A Review on Three-Dimensional Printing (3DP) Strategies to Induce Bone Regeneration in Bone Tissue Engineering

By

Saima Jahan
18146098

A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons.)

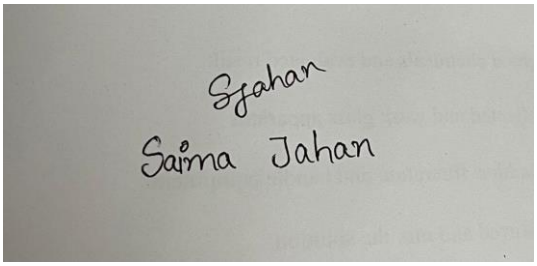
School of Pharmacy
Brac University
February 2022

Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

Student's Full Name & Signature:



Saima Jahan

18146098

Approval

The thesis/project titled "A Review on Three-Dimensional Printing (3DP) Strategies to Induce Bone Regeneration in Bone Tissue Engineering" submitted by Saima Jahan (18146098) of Summer 2021 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons) on [February 28, 2022].

Examining Committee:

Supervisor:
(Member)

Farzana Islam
Lecturer, School of Pharmacy
Brac University

Program Coordinator:
(Member)

Namara Mariam Chowdhury
Lecturer, School of Pharmacy
Brac University

Deputy Chair:
(Member)

Haina Yasmin, PhD
Professor, School of Pharmacy
Brac University

Departmental Head:
(Dean)

Eva Rahman Kabir, PhD
Professor and Dean, School of Pharmacy
Brac University

Ethics Statement

No human or animal tests are involved in this study.

Abstract/ Executive Summary

Three-Dimensional Printing (3DP) is an essential technology to build an ideal scaffold for bone tissue engineering which plays an important role in improving a patient's quality of life by reducing bone defect. The clinical results of the present traditional methods show that these cannot sufficiently treat bone injury, displacement or tissue regeneration but the recent advancements in tissue engineering have exhibited some beneficial means that can treat these conditions effectively. 3DP is used to construct customizable scaffolds with high performance qualities which are required for bone tissue engineering. This article provides a detailed review of progress in the research and development of bone tissue engineering scaffolds using 3DP, methods, existing materials and strategies to stimulate bone regeneration. Furthermore, it also discusses potential future scopes and tactics for enhancing the creation of personalized artificial bone structures and some of the technique's existing limitations.

Keywords: 3D-printing, bone tissue engineering, scaffolds, methods, materials.

Dedication

Dedicated to my parents who have given up their worldly joys and supported me in order to provide the best for me, as well as my beloved siblings and friends.

Acknowledgement

First of all, I would like to express my gratitude to my project supervisor, Farzana Islam (Lecturer, School of Pharmacy, Brac University) for her valuable input and enthusiasm in the work. She has been a great help and mentor to me during my studies and project writing. I am grateful for her excellent advice and ideas during my project work, which have greatly helped to the project's successful completion.

I would also want to thank Dr. Eva Rahman Kabir (Professor and Dean, School of Pharmacy, Brac University) for her devotion, contribution, and direction to both the students and the department.

Lastly, I would like to convey my gratitude to my parents, who continue to motivate me to push myself to new heights.

Table of Contents

List of Tables

List of Figures

List of Acronyms

3D	Three Dimensional
3DP	Three Dimensional Printing
TE	Tissue Engineering
VEGF	Vascular Endothelial Growth Factor
AM	Additive Manufacturing
UV	Ultra-Violet
DLP	Digital Light Processing
CDLP	Continuous Digital Light Processing
PPF	Polypropylene Fumarate
PLGA	Poly Lactic Co-Glycolic Acid
BMP-2	Bone Morphogenic Protein 2
MSLA	Microstereolithography
SLA	Stereolithography
PCL TCP	PolyCaproLactone-TriCalciumPhosphate
FDM	Fused Deposition Modeling
PGA	Poly Glycolic Acid
DED	Directed energy deposition
PNIPAm	Poly(N-isopropylacrylamide)
HAP	HydroxyApatite

