartilage. This controlled study was conducted on 72 New Zealand white rabbits which were further divided into four categories: cell-seeded scaffold, cell-free scaffold, sham operation, and a total meniscectomy. The regeneration of the tissue implantation and the deterioration of the articular cartilage were studied using gross and microscopic analyses (scanning electron microscope and histological) post-operatively at 12 weeks and then again at the 24-week mark. The mechanical performance (tensile and compression) was also studied.

In comparison to the cell-free group, the cell-seeded scaffold showed a smooth surface with shiny white color and overall showed a better gross appearance. The presence of fibro chondrocytes with extracellular collagen types I, II, and III was observed in both the seeded and un-seeded cell-free scaffolds at the 12-week check and 24-week check. Moreover, at the 24-week mark, the result was substantially better for the cell-seeded scaffolds and lower cartilage degeneration in both the femur and tibia was observed for the min comparison to the cell-free group and the meniscectomy group. The tensile and compressive properties of the implants again showed an improved performance in the cell-seeded scaffolds in comparison to the cell-free scaffolds.

From this study, it can be concluded that inserting MSCs in the PCL scaffolds leads to an increase in the regeneration of tissues and increased scaffolds mechanical strength which can provide a functional replacement as a protection for the articular damage which is caused as a result of a total meniscectomy. Moreover, the positive results of this study suggest the potential of using 3DP technology to develop PCL scaffolds that can be seeded with MSCs as a good alternate for the substitution of the meniscal. However, this method still needs additional modifications before being fully applied at the clinical level [17].

### 4.2.2 Hip Joints

Osteoarthritis, which is the wearing out of cartilage over time is a major concern in the population. For instance, it is reported that for the Russian Federation, 18 out of the 10,000 people suffer from this, with the greatest need reported in a patient below the age of 65. In addition to this, one of the major joints is affected due to osteoarthritis in the hip joint. Increasing hip joint replacements often follows with increased revisions due to recurring site infections and decreased aseptic nature of the added standard end prosthesis (artificial implants), which inevitably leads to bone mass loss from the supporting surroundings such as from the femur bone and pelvis bone. It also reported that out of 20% who report deconstructions in their

acetabulum, the removal of end prosthesis leads to increasing concerns of cavity defects leading to decreased supporting capability by the femur and pelvic bones, hence repeated hip replacements is a necessity to prolong the provided support [18].

Besides, it is also reported that a total of 10% of all hip replacement surgeries are total hip replacement (THR) revision operations, with the rate of revision being up to 17% by 13 years. With every additional surgery, the risk of huge bone defects and reduced motion in the pelvis is a major cause of concern especially for patients at an older age who generally have osteoarthritis as well [19]. Currently, the implants developed for femoral hip replacements are made from materials that have the stiffness higher than the bone. Due to this mechanical discrepancy, considerable bone resorption secondary to stress shielding arises, leading to per prosthetic fractures occurring during or after revision operations [20]. Moreover, the failure of using standard acetabular cup implants is also directly linked to accelerated dysplastic bone arthritis [21]. With the rise in patients with the damaged acetabulum, prevailing revision surgeries, and poor osteogenic ability, a more customized solution to better support the bodily functions and enhance movements is necessary.

Total hip arthroplasty (THA) is generally performed to remove discomfort and establish proper functioning of the body to enhance movements for patients with unsuccessful traditional treatments performed to cater to the hip joints. Even though THA has been successful, the risk of loosening aseptic and periprosthetic fractures still prevail. In a study conducted by Arabnejad et al. [20], a fully porous material with high strength and alterable mechanical properties was developed to be applied for a hip replacement design. The structure of the considered design is based on a "short stem taper-wedge" implant which can be fully compatible with marginally intrusive hip replacement operation. The internal structure is designed to be adjusted in a way that can tissue properties of the bone tissue at a local level, thereby minimizing the resorption of the bone secondary to stress shielding. Using 3DP (selective laser melting) technique, an entirely porous hip implant was fabricated and implanted. The performance of the implant was also monitored through in vitro experiments within composite femur material. The results revealed that in comparison to a standard robust implant, the 3D printed implant was capable enough to decrease bone loss secondary to stress shielding by 75%. The results also matched the results obtained in the in vitro quasi-physiological experimental model and the finite element model for both the porous and adequately solid implant. Through the in vitro test conducted in this study, a considerable reduction in the strain obtained at the femur surface was observed, which corresponds to the significant decrease in the stress shielding property. The results from this study suggest the potential application of an entirely tunable porous material used for bone implants and total arthroplasty procedures. Moreover, this study also highlights, the possibility of a substantial reduction in bone resorption could be based on material design and its alterable characteristics.

Recently, the use of computer-aided design-computed-assisted manufacturing (CAD-CAM) to develop prototyped implants has been considered as a potential alternative for the restoration of joints in critical salvage cases. Additionally, the insertion of autologous skeletal stem cells (SCCs) to enhance regeneration and

osseointegration of prototyped implants has been developed. In a research conducted by Gorinov et al. [21], 11 patients with extreme disability and major bone loss due to unsuccessful joint replacements surgeries were implanted with CAD-CAM prototyped implants with added SCCs were studied. Results from this study reported significant improvements in the overall clinical and radiological examinations of the patients. The improved results even in cases with critical revision hip arthroplasty (with a follow up in the third year) suggest a positive application of the customized 3D implants for joint revision surgeries. Additionally, the use of SSCs for enhancing bone regeneration and implant osseointegration is also validated through this study. Incorporation of such 3DP technology for preoperative planning allows for probe testing of the developed implant at a real scale and provides the ability to develop customized hip implants which can be considered as one of the most positive and cost-effective medicine advancement that can lower pain and discomfort for patients with osteoarthritis and revised hip surgeries. The ability to form intricate geometries is one of the key features AM technology. Not only has this influenced the plastic manufacturing industry, but it has also now become a leading alternative for metal fabrication as well. Incorporating 3DP in metal fabrication permits for alteration in the shape and dimension of the part as well as results in materials with improved mechanical and anti-corrosion properties. In a brief review collected by Popov et al. [21], research work from Polygon Medical Engineering in Russia and TedMed in Israel is discussed. Polygon assisted and monitored the 3D planning and 3D modeling for the implants, whereas TedMed was responsible for the optimization and manufacturing of the implants. One of the cases discussed was the use of 3D planning to assist the state of the walls and the possible positioning of the connection points between the bone and the acetabulum implant. The customized titanium implant was fabricated using plastic bone powder-bed additive manufacturing (PB-AM), (see Fig. 4.1b), polished and sterilized before implantation. Figure 4.2 represents the preoperative 3D planning illustrating the contour of the implant, the connection points along with the holes for the screws. Successful patient recoveries promote the successful application of such developments. Incorporation of tailored fit-solutions in complex cases makes AM an effective alternative to employ. However, challenges with its long-term reliability are still questionable.

In another study by Tserovski et al. [22], 20 patients were studied by using a 3D model of their acetabular part. After analysis of the 3D acetabular models, only two of the initially considered surgical procedures were altered. The use of the 3D models led to enhanced diagnostic accuracy and aided the surgeons to pre-assess the dimensions of the developed fit. The custom-tailored approach resulted in an excelled preoperative planning which led to an exact reconstruction of the joint. Moreover, this study suggests that the 3DP improves preoperative planning which can also aid in reduced anesthetic exposure. Although this method does have some limitations such as the need for technical skills, failure occurrence during printing increasing the time consumption, it can still be safely said that the approach was successfully employed and resulted in patients with satisfying results. As primary hip joint replacement surgeries are rising, consequently, the need for revision surgeries is also rising. Another study [18] on the development of customized

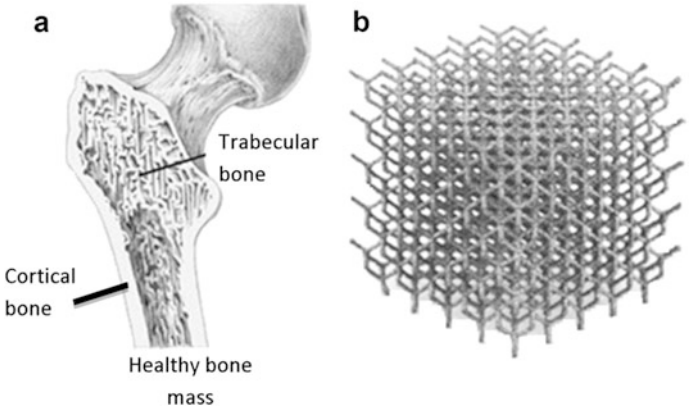


**Fig. 4.1** Hip replacement using 3DP: (**a**) Healthy human hip bone (**b**) proposed lattice structure replacement from 3DP [21]

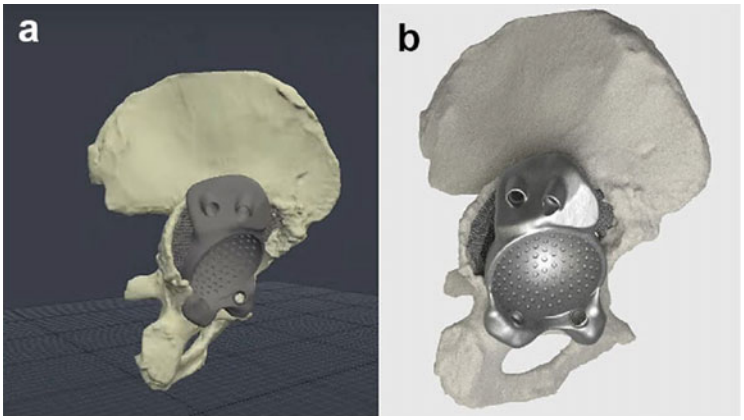


**Fig. 4.2** Pre-operative planning using 3D modeling (**a**) 3D model anatomal design (**b**) Titanium implant with plastic model [21]

acetabular parts focuses on the filling technique of the pelvis using 3D printed medical-grade titanium implants. Using the Hounsfield scale, to measure the density of the surrounding bone tissue, the implant is fabricated. Out of the 57 patients with a dislocated pelvic girdle, 46 received the customized acetabular component that stood stable within and did not require any revised procedure. Moreover, as the customized implant was able to model the correspondence to each individual's bone defects, the duration of the reconstructive restorative surgeries with serious defects in the acetabular cavity was reduced. Besides, the similarity between the bone and developed components was extremely high as highly specialized software was used to develop the fits. This study presents a promising combination of using Hounsfield units to assess bone tissue density along with software to customize the contouring

pelvis plasty resulting in a highly accurate geometry of the desired implant. Results also promote that the preoperative planning of tightening screws was also achieved easily through this approach.

Hip movement and motion-related data are now also being studied in aims to provide better and prolonged implant fit. Since the movement of every patient is different, surgeons are now also considering this fact while performing hip replacement surgeries. A novel technology developed by the Corin group claims to assist in locating the best placement of the implant and its components based on the motion-related data provided by the patient. Surgeons can now decide the most suitable orientation and placement for the hip implant with the help of preoperative planning and functional simulation accompanied by an intra-operative positioning system that uses 3D printing and laser guiding along with the optimized positioning system (OPS). Even though hip replacement surgery is mainly associated with positive outcomes, medical advancements are always finding new ways and techniques to improve patient satisfaction and comfort and to decrease further displacement and early breakdown of surfaces. Through this OPS, a more patient-specific solution will promote further for an optimal hip replacement [23].

On-going studies on clinically representative animal models are always done as they are essential for progressing translational orthopedic research into areas associated with the replacement of joints, metal on cartilage wear, and infections caused due to prosthesis. In a study conducted by Paish et al. [24], micro-computed tomography was employed to obtain measurements of the rat proximal femur to develop parametrized hip implants that could be implanted in a replacement joint of a clinically small animal. To determine the scaling of implants for various sizes, the correlation technique was used. 3DP technology using medical-grade metal alloys was used to develop the implants. Results showed that the animals were able to withstand the implant installations and were capable to move using their limbs post-operation. The importance of this research work lies in the fact that using a simple approach of CT images and iterative techniques, developing customized 3D printed implants using metal alloys is very much possible, which positively promotes using 3DP technology as a simple, cost-effective method for rapid production.

### 4.2.3 Temporomandibular Joint (TMJ)

Another part of the human body that has seen the use of 3DP technology is the temporomandibular joint (TMJ) which is a horizontal hinge-like articulation located in between the mandible and the temporal bone connecting the jawbone to the skull. This joint is necessary for speech, chewing, swallowing, and other movements of the muscles in this area. Up to 86% of the people suffer from pain and discomfort with the TMJ and surrounding areas. Degenerative diseases of the TMJ include osteoarthritis, ankyloses, and condylar resorption as well as trauma and cancer, which often have to resort to using a prosthetic total joint replacement when conventional treatments have failed [25]. The morbidity of TMJ arthroplasty is significantly decreased as no donor is required for this procedure, making the recovery faster

and providing immediate relief as no post-operative procedure is further required. However, a spike of up to 14% reported in the complication rate of the TMJ arthroplasty mainly due to unsuccessful screw tightening, nerve damage during operation, fracture in the implant, and infection build up which continues to decrease the longevity of the prosthesis.

Currently, the standard TMJ device is manufactured using a traditional computer numerical control (CNC) method. It is mainly developed using a titanium device with a cobalt-chromium-molybdenum condylar head that interacts with the occlusal surface which is made from ultra-high-molecular-weight polyethylene (UHMWPE). Although these devices have shown a decrease of 76% in its mean pain score, a 68% rise in their mean mandibular function and diet consistency score, and a 30% in its mandibular range of motion over 10 years post-operatively, 10% of the patients have still reported being dissatisfied and have reported a loss in the transient facial nerves [25]. Additionally, due to the proximity of the condylar fixation screws, mandibular nerve damage remains a cause of concern in TMJ arthroplasty. Also, the limited sizes of these screws make it in appropriate in cases where congenital deformity, large bone loss due to condylar resorption, and tumor resection can occur. However, despite many drawbacks related to TMJ rise, the ability to customize joint replacements has emerged as a cutting-edge solution, as the shape and size for conventional prosthesis may not be the right fit for the patient compared to custom-tailored fits.

Recently a team at Melbourne developed a TMJ prosthetic design called the "Melbourne" prosthetic TMJ [25], which is a customized design of the condylar part of the patient and has its fixation screws located in positions that prevent intra-operative damaging of the mandibular nerve. This implantation study was examined on a 58-year-old female patient, with a final stage of TMJ osteoarthritis. The loading results of the implanted joint were recorded during normal range chewing motion and maximum force-biting motion, using a personalized musculoskeletal model of the masticatory system, fabricated using medical images. Trial implantation of a traditional stock implant (Biomet©) was performed, and a similar range of motion was repeated to study. Results revealed that at maximum force-bite, the Melbourne prosthetic reported a lower value of maximum condylar stresses (259.6 MPa), mandibular stress (198.4 MPa), and screw stress (312.9 MPa) in comparison to the Biomet implant (284 MPa, 262.2 MPa, and 416 MPa, respectively) at the interfacial surface of the screw and bone. Similar results were also obtained with chewing motion. After a duration of 6 months, the customized 3D implant was surgically implanted into the patient. With the new implant, an increased opening of the jaw (40 mm) was reported by the patient. Additionally, no complications or discomfort was also reported. The novel design of the customized implant suggests enhanced biological and mechanical operation and has also contributed to prevent the post-operative damaging of the mandibular nerve in comparison to the conventionally used stock design. This customized approach presents great potential in using 3D printed implants for treating incapacitated joint and bone conditions.

In another study conducted by Ackland et al. [26], a customized total joint replacement for a 48-year-old female patient with last-degree TMJ osteoarthritis

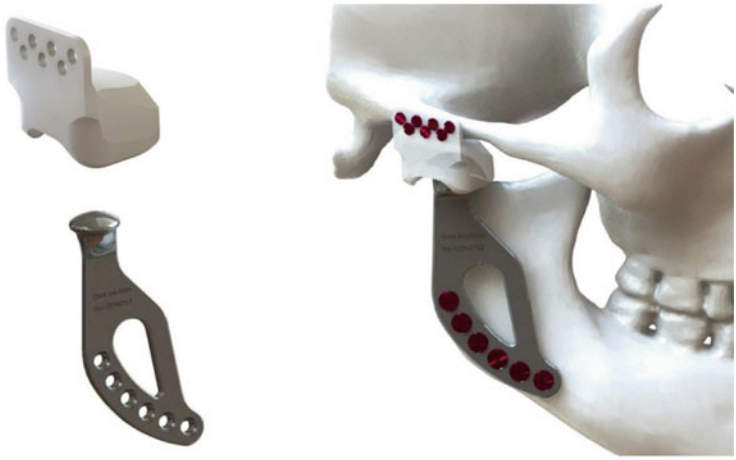

**Fig. 4.3** OMX© TMJ prosthetic replacement: UHMWPE fossa, titanium alloy ramus condyle unit (left) and titanium alloy screws used for securing to the bone with (right) [26]

(to the left) was developed. The post-operative clinical results after a year were also examined. As per the conventional procedure, the implant was 3D printed, sterilized, and then implanted into the patient. The outcome of the functioning and pain was examined a year post-operatively. A similar analysis using a personalized musculoskeletal model was examined, as reported in their previous study [25]. Additionally, the condyle thickness, head sphericity, and neck length were perturbed, and simulations of chewing and force-bite were examined. Results showed that the change in the condylar thickness had a significant impact on the stress response of the fixation screw. Moreover, the unusual condylar head movement increased the joint-contact loading of the prosthetic. On a visually based comparable scale of 1-10, a decrease in the pain from 7 to 1 and an increase in the opening distance from 22 mm to 38 mm were reported post-operation. Through this study, it is quite evident that the customization of the implant resulted in significant improvement in the functioning of the TMJ and promotes clinical employment to treat end-stage TMJ and TMJ-related joint concerns.

In another similar study conducted by Dimitroulis et al. [25], a preliminary clinical study on the OMX© TMJ total joint replacement was collected. The novel OMX© TMJ 3D printed implant is fabricated using a fossa made of UHMWPE, a ramus condyle unit of titanium alloy which is fastened to the bone using screws also made from titanium alloy. Figure 4.3 represents the OMX© TMJ prostheses used in this study.

In this study, 31 females and 7 males participated ranging between 20 and 66 years with a mean of 43.8 years (±14 years). Thirty-eight out of the fifty patients were implanted with implants. Ten out of the fifty patients received the customized OMX© implant, whereas the rest of the group had implants that were matched based on a virtual planning software, with 12 the patients receiving the prosthetic joints in

the bi-lateral direction. A preliminary set of data was obtained on a mean follow up period of TMJ implantation, of around 15.3 months (ranging in between 12 and 24 months). Results revealed a significant positive effect of the OMX© customized TMJ prosthesis on the patients. Drastic improvement ($p < 0.05$) was reported in jaw opening (30.8%), pain reduction (74.4%), diet intake (77.1%), and functioning (59.2%) based on the visual analog scale. Moreover, a 0% device failure rate was reported throughout this study. Again, the customization 3D printing of TMJ prosthesis proves to be a promising solution to provide a fully functional, safe, and dependable technique to treat final-stage TMJ conditions.

In a review collected by Popov et al. [21] one of the cases represented was on the development of a mandibular implant. As mentioned earlier, mandibular implants experience huge amounts of cyclic fatigue loading. The implant was developed using an electron beam melting technique. The implant was also treated with hot isostatic pressure (HIP) treatment to augment the resistance due to fatigue. Additionally, to improve binding at the joint, a 3D printed bone model was also developed from plastic. The result suggested that the use of a 3D printed plastic bone along with a 3D printed metal (titanium) implant promoted the surgical procedure and provided better suitable fixation solutions.

Another similar study conducted in China, by Chen et al. [27], the biomechanical functioning of a customized 3D printed TMJ prosthesis was studied using the finite element analysis technique. The model was first designed in the Mimics 18.0 setup and then its corresponding stereolithography (STL) format was obtained. A set of two models were developed to do a comparative study. One of the models was used to study the strain behavior on an intact mandible, whereas the other was used to study the same behavior on a mandible implanted from one of the sides. The computer-aided finite element analysis was performed using Hypermesh and LS-DYNA software. Using a maximum masticatory force, the stress–strain distributions were studied on the customized implant. The results from this study reported that the maximum stress obtained on the surface of the UHMWPE was around 19.6 MPa. The maximum stress of 170.01 MPa was found to be at the front and back surfaces of the condylar neck for the mandible component. Additionally, the peak von Mises stress (236.08 MPa) was obtained at the top screw of the mandible. However, asymmetric stress–strain distribution was observed for the intact mandible comparatively. With these results, it can be concluded that asymmetric stress–strain distribution can be obtained without altering the functioning of the natural joint on the opposite end. Such uniform functioning of implants promotes the potential of enhanced designing and modeling of 3D printed implants for effective pain relief and treatment.

### 4.2.4 Upper Limb Joints

It is estimated that in the USA, in another 30 years, an estimate of around 3.6 million people will have had amputation procedures done of some sort. Additionally, it is reported that in 2016, 22% of the people who received amputations was an upper

limb amputation. Moreover, a staggering 52% of the amputees are reported to abandon their prosthesis due to discomfort and added bulkiness. However, the incorporation of the transitional prosthesis within 4 weeks of amputation reduces the load on the contralateral limb which results in increased functioning and assisting in overall body coordination [28].

Another major concern is associated with providing upper limb prosthesis for children. Over the past 20 years, a spike in the number of children born with congenital upper limb shortcomings or developed traumatic amputation is reported. A reduction of the upper limb varies from 4–5 children in 10,000 to 1 in 100 live births globally. Furthermore, estimated 1500 children in the USA are born with upper limb reductions annually, and over 32,500 children experience a pediatric amputation.

Currently, these temporary devices are hand-made, which need a longer fabrication time as well as require highly trained specialists to develop them. Moreover, the patients that use the socket-type prosthesis are reported to experience skin disorders that are easily prone to infections. The resulting skin conditions impact the quality of life and reduce the daily functioning of the patient [28]. Therefore, the need to develop a more customized cost-effective solution to provide upper limb prosthetics especially for children is vital to incorporate the part as early as possible.

A study led by Zuniga [28] was focused on developing a low-cost upper limb prosthesis for human finger, and shoulder prosthesis [29] using 3DP technology, as using a customized prosthesis can help improve bi-manual activities and unilateral activities with the function of grasping and holding. For the developed shoulder prosthesis, manually adjustable options increased wrist movements, elbow extensions, and shoulder rotations as well. For this study, PLACTIVE™ with 1% antibacterial nanoparticle additive was purchased as the ready-made 3DP material. The PLACTIVE™ is a pure high grade of polylactic acid (PLA), which incorporates copper nanoparticles as the key material to provide the antibacterial effect. Copper nanoparticles are highly effective in killing bacteria as well as other pathogens including fungi and viruses. The customized prosthesis was designed and developed using extrusion and FDM. The results showed that the surface of the developed prosthesis was effective against 99.99% to *S. aureus* and *E. coli*. The study also states that the antibacterial characteristics of the 3D printed PLA filament were not altered after addition of the nanoparticles or after the extrusion which supports in the positive post-processing modifications needed for customization of any protheses. Through this study it is highly evident that using PLACTIVE™ in 3DP technology can provide a simple inexpensive method to develop customizable patient-specific prostheses and potentially medical devices. Additionally, the fabrication of cost-effective personalized implants for children would have a major impact on the developing child both physically and mentally.

### 4.2.5 Ankle and Talus Joints

Failures and dissatisfaction with joint replacement are continued to be reported even after a successful surgery. Several different approaches have been tried, such as computer-assisted surgery and in-silico simulations. Extensive research has been conducted for the most common joints such as the knee joint and the hip joint. However, the smaller joints of the body such as the wrists and ankles have had lower research attention. With the limitation of sizing and insufficient supply being the major concern for smaller joints, the rapid prototyping provided by the 3DP technology is much more suitable to develop the smaller intricate customized fits better than conventional manufacturing.

In a study conducted by Belverdere et al. [30], a novel customized 3D printed ankle replacement was developed after careful medical imaging, modeling, and designing of the joint. Using CT scanning and 3D bone models of the shank (tibia, fibula, talus, calcaneus), the study specimen was developed. Based on this specimen, the designing of the implant was conducted followed by the 3D printing of the implant using a powder mix of cobalt-chromium-molybdenum. To test the overall accuracy of the performed procedure, distance map comparison was set between the original anatomical implant and the final implant. Moreover, joint torques in the front and axial planes were imposed at three different locations on the joint. The results from this study reported a mean fabrication error to be as low as 0.08 mm with repeated motion patterns consistently being observed with a corresponding standard deviation being less than 1°. Additionally, the stability and mobility of the replaced joints were quite comparable to the original joints. This study promotes the use of 3DP technology for smaller joints such as ankles and wrists and adds significant value towards the development of entirely personalized smaller sized prostheses for patients.

Avascular necrosis (AVN) is the temporary or in some cases permanent loss of blood supply to an area of the bone. As a primary result of AVN, the tissue surrounding the corresponding bone starts to disintegrate. Additionally, if AVN affects an area surrounding a joint, damage to the cartilage can occur and contribute to severe pain and arthritis. In a study conducted by Tracey et al. [31], in the lack of surrounding joint pathology, third-generation total talar prostheses (TTPs) are suggested as a feasible alternative for talar AVN. In this work, 3DP technology is used to reproduce a synthetic talus, which claims to reestablish and retain the normal radiographic configuration of the ankle including the subtalar and forefoot joints in the presence of talar AVN. In this study, talar and TTP implant dimensions and the radiographic measurements for the ankle, forefoot, and hindfoot of 14 patients were taken for analysis, both pre- and post-operation. Results revealed that there were no alterations in the length of the arc and width for the talar joint. However, there was a significant increase in the height of the talar with the addition of the TTP. Additionally, only the talar tilt angle was majorly impacted out of the five alignment measured dimensions. However, the occurrence of corrections was also reported in the Meary's angle, in the cases of planus and cavus deformation of the foot. The positive outcomes of this research indicate the successful employment of 3D printed

TTP which was able to restore the height and tilting angle of the talar in the presence of AVN. Moreover, the use of TTP, in this case, exhibited a normal configuration in nonpathological joints, which promote the use of TTPs to reestablish a regular anatomical orientation.

## 4.3 Bone and Joint Tissue Regeneration

With the need to replace and repair human skeletal joints on the rise, newer materials are introduced and developed to discover the limitations and possibilities of the current joint replacement technologies. Extensive research on the use of 3D printed metal alloys, polymers, and ceramics to enhance joint materials has gained much attention over the years. The interest to incorporate these materials in 3D printing lies in the capability of gradient integration of these parts within the developed components. For instance, porous bone-like ceramic arrangements can be developed with metallic structures that are able to integrate well within the tissues of the bone. These possibilities are just beginning to be explored and show great potential in the near future in the field of bone and tissue regeneration that can facilitate joint replacements and injuries [32].

In various surgical operations, for instance, in orthopedics, dental surgeries, and neurosurgery, regeneration of the bone plays a critical role in the recovery of the patient as it aids to rebuild any flaws and deficiencies in the bone. However, the production and extraction of an autogenous bone from the patient many times can be restricted accompanied by a rate of 25% morbidity from the donor's site. 3DP technology allows for efficient prototyping and experimentation of materials leading to advancements in 3D printed porous scaffolds which are proving to be a promising technique in the field of bone regeneration.

In a study conducted by Carrel et al. [33], tricalcium phosphate (TCP) and hydroxyapatite are used to 3D print layers of strands called Osteoflux (OF) which can be used an alternative to traditionally developed medical scaffolds. The porosity and interconnectivity of this 3D printed material highly specified and can easily be shaped to adopt the shape and dimensions of the bone bed. In this study, a total of six hemispheres made of titanium were packed with OF along with Ceros (particulate TCP (CO)) and Bio-oss (particulate bovine bone (BO) for comparative analysis. A total of 6 hemispheres were inserted in the calvaria of 12 mature sheep and histomorphometric analyses were conducted at the 8-week mark followed by at the 16-week mark. Figure 4.4 illustrates the developed scaffold.

Results of this testing revealed that substantial growth in the vertical bone was observed within 8 weeks with OF. Moreover, new bone formation was observed to develop four times faster with OF in comparison to CO and BO. However, new bone formation was observed to be similar at the 16-week mark for OF, CO, and BO along with mild bone degeneration for all the bone alternatives. This 3D developed material was concluded to improve the growth of the bone in the vertical directions in a sheep calvarial system within a couple of months of the implantation in comparison to the other bone implantation substitutes. The 3D printed block of

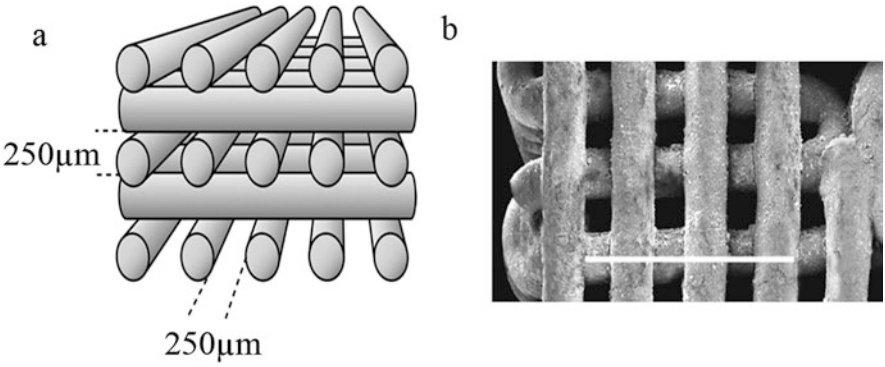


**Fig. 4.4** (**a**) Schematic of the microsctructure of the 3D blocks of OF (**b**) SEM view of OF [33]

layered strands promoted excellent osteoconductivity, with an increase of bone mass of 3 mm above the bed of the bone which is greater than the conventional bone alternatives by fourfolds. Even though the results obtained from this study still require further clinical testing, the results are very promising and show great potential in the use of 3DP technology as bone replacement material [33].

Subchondral bone restoration with the simultaneous rejuvenation of the articular cartilage which opposes vascularization and endochondral ossification is required by osteochondral (OC) defects. Conventionally tissue engineering of bone and articular cartilage requires encapsulating cells or growth elements into layered structures such as hydrogels or scaffolds. Postnatally, the articular cartilage which acts as a growth plate surface during the skeletal growth is substituted by more spatially intricate bone-cartilage interfaces. In research conducted by Critchley et al. [34], the regeneration of the subchondral bone articular cartilage in the osteochondral defects in caprine joints is expected using fiber-reinforced cartilaginous structures. A range of materials such as PCL PLA and PLGA were 3D printed as fibrous mats which were then studied for their in vitro mechanical ability to reinforce hydrogels alongside being able to provide the required support to the mesenchymal stem cell (MSC) chondrogenesis. By incorporating a co-cultured infrapatellar pad of fat derived from a stem or stromal cells (FPSCs) and chondrocytes to formulate the superimposed chondral phase, MSC loaded alginate hydrogels were articulated to build an endochondral bone formation with a two-phase osteochondral built structure. The implantation was primed chondrogenically and executed within mice. The bi-phasic constructs of cartilage resulted in supporting the development of vascularized endochondral bone. Furthermore, restoration of the osteochondral flaws was then clinically studied with the developed hydrogels.in a larger animal model (caprine). Even though the variation of results in terms of the repair was witnessed within different animals, it could be concluded that after observation of 6 months, a smoother cartilage formation was observed in animals treated with the developed constructs in comparison to the animals treated with conventional scaffold structures. Due to the variation in results obtained at different locations, further

advances in the development of 3D printed implants for the regeneration of joints are still very much needed. However, with on-going developments in 3D bioprinting, these 3D printed structures can soon pave the way for a new category of implants for regeneration in orthopedics.

Through prior discussions, it can be clearly seen that the damage caused to cartilages especially around the joints is very common. However, continuous development in the hopes to treat cartilage damage around the joints led to the development of a novel three-part 3D printed joint plug kit [35]. The kit includes three required parts alongside an optional part within the plug. One of the parts is a 3D printed scaffold which is hard similar to the bone which is developed to be able to grow the cells of the bone. The second part consists of a 3D printed scaffold which is soft similar to the cartilage of the bone, which will superimpose the bone and cater to the chondrocyte development. The third part of the three-part plug is a permeable membrane which is to conceal all the parts together to be able to provide the needed synchronized sliding motion during the rejuvenation of the cartilage. The membrane is also responsible for holding the chondrocytes in place and allowing for successful nutrient flow. The additional fourth part is a membrane barrier to prevent chondrocyte loss from the bone to the cartilage. This study presents the detailed design and material usage to develop the three-part joint plug and promotes how 3DP technology is capable to develop a customized solution to fit individual geometries and requirements for targeted joints recoveries.

Surgeries are specifically designed to integrate surfaces that promote osseointegration between the bone and implanted device. Among several orthopedic surgeries, implants associated with the spine endorse the need to include such surfaces to speed the recovery. With the numbers of older people continue to rise, and the reduction in their bone abilities being inevitable, advances in spinal implants that promote osteoconductivity are much needed. With a rise in 3D printing, enhanced bone integration could be approached by generating biomimetic spinal implant surfaces that mimic bone morphology. These biomimetic surfaces are proven to promote the response of cells in comparison to the conventional processes used for surfacing implants. In a study conducted by Macbarb et al. [36], AM technology was used to develop trabecular-like titanium surfaces of the implants which were compared to biological osteoblasts and conventional material with titanium plasma spray-coated surfaces (TPS) with and without the coating of nanocrystalline hydroxyapatite (HA). The coating of AM discs was such that the titanium powder was 3D printed to form a strong foundation that mimicked a porous trabecular-like surface. The coating for the TPS material was a simple layer of TPS coating and for the coating of HA material, a dip-spin technique was used. After characterization, the results revealed that the proliferative growth of cells on AM discs was higher in comparison to the other two sets of material. Besides, the production of calcium on the AM discs was higher by 48% in comparison to the TPS and HA surfaces. However, no variation in the alkaline phosphatase (ALP) activity was observed between AM and TPS discs. Furthermore, the HA addition to the surface did not enhance the activity of the cells in both cases. The results concluded that the proliferative cell growth and calcium production can be enhanced

using AM technology for coating. The ability to fabricate a controlled porous titanium material which has the ability to boost osteoconductivity can be an effective alternative to the traditionally used TPS coated implants used in implants, especially for complex spinal surgeries.

Neurosurgery requires an intricate mix of hard and soft materials to accurately mimic the parts of the skull and the brain tissue underneath. Even though advances in 3D printing associated with neurological conditions are relatively slow, interests in stereolithographic printing to develop such material in the form of hydrogel using poly(ethylene) glycols which can give some control in the mechanical ability of the material are being explored. Tissue engineering can greatly benefit from the similar wet nature of the printed hydrogel to an actual biological tissue. Although hydrogels have not yet been used in developing models for surgical simulations, the on-going research of 3D bioprinting hydrogels shows a promising future for neurology [37].

## 4.4 Use of 3D for Drug Delivery for Joint Replacements and Support Systems

### 4.4.1 Periprosthetic Joint Infection (PJI)

The surrounding area of an implant is easily prone to periprosthetic joint infection (PJI) which is generally caused by *Staphylococcus aureus* (*S. aureus*). It is reported that after a total joint arthroplasty (TJA), the rate of infection varies between 1% and 4%, with a higher infection rate for unsuccessful revision surgeries due to the extended procedure time. Although the infection rate is low, the occurrence of the infection is on the rise due to increasing arthroplasties performed with time. Currently, to treat such infections, antibiotic releasing temporary bone-cement spacers, usually made of poly (methyl methacrylate) (PMMA) are used to replace the primary implant. However, this sort of pacer lacks good mechanical properties and still releases antibiotics ineffectively.

Advances in 3D printing have tried to address this issue has 3D printing is able to develop reservoirs that have improved mechanical strength and can perform a controlled and effective antibiotic release for a prolonged duration. In this study conducted by Allen et al. [38], calcium sulfate hemihydrate (CSH) implanted with gentamicin powder was mixed and injected into reservoirs that were 3D printed mainly using rigid polyurethane (RPU). After the settling of the cement, the reservoirs were then submerged in saline solution and an antibiotic concentration measurement is conducted at varied time intervals. Figure 4.5 illustrates the design of this 3D printed reservoir.

By varying the parameters of the reservoir, such as the diameter of the channel, the length of the channel, and the quantity, the in vitro release of the antibiotic was optimized. Additionally, effective prediction of the antibiotic release curves was developed using a computational model to develop designs with the desired profile of release. Results revealed that in the first 24 h, there is a spike in the release of gentamicin from PMMA was noticed followed by a very minimal release. However,

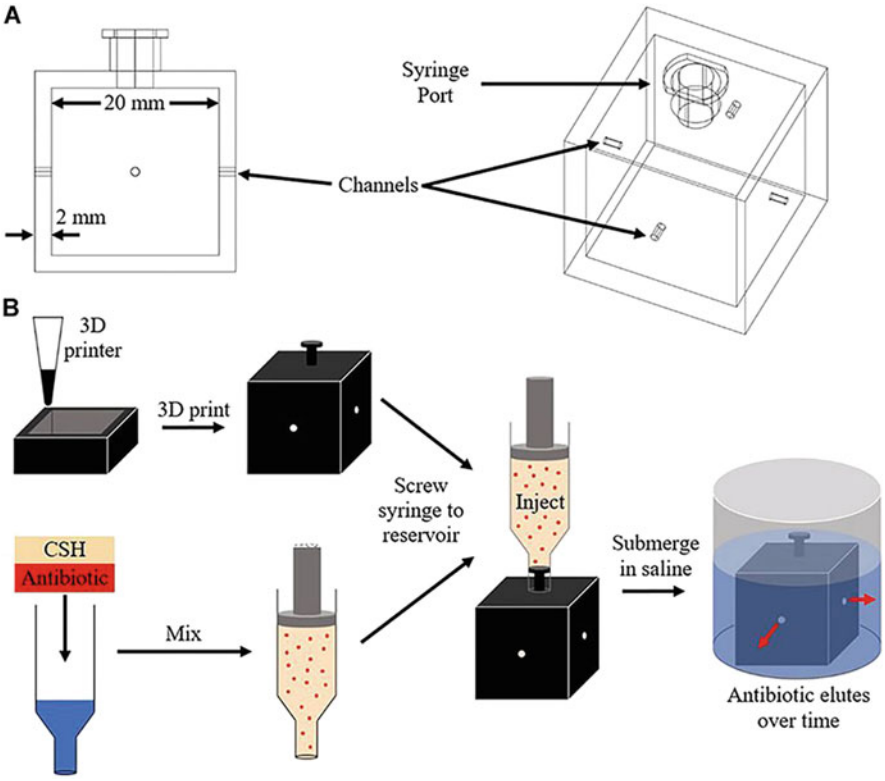


**Fig. 4.5** Design of 3D printed antibiotic reservoir [38]

the spike in the release of gentamicin from the CSH was high in the first 5 days, followed by a consistent release of lower levels of gentamicin over the next month. It was also concluded that the release by the reservoir loaded with CSH was at required therapeutic levels of gentamicin for a considerably longer duration of time in comparison to CSH or PMMA individually. The positive results of this study highlight the potential to use 3D printing in developing a mechanically enhanced reservoir capable of a prolonged antibiotic release that can improve the recovery rate from PJI and treat joint infections more effectively.

As previously stated as well, knee and hip replacement surgeries are on the rise every year, with up to 3.3 billion knee surgeries expected annually in the USA itself. The general procedure is a dual exchange process, which first includes removal of the prosthesis, followed by material implantation of polymethylmethacrylate (PMMA), which is an antibiotic spacer placed to provide a high dosage of the antibiotics to the joint, and finally the implantation of the replacement. Unfortunately, the PMMA material has its limitations concerning mechanical performance and its ability to deliver drugs. The brittle nature of PMMA results in a large amount of the antibiotics being eluted within the initial days. Additionally, the

polymerization process of PMMA is high—exothermic in nature, which limits the use of heat resistant and heat-stable antibiotics. To tackle these limitations, the use of 3D printed polymer-based liner developed from polylactic acid (PLA) has come into existence. Due to the increased mechanical and thermal stability of PLA, better performance is reported with the use of this material compared to the traditional PMMA spacer. Moreover, since the 3D printed PLA liner can control drug delivery through in-built microchannels by integrating antibiotics at the development phase, it can deliver better results. The most unique characteristic of 3D printing is the ability to customize according to the patient's anatomy, making this approach to treat periprosthetic joint infections a more beneficial one [39].

### 4.4.2 Support Cast

In an average lifetime of a person, at least one or more fractures is estimated to affect 2 out of 100 people. Big conventional cutaneous orthopedic casts are normally developed using a body-contact approach. With the increase in the number of fractures occurring, the demands for developing a hygienic cast are also on the rise. The approach of this research focused on using 3DP technology to fabricate an effective smart modeling method to develop orthopedic casts that are patient-customized and highly hygienic [40]. The incorporation of capturing the patient's image to fabricate the customized design along with computer modeling is used. Figure 4.6 represents the 3D printed cast based on an intelligent system that uses rapid modeling techniques.

A funnel-shaped design is employed for the cast, which contributes to the edges of the cast being smoother to avoid bruises from movements of the wounded limbs. The gaps within the surface arrangement are aimed to provide proper ventilation for hygienic reasons and provide comfort to wear. Additionally, the developed cast can adapt to adjust in case of swelling from the injury. The validation of the mechanical properties of the cast was performed using finite element analysis (FEA) which is important as through this examination the risk of collapsing due to the stress concentration of the developed model can be evaluated. The results from this

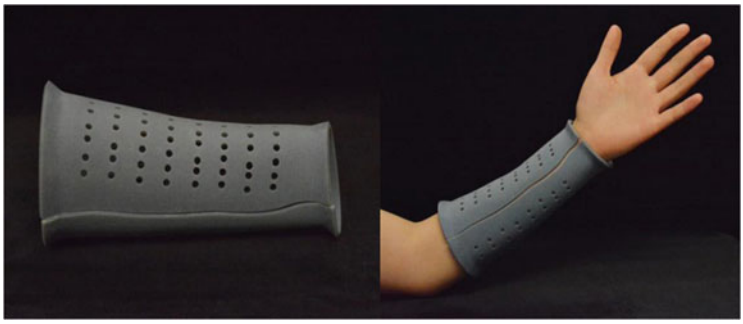

**Fig. 4.6** Intelligent system based 3D printed cast [40]

study showed that the design of the 3D printed cast was very lightweight with 1 out of 10 in comparison to the weight of the traditional substitutes. Furthermore, technicians with limited technical experience were also able to design the cast using the suggested method within 20 min. The customized geometry developed on the patient's image-basis reduces the distortion while healing, and promotes a speedier recovery. Additionally, the enhanced ventilated arrangement of the 3D printed cast promotes hygiene and reduces the potential of occurrence of cutaneous infections thereby improving effective treatment and patient comfort.

## 4.5 Conclusion

Past training and experiences accompanied with visual aids from medical imaging techniques, for instance, CT or MRI, play a crucial role in guiding surgeons for preoperative planning of surgical procedures. However, it is quite possible that virtual images or two-dimensional images are not enough to provide the intricate details required due to the complexity in the anatomy of the surgical site. In such cases, the patient's anatomy expressed using a 3D printed model enables customized preoperative surgical planning effectively. As highlighted in this book chapter, employing 3D models of replacement joints and cartilages are starting to gain much momentum in the medical field. 3DP is now considered the preferred alternative to replace both bone and cartilage, by creating replacement joints that move smoothly and have high bio-functionality. Additionally, 3DP is providing critical aid to surgeons to operate on intricate joint systems and assist in implantations. The simple procedure of 3DP from imaging scans to developing 3D-image files that can be easily created and sent to a specialized 3D printer, to finally produce the outcome makes it an effective fabrication technique. The surgeons are now using these models to plan pre-surgical treatments, as well as for consulting during surgical operations. 3DP can provide a different perspective to the surgeons, which is well appreciated in the medical field. In the upcoming years, 3DP is set to play a crucial role in medical simulation and education as well as be heavily adopted in the design of customized orthopedic implants and other intricate medical structures. The increased "patients" satisfaction rate with the use of 3D printed implants makes it a promising alternative in advance medical science in the near future. Moreover, through close collaboration between medicine and engineering, fully functional 3D bioprinting of cells and organs can be expected to be developed soon, especially focusing on using 3D Printing biomaterial [41] for manufacturing joints with the aid of using digital 3D scanners [42] that would make a perfect replica of the damaged joint that could be verified experimentally through the mechanical properties [43] of the tested joints that could be implemented using different types of the 3D printers [44] of different 3D printing capabilities [45] that have the capacity to produce numerous medical prosthesis for different applications [46].

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# Design of Patient-Specific Maxillofacial Implants and Guides

5

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**Abstract**

With the development of three-dimensional (3D) design and manufacturing technologies, it is possible to easily manufacture various computer-aided patient-specific instruments. In the maxillofacial region, treatment of facial defects, asymmetries, and dental disorders can be done efficiently by using custom-made implants. In addition, reconstruction of the jaws even including temporomandibular joints can be performed by today's 3D technologies. One of the most popular subjects is the use of computer-aided design and manufacturing techniques in orthognathic surgery. Postoperative outcomes of maxillofacial surgeries can be improved by integrating the patient-specific implants (PSIs) into the treatment protocol. With this novel approach, the contouring that is required to ensure the geometrical compatibility between the patient's anatomical

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form and the implant is eliminated. Screw positions can be planned during the preoperative simulation so as not to damage any anatomical structure. These preoperative preparations shorten the operating room time. Also, customized osteotomy and drill guides can be used to fixate the implants in the planned position, which minimizes damage possibility over the maxillofacial region and makes surgeries more accurate. The fabrication stages of such implants include (1) obtaining a three-dimensional solid body model of anatomical structures from the patient's two-dimensional scanning images, (2) simulation of the operation on the anatomical computer model, (3) design of the PSI according to the patient's model, (4) manufacturing of implants by using proper additive production methods. In this chapter, we described state-of-the-art studies about the development of patient-specific maxillofacial implants and guides, highlighted current insights, and focused on reported clinical outcomes. Besides, we presented the design stages of a PSI and guide for a bimaxillary orthognathic surgery.

**Keywords**

Patient-specific implant · Patient-specific guide · Custom-made miniplate · Customized miniplate · Custom-machined miniplate · Orthognathic surgery · Maxillofacial surgery

## 5.1 Introduction

In the last two decades, patient-specific implants (PSIs) have become widespread with the advances in three-dimensional (3D) computer-aided design (CAD) and computer-aided manufacturing (CAM) technologies in different fields of medicine [1]. Hip and knee arthroplasties in orthopedic surgery and cranial surgery are some of the implementations of PSIs [1]. PSIs are also used in oral and maxillofacial surgery for reconstruction of orbital defects, facial contouring, reconstruction of the mandible, dental rehabilitation, temporomandibular joint prosthesis, and orthognathic surgery.

Oral and maxillofacial surgeons applied PSI for the first time in the 1940s. These were subperiosteal implants for dental rehabilitation [2]. But two operations were needed for this procedure due to the absence of modern imaging techniques and CAD-CAM technology. In the first operation, the soft tissue over the edentulous jaw was incised and raised to take an impression of the alveolar bone. After the manufacturing, the flap was raised again and the PSI was fixed to the jaw with a second operation [2].

Now, we can collect data about the form of any internal tissue with current imaging techniques. We can design PSI on the virtual 3D model and we can manufacture the design with different techniques. So in maxillofacial surgery, the additional operation to take the impression is not needed anymore and PSIs became an easier treatment option than before.

In recent years PSIs and patient-specific guides (PSGs), which are used to determine the position of the implants during the orthognathic surgery, have been presented [3–19]. Le Fort I osteotomy for the maxilla and bilateral sagittal split osteotomy (BSSO) for the mandible are some of the most commonly used techniques for orthognathic surgery [20]. In the traditional orthognathic surgery technique demonstrated by Harris and Hunt [21], intermaxillary occlusal splints are used to move the maxilla and distal segment of the mandible to the planned positions. Intermaxillary occlusal splints are a pair of acrylic bite guides that have imprints of teeth of both jaws on either side. During the operation, intermaxillary occlusal splints show the planned position of the relevant jaw according to the other one. To fabricate intermaxillary occlusal splints, dental plaster models of both jaws are produced. Then plaster models are placed in a semi-adjustable articulator with a face bow transfer. The plaster model of the maxilla is cut and fixated to the planned position. The intermediate intermaxillary occlusal splint is produced, which guides the segmented maxilla to its planned position according to the mandible's initial position. Then the plaster model of the mandible is cut and fixated to the planned position. The final intermaxillary occlusal splint is produced, which guides the distal segment of the mandible to its planned position according to the final position of the maxilla. This process is called model surgery. Intermediate and final intermaxillary occlusal splints are produced in reverse order for operations where first the mandible and then the maxilla are operated [21]. Also today intermaxillary occlusal splints can be designed by using some planning software and manufactured by 3D printers to overcome errors that can occur during the model surgery [13]. However, traditional orthognathic surgery technique has some disadvantages. An intermediate intermaxillary occlusal splint guides the maxilla according to the initial position of the mandible, but in the operating room, condyles of the mandible are manually positioned in centric relation by the surgeon according to his or her experience [19]. The final intermaxillary occlusal splint guides the distal segment of the mandible according to the final position of the maxilla [9]. But during the fixation of the mandible, proximal segments are manually positioned in a normal condyle-fossa relation, ideally in the centric relation. So, due to potential errors during the positioning of the condyles, the maxilla and mandible are not perfect references for the fixation process. Also, intermaxillary occlusal splints do not guide the maxilla vertically [14]. The surgeon places the maxilla vertically according to the operation plan by taking the measurements of some marks or reference points [10]. Most of the time these measurements are not geometrically perfect. In the traditional technique, conventional miniplates are used for fixation. These miniplates are contoured manually and are not always in a perfect fit. Some minor positioning errors can occur if the miniplates are not contoured properly. Contouring takes some time in the operating room. Besides, there is a risk of deformation of the conventional miniplates due to the forces applied to them.

The novel 3D printing technologies allow for the fabrication of PSIs for fixation and PSGs for osteotomy lines and screw holes. PSGs enable a surgeon to (1) cut planned osteotomy lines that do not interfere with planned screw holes of PSIs, (2) drill the screw holes in planned positions and depths, which are in a surgically

safe area, and a large and dense bone for better anchorage, (3) cut the osteotomy lines and drill the screw holes at once and save operating room time, and (4) fixate PSIs in preplanned positions. So, this technique has some advantages over the intermaxillary occlusal splint. PSIs and PSGs position the maxilla according to the cranium and position the distal segment of the mandible according to the proximal segments of the mandible. Therefore potential errors in the positioning of condyles can be eliminated. PSIs and PSGs are also used to position the maxilla vertically. Since PSIs have a good match with the bone surface, they do not result in a high level of residual stress and there is no need for contouring of PSIs. PSIs can be manufactured highly rigid, unlike the conventional miniplates which are contoured manually during the operation.

In this chapter, we present the design stages of a set of patient-specific implants and guides used in bimaxillary orthognathic surgery.

## 5.2 Material and Methods

### 5.2.1 Operation Planning

The preoperative virtual planning process was carried out for Le Fort I osteotomy and BSSO operations. Computed tomography (CT) data of a skull with skeletal class III malocclusion, mandibular prognathism, and maxillary retrognathism were acquired from a cadaver. The head position was set according to the Frankfort horizontal plane. We planned a 5 mm set back in the mandible with some yaw rotation to fix the midsagittal line and 5 mm advancement in the maxilla along the anteroposterior axis.

### 5.2.2 Three-Dimensional Models of the Maxilla and Mandible

The pixel spacing of CT data was 0.48 × 0.48 mm with an interslice dimension of 0.625 mm. The maxilla and mandible with temporomandibular joint and zygomatic process were segmented by taking into account the Hounsfield Unit (HU) thresholds of bone tissue between 226 and 3071 HU [22] and then, 3D models of the maxilla and mandible were created from two-dimensional CT images (Fig. 5.1). To do this, an open-source and non-commercial software (3D Slicer, Slicer Wiki) was employed.

### 5.2.3 Design of the Patient-Specific Implants and Guides

The model of the PSG for Le Fort I osteotomy was developed according to the surface of the maxilla. The outline of the surface was drawn and the surface of the PSG model was extracted in accordance with the design criteria determined in the operation planning stage. The same procedure was repeated for the PSG to

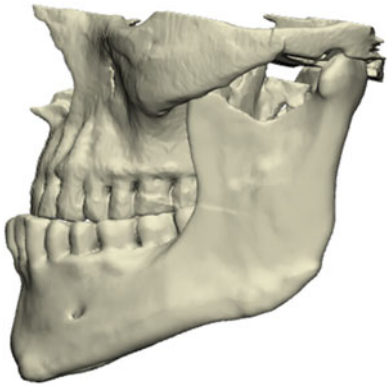

**Fig. 5.1** Sagittal view of the three-dimensional model of the maxilla and mandible

be used in BSSO of the mandible. The PSG for BSSO was modeled to set back the mandible 5 mm along the anteroposterior axis with some yaw rotation. The surfaces of the PSGs for Le Fort I osteotomy and BSSO were in full contact with the maxilla and mandible, respectively. The surfaces of the PSGs for Le Fort I osteotomy and BSSO were exploded such that the 3D model had a thickness of 1 mm. The 3D model which is in the STL format was exported to SolidWorks (DS Solidworks Corp., Waltham, MA) to create drilling and fixing holes. After the export, the STL format was converted to Standard for the Exchange of Product (STEP) file. The screws to be used for fixing the PSGs to the maxilla and mandible had a diameter of 2.3 mm (head diameter was 2.6 mm). Fixing holes with a diameter of 2.3 mm were created on PSGs. For embedding the head, the screws of PSGs have a 0.3 mm countersunk. The screws with 2 mm diameter were used for fixation of the PSIs to the maxilla and mandible. Holes with the same diameter were formed on the PSIs. While creating a hole in the mandible, 1 mm protrusions were formed on the PSIs to create a hole in the right direction (Fig. 5.2).