β-TCP	Beta Tri Calcium Phosphate
BG	BioGlass
CPCs	Calicium Phosphate Ceramics
NPs	Nano-Particles
BMSCs	Bone Marrow Stromal Cells
β-TCP	**β**-Tricalcium Phosphate
HCA	Hydroxy Carbonate Apatite
ECM	Extra Cellular Matrix
SF	Silk Fibroin
PLA	Poly L-Lactic Acid
HA	Hyaluronic Acid
Ppy	PolyPyrrole
PANI	Polyaniline
PEDOT	Poly(3,4-ethylenedioxythiophene)

Chapter 1

Introduction

Bone tissue engineering is a holistic method to tissue restoration that uses cells and a combination of biomaterials. In the development of bone tissue with their normal functioning, scaffolds play an essential role by enhancing cell-attachment and migration so that a well-built bone structure similar to human body is made by keeping its biochemical properties. Pore size, pore volume, mechanical strength, chemical characteristics etc. characterize the performance of scaffolds. It is designed for bone tissue remodeling based on current knowledge of bone mechanics, its structure, and formation of tissue. (Haleem et al., 2020) Bone damage, malformations, inflammatory diseases, and accidents can damage tissues and lead to the loss of function in the organs and joints of the human body. Restoring the function of damaged tissue, organs, or joints often requires expensive surgical procedures such as transplantation, repair, and replacement of implants or scaffolds. In medical procedures, transplanting or replacing a damaged organ and joint causes a serious difficulty. Tissue Engineering (TE) techniques were developed by scientists and researchers as a promising and possible option for restoring the functionality or regeneration of damaged tissues and organs. TE is an emerging research field in which three-dimensional (3D) tissues were created by integrating bioactive materials scaffolds with human cells for the regeneration of tissue or organs.(Poomathi et al., 2020)

1.1 3D Printing (3DP):

3DP is a technology aids to produce complicated shapes without the use of manufacturing for certain parts which is done by alternating layers. Charles Hull invented the first 3DP technology commercially in 1988, termed "Stereolithography"(Bandyopadhyay et al., 2020). 3DP was originally used to make the first model for the development of unique industries' products(Haleem et al., 2020). Many industries can benefit from 3D printed objects, including turbine blade manufacturing, jewelry creation, construction etc. (Wang et al., 2020). Its uses are expanding, and it is now being used in bone tissue engineering also. This method is useful for printing porous scaffolds with the desired size and shape adopting an alternating layers process. It is gaining popularity due as its capacity to produce customized parts can be at a cheaper price and in a shorter amount of time. 3D printing is a customizable process for fabricating a variety of materials with specific shapes and dense or porous

architecture, such as- ceramics, polymers, metals, and composites. The following properties of 3D printed materials and goods are advantageous: customized shape, adjusted pore size or porosity, controlled mechanical properties, and so on (Wang et al., 2020).

The first step in printing any item is to import a 3D digital CAD file and convert it to the Standard Triangulate Language (STL) file format, which makes 3D printing easier. Afterwards, in 3D Print software, configuration files for printing are supplied, together with the right direction of the part. Lastly, the portion is printed with a desired material. Post-processing (cleaning, surface finishing, painting etc.) is carried out in accordance with the specifications of part's quality and strength (Haleem et al., 2020; Park et al., 2022).

1.2 Bone Tissue Engineering:

TE has evolved as a prospective technique for dealing with a variety of clinical issues. Tissue and organ regeneration using cells, bioactive compounds, and scaffolds are among the techniques used in this field (Bittner et al., 2018). It is a significant approach in which a cell scaffold is formed from a biomaterial with appropriate biochemical and physicochemical properties. It's a procedure that replaces or improves bone by combining it with the surrounding tissues. It is used to make progress in the field of orthopaedics. As a result, bone tissue engineering, itself with vast ability to build bone structure, marks a substantial advancement. (Haleem et al., 2020)