The magnitude of advancement was adjusted by measuring the distances between the maxilla and mandible along the anteroposterior axis. Le Fort I osteotomy was simulated on the maxilla model by using the maxillary PSGs. The maxilla was horizontally cut and the detached part of the maxilla was advanced 5 mm along the anteroposterior axis. BSSO was performed on the mandible by using mandibular PSG in accordance with design criteria. The osteotomy area was oriented based on the sketch of the PSG. Then the detached mandible was set back 5 mm along the anteroposterior axis with some yaw rotation (Fig. 5.3).

The process continued with modeling PSIs for the maxilla and mandible. The modeling stage of the PSIs was similar to that of PSGs. Models of the maxillary PSIs and mandibular PSIs were designed by taking into account the surfaces of the maxilla and mandible, respectively. The surfaces of the PSIs were extracted in accordance with the design criteria. The surfaces of the maxillary and mandibular PSIs were in full contact with the maxilla and mandible, respectively. The surfaces of the maxillary and mandibular PSIs were exploded such that the 3D model had a thickness of 1 mm. The 3D model in STL format was exported to SolidWorks

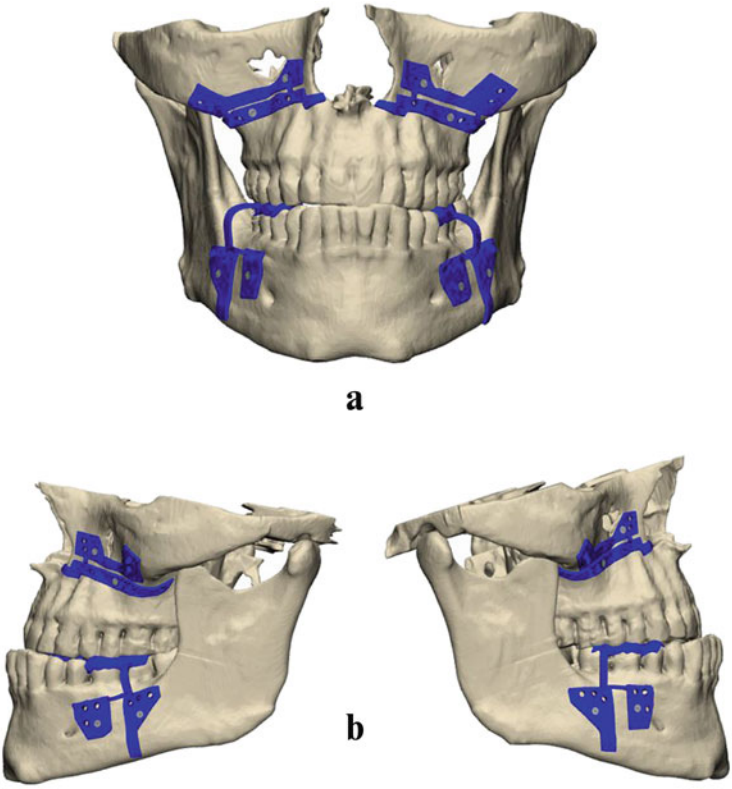


**Fig. 5.2** Computer-aided design models of the patient-specific guides (PSGs) for the maxilla and mandible. (**a**) Coronal, (**b**) sagittal views

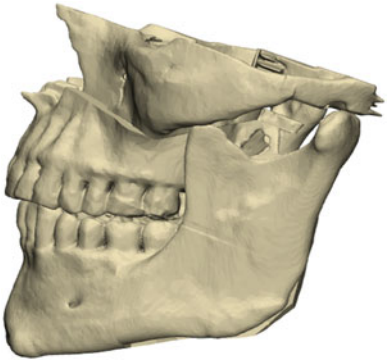

**Fig. 5.3** The dentoskeletal model with the advanced maxilla and set back mandible

(DS Solidworks Corp., Waltham, MA) to make fixing holes on the PSIs which overlap the drilling holes on the PSGs. Then the STL format was converted to the

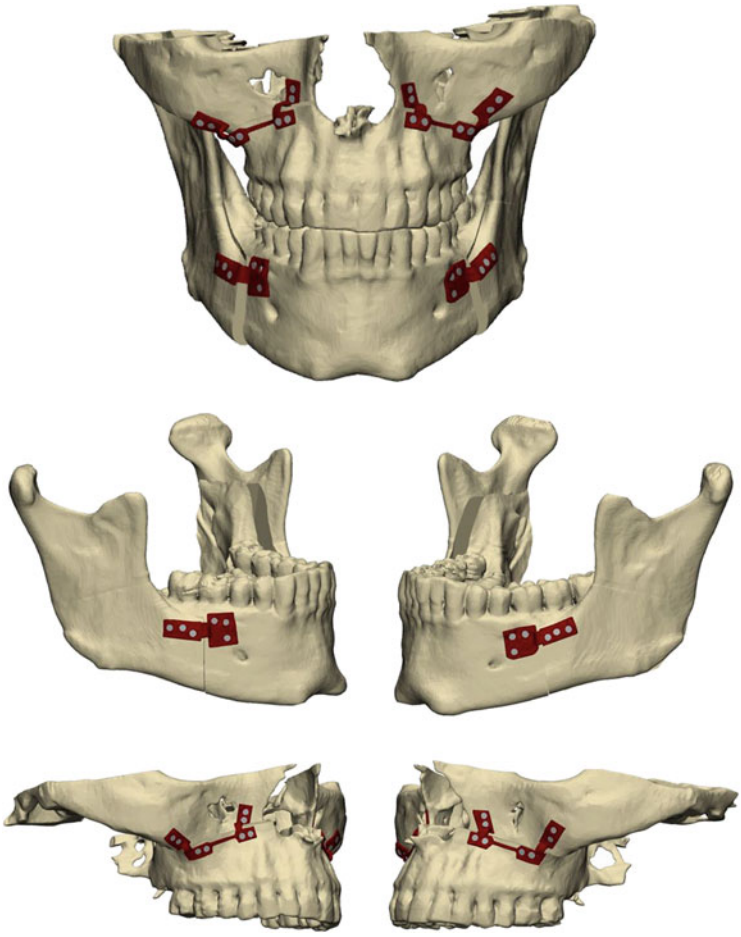

**Fig. 5.4** Computer-aided design models of the patient-specific implants (PSGs) for the maxilla and mandible

STEP file. All holes were created to have a diameter of 2 mm with 0.3 mm countersink for embedding the head. The screw to be used for fixation of PSIs to the maxilla and mandible has a diameter of 2 mm and their head is 2.3 mm. The maxilla and mandible were fixated to their new positions by using PSIs (Fig 5.4).

Fixation screw holes were planned in the positions such that probable damage to the inferior alveolar nerves, roots of adjacent teeth, and possible osteotomy lines would be avoided. The maximum possible length of each screw was noted but screws on the proximal segments of the mandible were planned monocortically. Stock screws were used for fixation.

## 5.3 Implications of Patient-Specific Implants and Guides

The use of PSI in orthognathic surgery is still a new technique. As far as we know the first English report regarding this content was published in 2013 [14]. In this very first design for Le Fort I osteotomy, PSGs were indicating only the osteotomy lines. After the down-fracture of the maxilla, PSI was positioned on the surface manually. Even though Philippe had satisfactory results, the use of PSGs, which also indicate screw positions, are common today [14]. We consider that PSG designs increase the accuracy of the operational interventions. But there are no well-accepted PSI or PSG designs for orthognathic surgery, yet. More studies are needed to compare the accuracy of different designs.

Various PSI designs for Le Fort I osteotomy were presented by previous authors. Some designs consist of a single unit [6–8, 14, 19]. Two-unit PSIs for each side of the maxilla were used as well [3, 4, 11, 13, 16, 18]. Gander et al. designed three-unit PSI for a two-segment Le Fort I osteotomy patient. Two of the units were positioned at the zygomaticomaxillary buttresses but the other U shaped PSI was fixated to paranasal buttresses of both segments [5]. Some authors preferred four-unit PSIs which are similar to L shaped conventional miniplates [9, 10, 12]. We prefer a two-unit design to avoid the larger incision need.

All previous PSI designs for BSSO were two-unit for each side of the mandible [3, 7, 11, 17, 23]. Most of the PSI designs have 6 screw holes [3, 11, 17, 23]. But PSI with eight screw holes also have been used [7]. We recommend that 4 screws would be enough for fixation of BSSO but we used PSI with six holes to eliminate problems in case a hole was over-drilled.

Different PSG designs for Le Fort I osteotomy were presented in the literature [18]. PSGs are positioned by a surgeon manually. There are some landmarks and anatomic curves on the maxilla which lead PSGs to the correct position. These references are piriform aperture, anterior nasal spine, zygomaticomaxillary buttress, alveolar bone curves around the roots, and the surface of the anterior wall of the maxillary sinus. Some authors presented two-unit PSG for each side of the maxilla and this design enables a surgeon to position the PSG from a small incision [4, 5, 9, 11–13]. This is an advantage especially for craniofacial deformity patients with poor vascularization in the region. Liu et al. used a two-unit PSG design for microsomia patients [12]. But one-unit PSG is commonly used as well [3, 6–8, 10, 14, 16, 18, 19]. We prefer a two-unit PSG design with extensions to zygomaticomaxillary buttress and piriform aperture of the maxilla to avoid misfit.

For BSSO, Brunso et al. used a single-unit PSG with a full-arch occlusal plate over mandibular teeth [3]. Also, some PSG designs were two-unit with a smaller occlusal plate [11, 17]. Ho et al. used a large two-unit PSG design which partially covers the external oblique line and base of the mandible for edentulous patients [7]. We use a two-unit design which has an extension to the occlusal sides of adjacent teeth.

Accuracy of PSIs with PSGs for Le Fort I osteotomy was evaluated by many authors with different methods and considered accurate in the literature [3, 5–7, 9–14, 18, 19]. PSIs with PSGs for BSSO were considered accurate by some authors as

well [3, 7, 11]. But according to a study with 30 patients, the PSI technique for BSSO was limited and the fitting of the PSI was unpredictable due to the positioning of the condyles and bony interferences [17]. To overcome these problems, we recommend making the osteotomy plane close to the sagittal plane, removing bony interferences, and using the secondary osteotomy technique of Ellis III [24]. We have also designed a PSG to determine all of the osteotomy lines for BSSO [25, 26].

Guides and osteosynthesis miniplates are generally manufactured by using Ti-6Al-4V and pure titanium materials because they are biocompatible and prevent oxidation, and also provide toughness and stiffness to the dental devices [27]. 3D printing or direct metal laser sintering technology is generally used for manufacturing subject-specific models [5–7, 11, 13–15, 18]. Some others used PSIs that were machined from titanium blocks [3, 9, 17, 23, 28]. Liu et al. used electron beam melting [12]. Carnerio et al. manufactured PSI by laser sintering and put a machine milled ultra-high molecular weight polyethylene graft on it [4]. Brunso et al. criticized the laser sintering for a high risk of contamination and lower rigidity [3]. Suojanen et al. reported that there were no differences in infection rates between PSIs and conventional miniplates for Le Fort I osteotomy and BSSO. But they did not mention their manufacturing method [23, 29]. We recommend the manufacture of PSIs and PSGs from titanium alloys to avoid the dust of other materials during the operation.

PSIs for orthognathic surgery can be manufactured with high toughness and strength. Their mechanical characteristics can be analyzed by using finite element approach and the design parameters can be improved by various optimization techniques. These improvements can reduce the size of PSIs which would lead to a cost-effective manufacturing process.

PSI and PSG technique for orthognathic surgery have also some disadvantages. Most of the companies producing such devices do not provide service in developing countries, which increases the cost. Hence we recommend the production of PSIs with the cooperation of local manufacturers to overcome this issue. Also, this technique needs a CAD operator and the CAD process is time-consuming. But these disadvantages may be eliminated with repetition and standardization of the protocol soon.

## 5.4 Conclusion

PSI and PSG applications in oral and maxillofacial surgery have been widespread thanks to the developments of 3D design and manufacturing technologies. PSIs and PSGs for Le Fort I osteotomy are accurate and reliable. These novel devices also eliminate the need for manually positioning of the maxilla vertically and mandible in the centric relation during the fixation of the maxilla in the operating room. Such interventions in a conventional way are possible causes of positioning error and dependent on the experience of the surgeon. Therefore, PSIs for Le Fort I osteotomy are promising and the use of these devices would increase in the near future. But PSIs for BSSO have some difficulties. The osteotomy is carried out as a controlled

fracture and hence the fracture surface cannot be predicted during the segmentation of virtual operation planning. Bone interferences appear as a result of an unpredictable irregular fracture surface and movement of the mandible. In this case, the successful alignment of the segments depends on the experience of the surgeon. In the future, the whole osteotomy surface may be transferred to the operating room with dynamic surgical guides to solve this problem.

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# Design and Development of Surgical Guide for Dental Implant Surgery

6

Varun Arora, Sanjeev Kumar, Parveen Kalra, and Vivek Sharma

**Abstract**

Use of dental drill guides helps surgeons in improving pre-surgical planning. It could lead to a decline in surgical mistakes and more accurate results. Other uses of these guides are simulation and training. Use of dental drill guides can ensure placement of an implant at the location where there is insufficient bone, chances of collision of drill with the nerve and the drilled hole. Cavities prepared using drill guides are of an exact size as desired, which further results in fewer chances of implant failure and less healing time. A computer-generated surgical guide can provide a link between treatment plan and real surgical treatment by passing on the simulated plan precisely to the surgical site. The targets for the design of dental drill guide are as follows:

1. The accuracy of drilled holes.
2. Temperature control of bone by better circulation of irrigant.
3. Easy to use and adaptable by the surgeons.
4. Economical.
5. Easy to fabricate.

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6. Universal and can be used in combination with drilling system of any implant provider.

Design and development of dental drill guide can be divided into four major stages.

1. Diagnosis.
2. Image/data acquisition.
3. Image/data processing.
4. Fabrication.

**Keywords**

Dental implant design · Jig · Dental surgery · Computer aided design · Surgical guide

## 6.1 Introduction

Until now, surgeons are making use of radiographic imaging for preoperative planning and execution of the surgery. This may cause potentially higher amounts of risk to patient safety. Hence, there is a need for an approach that provides surgeons with the capability to enhance their pre-operative planning and assist them in improving their intra-operative procedures. A precise 3D printed aid may help surgeons in choosing the optimum approach and achieving the best results. As dental implants are now recognized dental treatment, the variety of its applications to challenging situations: having a limited amount and quality of bone has enhanced. In these kind of situations, proper estimate of the bone quality, decisions regarding the positioning of the implant as well as accurate drilling into the bone are crucial for guaranteeing the successful placement of a dental implant. To conquer these types of complexities and successfully achieve risk-free and precise procedures, CAD / CAM guides are required.

Use of surgical guides for dental implant surgery can increase the bone temperature while performing surgery because of the restriction introduced by the small clearance between the drill bit and the internal diameter of the guiding cylinders/bush/sleeve. This restriction can severely limit the required amount of irrigate flow to the drilling site, leading to increase in bone temperature. The rise in temperature can result in thermal necrosis and may induce many complications like bone cell death, longer healing times and weak bond between the implant and the jaw bone.

### 6.1.1 Diagnosis

Dental treatment of patients starts from planning starts with a detailed consultation with the patient, which is used to figure out the state of mind of the patient and to

know exactly what he demands from the treatment, accompanied by a detailed general and specific health background and intraoral diagnosis with analysis of the initial anatomical condition [1]. Bone sites are considered as a very important issue, which needs to be addressed in the presurgical evaluation and needs to be determined reasonably good in quality and quantity. Grafted locations need to be totally regenerated to a mechanically stable condition before preparation. The treatment plan is made after examination and assessment of every diagnostic information. The plan includes the estimate of number of implants to be placed, their diameters, length, angulations as well as set-up. After making a decision of using surgical guide, the doctor decides which type of guide will be best for the patient.

Previously dentists were supposed to place implants at the locations, where the quantity of the bone is highest, with lesser concern about the final positioning of definitive restoration. In most of the occasions, the placement of implant was less accurate than expected. Even a slight deviation compared to ideal placement can lead to challenges in the manufacturing of final prostheses. Precise placement of implant is needed to accomplish better functional and aesthetic result. As the oral cavity is comparatively restricted space, therefore, high level of precision in the placement of the implant is essential for the success of the prostheses. It can be attained with the aid of a surgical guide which provides sufficient information regarding implant placement and during surgery, it sits onto the present dentition or on to the edentulous span [2].

### 6.1.2 Selection of Dental Drill Guide to Be Used

Based upon the requirement, surgical guide can be used on different contact surfaces like patients jaw bone, teeth or gum. These are known as bone supported guides, mucosa supported guides, and tooth supported guides. There is another kind of guide meant for the placement of special implants such as the zygoma implant.

#### 6.1.2.1 Bone Supported Surgical Guides

Bone supported surgical guides are utilized for partially and fully edentulous patients. A bone supported drill guide is a customized guide made to fit on the jawbone, as its name indicates. For the use of this guide, an incision is created and mucoperiosteal flaps are raised to free the bone surface. Bone supported surgical guides offers improved visibility while performing surgery because of the free bone surface.

#### 6.1.2.2 Mucosa Supported (or Tissue-Borne) Surgical Guides

These guides are used for fully edentulous patients. The key benefit of this technique is the probability of carrying out minimal invasive flapless surgery, which leads to a less difficult intraoperative and postoperative period. These guides are designed for firm fit on the soft tissue. A scan of the patient along with a scan of the prosthesis is essential to visualize the desired setup for improved implant planning [3]

#### 6.1.2.3 Tooth Supported Surgical Guides

Tooth-supported surgical guides are supported by the patient's remaining teeth. These are used in the treatment of single tooth or partially edentulous areas. There is no need to raise a flap to perform the surgery.

#### 6.1.2.4 Surgical Guides for Zygomatic Implants

Unlike conventional implants that are inserted into the jaw bone, zygomatic implants are the long rods which are placed into the cheekbone under the eye socket to support a complete set of upper replacement teeth. Accurate positioning and minimum angle deviation are very important in these kind of implants. For the placement of zygoma implants, drill guide can be either bone or mucosa supported [3].

### 6.1.3 Data Acquisition

The main goal of this stage of treatment is to create and apply a treatment plan for the patient which allows re-establishment of the patient's functionality and looks by the utilization of adequate and ideal needs being transformed physically into a three-dimensional diagnostic template [4]. Appropriate radiographic evaluation is a necessary part of dental implant planning. Two 3D imaging techniques computerized tomography (CT) or cone beam computerized tomography (CBCT) are used to assess the bone quantity and anatomic structures with regard to the proposed implant site. Outputs of both the 3D scanning techniques (CT and CBCT) are compatible with the image processing software, used for drill guides design. Acquiring a CT scan with the correct parameters and protocol is the base for an accurate planning of implants. CBCT commonly provide a less dose of radiation to the patient in comparison to CT and also delivers fairly sharp images with three-dimensional information. The theoretical resolution of CBCT is more than CT [5]. However, the variation might not be as significant because of the influence of patient movements as a result of the higher scanning times [6].

### 6.1.4 Data Processing

The image processing software MIS (Mimics Innovation Suite) by Materialize is used for data processing and drill guide design. MIS consist of three modules MIMICS, 3-matic, Magics. Different modules are used for performing different task for designing surgical guide for dental implant surgery. One of the basic requirements of the software is CT or CBCT data in DICOM (Digital Imaging and Communications in Medicine) format. Therefore, CT scan data of the patient was acquired and saved as per the software requirements.

#### 6.1.4.1 Segmentation of Mandible from the Full Face CT Scan

After acquiring the scan, the data are processed for editing the images by eliminating the scattered and unnecessary parasite images such as spinal column, antagonist

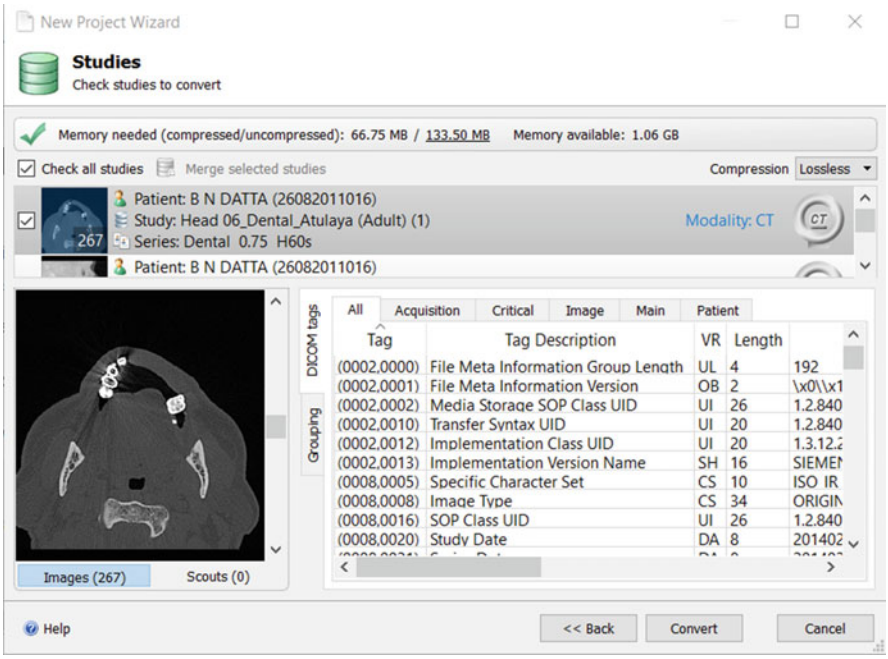



**Fig. 6.1** CT data loading and information about scanning

teeth, and projection of the upper extremities of the mandible for maxillary analysis. Each of the masks can be toggled on or off to enable separate visualization and understanding [7]. Below is the sequence of operations needs to be performed.

Import Dataset: It is required to import the whole data set containing all the slices. After importing the data set, the basic information regarding the scanning including the number of slices, slice thickness, and some other parameters appears on the screen (Fig. 6.1). The moment dataset is imported, it is visible in three diffident views axial, coronal, and sagittal (Fig. 6.2).

1. Creating a mask: Thresholding is a first step towards creating a mask. Thresholding implies that the segmented item (visualized by a colored mask) only contains those pixels of the image with a value greater than or equivalent to the threshold value specified by the user. The segmented mask includes all pixels between specified values (Fig. 6.3). After performing thresholding operation new mask is created. To see what had been created in a mask, there is a need to calculate 3D (Transform data from the 2D images into a 3D model) Fig. 6.4.
2. Region growing: In order to separate out floating pixels and remove unwanted areas, which were not of our interest region growing tool was used. With the use of this tool it is possible to separate the segmentation generated after thresholding into various objects and also eradicate floating pixels.
   How this command works: Select the Source (Green) and Target mask (New Mask). The program begins to calculate the new segmentation, all the points of

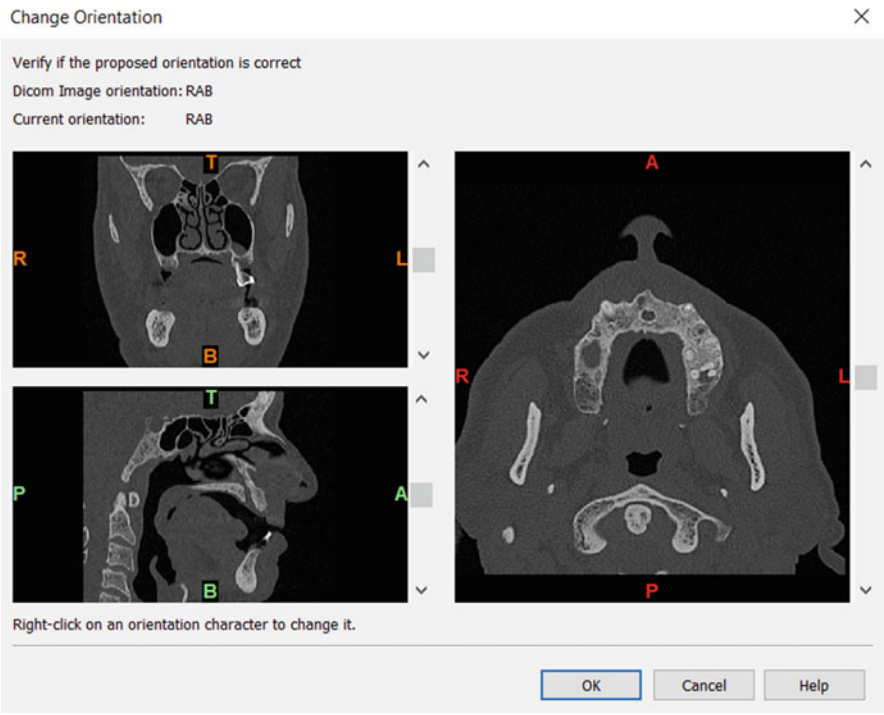



**Fig. 6.2** Visualization of CT data in axial, coronal, and sagittal views

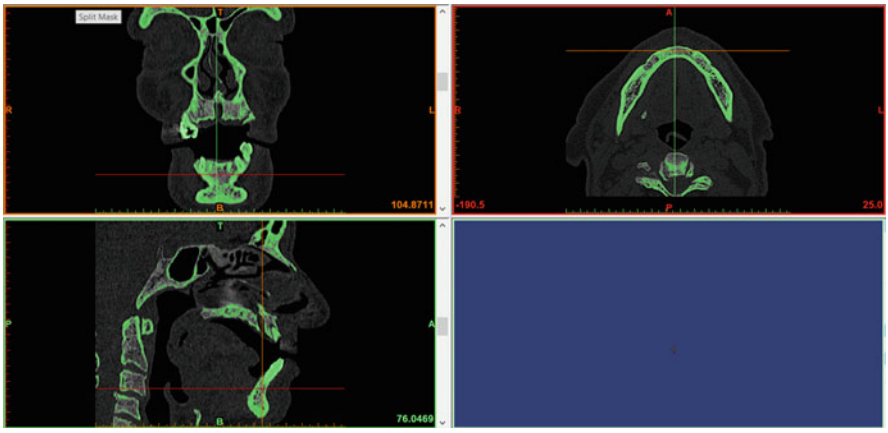



**Fig. 6.3** Thresholding and segmentation

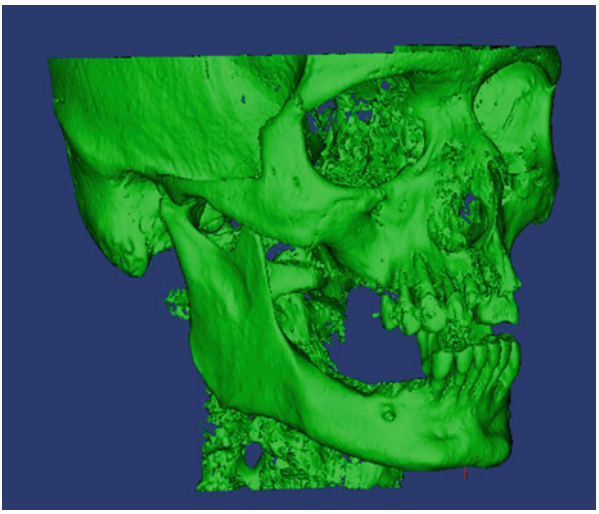


**Fig. 6.4** 3D model creation

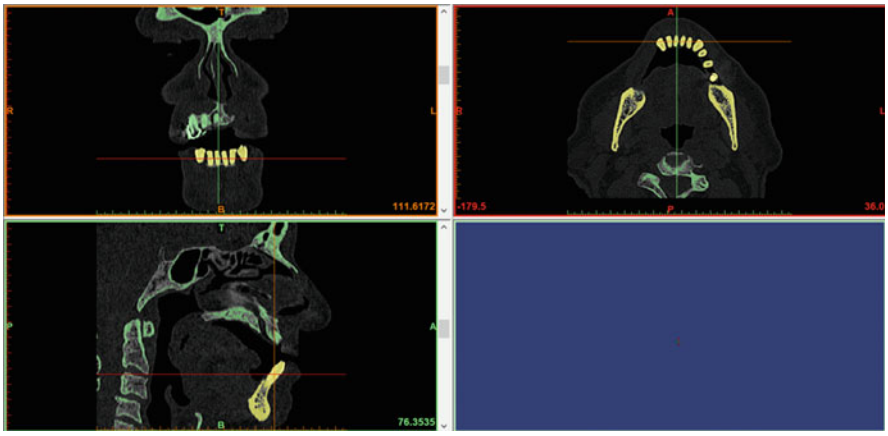


**Fig. 6.5** Region growing

the current segmented object, which are attached to the marked point, will likely to form a new mask. The new segmentation will be yellow in color (Fig. 6.5).

In order to see, what had been created in a new mask after region growing, there was a need to calculate 3D. This new 3D model was further wrapped and smoothened. The wrap function creates a wrapping exterior of the picked entities. This tool is particularly used to filter tiny inclusions or close small holes. In addition, this function is a helpful tool for Finite Element Analysis, where an enveloping surface is required. This 3D Object is quite coarse. Therefore, smoothing is performed to

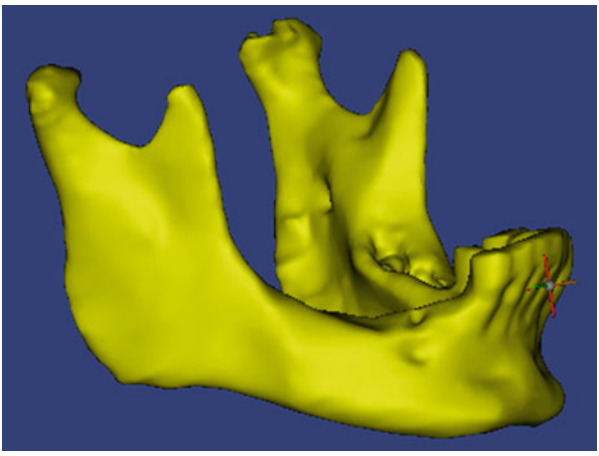


**Fig. 6.6** Wrapping and smoothening

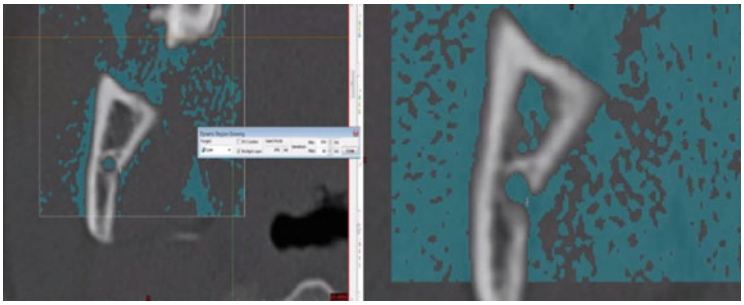


**Fig. 6.7** Dynamic region growing of nerve

remove sharp edges and to smooth 3D object. 3D model of mandible created after region growing and performing wrapping and smoothening is shown in Fig. 6.6.

#### 6.1.4.2 Segmentation and 3D Model Creation of Nerve

To minimize the potential risk of damaging the nerve while drilling and placement of the implant, it is necessary that nerve must be clearly visible at its exact location while planning the surgery and for that segmentation of nerve was required and the sequence of commands used are: Dynamic region growing: It is used to grow a mask from a selected point without performing thresholding operation. This tool is extremely useful for vessels, nerves, and arteries.

After dynamic region growing, the nerve was visible but there were some other regions which were under consideration and there was a need to get rid of them (Fig. 6.7). Therefore, it was required to use region growing but before that, the nerve has to be separated from the outside portion. So, the connections between the nerve

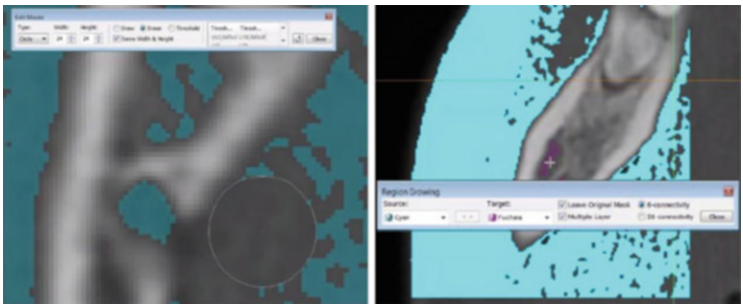


**Fig. 6.8** Mask editing of nerve

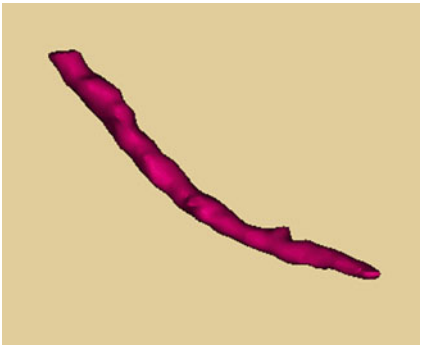


**Fig. 6.9** Wrapped and smoothened nerve

and the unwanted region were deleted using commands like edit mask and edit mask in 3D (Fig. 6.8).

In order to visualize the nerve in a better way, wrapped and smoothened 3D model of the nerve was created using different tools (Calculate 3D, wrapping and smoothening) which is shown in Fig. 6.9. This nerve provides a reference for implant placement. Figure 6.10 shows the 3D model of the segmented nerve inside mandible while keeping the transparency of the mandible on and keeping the nerve opaque.

The volumetric representations shown above are the outcomes of user-entered threshold values depending upon visually segmenting various tissues.

### 6.1.4.3 Implant Placement

Placement of implant was performed in 3-matic. Therefore, both nerve and mandible were transferred into 3-matic. This can be done by just coping (Clrt+C) both the object from mimics and pasting (Clrt+V) 3-matic. The relative position of both the objects with respect to each other remains same.

New primitive (cylinders of required length and diameter) were created in 3-matic which acts as an implant. Then it was required to place the cylinder at the position of implant (Fig. 6.11).

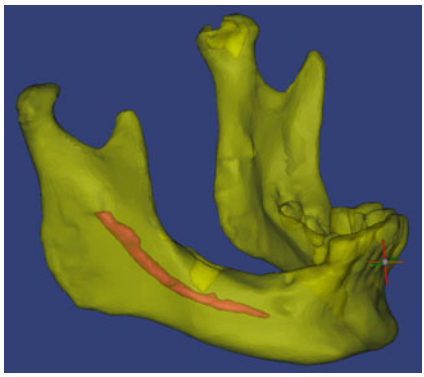


**Fig. 6.10** 3D model of the segmented nerve inside mandible

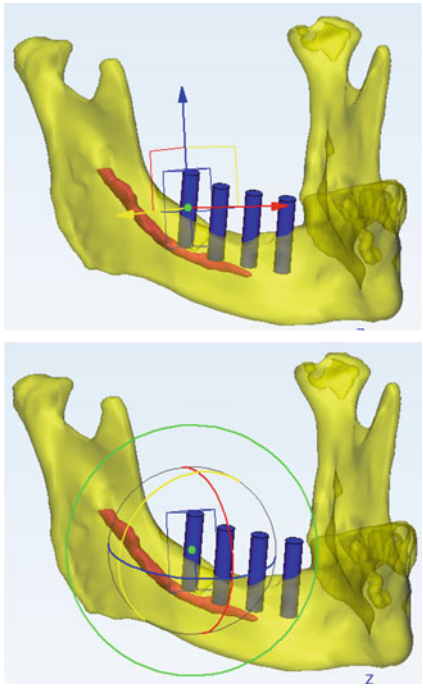


**Fig. 6.11** Implant Placement

### 6.1.4.4 Implant Position Verification in Mimics

The moment, final placement of the implant was achieved, it was now required to confirm the placement in 2D. Cylinders were copied from 3-matic again and pasted in mimics. Contours of all the cylinders were turned on, they were best viewed in axial view (Fig. 6.12). It was required to check the placement in all the three views,

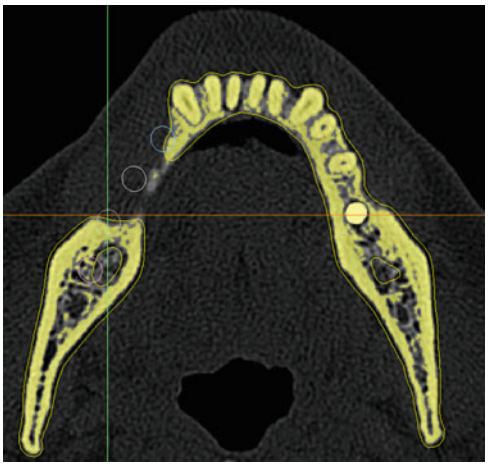


**Fig. 6.12** Axial view of cylinder/implant placed in mandible in Mimics

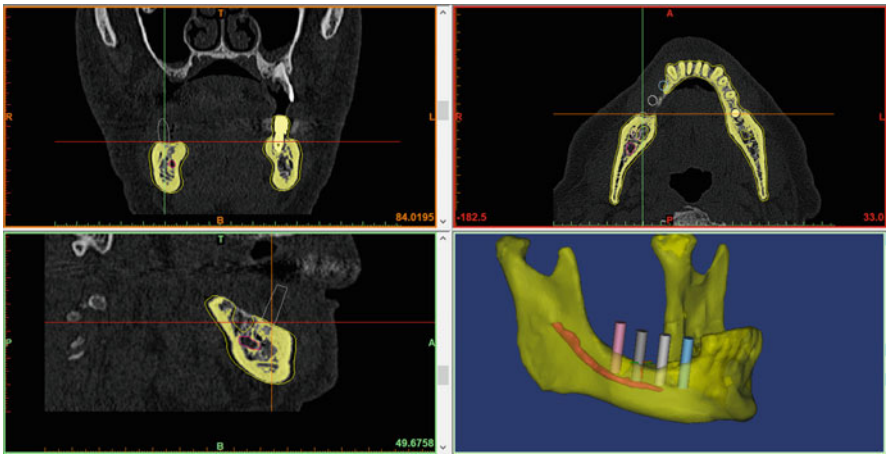


**Fig. 6.13** Confirmation of implant positioning in Mimics

to confirm that implant is not hitting any of the nerve and has sufficient bone around it for proper support (Fig. 6.13). After finalizing the placement of the implant, it was the time to use it, for creating patient-specific dental drill guide.

### 6.1.4.5 Designing of Template

Template is a 3D printed part, which has to seat above the bone or the tissue of the patient depending upon the type of guide. Therefore, it has to adapt very precisely above the meeting surface. In order to design a template, a curve was created to mark a region for the guide using create curve command, while creating a curve designer must take care of undercuts. Marked surface was converted into new surface by

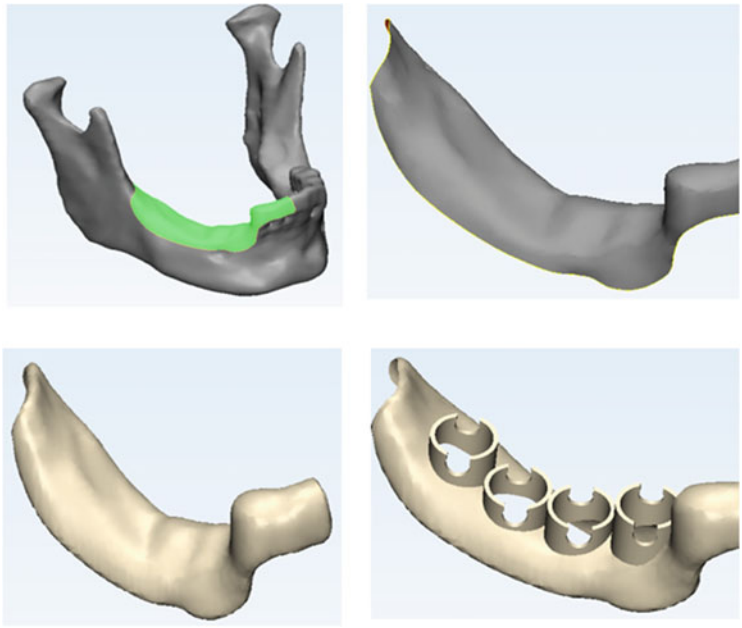


**Fig. 6.14** Designing of template

using a tool named split surfaces. The surface shown in green was a new surface and was further copied as a new part. This new part was further processed using different tools. The template as shown in Fig. 6.14 needs to have place for the placement of stainless steel guiding cylinder. To achieve perfect fit over the meeting surface, in this case which is bone, mandible was subtracted from the template by the use of Boolean subtraction command. After finalizing the design of template, the next step was to perform fix wizard. This step was performed to determine, whether STL file has any problem or it is ready for 3D printing. Based on the diagnosis, the fixing wizard advises actions. Fix wizard perform diagnostics regarding: inverted normal, bad edges, bad contours, near bad edges, planner holes, number of shells, possible noise shells, overlapping triangles, and intersecting triangles, etc. (Fig. 6.15). There are two ways to fix problematic STL file automatic and manual.

### 6.1.5 Designing of Bush/Sleeve

In order to achieve primary target, i.e., to increase the circulation of irrigant at the drilling site for controlling the bone temperature, various brainstorming sessions were planned and our team came up with an idea of providing irrigation channels in the internal diameter of bush through grooves. The supply of irrigant to these irrigation channels is through inlet pipes brazed in the transverse direction of the bush (Fig. 6.16).

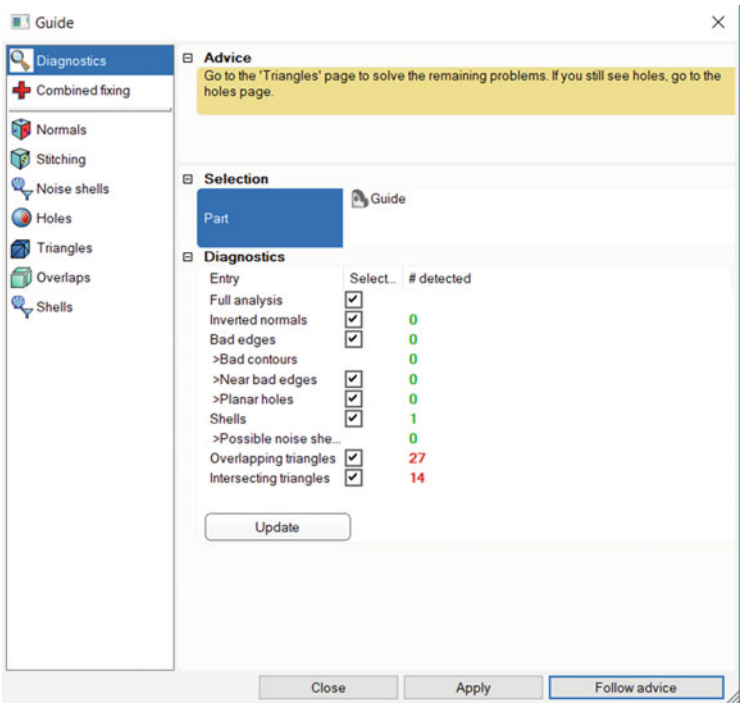



**Fig. 6.15** Fix wizard to ensure problem free stl file for 3D printing

### 6.1.6 Fabrication of Template

After finalizing the design of template in 3-matic. Now, it is time to manufacture the template using FDM (fused deposition modelling) based 3D printing technology. In order to do so, the output from the designing software (3-matic) was saved in STL (Standard Triangle Language) format. 3D printers do not use STL file as an input, these files are needed to be processed through some specific software's, which generally depends upon the printer being used. In this case Fortus 400 MC was used (FDM based 3D printer by Stratasys) and the software compatible with it is Insight.

Insight prepares CAD programs STL output for 3D printing. This software has the capability of automatically slicing and support generation. However, to give control to the user, there are manual options also, with the use of which, it can be possible to figure out the appearance, strength, and accuracy of parts along with the printing speed and material consumption. By using Insight, following task can be performed:

1. Optimization of build orientation for highest possible strength and surface finish.
2. Customization of support material for fast printing, easy removal of support

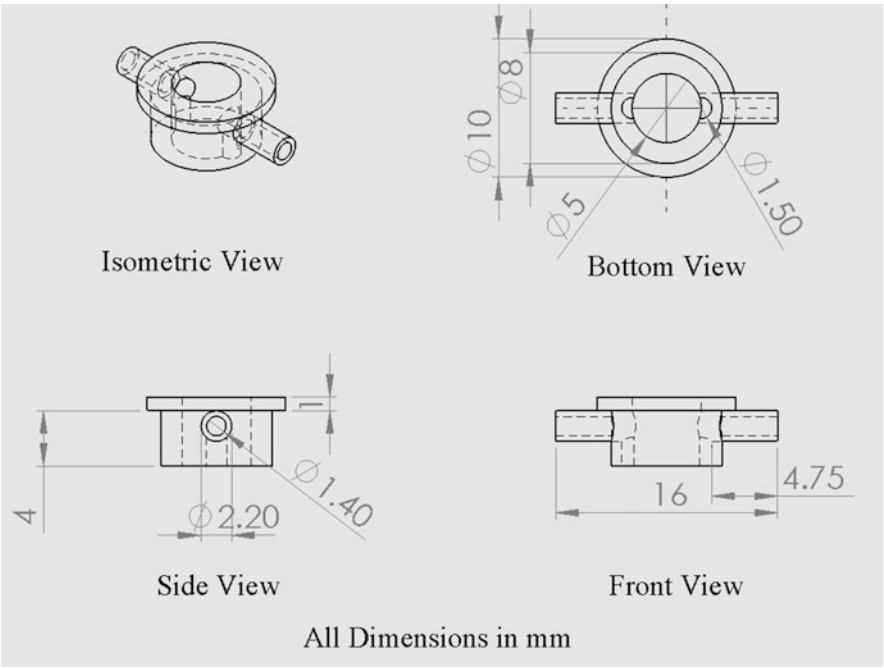



**Fig. 6.16** Different views of improved bush / sleeve, used in dental drill guide

material, and ideal utilization of the material.
3. Program can be paused during the printing process to insert any hardware into the 3D printed component or for any specific reason.
4. Manipulation of the tool paths for better control over the properties of 3D printed component.

Insight software works in combination with the control center. It is a sophisticated software which communicates with the 3D printing machine to manage jobs and to production status (Insight 3D printing software). The step by step procedure from importing a STL files to sending a command to the printer for printing the job is discussed below.

First of all STL file is imported into the software followed by checking the configure modeller, in the window it is possible to check material and the tip currently loaded on the machine. This machine has the option of two material ABS M30i and PC-ISO both the materials are biocompatible materials and can be used for making the dental drill guide but it was decided to use PC-ISO because of its better sterilization capabilities specially autoclaving.

After checking the material and the tip, next step is to set the tool path parameters, here different changes can be made which can affect the build quality, surface finish, material consumption, strength, build time, etc. (Fig. 6.17). The major changes include number of contours, contour width, part interior style, contour to contour

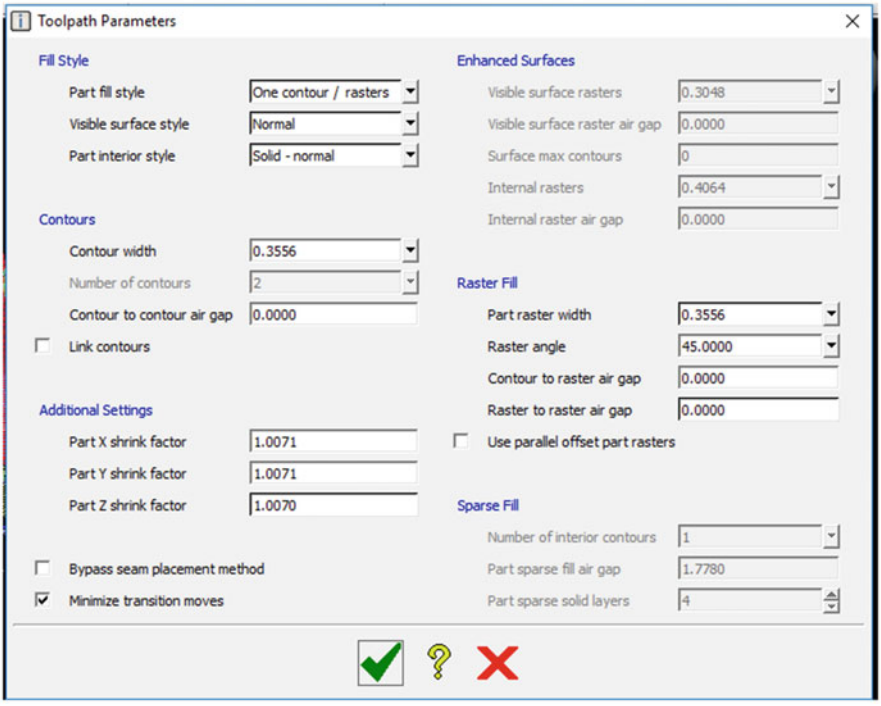



**Fig. 6.17** Toolpath parameters

air gap, raster size, raster air gap, and raster angle. Changing any of the above mentioned parameters affects the tool path.

After finalizing the parameters, orientation has been performed. Placement of the part affects the build time, material consumption, and the appearance of the part to be printed. The next step is to slice the 3D STL model into a stack of discrete 2D part curves (slices) (Fig. 6.18). The slicing operation computes part contours by analyzing cross-sections of the STL file. It starts from the bottom of the model and advances sequentially to the top at fixed slice height. Slice height depends upon the type of material and tip size being used.

Slicing is followed by support generation. Outward extended and hollow sections of the part may require supports to prevent them from sagging or collapsing during 3D printing. Before generating supports, it is essential to determine the support type because it affects the supporting strength, the quantity of support material to be used, and the part build time. There are five inbuilt support types in the software and in addition to that manual options are also there. Toolpaths can be generated any time after slicing the STL model. This converts the 2D part curves into a series of discrete line segments which could be translated into commands for the modeller. Toolpath is generated as per the current toolpath parameters. As part of toolpath creation, the seam can be located on the part slice curves. The basic controls for toolpath

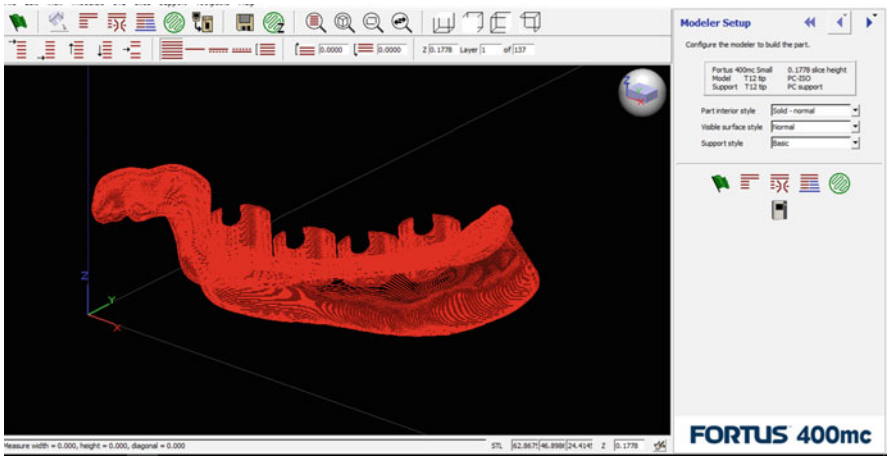



**Fig. 6.18** STL file slicing

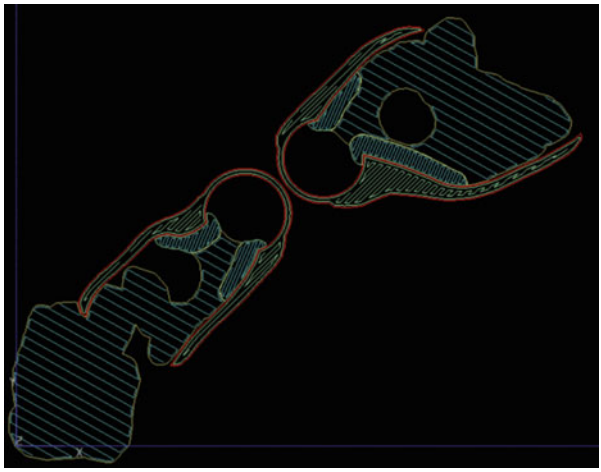


**Fig. 6.19** Wireframe toolpaths

generation are: toolpath setup, estimate time, insert pause, seam control, custom groups, and shade toolpaths. Tootpaths can be visualized as wire frame (Fig. 6.19) and shaded (Fig. 6.20). Shade tootlpath option handles the display of the toolpaths on screen. Toolpath generation is the last operation to be performed in Insight software.

After toolpath generation, it is time to hit the build button to build the current job. At the moment software is instructed to build a part, software generates a CMB file. This file contains the instructions to build the part. When Insight creates a CMB file, it is processed for a specific modeller type, model tip size, model material, support

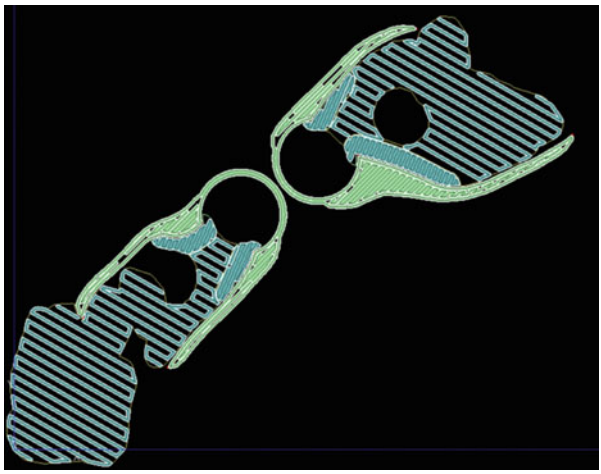


**Fig. 6.20** Shaded toolpaths

tip size, and support material. A CMB file created for one configuration is different from a CMB file created for another configuration. An existing CMB file cannot be modelled if it was processed for a configuration that is different from the current configuration. The original STL file will need to be opened within Insight and processed for the current configuration. The final instruction to build a part is given through control center.