True biomimetic scaffolds must consider the homotypic and heterotypic cell–cell interactions, vasculature and mechanical attributes specific to each tissue to enable successful tissue regeneration. More complex processes, like synergistic effects on growth of neighboring tissue layers and restoration of the interface between these layers, must also be included within implanted scaffolds. Combining these attributes into produced scaffolds in the same way they occur in original tissue has a significant impact on new tissue growth and development. (Bittner et al., 2018)

1.3 Importance of 3DP in Bone Tissue Engineering:

Bone is a tissue as well as an organ. Congenital malformations, trauma, metabolic diseases, infections, and tumor removal can all cause bone deformities. Bone, unlike many other skeletal tissues, has some natural regeneration potential for self-repair. Many bone abnormalities, unfortunately, do not repair and termed as "critical size bone defects," requiring surgery. The therapeutic management of a significant diaphyseal defect, which is a huge discontinuity in bone that does not heal on its own, remains a serious difficulty.(Kausar & Naga Kishore, 2013)

A scaffold structure with cells and some biologics from the same patient are combined by them, thus the autografts which are natural bone tissue grafts are characterized as the therapeutic golden benchmark treatment for the abnormalities of bone. Nevertheless, donor-site morbidity, poor harvesting capacity, the need for the alteration in intraoperative course, and changes in the bone's structures from different parts of the body imply that new approaches are required. Also, scaffolds can be made in a variety of forms and sizes through structural allografts, however they are related with restricted revascularization, infection, and immunologic response. (Jariwala et al., 2015) Due to advancements in bone tissue engineering, the new scaffold materials and cells from the own body which are transplanted has opened up new treatment options for bone defects. To make a biodegradable porous bone scaffold that is loaded with growth factors, bone cells, and other materials before being placed into the human body is its essence. These scaffolds can breakdown and be absorbed by bone cells, resulting in the production of new bone. The fabrication of bone scaffolds is now becoming an important aspect of bone tissue engineering as a platform for the regeneration of tissues and planting a cell, but it is also a restriction which is also limiting the practical application of bone tissue engineering. (Ji et al., 2018)

1.4 Evaluation parameter of bone scaffolds:

1.4.1 Mechanical properties:

In order to be properly implanted, scaffold requires precise mechanical aspects. It must be strong enough to be easily handled at the time of surgical procedure or implantation. It must be suitable for either bone or cartilage (Haleem et al., 2020)

1.4.2 Biological properties:

To allow cells to grow and produces a new matrix, scaffolds must have biological and harmless qualities. It results in full and complete bone tissue regeneration. The ideal biological features of scaffolds strongly influence how they interact with organs and tissue. It encourages improved cell interaction, development, and migration, as well as the incorporation of cells into scaffolds (Haleem et al., 2020)

1.4.3 Chemical properties:

It is composed of bio-ceramics, polymers, and hybrid materials that help cells grow in scaffolds. These lead to the production of complicated shapes and improved cell attachment.

In bone tissue engineering, a scaffold with outstanding chemical characteristics overcomes a variety of issues (Haleem et al., 2020)

1.4.4 Biocompatibility:

Scaffolds must be made of a biocompatible substance that allows cells to conduct their normal functions. Following implantation, these materials diminish bodily reaction and boost efficiency (Haleem et al., 2020)

1.4.5 Biodegradability:

It must be biodegradable, as this aids in the formation of tissue from the body's cells. A scaffold is not a permanent implant as it permits cells to create their own extracellular matrix. It has a significant impact on clinical practice and research (Haleem et al., 2020).

1.4.6 Porous structure:

Scaffolds should be highly porous and they must contain an interconnected pore structure to ensure cellular penetration. From the cells, it forms an extracellular matrix and exits the body without causing any damage to the native tissue or organs. The high porous structure (90%) might be able to achieve greater cell attachment, new blood vessel formation and removal of waste products effectively (Haleem et al., 2020; Ji et al., 2018).