### 6.1.7 Fabrication of Guiding Cylinders/Bush/Sleeve

Figure 6.21 shows the detailed drawing of guiding cylinder in four different views. Guiding cylinder consists of two parts: SS 316 L cylindrical part having a flange and grooves in the internal diameter and the SS 316 L tubes attached perpendicular to the axis of the bush. There are two reasons for proving the flange on the bush: i. for the proper setting of the bush in the 3D printed template. ii. For the ease of changing the bush, as the surgery progresses there is a need to change the bush due to incremental drilling. Without the flange, it becomes very difficult to remove the bush during the surgery. This bush is manufactured from SS 316 L round of diameter 12 mm. This solid round is machined on the lathe to get the desired design and dimensions. The grooves in the internal diameter of the bush are provided for the supply of irrigant. These grooves are made by drilling blind holes in the direction parallel to the axis of the cylinder prior to machining internal diameter. After the creation of the inner diameter of making the cylinder hollow, the blind holes gets converted into irrigation channels. The reason for creating the blind holes before marching the cylinder for internal diameter is because it is very difficult to make channels in the internal diameter of the bush. What we have done is created holes, which after machining of the cylinder for creating internal diameter gets converted into grooves. As discussed above the supply of irrigant to these grooves is through

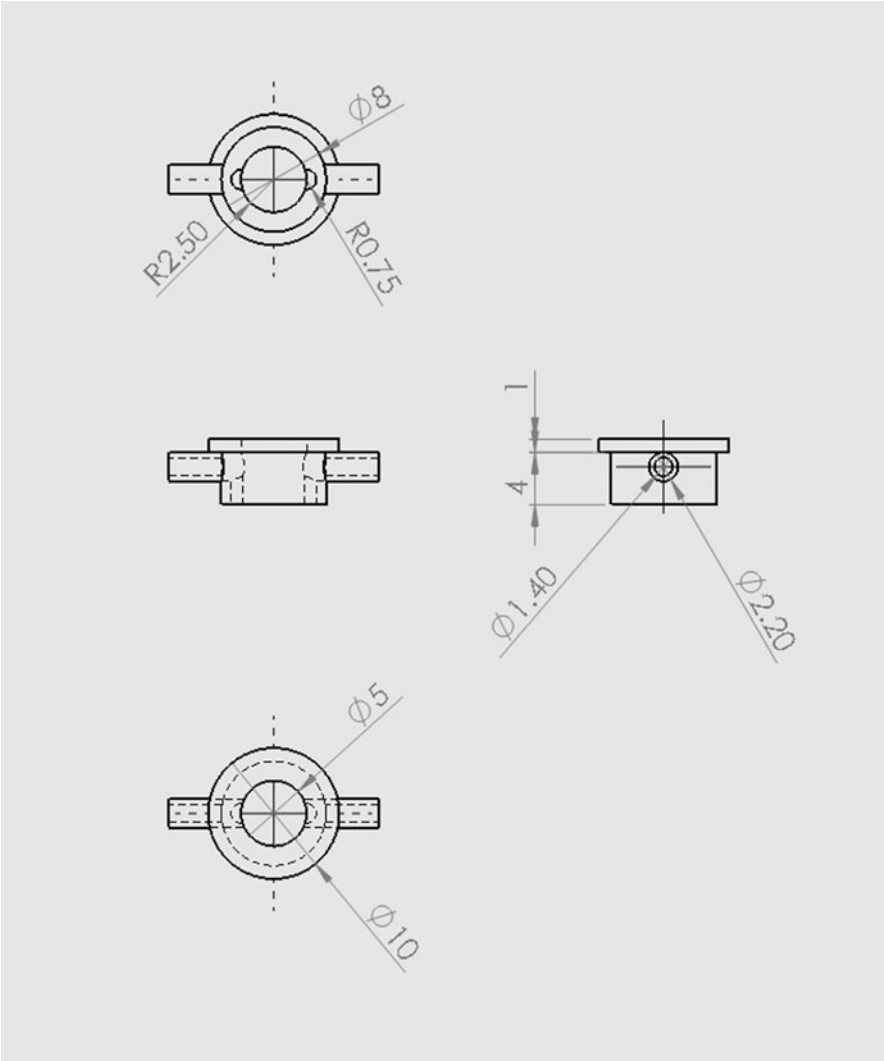
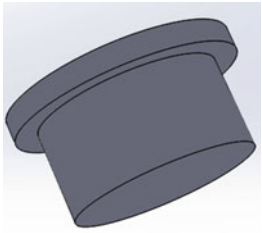
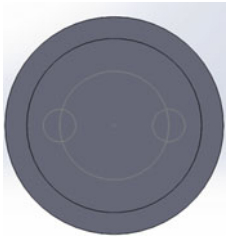
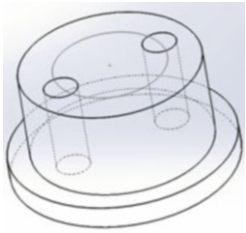
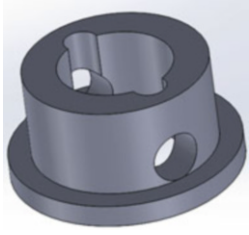



**Fig. 6.21** Detailed drawing of guiding cylinder

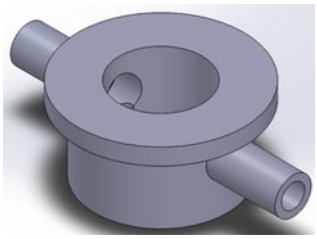
the SS pipes brazed perpendicular to the axis of the bush having dimensions. To make connection between the pipes and the grooves a through hole is drilled just below the flange. The step by step procedure for the fabrication of the bush is discussed in Table 6.1.

**Table 6.1** Procedure adopted for the fabrication of the bush

| Operation sequence | Operation | |
|---|---|---|
| 1 | Cutting of SS 316 L round to desired length (ϕ 12 × 5.5) mm | |
| 2 | Facing to limit the length to 5 mm | |
| 3 | Turning to the maintain the length and diameter of flange and body of cylinder |  |
| 4 | Marking of hole to be drilled |  |
| 5 | Blind holes drilling at 180° to each other (4 mm deep) |  |
| 6 | Through hole at the center of the cylinder, in order to provide internal diameter to it |  |
| 7 | A through hole is made just below the flange, perpendicular to the axis of the cylinder and intersecting the dead end of the blind hole |  |

(continued)

**Table 6.1** (continued)

| Operation sequence | Operation | |
|---|---|---|
| 8 | Brazing of SS 316L tubes |  |

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# Three-Dimensional Printed Drugs and Related Technology: A Potential Review

7

Vibha Bhatia and Jagjit Singh Randhawa

**Abstract**

Technological progression in the field of drug Three-Dimensional Printing (3DP) gives evidence of its mass adoption by the pharmaceutical industry. Due to the wide range of applications of drug 3DP methods, it is now a crucial branch of the healthcare sciences. Due to the new USFDA guidelines related to 3DP machines, a holistic approach is required to assess the knowledge about the drug 3DP for the smooth production of the intended drug. This chapter discusses the existing drug 3DP technologies and the risk involved in their manufacturing. It also discusses the expected regulatory norms to be followed by pharmaceutical manufacturers and challenges to establish production houses for 3DP drugs. The emphasis is given on the advantages, disadvantages, and applications of 3DP drug technology. To conclude the current review, research articles associated with drug 3DP steps and their outcomes are scrutinized. The conclusion of this review indicated that customized 3DP medicines may prove beneficial to the patients. On paper, the delivery of printable drug dose appears to be smoother than the powder printed drug dose. The drugs prone to polymorphism may be fabricated using 3DP. In general, the chapter summarizes the current scenario of research in the field of drug 3DP.

**Keywords**

Drugs · Pharmaceutical · Drug delivery

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## 7.1 Introduction

With time, the notion of drug delivery is altering from the conventional oral dosage methods to the drug delivery systems with the targeted release. The term drug delivery involves the technologies, methodologies, formulations, and systems to deliver a chemically active compound in the body to efficiently provide therapeutic action at the drug targeted area. The pharmacokinetics—the indicator of safety and efficiency of a drug can be modulated by controlling the drug release profile. The multifaceted drug therapies create obstacles in clinical scenarios due to the probable occurrence of side effects. Most of the drug bulk manufacturing is done keeping the mean population data into consideration leading to a higher probability of ill-effects in the geriatric and paediatric cohorts [1, 2]. Wide range advantages of customized pharmaceutical treatments have driven more attention to drug dosage delivery.

## 7.2 What is 3D Printing?

The rapid prototyping technique named three dimensional printing (3DP) was first introduced in the early 1990s, therefore, is considered as relatively new technology [3]. It involves the construction of a 3D physical model using computer-aided design and programming by sequentially layering the printing material onto the substrate. To create the foundation of the 3D model, the printing material is extruded from the printer head onto the x-y plane with subsequent movement in the z-axis direction. The object is bound with the ejection of the liquid binder up to a certain thickness of the 3D printed object. The model is created by using layer by layer deposition method using a computer-aided drafting technique. The binding material is removed to get the finished model. The other names corresponding to 3DP technology are additive manufacturing and solid freeform fabrication [4]. Since the emergence of 3DP technology, it has been used in a range of fields such as medicine, architecture, drug industry, etc. 3D printing technique provides flexibility in the design and creation of complex customized objects (Fig. 7.1).

## 7.3 Need for 3D Printing of Drugs

The individualization of the dosage forms can be achieved by the 3D printing area called the Polypill concept. 3DP plays a vital role in multi-ingredient formulations having sustainable release properties with single or multi-layered printed tablet blends. The inclusion of a variety of drugs mandatory for the prescribed treatment in a single unit of drug dosage is possible with 3DP. This may help in reducing the number of drug dosage units taken by the patient while having a treatment routine. In 1992, pharmaceutical formulation progress using 3DP at Massachusetts Institute of Technology has strategically directed the effective measures to overcome the drawbacks of conventional techniques of pharmaceutical manufacturing unit operations. The possibility of production of degraded quality of final dosages is

**Fig. 7.1** 3D CAD model and 3D printed model [5]


**Fig. 7.2** 3D printing technique (semi-solid extrusion) to make chewable drugs [9]

possible due to the uncontrolled limitations of conventional drug unit production operations like milling, grinding, mixing, grain formation, and compression during drug loading, release, stability, and dosage form stability [6–8]. Microchips, oral controlled released systems, pills, implants, and multiphase release dosage forms and immediate release (IR) tablets come under the range of drug delivery systems that have been created using 3DP techniques (Fig. 7.2).

## 7.4 History of 3DP Technology in Drug Manufacturing

In 1986, Charles Chuck invented and patented Stereo lithography (SLA), an additive manufacturing method (resin 3D Printing), used a curable vat of photopolymer resin. Chuck co-founded the 3D printer manufacturing company called the 3D Systems [10]. Fused deposition modelling, another 3D Printing technology patented by Scott

Crump in 1989, polymer filaments were heated to form semi-liquid material which could create 3D object platform in layers via extrusion through a heated nozzle [11, 12]. As the existential proof of 3D printing personalized drugs also dates back in the 1990s, which marks the proliferation of breakthroughs in 3D printed medical devices. The review and approval of 3D printed medical devices were done by the Centre of the Device and Radiological Health (CDRH), sub-branch of the Food and Drug Administration (FDA) (the authority of additive manufacturing). The first-ever pharmaceutical 3D printing method was patented by MIT in the early 1990s. The inkjet 3D printing was used as a base technology to bind the particles together using binding material on the powder bed. The layer by layer repeated deposition of the material was done to obtain the final solid object [13].

FDA had approved Spritam (Levetiracetam) in 2015 as the first drug manufactured using 3D printing technology and started a new era of 3D printed drugs in the pharmaceutical sector [1]. Inkjet printing technology was used to manufacture Spritam. In Pharmaceutical the 3D printing technology is still in the initial phase in comparison to the advancements in other fields like aerospace, automobile, tissue, and biomedical sciences. Risk-based approaches are adopted by FDA and motivate the progression of the latest manufacturing methods like 3D printing of drugs.

## 7.5 Applications and Advantages of 3DP Technology in Drug Manufacturing

It is undeniable to say that 3DP technology holds an edge over the other conventional manufacturing methods in terms of a wide range of application areas and advantages, such as

a. efficient operating systems enable fast production rates,
b. Cutting off the cost of production due to minimal material wastage,
c. Capable of inducing intensive drug loading both precisely and accurately in dynamic medicinal drugs,
d. Compliance solubility and bioavailability to a broad range of pharmaceutical effective drug constituents like peptides, proteins, inadequately water soluble, also the drugs with the constrained therapeutic windows [5, 14–16]. This is due to the possibility of creating variable and desired geometric structures which lead to a change in porosity and other physical properties of the drugs [17, 18]. The use of porous pills is advantageous and amenable to the patients having drug swallowing difficulties like the elderly, children, Alzheimer's disease, strokes, tumours in head and neck, etc.[18, 19]. Therefore, the proper control over the spatial distribution of active pharmaceutical ingredients in the drug dosages gives the flexibility of making complex designs and customized drug release profiles.
e. Pharmacy practice is witnessing an era of individually customized medicines whereby "one size does not fit all". Careful tailoring of the medications taking age, race, genes, gender, environmental factors, and epigenetic in mind is possible

using 3D printing techniques in pharmaceutical drug delivery. In the case of chronic diseases, it is anticipated that patients must adhere to complex treatment regimens with multi-drug, high-frequency dosages, and resulting in an umpteen number of side effects. The 3DP of individualized drugs (single or multi-layered) and constrained-release layers may support the patient's complicated medical situations [20]. 3D printing of drugs introduces the concept of prior investigation of pharmacogenetic profile to the health practitioners before giving treatment regimen advice [6, 14, 21–23].

f. 3DP technology is expected to benefit implantable drug delivery systems particularly in providing effective methods in case of several constraints related to implant preparation using batch to batch variation of the drug-excipient blend. Well-defined micro and macroarchitecture of implants using 3D printing techniques will benefit the complex drug release. Improvisation of drug efficacy, minimal drug toxicity, and side effects could be achieved by optimizing the drug concentration with the use of 3DP methods [24, 25].

## 7.6 Regulatory Expectations [26]

In 2017, advisory on technical considerations for 3D printing manufactured medical devices was issued by the US FDA. Range of parameters requisite for standardization and safety of the 3D printed drugs were enlisted including design considerations, manufacturing process guidelines, device testing, and labelling considerations. The validation process for providing maximum assurance following the pre-existing procedures was included in the guidelines. Additional documentation must be filed to adhere to the guidance outlines for the quality system maintenance regulation for validation of medical devices. Components and devices manufactured in a single build cycle, within build cycles, and between machines, where the test and inspection cannot fully verify the output of the process (i.e. result specifications), validation of the process is necessary to maintain and ensure the quality of all built devices. According to the standard protocol [26, 27], the validation of the software for its specific use must be performed. Some of the examples are listed below in light with the powder bed fusion methods:

- Parameter's monitoring within the process: melt pool data, the temperature at the beam focus,
- Environmental conditions of the build-space: temperature, humidity, pressure, etc.,
- The power associated with the energy delivery system: laser, extruder, electron beam, etc.,
- Printing technology and status of the mechanical elements: gantry, recoater, etc.,
- Defined acceptance criteria for visual inspection (both manual and automated),
- Evaluation of test coupon, and
- Non-destructive evaluation.

Identification and analysis of the device changes, process deviations, or manufacturing process for the introduction of the potential risks. It may be needed to revalidate the process due to the deviation or change depending upon the assessment results [26]. For the devices manufactured using Additive manufacturing which has been pre-approved by the FDA, manufacturers are supposed to rely on the pre-existing FDA regulations. Some of the examples are listed below in light with the revalidation associated with additive manufacturing [26–29]:

- Material change (e.g., supplier, reused powder, incoming material specification, and new formulation) or material handling,
- Change in software (e.g., modification or update in build preparation software),
- Changes to the workflow of build software,
- The physical movement of the machine to the new location, and
- Changes to post-processing parameters or steps.

Safety of the pharmaceuticals has been highlighted with the tragic happenings like in 2012 New England Compounding Centre (NECC) and many menacing safety problems related to compounding pharmacies. This raises the primary question of defining the difference between the manufactured and compounded drugs associated with the guidelines of 3D printed drugs [30–32]. For achieving the existing chemistry, manufacturing, and control (CMC) standards enlisted in 21 CFR 200 and 300, the 3D printed object has to be manufactured in coherence with established regulations and guidelines for the manufacturing of drug products [33, 34]. It becomes herculean to meet existing regulatory measures of FDA which may pose a hindrance to introducing 3D printed medicine to the current market. To protect users and manufacturers, it becomes a necessity to put forth the main issues related to 3D printed drugs such as intellectual rights and tort liability [35]. FDA's functional performance and device use laboratory and the laboratory for solid mechanics, subsections under the FDA's Office of Science and Engineering Laboratories (OSEL) are serving the purpose of research to study the prospective effects of additive manufacturing [36].

## 7.7 Association between Customized drugs and Healthcare Network

There exists an enormous possibility of an association between customized drug and Healthcare Network. A wide range of 3D software available is used to generate commands to manufacture 3D printed model. The autonomous computerized fabrication process is performed by the 3D printers to meet the product requirements. The patient's next doses can be created by a healthcare professional based upon the patient's recent physiological data. The physiological data can be recorded clinically using biomedical sensors and can subsequently be stored in the healthcare network. The proper placement of the sensors is crucial for accurate data collection. The changes in the patient's physiological data may be reflected and are taken as a basis

to manufacture customized medicine. Therefore, with the usage of such a concept, the improvement in patient's compliance and treatment requirements can be met, further reducing the response time for any clinical action [18]. In-depth knowledge and research are mandatory for the appropriate and practical implementation of the 3D printed drug dosages into the current drug dispensing system. First, immersive improvement and further optimization are possible in the existing 3D printer software. Second, bonding agents have to be developed to meet the needs of the advanced 3D formulations in a better way. Third, optimization and development in the manufacturing process for the efficient production of a wide range of drug products can be achieved with technical advancements. Fourth, as the 3D printing systems provide flexibility to manufacture, safety, stability, and potency of the contemporary 3D centred formulations need to be looked into to maintain the quality and safety of the drug. 3D printing in 2D is repeated and the principle employed in 3D printing drug delivery methods is layer formation and construction of 3D printed models. The technology name is kept usually after the technique involved in layer formation [18].

## 7.8 Advancements in drug 3D Printing technology

Drug tablets with different drug release profiles are attributed to variations in materials and structures [18]. 3D printing technology enables research scientists to manufacture controlled-release medicines [11]. Doughnut shaped and layered drug tablets having a linear drug release profile were fabricated by using PB 3D printing [38–44]. The formulation composition determines the drug release profile and the SLA 3D printer was used to fabricate drug-loaded medicines with modified release profiles [45]. SLS printer was used to fabricate the print lets with different geometries with a model of calibration to forecast the drug composition of varying geometries [46]. Recently, FDM 3D printing has become the most widely used method and has attracted large interests in the pharmaceutical industry to manufacture customized drug products [47]. FDM 3D printing technologies are showcasing the promising capability to fabricate drugs. FDM printers are easily operatable, relatively cheaper, and can cleanse hollow objects [48]. The antimicrobial metals having a wide range of antimicrobial properties when incorporated can fast forward the wound healing process [49, 50]. With the assistive 3D scanning technology, models of ear and nose were constructed and 3D printed an individualized wound dressing with antimicrobial metals mixed to polycaprolactone (PCL) to create filaments used in 3D printing techniques [51]. The FDM technique is capable of manufacturing complicated geometries and shapes to obtain various release profiles in customized drugs [25]. Sustainable, immediate, and time-released drugs can be developed using FDM printing [1]. Umpteen numbers of applications related to the FDM 3D printing technique has been stated by various researchers. The huge potential was discovered to create oral capsular equipment for pulsatile release [52]. It was also stated that the different geometrical shapes of the drug tablets can result in different drug release profiles. The demonstration was given by Goyanes

et al. [48] by producing 3D printing drugs with various geometrical shapes using FDM technology and analysed that the tablet shape can change the release profile. The potential of FDM 3D printing was explored to produce oral capsular devices for pulsatile release. Prednisolone sustained-release pills were produced by using the FDM printer. The concept of long duration sustained release of medicine in the stomach was explored for its efficacy using FDM 3D printing technology [47]. Different structures were created by using FDM printing and the relationship between the different shapes and drug release profiles was explored [53]. Different experiments like using safe and non-toxic pharmaceutical excipients as a forming agent like Hydroxypropyl methylcellulose (HPMC) and different binding additives to get a sustained release [54–57]. Drug impregnated and drug-free printed tablets and filaments with different infill patterns and densities were prepared using diltiazem and HPMC. The bilayer pills with a definite release profile were created by 3D printing through the hydrated HPMC gel layer [15]. However, the shape of the printed tablets could be affected by the shrinkage in the gel models [47]. Medical drug delivery devices were FDM 3D printed by using ethylene vinyl acetate (EVA) as the new feedstock material [43]. In the hot-melt extrusion (HME) of FDM 3D printing, the layer by layer deposition of thermally softened material can be achieved by extrusion through the nozzle. In various studies, polyvinyl alcohol (PVA) is used as a water-soluble synthetic polymer in various applications [58]. The good biocompatibility of PVA has backed up its use as a drug carrier and benchmark polymer in 3D printing technology [59–61]. The filaments of PVA containing paracetamol (APAP) were created by using filament extruder and PVA based caplets with particular release profiles were manufactured by FDM 3D printing [62]. The application of polyvinyl alcohol (PVA) in modern pharmaceutical technology was stated by Pluta et al. [63]. FDM 3D printing with dual nozzles was used to create composite tablets with PVA or PLA filler component and drug incorporated PVA component [64].

## 7.9 The Concept of "Polypill"

Due to the age factor, the patients belonging to the geriatric population are susceptible to multiple diseases and are often undergoing multiple therapies. The combination of multiple drugs into a single tablet establishes the "polypill" concept [2]. The polypill technology got recognition with the research done by Khalid et al., the single 3D drug dosage form formulation comprising of five pharmaceutical ingredients with different drug release profiles was done [21]. 3D printing of pravastatin, ramipril, and atenolol was done in the extended-release compartment. Hydrophobic cellulose acetate, a permeable membrane was used to separate the drug compartments physically. On the top of the extended-release compartment, hydrochlorothiazide and aspirin were deposited as an immediate release compartment [21]. The medicinal combination (captopril drug with sustained-release compartments of nifedipine and glipizide drugs) created was used to produce a polypill to treat diabetic patients with hypertension. The extrusion-based 3D printing

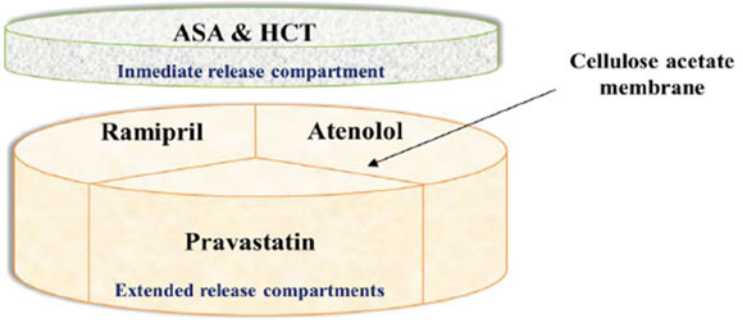


**Fig. 7.3** Polypill created using 3D printing [65]

technology was used to produce "polypill", so that complicated medicinal formulations can be manufactured in the single tablet form. The concept promoted the idea of customized medicines. The osmotic pump was used to demonstrate this concept [21] (Fig. 7.3).

## 7.10 Spritam

3D printing technology was conventionally used to print medical devices. In August 2015, the pharmaceutical manufacturing witnessed the new era of 3D printed drugs with the FDA's approval of Aprecia's SPRITAM® (levetiracetam) as the first 3D printed drug. ZipDose® Technology (originated at Massachusetts Institute of Technology) platform of Aprecia was utilized to produce SPRITAM [37]. It is the groundbreaking advancement of 3D printing technology which is capable of producing porous formulation. A sip of water activates the SPRITAM to disintegrate rapidly (1000 mg of SPRITAM disintegrates in a few seconds) [1, 66]. A three-dimensional structure is created by using ZipDose technology in which multiple layers of 3D printed drugs are stitched together to create a porous and water-soluble matrix. A powder bed fusion system with layer by layer deposition based on the ZipDose technique is used in making SPRITAM. Active pharmaceutical material needed for the matrix tablet and excipients needed comprises of the first layer following a binding layer deposition for perfect integration of all the subsequent similar layers [67]. The highly soluble drug form is advantageous to patients or children with swallowing problems promoting their adherence to therapy [12, 19].

## 7.11 Inkjet 3D Printing Technology

The two types of technologies associated with Inkjet Printing; (1) Continuous Inkjet Printing (CIJ) (2) Drop-on-demand (DOD). In the CIJ method, a persistent stream of ink is created and ejected through an orifice having 50–80 μm diameters at a very

high pressure using a pump. Comparatively, smaller droplets (10–50 μm) are produced with the DOD method having 1–70 pL volume [68]. The control parameters in both the technologies are size, speed, fluid viscosity, and interval of drop formations. Both the Inkjet Systems comprise of printer heads which are categorized as Thermal Head or a Piezoelectric Crystal [69]. The thermal DOD is also called Bubble Jet Printing. The ink bubbles are ejected after the local heating of the ink. In thermal DOD, the sudden change in volume is noticed after the change in the shape of the piezoelectric crystal. This produces the acoustic pulse signals which stimulate the needed ejection of the ink [70]. Both technologies showing their individual qualities and applications, the piezoelectric DOD method can be used with a diverse range of liquids, with the thermal DOD technique being limited to volatile liquids. Also, the high temperature of up to 300 °C can be achieved in thermal technique which may be responsible for the degradation of drugs whereas the piezoelectric DOD technique seems a promising option for pharmaceutical applications as at room temperature it can be operated with biocompatible and less volatile liquids. Further, the DOD method is categorized as a drop on the drop and drop on solid deposition [69]. Various research studies are done which validated the characteristics of both the DOD methods. In the drop on drop method, the solid layer is formed as a result of the ejection of droplets one onto the other which results in higher resolution 3D structure. Fabrication of a microscopic drug delivery system having complex geometries with a droplet size of about 100 μm diameter is possible by direct IJ-printing technique. The formulation of the printable fluid should be suitable for rapid solidification and jetting. Maybe due to solvent evaporation, surface wetting, or shrinkage, the layer thickness is usually smaller than the droplet size [1]. To prevent limitations of printable fluids like fluid leaking, coffee ring effect, and nozzle clogging associated with the properties like viscosity and volatility [69]. The stability of the drug is related to the therapeutic properties of incorporating drugs. Drop on solid deposition is comparatively more suitable for the pharma printing of a variety of drugs. The solid material is sprayed on the platform and the binding material is sprayed on the powders. The other names for the drop on solid deposition are drop on the bed, drop on powder, binder jetting, powder bed 3DP, or plaster printing [69, 71]. The height of the platform is lowered to spread the next powder layer, repeating it until a 3D structure is formed [69]. Good adhesion between the layers is achieved by optimizing layer thickness and spacing. The 3D object is created when the particles of size range 50–100 μm are adhered to each other using a binding ink [71]. The quality of the final product depends on the powder's topological features and the reaction of powder bed with the bonding material (ink) [69].

## 7.12 Laser-Based Writing Systems

The first free form fabrication method for solids became commercial with the emergence of the technology based on the laser, known as the Stereolithography (SLS). The photo polymerization method is used for the controlled solidification of

the liquid resin to create a 3D object by SLA [72]. The movement of the platform takes place in the vessel filled with a liquid photopolymer. The first proper laser is given when the lifting platform initiates close to the surface of the liquid photopolymer. The height of the platform is further lowered into the vessel after the application of the first laser to an equivalent depth of the thickness of the next polymerized layer. The repeated process takes place until the required 3D geometry is achieved. The SLA is compatible to produce thermo-labile drugs as due to the high resolution of the process it minimizes temperature rise while printing [68]. The photopolymer material should be chosen such that it is approved for human use and gets solidified instantly on exposure to the ultraviolet (UV) light. Undoubtedly, SLA is immensely used in the field of tissue engineering, SLA offers comparatively less number of applications due to low drug loading capability and availability of a few FDA approved photosensitive polymers [73]. The similar 3D printing technique is which object creation using a laser beam and liquid photopolymer is possible is digital light projection (DLP) [74], unlike SLA, curing of the single layer can be done by controlling multiple mirrors. This significantly reduces the layer production time [71]. Therefore, using DLP is advantageous as it offers smooth adjustment of the layer thickness and provides quick fabrication process. High power laser energy source selectively fuses the powder photopolymer in the technology called Selective laser sintering in which the polymer supporting bed lowers to refill the powder. SLS is advantageous as it offers high strength, speed, and chemical resistance. SLS applies to a range of materials like metals, ceramics, and polymers [71], whereas for specific metal applications, direct metal laser sintering (DMLS) is a similar technology. Two more technologies similar to SLS are selective laser melting (SLM) and electron beam melting (EBM). Melting of metal takes place during the layer-based process in both EBM and SLM technologies. In SLM, the energy from the laser is used to provide high temperatures above the melting point of the metal to fuse the metal powder particles whereas in EBM high-intensity electron beam in a vacuum environment is used. The primary application of both EMB and SLM is in drug-loaded implants [75]. Uniform thermal field distribution is achieved using EBM while the surface quality and accuracy are compromised [76].

## 7.13 Nozzle Based Deposition Systems

Inkjet printing method has drawbacks like a rough surface, insufficient hardness, and low drug loadings [77]. The solution to these limitations can be sought by using nozzle based deposition systems. Nozzle based deposition systems mix the solid base material with the binder before the 3D printing and deposit the material directly by extruding it through the nozzle to create a 3D geometry [78]. This method is categorized into two types, fused deposition modelling (FDM) in which melted material is used and pressure-assisted micro-syringes (PAM) in which there is no need to use melted material.

## 7.14 Fused Deposition Modelling

In FDM, extrusion of the thermoplastic filament via high-temperature nozzle occurs which gets converted to the fused semi-solid filament where deposition takes place in layers. Dedicated computer software is used to guide the extrusion process from the head of the printer where extrusion takes place in particular directions in layers of melted thermoplastic filament. FDM is also known as Fused Filament Fabrication because the softened material after being heated at a certain temperature is extruded through a nozzle, to form layers and solidifying within seconds [45]. By incubating drugs into the organic solvents drug is loaded in the filament. Improper drug loading may lead to its limited use in low dosage drugs. Domperidone tablets, intragastric plus with sustained-release type, were created using fused deposition modelling. Before printing the hollow structured tablets, hot-melt extrusion was done to load drugs into hydroxypropyl cellulose filament material. Later, shell numbers are changed and the infill percentages are given to produce structured tablets [79]. 5-aminosalicylic acid (5-ASA) or 4-aminosalicylic acid (4-ASA) showed the capability of an extended-release profile when FDM was used [48]. The extended-release profile of the prednisolone oral drug for 24 h was also observed when PVA filaments were printed using the FDM technique [47]. The primary limitation for FDM is the requirement of high temperature (~220 °C) for its operation which may reduce a large number of active drugs and excipients [48].

There are methods to increase the use of a range of polymer materials that can be adopted with higher drug loading and Fused Deposition Modelling. The FDM 3D printing can be bridged with a hot-melt extrusion process. Extended and immediate, cellulosic, or methacrylic polymeric filaments having 50% drug loading showed the possibility of extended and immediate theophylline caplets [80]. The rheological material properties mandatory for processing are influenced by factors like the pressure drop, nozzle diameter, the feed rate, and the thermal properties are affected by factors like density, thermal conductivity, or glass transition temperature [4].

**Pros of Fused Deposition Modelling [68]:**

- Creates mechanically stronger parts.
- Multiple release profiles of the dosage forms can be obtained after printing by making changes in the infill percentages, the surface area of the dosage form, or the 3D model design.
- The correct dosage and better resolution than technology like powder bed printing.

**Cons of Fused Deposition Modelling [68]:**

- High temperatures of the process limit the use of APIs.
- For the extrusion process, a limited number of thermoplastic materials with optimally good melting viscosity are present.

## 7.15 Pressure-Assisted Micro Syringe Technology

Pressurized air piston is used to deposit viscous material with the help of a syringe extruder. The deposition takes place in layers in the predefined 3D form. The robustness of the technology is estimated by taking factors like elastic limit, viscosity, and viscoelasticity in consideration. The technology is used in printing tissue scaffolds and substitutes of soft tissues [41]. The technology is advantageous as it allows continuous flow and is suitable at room temperature. The active pharmaceutical ingredient can be degraded as a result of the usage of solvents that could be responsible for the health problems [41]. Layered deposition of semi-solid homogeneous paste on the moveable platform via extrusion to form a final product. Tool head with a syringe is used to layer semi-solid material (paste or a gel), usually a mixture of solvent and polymer in an appropriate ratio suitable for making it consistent with 3D printing [81].

The use of paste or gel form of material is mandatory due to the extrusion process involved in the method. The material is usually prone to shrinking and deformation. The dosage form should be able to bear the weight of the further layers, therefore, they should be sufficiently hardened in advance to overcome the possibility of collapse while 3D printing the drug [18]. Khaled et al. has done multiple studies on semi-solid extrusion 3D printing technology. In 2014, semi-solid extrusion was used to manufacture guaifenesin bilayer tablets and was compared to the dosage forms available in the market. Apart from providing a smooth approach to drug production, 3D printed branded drugs and tablets showed similar release profiles which supported the fact of the versatile nature of semi-solid extrusion 3D printed technology [81]. Alternatively, the method was used in multi-active drug tablets which can deliver three drugs using two release mechanisms; diffusion through the gel and shell layers and osmotic release mechanisms [81]. The feasibility of the technique was further demonstrated by constructing a polypill having multiple compartments with five actives and showing a well-defined and sustained-release profiles. The work by Okwuosa et al. [82] ascertains the possibility of establishing the usage of FDM3D printing for a wide temperature range for on-demand manufacturing of drug release products. The approach supported the fact that patient-individualized tablets can fabricate immediate release profiles at lower temperatures via 3D printing by using solubility enhancing and pharmaceutically approved drug-polymer [82].

## 7.16 Limitations and Challenges of 3D Printing Technologies in Drug Manufacturing

1. The capability of FDM 3D printers which are available in the market is restricted to a limited number of materials like convertible thermoplastic polymers, which usually are not ideal for optimizing the performance of less soluble compounds of various dosages and the materials which are not pharmaceutically approved. The

scarcity of adequate pharmaceutical materials for making FDM filaments is the primary daunting factor to make adequate use of FDM technology.
2. Due to the gigantic potential of the FDM technology in manufacturing drugs, it cannot be ignored and is under widespread investigation.
3. Many drug 3D printing techniques bear limitations like the high cost of 3D printers, restrictions of raw materials and size, intellectual property-related issues, and unchecked manufacturing of harmful products.
4. The active drugs may deteriorate at elevated temperatures during printing and extrusion and therefore, the 3D printing technique is not suitable for the thermo-labile drugs. This accounts for the major limitation of the FDM drug 3D printing [49, 56, 83].
5. Repeated efforts made by researchers to address the issue of limited use of 3D printing in drugs at high temperatures. FDM printing temperatures were reduced to 90 °C and Ramiprilprint lets were printed. At a printing temperature of 58 °C, polycaprolactone (PCL) was used as the coating and dual extrusion printing was done using three-part 3D printing designs to print dual-coated drug tablets [57].
6. During the process of 3D printing of drugs, the polymeric filament is mixed with the drug, and the drug-loaded filament is heated at high temperatures, for the layer by layer deposition through the extrusion process.
7. The process to prepare tablets using an insoluble container is categorized as a complex manufacturing method and acts as a limitation for the usage of FDM 3D printing in the drug printing process.

## 7.17 Identification of Risk While Using 3D Printing Technology

The quality of the 3D printed product can be controlled by controlling the quality of the related technology factors like a content, appearance, uniformity, etc. Quality assurance of the process variables ensures failure prevention. Norman et al. [1] had done one such critical assessment of the 3D printing process:

- The software should be able to control the printing process when the printer becomes incapable of 3D printing a given design.
- Monitoring the parameters like print head speed and height can prevent the inaccurate positioning of the print head during 3D printing process.
- Keeping a check on the particle size distribution of the powder and water content in the powder.
- Monitoring inkjet flow and particle size distribution can prevent clogged print heads.
- Employing real-time monitoring of layer thickness to reduce the variability of layer thickness.
- The humidity level and temperature of the manufacturing area can be controlled to prevent environmental conditions leading to improper layers.
- Variation in the surface tension or viscosity of the binder material can be the responsible factor for irregular binding or agglomeration.

## 7.18 Prospects and Future Perspectives

If 3D printing technology is optimized and merged with novel technologies, it is anticipated that it will emerge as an efficient technology in the pharmaceutical industry. The merge of the 3D printing technology with conventional pharmaceutical technologies will broaden the usage and applications. The hybrid systems will be advantageous and are expected to provide the benefits of 3D printing for precision, customization, reduction of material wastage, and its association with conventional drug methods will exploit all the pre-proven benefits. Vision, time, and money are three requirements for the persistent clinical progress in 3D printing. It is expected that the clinical progress in 3D printing will inculcate (1) Development and assessment of new or old excipients for 3D formulation applications (2) Performance optimization of 3D printing software (3) Optimization and development of manufacturing methods related to drug products (4) Clinical assessment of 3D printed drug formulations for its safety, efficacy, and stability. Keeping safety in mind, not only the built-in flexibility but the cost of creating new formulations using 3D printing may be responsible for liability factors. It is compulsory to reject the tampered drug through some means to be sure that no mistake or no adulteration is there in treatment regimens of patients. It is also expected that regulatory norms for the 3D printing formulations will be strict to stop the illegal 3D printing of drugs. It is anticipated that, depending upon the type of the drug product, broad-based application of 3D printing pharmaceutical drug delivery will need to have tamper-resistant strategies as they may be seriously affected by the regulatory concerns. It is widely accepted that the therapeutic potency of the drug is dependent on properties like polymorphic changes, drug-excipient interaction, and stability in the dosage form. Undoubtedly, for the wide range of pharmaceutically active ingredients, 3D printing is an acceptable technique. The impact of the 3D printing on the physiochemical drug characteristics must come into effect on a regular case basis. Adoption of the 3D printing technology might be difficult at first due to the imposition of manufacturing norms and state board requisites. A noticeable difference should be defined to differentiate drug printers as compounding or manufacturing technologies. Implementation of the 3D printed dosage forms is difficult and extensive randomized controlled clinical trials need funding amount and much time. The cutting edge technology and potential applications of 3D printing in pharmaceutical practice seem promising enough to invest time and funding.

## 7.19 Conclusion

This book chapter summarizes the available literature on 3D printing technologies related to the pharmaceutical industry in a structured manner. After the literature survey, it is observed that powder-based printing and inkjet printing are primarily used technologies for drug manufacturing and development. Nevertheless, 3D printing techniques, in general, are used to develop customized drugs, multiple release drug dosages, manufacturing porous materials that can limit the instant

degradation of biological compounds, precise drug droplet formation, and promoting the solubility of the insoluble drugs by developing amorphous forms. Appropriate considerations and modifications in the regulatory norms related to pharmaceutical drugs produced by 3D printing techniques are requisite for the positive acceptance of this innovative technology. It is believed that 3D printing technology will revolutionize the creation of effective and safe drug formulations. The versatile nature of 3D printing technology is evident as it provides a speedy production process with precision and the drugs manufactured can be patient-specific. The continuous refinement and improvement in the 3D printing technology of drugs will make the technology more accessible to accelerate its use in clinical practice to produce patient-friendly pharmaceutical products.

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# Product Sustainability Assessment

8

Harmanpreet Singh and Sagarika Bhattacharjee

**Abstract**

The manufacturing sector has seen much advancement from the first industrial revolution to the modern twenty-first century. The primitive manufacturing techniques include mostly the human effort but with the pace of time, all the processes were automated through the various programmable codes. Manufacturing earlier was limited to cutting, shaping, casting processes where the required objectives were completed using various dies, jigs, and fixtures. 3D printing, also known as additive manufacturing, is the process of making the object by addition of the material which was different from the earlier process which usually removes the materials by various manufacturing and advanced manufacturing techniques. Additive manufacturing was originally discovered and started for the development of prototypes rapidly in a slice wise fashion imitating the new products for display purposes, which earlier were made using wood manually. The research and development in 3D printing made this process popular and has found many applications in the aerospace, pharmaceutical, biomedical and food industry, etc. Additive manufacturing is the process of making the product layer by layer by the deposition of the material on the substrate. Rapid prototyping is done by various methods and using different materials, even metals and other biomaterials are used nowadays for making products, drugs, and bioimplants. Sustainability is the demand of time and all the aspects of sustainability are affected differently during production. 3D printing

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techniques and sustainability evaluation can give the new course of action for the development. This chapter will include an overview of the 3D printing technique and the materials used for the production. Further, the sustainability and its pillars are discussed along with the additive manufacturing techniques. Life cycle design and assessment of the product can help in achieving sustainable development. The evaluation at every step of the production can lead to the achievement of sustainability.

**Keywords**

Sustainability · Life cycle assessment · 3D Printing

## 8.1 Introduction

Product, the outcome of the production process goes through the many phases before being manufactured into its current state. The need for a product for any task or its use is the first step that makes to think about the product. The use/need decides the kind of product to be made and its required dimensions are also defined accordingly. Designing is the first part after the decision for making a product that provides the drawings and soft 3D models to be formed into the real product. Usually, some prototypes are also made before the actual product that intimates the product and can be used for physical examination of the same. Production involves various manufacturing operations that according to the demand of product and kind of material are done, mostly which involves the material removal and joining processes.

Rapid Prototyping which was initially used for prototyping is the material addition process also referred to as Additive Manufacturing. RP process is the new boon for the industries that can be used to make complex shapes which are otherwise a tedious task. RP process gained the importance after the development of the computers or particularly the computer-aided designs and manufacturing [1–3]. The basic process chain in which the entire working of RP can be divided includes

- Creating a geometric model, which requires suitable dimensions.
- Converting the geometric model into .stl format where the model is divided into the triangulated pieces.
- Pre-processing/slicing of the model.
- Fabrication of the part is done in the machine with 3dimensional moments.
- Post-processing or finishing of the product.

The benefits of the RP process are not limited to the complex shapes but it also plays an important role in fast and cheap production, with no or minimal tooling and labor needs. RP process opens new opportunities for the company to earn a good profit in the market by introducing the new products. The application of the RP process is very wide from the engineering and aerospace industry to the jewelry, tableware Industry, and the recently emerging field of Biomedical engineering.

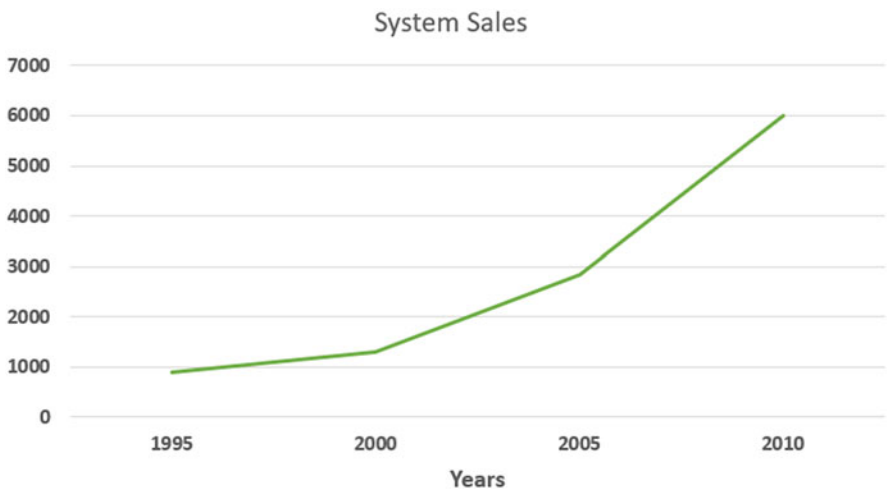


**Fig. 8.1** The rise of RP system yearly [6, 7]

Earlier there was the limited use of the RP process just for the prototypes to showcase the models but in recent years the technology is developing and efficiently making the product, the record growth in the year 2010 was about 24.1% and the combined growth rate till this year was nearly 26.2% which is quite good [4, 5]. Figure 8.1 represents the sales growth data up to the year 2010, it can be observed that year-wise the sales are increasing [6, 7]. The growth rate depicted herein is the combined growth for the RP processes including biomedical, metal industries, and even the food industries.

There is a growth of technology with the rate of usage as various other aspects are researched and improved accordingly. Sustainability for any industrial production solely depends upon its nature of the operation, kind of resources it is using, and the social adaptability of the product and process remaining in the economical stable conditions. RP process is of different kinds but with the basic idea of CAD drawings made in computer and converted to the required format (.stl) which is easily interpreted by the machine and the accordingly product is made. RP processes can be distinguished according to the type of starting material that can be in any form, viz. solid sheet, liquid-based, paste based, or in powder form. According to the starting material the appropriate kind of technology is used to make the final product. These products may find their different uses according to their built quality and design. Overview of the RP processes based on their starting material, techniques, and application is depicted in Table 8.1.

## 8.2 Sustainability

This term is widely used and is a demand of an hour, which means to sustain constantly for long duration with minimal impact on the environment, political justice, and economy conditions with the help of good decision-making techniques

**Table 8.1** Various Rapid Prototyping techniques with working principles [8]

| State of starting material | Process | Material preparation | Layer creation technique | Phase change | Typical materials | Applications |
|---|---|---|---|---|---|---|
| Liquid | SLA | The liquid resin in a vat | Laser scanning/ light projection | Photopolymerization | UV curable resin, ceramic suspension | Prototypes, casting patterns, soft tooling |
| | MJM | The liquid polymer in jet | Ink-jet printing | Cooling & photopolymerization | UV curable acrylic plastic, wax | Prototypes, casting patterns |
| | RFP | Liquid droplet in a nozzle | On-demand droplet deposition | Solidification by freezing | Water | Prototypes, casting patterns |
| Filament/ paste | FDM | Filament melted in a nozzle | Continuous extrusion and deposition | Solidification by cooling | Thermoplastics, waxes | Prototypes, casting patterns |
| | Robocasting | Paste in nozzle | Continuous extrusion | – | Ceramic paste | Functional parts |
| | FEF | Paste in nozzle | Continuous extrusion | Solidification by freezing | Ceramic paste | Functional parts |
| Powder | SLS | Powder in bed | Laser scanning | Partial melting | Thermoplastics, waxes, metal powder, ceramic powder | Prototypes, casting patterns, metal and ceramic performs (to be sintered and infiltrated) |
| | SLM | Powder in bed | Laser scanning | Full melting | Metal | Tooling, functional parts |
| | EBM | Powder in bed | Electron beam scanning | Full melting | Metal | Tooling, functional parts |
| | LENS | Powder injection through a nozzle | On-demand powder injection and melted by laser | Full melting | Metal | Tooling, a metal part repair, functional parts |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 3DP | Powder in bed | Drop on-demand binder printing | – | Polymer, metal, ceramic, other powders | Prototypes, casting shells, tooling |
| Solid sheet | LOM | Laser cutting | Feeding and binding of sheets with adhesives | – | Paper, plastic, metal | Prototypes, casting models |

using the state-of-the-art technology and the equipment [9]. Sustainability has various aspects according to different people and is very difficult to define precisely, but it is mostly considered as the combination of the environmentally protected, economically and socially developed regime as described in Fig. 8.2.

### 8.2.1 Product/Process Sustainability

The sustainable product or process is the one that proves itself according to the three pillars of sustainability. There is always one or the other improvements that were done in the R&D for improving the production which often glides towards sustainability.

#### 8.2.1.1 Environment

This is the most crucial part of sustainability, with multiple dimensions that add to the various environment governing factors either for the product or the process of the production. Environment-friendly products are the one which uses the minimal environmental resources, their production and further uses pollute the environment least. Complete achievement of the environmentally sustainable product is impossible, however, the gap between them can be reduced to much extent by using the new techniques and methods.

Pollution in the environment in three ways including Soil, water, and Air are a major concern, due to which various types of diseases are attacking the flora and fauna. The pollution caused by the industries has the derivatives of $CO_X$ and $NO_X$ which is transferred in the air leading to various respiratory diseases. The following factors in industries are responsible for accelerating the pollution during production

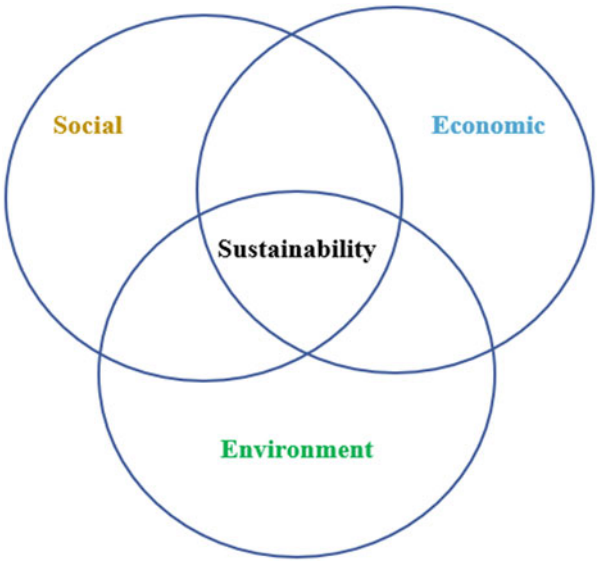


**Fig. 8.2** Venn diagram depicting Sustainability and its three pillars

- Excess usage of electricity in the industry, reducing the power resources.
- The kind of process used for manufacturing; some process may cause high exhaust discharge.
- The material used for production, it is a complex (non-biodegradable) material can increase the air, water, and soil pollution.
- The design of the product is also a major factor that can cause pollution in terms of its shape and size that can delay getting degrade when dumped off.
- Re-use and Recycling properties of the material are of main importance as if they are absent in material probably can add to the pollution in many aspects.

#### 8.2.1.2 Economic

This is the second most important pillar of sustainability, wherein the cost plays a significant role. Generally, all pillars are interlinked, the high cost of any product can be related to its uniqueness or the difficult process to obtain or produce it. The uniqueness of the material can be due to its less availability and if a less available item is used more often that tends to scarcity or extinction of that material (resource), causing harm to the environment. The few cost-increasing factors include

- The material's availability is inversely proportional to the cost, where the lower availability increases the cost.
- The unique product design requires the various steps of production which increase the production cost.
- The process if is unique, for production increases the cost.
- The quality of process/production also adds on the cost, as higher the accuracy (shape, design and finish) higher will be the cost.
- Design is also the most important part which varies the cost, the good design can reduce the excess material cost-reducing the wastage of material but the design cost goes up which can be accepted as the positive cost rise.
- The use of new tools and techniques can serve best in reducing the cost.

There are a few factors which though increase the cost initially but in the long run, help to reduce the excess cost due to wastage of material, energy, and improper use of manpower. These costs can be termed as a positive cost rise, which may include

- High investment in Design activities.
- Investment in new machine tools.
- Investment in the gadgets and equipment which are power efficient.
- Investment in the machines using natural resources (solar products).
- The investment in the automation of machines (robotic handling) and smart devices (sensors).
- Investment in safety and occupational health.

#### 8.2.1.3 Social

This is the most neglected and difficult to explain pillar of sustainability, which involves the social life of the people, their behavior, and also the effects in various

forms due to the production processes [10, 11]. These qualitative changes can also be due to certain product, area, or resource which can create physiological and, in some cases, the anthropological impacts. The social issues are mainly related to health, safety, justice, culture, and few with the rights of the labor [12]. The few social issues related to sustainability include [13].

- Safety issues: High risk in developing a product, some processes are hazardous.
- Transparency issues: Details of the process, employment, and facilities are hidden.
- Import issues: Dependence on the materials produced outside nations, reducing employment in the country.
- Hackneyed of resources: The non-renewable energy resources are used at a high pace making the shortage for the coming generations.
- Justice: The unethical governing of labor laws causes disputes related to wages, medical leave money.
- Adulteration in cultural products/process: The changes in cultural products leading to disputes in the community and also the employment opportunities of rural livelihoods.

The social issues are numerous and vary differently according to the regions, culture, and local laws. The product/process is regarded as socially sustainable if it is accepted socially or if its existence is causing minimal harm to the social behavior of the people. The few steps which can help in maintaining the social sustainability aspects include

- Maintaining the health and safety in the industry reducing the risk to life.
- Developing the product, which is accepted socially according to customs and beliefs.
- Adapting to free, renewable energy sources, so future generations can enjoy all the resources available.
- Creating opportunities in the undeveloped region, creating the source of employment for local people.
- Providing satisfaction to the workers in terms of good salaries, and health benefit schemes.

## 8.3 Sustainability in Various 3D Printing Processes

There was a brief overview of the 3D printing processes in Table 8.1 and sustainability in the previous section. The sustainability of the 3D printing processes with their respected state of starting material is of great importance, as the different process requires the different techniques and machine tools contributing differently to the sustainability.

### 8.3.1 Liquid-Based Materials

The liquid-based starting materials have various kind of 3D printing process setup, based on the kind of preparation of material the processes here are defined in three main categories along with their sustainability.

### 8.3.2 Stereolithography Apparatus (SLA)

The process imparts the laser for the solidification of the liquid resin layer by layer according to the .stl file generated from the CAD model [14]. The platform on which the product is solidified is kept just under the surface of the liquid resin present in the process chamber, which lowers itself for spreading the layer of resin on the previous slice which is solidified. The main apparatus apart from the computer and control panel is the photopolymer resin and the laser. Table 8.2 represents the sustainability analysis of the SLA process with its positive and negative impacts.

### 8.3.3 Multi-Jet Modeling (MJM)

The process includes the design from the CAD model and its converted (.stl) format that is used by the software to drive the hardware combinations of the machine to complete the 3D modeled product. Here the material (thermo-polymer) which is in liquid form is provided in tiny droplets by the print head as per the need of the model which is cured by the UV lamp. The product is made in steps wherewith completion of every step the build table is lowered and the next layer starts to develop. The process is also known as ink-jet printing. The method is mostly used for prototype models as the build volume is small for the process and is used mostly for jewelry, molding, and casting patterns. A few of the aspects of sustainability are discussed in Table 8.3.

**Table 8.2** Sustainability assessment of the SLA process

| Sustainability factor | Environment | Economic | Social |
|---|---|---|---|
| Positive impacts | No release of harmful gases | • Continuous production<br>• Different sizes of products<br>• Good surface finish<br>• Accuracy is good<br>• Variety of materials available | • Requires less attention<br>• Safe process |
| Negative impacts | Requires support structure | • Cost of post-processing<br>• Support structure<br>• Requires post-curing in some cases | • Automated process reduces employment<br>• The tedious task of post-processing |

**Table 8.3** Sustainability assessment of the MJM process

| Sustainability factor | Environment | Economic | Social |
|---|---|---|---|
| Positive impacts | Environment and office-friendly process | • Fast process<br>• Highly precise<br>• Material is inexpensive<br>• Variation in quality is possible | • Easy to use.<br>• High flexibility in terms of connectivity of the machine |
| Negative impacts | Requires support structure | • Only fit for small prototypes<br>• Materials are limited | The downfall in opportunities for skilled artisans |

### 8.3.4 Rapid Freeze Prototyping (RFP)

The process is similar to other liquid processes with a change of material (water) with less viscosity and the operating temperature of the production is very low [15]. The CAD designed file in (.stl) format is converted to the slices and stored in the CLI file. The temperature of the substrate is lowered on to which the nozzle discharges the material with controlled speed as per the need. The process game here is of the controlled supply of materials and its heat transfer with fluid flow strategy. Due to the property of water, it does not get solidify immediately rather the continuous line of water is formed. The material is extruded either continuously or on a need basis in droplet form [16]. The process is efficient for creating the investment casting, sculpture of ice, and also the prototypes for visual confirmations. Table 8.4 highlights the aspects of sustainability.

### 8.3.5 Filament/Paste Based Materials

The Filament/paste based starting materials have three main categories of 3D printing processes. Here the processes use different techniques for the production of the material. These are discussed briefly in subsequent points.

### 8.3.6 Fused Deposition Modeling (FDM)

Fused deposition modeling is the technique where the material which is converted in the semi-liquid state (paste) form is extruded from the nozzle which works according to the (.stl) or (IGES) format file created by CAD software after slicing into the horizontal layers [19, 20]. Here the material is actually in spool from where the continuous supply is maintained and transferred to the nozzle. The nozzle deposits the material on the substrate according to the product being formed in layers. The

**Table 8.4** Sustainability assessment of the RFP process [17, 18]

| Sustainability factor | Environment | Economic | Social |
|---|---|---|---|
| Positive impacts | • The material used is non-toxic<br>• Support material is non-toxic | • Energy utilization is low<br>• Material is cheaper<br>• Method is accurate<br>• The processing speed is high | • Easy to handle<br>• Safest process |
| Negative impacts | The requirement of artificial cold environment | • Low repeatability<br>• Post-processing required | Tedious task as requires additional processing |

**Table 8.5** Sustainability assessment of the FDM process

| Sustainability factor | Environment | Economic | Social |
|---|---|---|---|
| Positive impacts | • The wastage of material is minimal | • The functional prototypes can be made<br>• The manufacturing volume is high | • The material changing process is easier<br>• The support material is easy to remove |
| Negative impacts | The process is slow, consuming more energy | • The built accuracy of the product is low<br>• The product formed tend to shrink | Tedious task |

layers get solidified as they cool subsequently. The sustainability assessment for the process is shown in Table 8.5.

Certain aspects cannot be segregated into different sustainability pillars, like shrinkage of material, the speed of the process, etc. Rather they give their combined positive or negative effects on sustainability.

### 8.3.7 Robocasting

This RP technique is kind of writing with the ink like a pen, but here the small nozzle extrudes the material (paste based) on the base according to the CAD drawing (.stl format) after slicing which gets solidified and forms the product [21]. The movement of the nozzle is according to the required shape/geometry of the final product. The product is a built-in steps layer by layer and there is a continuous supply of ink with a specific rate which is set according to the need [22]. The name itself represents the use of automatic systems (here Robotic) for the casting process which is usually the use of liquid molten metal into the solid product. Table 8.6 gives the sustainability assessment for the Robocasting process.

**Table 8.6** Sustainability assessment of the Robocasting process

| Sustainability factor | Environment | Economic | Social |
|---|---|---|---|
| Positive impacts | • The wastage of material is minimal<br>• No requirement of support material | • The real products with good strength can be made<br>• The manufacturing volume is high | • The material changing process is easier |
| Negative impacts | • The process is slow, consuming more energy<br>• Use of a lot of chemicals in preparing ink | • The built accuracy of the product is low<br>• The product formed can have directional defects due to shrinkage | Health and safety issues in preparing ink |

### 8.3.8 Freeze-Form Extrusion Fabrication (FEF)

The process uses the freezing technique where the temperature is below the freezing point for water, for solidifying the paste [23, 24]. The paste material can be prepared according to the chemistry of the required product. The multiple mixtures of the material can be done by increasing the number of extruders [25]. The paste is passed from the nozzle and placed on the bed according to the CAD file feed after slicing to the machine. The paste gets solidifies and finally, the layer by layer product is formed. The sustainable assessment for the FEF process is shown in Table 8.7.

### 8.3.9 Powder-Based Materials

Here the starting material used for the production is the powder. The broad five categories are described below which uses the powder as a material. All these techniques differ in operations performed and the sustainability assessment of the processes.

### 8.3.10 Selective Laser Sintering (SLS)

This process involves the laser and the heat fusible powder to make the products. Here the file (.stl format) is input to the machine after the slicing into equal parts. The machine gives the motion of laser light according to the geometry of the product. Powder on the fabrication bed is kept loosely and the sintering is done by laser layer by layer and after each step, the rollers spread the powder on the part that is solidified [26]. The remaining powder provides support to the structure which gets easily separated when the part is removed from the bed. The necessary post-processing is done according to the need for the application of the product. Table 8.8 evaluates the sustainability of the SLS process.

**Table 8.7** Sustainability assessment of the FEF process

| Sustainability factor | Environment | Economic | Social |
|---|---|---|---|
| Positive impacts | • The material used is non-toxic<br>• Support material is non-toxic | • The functional prototypes can be made | • The material changing process is easier |
| Negative impacts | • Use of wide varieties of chemicals<br>• The requirement of artificial cold environment | • The equipment material requires good strength<br>• Formation of a bubble in a material can make product inferior | Tedious task |

**Table 8.8** Sustainability assessment of the SLS process [27–30]

| Sustainability factor | Environment | Economic | Social |
|---|---|---|---|
| Positive impacts | • The recycled powder is used<br>• No extra material for the support structure | • The parts formed are stable<br>• Variety of material available.<br>• Minimal post-processing required | • Time-efficient |
| Negative impacts | • Unit is large requiring big space<br>• Power consumption is high | • Requires nitrogen supply<br>• Poor surface finish<br>• High maintenance cost | • Requires skilled labor |

### 8.3.11 Selective Laser Melting (SLM)

The process is quite similar to the SLS but with the difference of the metallic powder used here and the sintering here is replaced with the melting process. The powder is melted and made a solid with good mechanical strength and accuracy in terms of geometry [31, 32]. Rest the process is made similar to the other process layer by layer and using the CAD generated files. The machine controls the moment of the laser beam passed through the optic lens, which melts the powder. The certain kind of environment, economic, and social aspects are discussed in Table 8.9.

### 8.3.12 Electron Beam Melting (EBM)

The mode of heating /melting the powder in a vacuum is changed with the electron beam whose firing intensity along with the directions is controlled by computer and by the CAD file [34]. The process is the same as the other processes where the parts are formed layer by layer. Here we have the option of producing multiple parts on the same bed. Rest the process of applying the powder on the previously built layer is the same as SLM. The process is efficient in terms of the quality of the product, having

**Table 8.9** Sustainability assessment of the SLM process [33]

| Sustainability factor | Environment | Economic | Social |
|---|---|---|---|
| Positive impacts | • The recycled powder is used<br>• No extra material for the support structure | • Products are of good quality<br>• Choice of different metallic materials<br>• Cost-efficient process<br>• No post-processing required | • No skilled labor needed<br>• Safe process |
| Negative impacts | • Power consuming process<br>• The unit requires a large space | • Slower process | • Causes fatigue to labors |

**Table 8.10** Sustainability assessment of the EBM process

| Sustainability factor | Environment | Economic | Social |
|---|---|---|---|
| Positive impacts | • No extra material for the support structure | • Accurate parts.<br>• Product has the best-built quality<br>• Ultimate product finishing<br>• High production rate | • Time-efficient |
| Negative impacts | • Power consumption is high | • Poor surface finish<br>• High maintenance cost<br>• Need a good vacuum chamber | • Requires skilled labor<br>• Gamma rays are produced |

good mechanical properties [35]. Table 8.10 gives the details of the EBM's sustainability.

### 8.3.13 Laser Engineered Net Shaping (LENS)

This is the unique process where the material (metallic powder) is delivered through the outer circumference of the nozzle with instantaneous melting through Nd: YAG laser beam focused through the same nozzle part located in the center [36, 37]. This setup gives the freedom to accurately place the material on the substrate. The moment of the nozzle is governed according to the CAD file (.stl format) after slicing. The laser beam is focused on the group of lenses under the controlled argon atmosphere for avoiding oxidation. The process is suitable for giving the quality product where the grain structure formed is also good [38]. The Sustainability assessment of the LENS is described in Table 8.11.

**Table 8.11** Sustainability assessment of the LENS process [39–41]

| Sustainability factor | Environment | Economic | Social |
|---|---|---|---|
| Positive impacts | • The material is not used in excess | • Products are of superior quality<br>• Complex parts/ shape can be produced | • No skilled labor needed |
| Negative impacts | • Power consuming process<br>• The unit requires a large space | • Slower process.<br>• The materials are limited<br>• Use of special inert atmosphere | • Safety risks for operators<br>• The process reduces the employment opportunities for labor |

### 8.3.14 Three-Dimensional Printing (3DP)

Unlike other processes of powder, the product here is formed by the powder and binder. The loose powder on the build platform is spread and according to the geometry from CAD files the print head prints, the binder solution on the powder, and that makes the layer solid [42]. The neighboring powder act as the supporting structure. Here the multicolor facility is available, where the binder of a different color can be printed on the powder [43, 44]. The layer by layer product is formed following the same steps of spreading the powder and printing the binder. The sustainability of the 3DP is discussed in Table 8.12 below.

### 8.3.15 The Solid Sheet as Material

A thin sheet of metal is used as a starting material for making the product. The operation done on the sheet is a bit different than any other sheet metal operations done in the industry. The main process in Rapid prototyping or 3D printing techniques is the laminated object manufacturing.

### 8.3.16 Laminated Object Manufacturing (LOM)

The process consists of the production process of similar fashion like other RP processes of making the CAD file and feeding it in the machine after slicing. Here the CAD file drives the motion of the $CO_2$ laser that is used for cutting operation [46–48]. The material here in the form of the sheet also consisting of the binding ingredients is feed regularly where the one end has the supply roll and the other end has the used sheet collecting roll. The material is cut from the sheet by the laser after the roller on the previously made part has bonded the current sheet. The process can be related to the scrapbook where the pictures of different shapes are cut and pasted on the pages. Here, the platform bed is lowered with each step, and finally, the

**Table 8.12** Sustainability assessment of the 3DP process [45]

| Sustainability factor | Environment | Economic | Social |
|---|---|---|---|
| Positive impacts | • No extra material for the support structure<br>• The powder used can be recycled | • High production rate<br>• Multiple properties can be achieved<br>• Accurate parts<br>• Good built quality<br>• Used for multiple applications | • No skilled labor required<br>• Easy to operate |
| Negative impacts | Use of a lot of chemicals in processing and post-processing of a product | • Poor surface finish<br>• Products are weaker in strength<br>• High maintenance cost | • Downfall in opportunities for skilled artisans |

product is made. The process has multiple aspects of Sustainability that are discussed in Table 8.13.

The material here used in sheet is used inefficiently leading to the wastage in terms of money, pollution for dumping, etc. The process needs to be adjusted accordingly that it minimizes the wastage of the material.

## 8.4 Sustainable Design

For considering the sustainability concerning the products, the target should be the design stage. Although there are three aspects of sustainability (Fig. 8.2) there is a strong correlation between them. The environment being the most important and the major concern for sustainability as it includes the input and output for every single product being produced. The Fig. 8.3 describes the pre- and post-production effects due to inputs and outputs of the production. There will be a lack of resources for future generations if the extraction is continued at a rapid rate. The production which is carried out in an unplanned manner not only reduces the resources and energy system but also the economic and social life is affected. The degradation of the environment due to waste production, deforestation for industries and inventories, accidents associated, etc. all are the impacts that are related to the exchange of substances in terms of inputs and output with nature. Sustainable design or the Ecodesign is the need of an hour that, however, cannot eliminate the ill effects due to the industries in terms of pollution but yes can reduce it to a certain extent. The effectively Eco designed product/process with the use of proper guidelines from the

**Table 8.13** Sustainability assessment of the LOM process [19, 49]

| Sustainability factor | Environment | Economic | Social |
|---|---|---|---|
| Positive impacts | • No extra material for the support structure | • High production rate<br>• Multiple choice of materials<br>• Precise products<br>• No need of post-curing | • Easy to operate |
| Negative impacts | Wastage of material | • Need the power adjustment equipment<br>• Products are weaker in strength | • The tedious task for labor<br>• Need skilled labor |

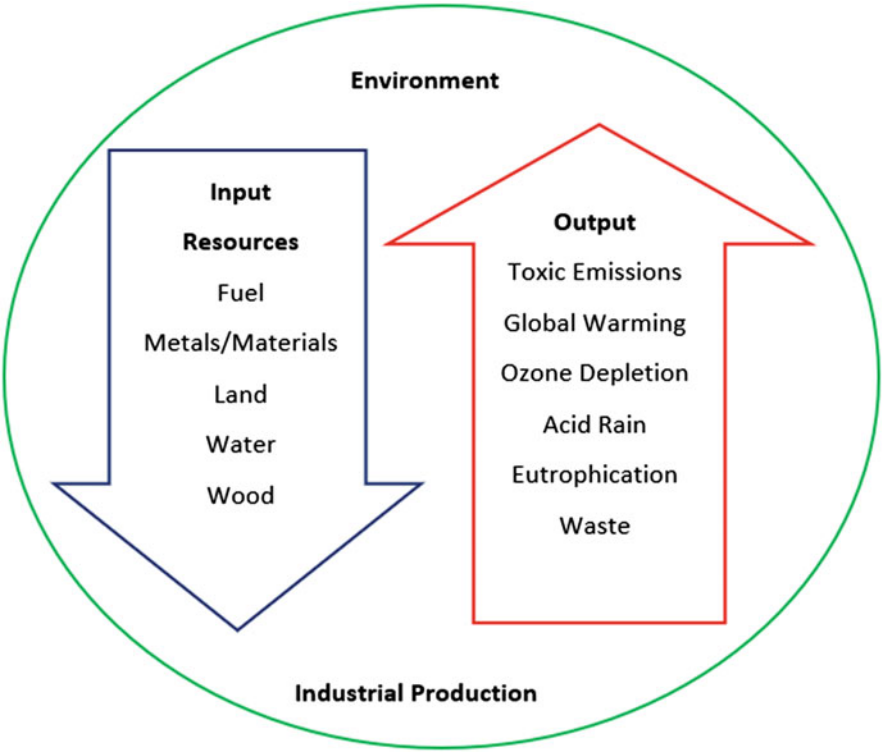


**Fig. 8.3** Diagram for the exchange of substance between Environment and Production Systems

previous set of rules and current innovations can make the Additive manufacturing a sustainable process.

## 8.5 Product Life Cycle

Considering just a product and its production for sustainability is not the right approach as many other phases are associated with the product. The complete life cycle of the product needs to be considered for evaluating sustainability. The life cycle of the product consists of the following phases [50].

- Ante Manufacturing.
- Manufacturing.
- Dispensing.
- Usage.
- Discarding phase.

All these phases have equal importance, as all require the use of resources that can be in terms of energy, materials, land, etc. and they all release some of the other sorts of harmful outpouring to nature.

## 8.6 Ante Manufacturing

This phase particularly deals with the arrangement of the raw material from the resources, which requires proper conversion after transportation to the suitable places of the manufacturing plant. The raw material is of two types which can be fresh material that can be extracted from nature for the first time and the other is the recycled material. The recycled material further has two types, one which is unused scrap material discarded due to the inferior production or fit and the other one is the completely used material which has completed its whole life cycle and is discarded after the end of its life. The resources can be obviously of two types, viz. renewable and non-renewable energy resources which depends upon the kind of products and production system used. For the sake of sustainability on every step, prior documentation and planning should be done for getting the more out for these words.

## 8.7 Manufacturing

Manufacturing itself is a complex process, which can have different junctures involving the transformation of product, assembling of various components, or the combination of both. The consumption of resources here is not limited to the raw material for the products to be produced but it can vary immensely. The machine tools, their accessories, and power needs are also covered in the manufacturing phase which is often neglected but is unintended resource needs. The dedicated machinery and tools along with suitable operations to be performed according to the product requirement demand customized resources.

For example, a camshaft is being manufactured in a firm for various automobiles, for ease let us suppose the material used is of the same grade of steel but still the

design, strength of the particular area in camshaft and size will differ according to the vehicle and model for which it is being manufactured. These differences require different cutting, grooving, and heat treatment operations to be performed which will require different kinds of resources in terms of power, machinery, and tools.

## 8.8 Dispensing

Dispensing or delivery of the product is of main importance and is also associated with the various marketing and sales affairs. Although certain manufacturing techniques like just in time manufacturing etc. are developed but cannot be applied to every product and in every region, which also requires a certain amount of inventory space be it for raw materials or semi-finished goods. Inventory requires certain resources like good illumination, cover from natural conditions, safety from the attack of insects or pests, and in some cases the controlled environment for storing the raw materials, products and tools, etc. inefficient and protective manner.

Transportation is the other main thing in the delivery of the products which can be done by various means including the roads, rail, water, and airways. Transporting the goods requires the energy sources not only to drive the means of transports but also to produce these vehicles, in some cases the dedicated vehicles like controlled environment chambers, pressured chambers, etc.

Packaging of products is of main importance that is required for the safe delivery of the product to the customer. The kind of product decides the way of packaging, ultimately the goal is to save the product from damage during freight and storing. The packaging is also played emotionally by certain design engineers to make the product in demand. The storing containers or design of the packages are made to attract most of the customers. All these require the resources in some way or the other. Sustainability can be achieved by researching and optimizing the material, quality needed for the particular product.

## 8.9 Usage

Usage of the product is dependent on the quality requirement of the user, where the product life for usage is already defined by the user and is discarded as waste after that particular time. The other case is dependent on the actual life of the product, adding to which for some users the servicing or repairing comes into play, and the life of the product is extended for further use. The psychological aspect of the user is also associated with usage of the product, wherein for some products, for example, mobile phones are changed according to the will of the customer rather depending on its actual life or functioning. All these usages aspects affect the sustainability a lot like the product which was bought is used to its optimal efficiency or it is discarded before its actual life cycle.

## 8.10 Discarding of Product

Discarding is ceasing the use of the product from the user end. The product which is dumped can be handed down again by some repair of the whole component or its parts. This can be reused for the same purpose. In some of the cases, the product can be remanufactured where some of its parts can be replaced with new components and can be delivered back to service to the customers. The product can also be recycled where the product components which are in good condition can be employed in the making of the new product of a similar kind. This can save a lot of resources that are employed in fabricating new components. There can be conditions where the dumped products or its components are not that suitable to be replaced in the new product, so the components are disassembled and melted to be used in some other applications requiring the same material.

Lastly, the remains from all the processes or the different discarded products are used to an optimal life cycle that there is a non-other option available than to finally dump into the environment. There can be few treatments or break down of the components into suitable forms that affect the soil or the environment least.

The above mentioned all the phases are attached to nature in somewhat or the other in terms of input and output of the materials. There is a continuous exchange of resources in every phase of the life cycle of the product. Sustainability of the product and process is still far to be achieved in complete sense, as there can be numerous other factors that can be examined and improved to get the optimum results.

## 8.11 Utilitarian Approach

The working of the product is the other major criterion that can be accessed for sustainability in terms of environment and economy. There can be instances that product may be considered less sustainable in terms of its manufacturing due to its size or shape but in a functional approach, it can prove itself to be more fruitful as it serves the need for long hours and benefits more. Similarly, there can be a product that might be sustainable in its manufacturing stage but using the product has less capacity and cannot work in the long run that conflicts the approach. The working conditions, therefore, are of great importance and should be evaluated along other processes to get the best result for sustainability.

The approach should be not that the impact of the product on the environment is reduced at one step but that increases exponentially on others as that will not solve the problem. There can be certain strategies that can be followed based on the products, their delivery, and packing. The need should be incorporated appropriately and excess resources or operations should always be lowered.

## 8.12 Economic and Environmental Aspects Related to Discarding

Sustainability discussions manifest the procedure of re-use, recycling of the products after they are discarded and that is quite an unerring approach. Unfortunately, in the current scenario, the possibilities of recycling and re-using the product by the act of repairing is getting difficult due to the elevating costs associated. It would not be wrong to say that buying and using a new product seems more economic than repairing the one that can be considered the boon of the manufacturing sector or the available technology but is affecting the environment by creating the large heaps of waste products. The simple example that can be taken of the pen that is used and thrown rather be refilled, this happens because the cost of another pen is so affordable that refilling has loosed its importance. One cannot even ignore the disposal of the pen as waste, a clear indication of wastage can be done by the number of users of a pen which is very huge and the material is usually thrown in the environment (land, water). The cost here is retarding the sustainable development.

## 8.13 Life Cycle Assessment (LCA)

The aspects of sustainability associated with the product in its complete life cycle as discussed above depend in multiple ways from antemanufacturing to the discarding of the product. LCA is the simple method that evaluates the complete life cycle of the product according to the environmental aspects, including the interaction with regards to input and output in between the production and environment. LCA is responsible only for the environment-related evaluations of the life cycle of the product, social and economic aspects are absent here. According to ISO 14000, the LCA has main four kinds of phases, viz.

- The complete definition of goal and scope for LCA includes the need for LCA and what can be performed using it and up to what extent it is beneficial.
- The life cycle inventory is the kind of data collection and analysis of the input and output of the resources.
- Life Cycle Impact assessment includes the classification of various problems there combined effect and then their normalized value is evaluated.
- Finally, the result for all the phases is evaluated and the suitable solution is concluded.

However, the process-wise and according to the product some databases have to be made for assessing sustainability in economic, social, and environmental front for Additive Manufacturing processes. This is because every process and product is based on the particular need and have different aspects accordingly some of the brief reviews of the Sustainability assessment of the RP process is described in Table 8.14.

**Table 8.14** Brief review of the Sustainability Assessment of few RP Processes

| S. No | Authors | Sustainability assessment |
|---|---|---|
| 1. | Huang et al. [51] | • RP machines are economical for small batch productions<br>• RP technology can produce customized surgical implants, putting an impact on health and well being<br>• Design innovation can be achieved, products with multiple properties can be produced in a single operation<br>• Leftover material can be reused<br>• No requirement of a cutting tool, jigs, fixtures, and coolants<br>• Less usage of energy in RP processes<br>• The scrap produced is very less<br>• Pollution (terrestrial, aquatic, and atmospheric) by the RP processes is low compared to other processes |
| 2. | Nannan et al. [8] | F1 gearbox developed using RP processes saves 20–25% weight and 20% volume. The torsion stiffness is doubled with less gear wear and also the power consumption is low |
| 3. | Optomec [52] | The company produced Ti6Al4V components including suspension mounting brackets and driveshaft spiders for racing cars using LENS yielding the more than 90% reduction in material, simultaneously reducing time and cost |
| 4. | Ackland et al. [53] | Patient-specific biomedical products are produced using RP processes including the products for hearing aids to various implants in the body |
| 5 | Cui et al. [54] | Even on using the temperature for 300 °C for the nozzle in ink-jet printing, the cells are saved from damage due to the micro 2-s time of heat stay rising the temperature from 4 to 10 °C |
| 6 | Erbel et al. [55] | • Use of biocompatible titanium alloys and biodegradable materials which are required just for the support of structures<br>• Mg stents have the optimum mechanical properties<br>• The degradation response of the stents is also safe with almost no side effects like inflammation on the body were observed |
| 7 | Cooley [56] | • A good combination of material can be made for aerospace missions<br>• The brittleness can be reduced with a combination of metal and ceramics, act as a thermal shield in earth's atmosphere |
| 8 | DUS Architects [57] | • In situ materials usage freedom in FDM<br>• The method can be used to create low-cost houses<br>• The technique can be used to make shelters outside the earth like on the moon |
| 9 | Xu et al. [58] | • RP processes can be used for making historic structures with a combination of materials and the latest techniques<br>• The cost-effective production of the structure is done using this technique<br>• Compared to the traditional method the current method is labor efficient |
| 10 | Domanski et al. [59] | • FDM, 3DP, and SLM use in the reconstruction of the disc with its geometry<br>• Computed tomography is used to make the bone design |

## 8.14 Conclusion

Rapid Prototyping earlier was used just for prototype manufacturing used for showcasing how the real product look likes, but with the growth of its usage in many fields, it has become an essential process. Sustainability for the rapid prototyping can be achieved if every step of production and usage, viz. from the design stage to the end of the life cycle the necessary forecast studies have to be made while selecting the resource, process, packaging transportation, and usage. The carbon footprint has to be reduced to make the earth available for the coming generations. The database of the sustainable assessment needs to be created as RP is the new technology and database can only help in attaining the Sustainability. The database must contain the details of the exchange of elements between the industrial activities and the environment. The Impact assessment of using a resource for the particular RP process should also be included in the database. Economic and social adaptability will be achieved by the use of technology inefficient way. Thus, all three pillars of sustainability are linked to each other and a combined approach can lead to sustainable development.

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# Successful Stories of 3D Printing in Healthcare Applications: A Brief Review

9

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**Abstract**

3D printing is extensively being used, nowadays, for mass customization and fabrication of complex products with different shape, size, and functionality behaviour. This led to the emergence of 3D printing in several medical applications such as tissue engineering, organ regeneration, prosthetic fabrication and customization, anatomical model construction, pharmaceutical investigations, and bioelectronics products. In this chapter, few successful stories of 3D printed products in biomedical domain are systematically reviewed and outlined. At first, the foundation of 3D printing and its processes are briefly introduced with appropriate set of examples. Thereafter, five subdomains, namely, tissue and organ generation, prosthesis fabrication, medical education, surgical planning, drug delivery and bioelectronics are considered to review the successful product developments in the healthcare sector. The flexibility of 3D bioprinting to print biocompatible products is observed at many places. Furthermore, the advantages, limitations, and future opportunities regarding use of 3D printing in medical sector are briefly discussed. At last, the contribution of this work is presented with the concluding remarks. This review will serve

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enthusiastic researchers to understand the true potential of 3D printing in healthcare applications.

**Keywords**

3D printing · Tissue and organ generation · Prosthesis fabrication · Bioelectronics · 3D bioprinting

## 9.1 Introduction

Additive manufacturing is a material addition technique, which has come forth in last four decades to fabricate objects. It offers the cost-effective solution for the designing and printing of complex shapes [1]. The conventional methods of formative technique, i.e. moulding and machining are not capable of frequent changes in final product/design as per requirement. They also have a drawback to form the complex shapes required in biomedical engineering applications. This led to the acceptance of additive manufacturing in industries widely [2]. Additive manufacturing, also known as fundamental 3D printing, was contrived in the 1980s and is basically a layer by layer material deposition technique in 3-D space using a computer model file. Furthermore, in 2010, printing biological living cells as complex functional products has given a plethora of medical benefits. This process is known as 3D-bioprinting. Thereafter, in 2013, the idea of 4D printing is proposed where the shape and size of manufactured bioengineering products can be altered using pre-programmed smart materials. This further leads to the 4D-bioprinting. Recently, in 2016, Mitsubishi Electric Research Laboratories (MERL) by William Yerazunis introduced the 5D-printing approach with a variation of five angles using a movable table [3]. The stepwise process of additive manufacturing and the development over the years is shown in Fig. 9.1 [4]. However, the content of this work is limited to the 3D printing and 3D-bioprinting processes of the additive manufacturing.

Several techniques have been discovered for layered printing of different materials since additive manufacturing has been developed. Defined by the ISO/ASTM 52900 standard [5], the well-recognized techniques are classified as powder based, material deposited, liquid reservoir based, sheet laminated, and nanofabrication based printing.

Powder based printing has two practices: first, binder jetting, which first covers the powder bed with binding material, then sprays a layer of powder; second, powder bed fusion, where laser is used to melt powder to print a layer by layer part. *Material deposition* technique utilizes a nozzle to deposit metal, thermoplastics, liquid resin, and cells suspended in bio ink to achieve 3D printed parts. (Bioprinting) [5]. Material (such as bio ink) to be printed should have suitable viscosity, shear-thinning property as well as printability for a wide range of processing parameters [6]. Fused deposition modelling, shown in Fig. 9.2a, is another name of material deposition technique. In liquid reservoir based printing, the print bed is submerged in a tub filled with

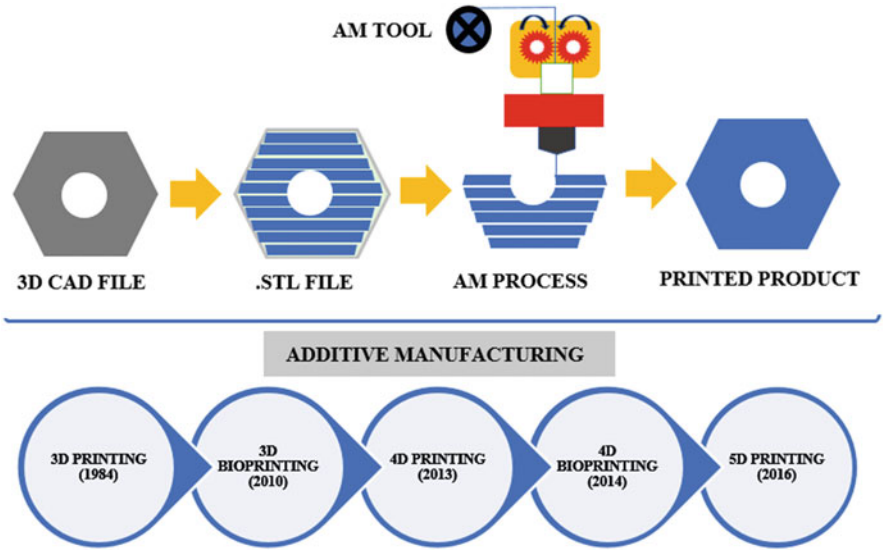


**Fig. 9.1** Schematic representation of additive manufacturing process and development over the years [4]

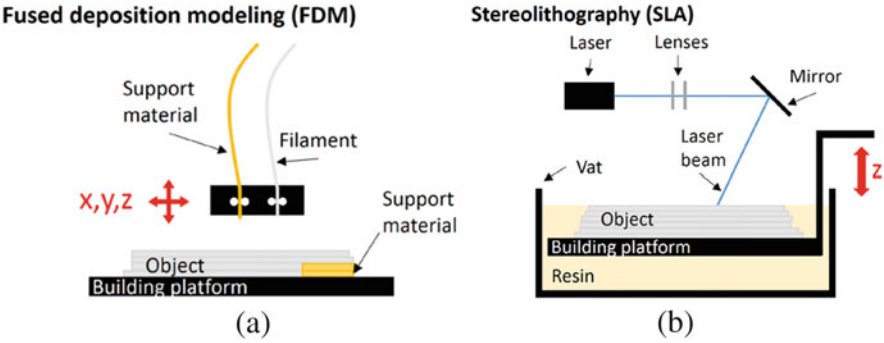


**Fig. 9.2** Schematic representation of (**a**) fused deposition modelling, and (**b**) Stereolithography process [31]

liquid photopolymer resin and either ultraviolet or visible light is focussed at the resin-bed interface to solidify the resin. This process is repeated to make the part layer by layer and is known as stereo lithography (SLA) [7], as shown in Fig. 9.2b. *Sheet lamination based printing* exploits a laser or blade to cut a stack of paper, plastic, or metal and confined by a binder. Thereafter, according to the CAD model, the excess material is removed, exposing the three-dimensional inner design. *Nanofabrication* is the process of making structures less than 100 nm in size, therefore, being suitable for application in a variety of fields inclusive of electronics and medicine. There are two methods: "top-down" approach and "bottom-up"

approach. In the former one, nano-sized components are produced from bulk material using machining process matched technique [8]. As the steps in this process are irrepressible, therefore, offering high complexity and limited variability in the printed products. The latter one prints by stacking up atoms or molecules to fabricate a nanostructure.

3D printing has been used by the manufacturing industry for decades to produce larger models and moulds within a less amount of time while comparing to traditionally machined products. A great amount of 3D printing designs are available in online repositories, many of them available for free to download, enabling consumers to print daily life products such as clothes, designer jewellery, guns, and car-parts. The 3D marketplaces like Thingiverse, Myminifactory, CG Traders, RepRap, and Pinshape are the websites that offer 3D model files free or with small charges to download [9–11]. Currently, thermoplastics, ceramics, graphene-based materials, and metals can be used as the printing materials in 3D printing technology [12]. The 3D printing technology has shown enough potential to improve the efficiency of different manufacturing sectors. At present, the 3D printing technology is being extensively used for mass customization and fabrication of complex designs in aerospace [13, 14], food processing [15], automotive [16], and healthcare sectors [17, 18]. 3D printing is playing a significant role, nowadays, in health care applications. In 2013, the application domain of 3D printing is covered by a $700 million investment with a share of 1.6% in the medical field. In the following decade, it is estimated to become an $8.9 billion industry, with a share of 21% in medical sector [19].

The application of 3D printing in healthcare sector can be categorized into tissue as well as organ fabrication, printing of customized prosthetics, implants, and anatomical models for teaching purpose, and pharmaceutical investigations like drug delivery and exploration [20]. The method of nanofabrication has a significant role in tissue engineering and biomedicine such as maintaining immunogenicity of compounds in vaccines, reducing transplant rejection through immune-isolation, achieving biomaterials with exclusive mechanical as well as biological properties, drug sequestration and delivery, and circulating waste toxin-binders [21]. The 3D printing technology is helping surgeons to enhance their skills and knowledge with more accurate anatomical models being developed using 3D printing. Bioprinting allows in vitro models for drug testing, disease modelling, and implantable tissue generation such as bone, cartilage, and skin cells in a 3D space. Current research on 3D printing technology in healthcare domain is focussed to 3D modelling of pathological organs for surgical planning and analysis, fabrication of customized non-bioactive implants, generation of local bioactive and biodegradable scaffolds, reconstruction of functional tissues and organs such as 3D printed bionic ear, 3D printed skin for burn victims [22].

Since many healthcare applications are available where 3D printing has been successfully incorporated; therefore, there is an emergent need to narrow down the list of such successful developments in the field of healthcare. In this work, five successful and well-established subdomains are systematically presented to realize the true potential of 3D printing in healthcare sectors. The considered subdomains in

this study are tissue and organ generation, prosthesis fabrication, medical education, surgical planning, drug delivery and bioelectronics. The benefits augmented with these developments are briefly reviewed and presented. At last, the future directions and possible opportunities are closely monitored and discussed.

## 9.2 Successful Stories of 3D Printing in Healthcare Sector

### 9.2.1 Tissue and Organ Generation

Although several procedures of tissue and organ transplantation are available to cure lesions and defects, however, they have few limitations. Auto transplantation has certain complications which can lead to secondary injuries, whereas xeno-transplantation has possibility of viral transmission and immunological rejection due to limited source donors. However, implantation of artificial mechanical organs is usually successful and making life of a patient better. 3D printing can solve unavoidable problems faced in traditional procedures by personalized fabrication of human bionic tissue and organs. Tissue regeneration is, nowadays, an extensive field of study due to the key process behind cell growth and reconstruction of organs. Since the introduction of tissue engineering in 1993, the replacement of damaged organs by application of biology and engineering principles has changed and improved many lives [23]. The guide component of a 3D printed tissue, scaffold, is responsible for cell interaction and physical stability of newly developed tissue. Scaffolds are also responsible for integrating the prime growth factors at required position to control and augment tissue growth [24]. 3D bioprinting is actively studied in the field of tissue engineering because of the higher control over cell distribution and scaffold fabrication. Bioprinting can be done in four ways: extrusion, inkjet, stereolithography, and laser-assisted. These techniques are briefly presented along with the process parameters in Table 9.1.

As bioprinting has printing resolution of wide range varying from 10 μm to 10,000 μm; therefore, it is found to be flexible compared to other assembly methods such as porous scaffold and moulding. Bio ink is an important aspect of research in bio printing, which controls the attributes like printability, printing fidelity, and mechanical properties. With the goal of improving the printing quality and incorporating a variety of biomaterials, Rees et al. [25] developed two types of nanocellulose bio ink, TEMPO and C-Periodate, to develop 3D porous structure with antimicrobial properties. The grid structures for both nanocelluloses are formed with a biopolymer component, as shown in Fig. 9.3a, b. Yu and Ozbolat [26] developed Alginate based bio ink using co-axial nozzle system to fabricate artificial organs. They achieved nearly 90% mouse TC3 cell viability and pancreatic tissues by coalition of umbilical vein smooth muscle cells and bio ink.

**Table 9.1** 3D Bioprinting techniques and associated process parameters

| | Printing apparatus | Printing process | Speed | Cost | Material |
|---|---|---|---|---|---|
| Extrusion | Nozzle | Extrusion of material with adjusted viscosity using temperature or shear thinning | Slow | Moderate | Nanocellulose, alginate, polyurethane (PU), poly(ε-caprolactone) (PCL) fibres, etc. |
| Inkjet | Nozzle with thermal or piezoelectric actuator | Droplet formation | Fast | Low | PEG-based bioink, soft hydrogel |
| Stereolithography | Beam projector | Polymerization of light sensitive polymer | Fast | Low | Poly(ethylene glycol) diacrylate (PEGDA), gelatin methacrylate (GelMA) |
| Laser-assist | Pulsed laser source | Laser induced forward transfer (LIFT) | Medium | High | MG63 cells, alginate, PCL electrospun scaffold |

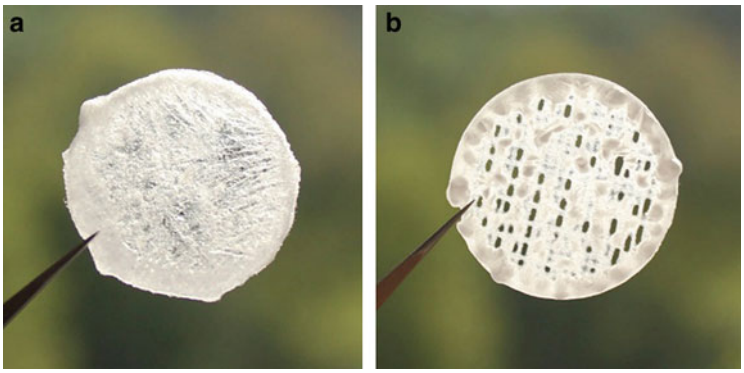


**Fig. 9.3** Grid structure representation of (**a**) TEMPO and (**b**) C-Periodate nanocellulose bio ink [25]

**Fig. 9.4** 3D printed prosthetic hand printed by a student [27]

### 9.2.2 Prosthesis Fabrication

3D printed prosthesis is revolutionizing the field of prosthesis as they are relatively less costly, consume less time to manufacture, and offer great customization benefits. The worth of 3D printed prosthesis can be observed in bone grafting to cure bones damaged by cancer or infection. 3D printed orthopaedic casts have been found to increase healing 40–80% faster than traditional casts [22]. According to a recent study [22], the limitations about symmetry and freedom to move with robotic lower body prosthesis are resolved with a 3D printed assistive suit. In another work [27], a $500 3D printed hand was developed by a high school student to replace a high cost prosthetic hand of $80,000. This hand, as shown in Fig. 9.4, was controlled by brain waves using EEG (electroencephalography) equipped headband. Other significant efforts in this field are: e-NABLE [28], an open source platform for innovators and enthusiasts to download, modify, design, and upload 3D printable prosthetics;


**Fig. 9.5** Open bionics prosthetic hand designs [30]

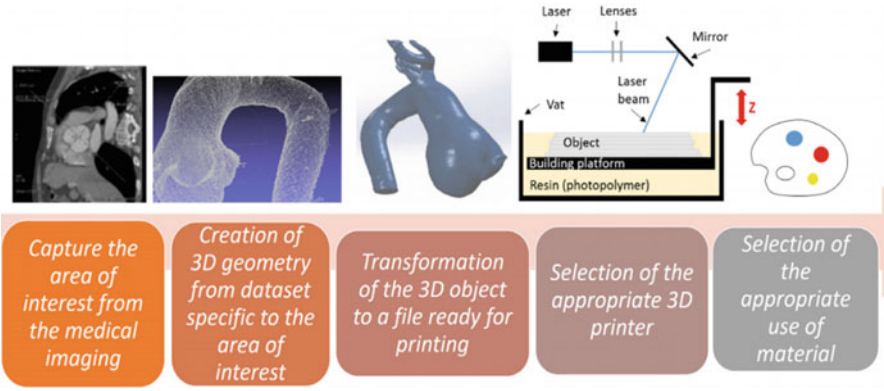


**Fig. 9.6** Step-by-step procedure for 3D printing for medical education [31]

NotImpossible [29], an open source platform with cost-effective 3D printed solutions for war victims and impaired poor individuals; and Open Bionics [30] for young individuals. The CEO of Open Bionic translated the business idea of printing prosthetics based on customers' demand. For young amputees, the company has introduced the printed designs inspired by Marvel, Disney Frozen, and Star Wars aesthetics, as shown in Fig. 9.5.

### 9.2.3 Medical Education

Personalized 3D printed models of patient's disease help the medical trainees get better at handling, understanding, and be confident. A step-by-step procedure for the required 3D printed model of the patient's disease is shown in Fig. 9.6 [31]. 3D printing technology has enabled the possibility to reproduce several models with more safety than the process of cadaver dissection, and supplying them to the

institutes with less resources as well as modelling other physiological and pathological based anatomy from a large dataset [32]. 3D printing can help doctors in communication with the patient by using 3D printed models rather than verbal or using 2D CT scan images. 3D printed materials, having a variety of densities and colours, can better simulate tissues used in medical education [33]. In study of cardiovascular diseases, 3D printed pathological cell status can be modelled with high-elucidation, and, therefore, spreading the efficient education related to heart diseases [34]. Generally, the rigid behaviour of 3D printed parts shows an incapability to fabricate flexible and softer tissue such as brain. This issue is addressed by a surrogate gelatin material, manufactured with a fusion of 3D printing and casting, which is carried out to demonstrate the effective training procedures [35].

### 9.2.4 Surgical Planning

3D printing is a cutting edge technology in manufacturing clinical equipment and surgical guides from clinical images of patients with efficacy. A diseased lever, as shown in Fig. 9.7, is printed with multicolours to differentiate among the tumour, portal vein, and hepatic vein for efficient surgical planning [31]. 3D printed stents have been applied in the different fields of surgery planning such as tracheobronchial, dentofacial, cardiovascular, orthopaedic, and spine [36]. The tracheobronchial stent, manufactured using SLS, has been implanted to cure collapsed bronchus [37]. Moreover, they are found to be suitable for anatomy while compared to the conservative implants. Bioresorbable implants have been tested to eliminate symptoms of severe tracheobronchomalacia pig model and found to be working effectively. VanKoevering et al. [38] utilized the 3D printing, for the first time, for successful development of craniofacial anatomy based complex foetal models in case of anomalies and to provide assistance in perinatal management. For surgical planning, many research contributions are available in which orthopaedics reports

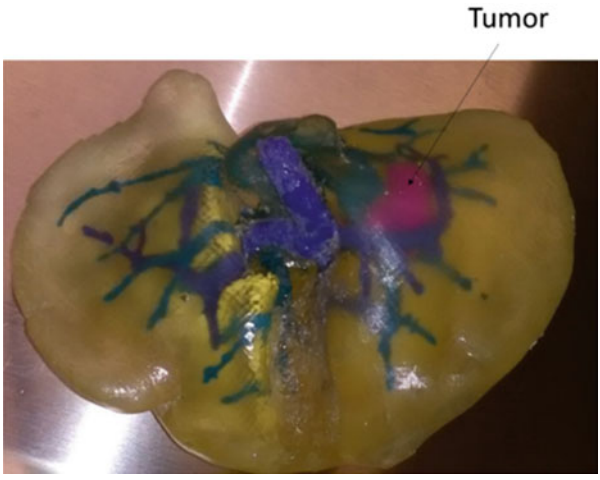


**Fig. 9.7** Multicolour 3D printed diseased liver with tumour, portal vein, and hepatic vein for surgical planning [31]

account for major portion, followed by maxillofacial surgery, cranial surgery, and spinal surgery [39]. 3D printed disease models are very helpful in preoperative planning. It offers better visualization to the surgeons and prevent harmful effect of longer surgery hours by taking quick decisions during surgery. Therefore, Vodiskar et al. [40] used a 3D printed congenital heart defect model for preoperative planning. In a study by School of Medicine, from Saint Louis University, the details of the 3D printing aided craniofacial and maxillofacial surgeries for 315 patients are presented. The study demonstrated that the surgical planning tools, splints, and implants took only mean time of 18.9 h and mean cost of $1353.31 for all surgeries [41]. The printings at laboratory and factory were carried out with high accuracy, minimal technical expertise, and at low cost. These can be produced commercially with incorporation of advanced virtual planning and suitable material.

### 9.2.5 Drug Delivery

Drug delivery, being an important aspect of medical research field, has witnessed several advancements with the application of 3D printing technology. A device has been developed using stereolithography for efficient delivery of doses of salicylic acid to treat acne [42]. Drug tablets of different shapes like torus, pyramid, cube, cylinder, and sphere were fabricated using 3D printing and their drug release profile was investigated. They have shown their dependence on the surface area to volume ratio of the drug release profile and stability factors irrespective of 3D printing method [43]. The 3D printing of drugs can revolutionize drug manufacturing by offering a cheap alternative to make tablets. A company named, Aprecia Pharmaceuticals, got FDA approval for the first time to print a pill of high drug dosage in a single tablet to cure epilepsy [44]. The printing technique, ZipDose, sutures the multiple layers of dosage in powdered form using the aqueous liquid to prepare a water-soluble and porous mixture. Although the production efficiency of the 3D printed pill is not value-added, the improvements while altering the composition of drugs are improved. In another application of 3D printing in drug transfer, Polypill [45] concept for printing a pill with composition of multiple drugs, having different release time, is proposed to serve the multipurpose aspect of the drug delivery. Polypill concept has been already applied for diabetes control.

### 9.2.6 Bioelectronics

The area of bioelectronics deals with the concurrence of electric circuits, electronic devices, and biological species. The heart pacemakers, prosthetic limbs, and grafted devices are being counted as the examples of bioelectronics. However, the issue of incompatibility with soft biological tissues can be observed in many bioelectronics based products due to the rigid configuration of the electronic parts. This, further, leads to tissue scars and infections after a prolonged use of the products. In a recent Bioprinting, a form of 3D printing can be exploited as a great saviour to address such issues. In a recent work by Shweta et al. [46], a versatile hydrogel based freestanding

platform is fabricated and tested with C2C12 murine myoblasts to ensure biocompatibility. This form of printing allows the different sizes of nozzles and regulates the pressure based on inks' viscosity.

In another work on Princeton, a bio-electronic ear is 3D printed by having living cells, gooey hydrogel, and silver nanoparticles based conductive ink [47]. The ink is capable to receive the radio signals by forming an electric coil. While printing the ear, the group of researchers prepared a cube of eight orange and green light emitting diodes. Furthermore, with the advent of multimaterial 3D printing, a variety of multipurpose, adaptable, and robust bio-electronic products can be created. For instance, Kong et al. [48] developed a Bluetooth enabled gastric resident electronic (GRE) system which can be send in the form of capsule and expands mechanically in the gastric environment. It can reside up to 30 days in the gastric zone and able to connect with wireless communication for approximately 15 days. The detailed step-by-step working process of GRE is shown in Fig. 9.8.

## 9.3 Discussion

The 3D printing technology has the potential to revolutionize the industries by changing the assembly line. This would give consumers more freedom over customizing final product according to their needs. As the distance between manufacturing site and consumer's place decreases, the need of global transportation will be decreased. This causes to use the fleet tracking technology for distribution and save energy as well as time. 3D printing will also modify the supply line of the companies for a better inclusive manufacturing and procurement. Bioprinting has developed very much in last decade, but still fully functional 3D printed organs have not been floated out in the market. The main challenges that exist in bio printing are bio-manufacturing and in vivo integration of printed cells. Another challenge is to achieve required stability or hardness of the scaffold so that tissue cannot fail due to deformation. These challenges can be addressed by carrying out the research in biocompatible materials. 3D printing of drugs in form of tablets can be dangerous, as it can increase the drug abuse by allowing anyone, with a list of chemicals, to print their own tablet. This can be controlled by proper distribution and approval of such products before implementation. Moreover, the research in bioelectronics offers possibilities of accessing the remote areas inside the abdomen and serves the purpose of accurate yet risk-free diagnosis and cure of diseases.

On the other hand, 3D printing technology has certain limitations associated with the illegitimate conventions. Countries, whose economy is relying mainly on less skilled jobs, will be affected the most. There could be misuse of 3D printing technology by terrorists to print weapons like guns and knives, illegally. Furthermore, it would be easy for the people having blueprint of the product design to do counterfeiting, as cost of material and the process would be cheaper [49]. Therefore, for abovementioned and other such problems like waste management and currency brummagem, national and international laws need to be strictly defined and mandated.

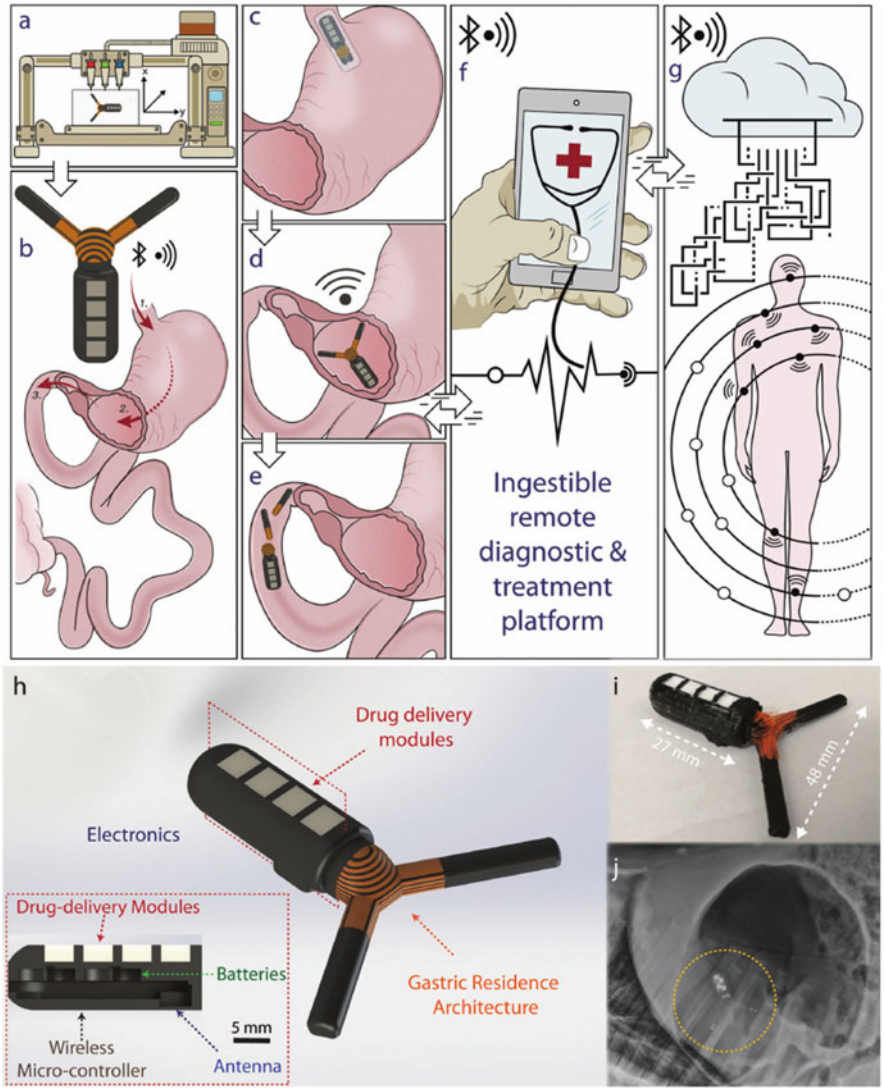


**Fig. 9.8** 3D-printed gastric resident electronics (GRE) (Labels: (**a**) patient-specific 3D printing, (**b**) designed GRE for oral delivery, (**c**) compressed into a capsule form, (**d**) device expansion activates gastric residence and offers wireless communication, (**e**) disintegration of the device, (**f**) compatible with smart phone, (**g**) interconnection with other electronics devices, and implants for responsive medication, (**h**) computer-aided design models of the GRE, (**i**) optical photograph of a fabricated device, and (**j**) X-ray image of deployed GRE stomach [48]

## 9.4 Conclusions

As the worth of 3D printed products are being recognized in the different industrial sectors, the applications of 3D printing in the healthcare sector are tremendously increased. In this work, the successful and significant contributions of 3D printing in healthcare sector have been carefully reviewed and presented. Five application subdomains from medical field, i.e. organ and tissue regeneration, prosthesis fabrication, surgical planning and deploying, medical teaching practices, drug supply distribution and bio-electronic innovations have been reviewed and highlighted with appropriate sources. The potential of 3D bioprinting has been noticed and explained with proper examples. The discussion regarding limitations and possible opportunities has been briefly summarized. This chapter will provide a rapid and crisp overview on the thriving developments of 3D printed products for healthcare applications.

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