1.5 Principles of bone tissue engineering:

Tissue engineering has gained popularity as a method of better recognizing abnormalities in infectious tissue form and effect and also for producing alternative ways to repair wounded tissues. It is used to cure diseases or injuries that cause bone deformities. The fabricated new bones have distinct shapes, sizes, and physiological properties due to the various sites of bone deficiencies. The usual way is in vitro which is done by putting osteogenic functional cells onto a 3D scaffold material and inserting them into the human body after the culture is done for a period to mend bone deficient tissue. To fix a bone damage by introducing growth factors or bone-forming biomaterials in vivo to stimulate mesenchymal cells for bone regeneration is another option (Ji et al., 2018).

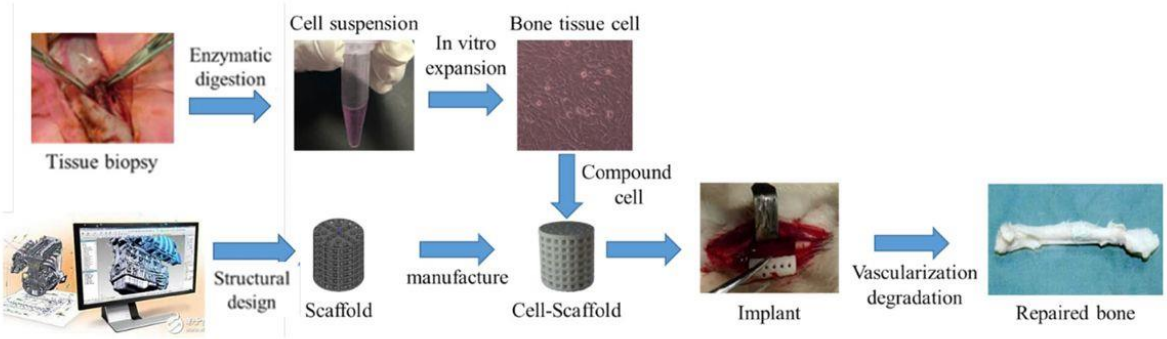


Figure 1.5.1: Principle of Bone Tissue Engineering (Ji et al., 2018).

1.6 Uses of 3DP for different sectors of bone tissue engineering:

The table 1.6.1 from the article of Haleem et al. discusses the major uses of 3DP in different sectors of bone tissue engineering which is helpful for forming the desired structure of bone tissue-

SL NO.	Uses	Description
01.	Bone defects repairment	• Bone fractures can be repaired along with the growth of new tissues. • Solving a variety of issues that arise during complicated surgery by constructing a precise replica of the bone. • Used to make patient-specific implants, prosthetics, slicing guides, fastening tools, etc.
02.	Cell planting	• In clinical applications, it is an important aspect of the process of generating new bone. • Offers excellent biodegradability, bio - compatibility, and mechanical properties. • To achieve the desired bone scaffold need, AM (Additive Manufacturing) by generating them from CT imaging data.

		• It appears to have uses in cancer cell biology, stem cell research etc.
03.	Biological functions	• Tissue engineering recognizes biological structure design factors in order to achieve proper biological functioning. • Artificial bone performance or resembling to human bone's structural design are used to create bone scaffolds. • After conducting the proper surgery, 3D printed personalised bone improves patient ease, effectiveness, and dependability.
04	Functions of tissue restored	• It aids in fixing bone abnormalities as well as reestablish tissue functioning. • To meet the demand of tissue engineering with precision and rapidity. • Has mechanical qualities that enable it to execute its desired purpose. • After being implanted in the patient, the qualities enhance.
05	Functioning scaffolds	• Tissue regeneration is a multidisciplinary method of constructing functional scaffolds that enhance cellular activity. • The formation of bone tissue with the necessary growth and surface qualities is a major challenge.

		•. By using a layer-by-layer process, 3D printing enables a fundamental revolution in bone tissue engineering, solving the difficulties of previous scaffolding methods.
06	A functional gradient of cells	• Using this method, completely functional 3D printed tissue with a functional cell gradient is achieved. • This technological development provides for more durable scaffold using new materials. • Medical research uses include 3D liver models, vascularized constructions, heart tissue, and other clinical applications.
07	Desired mechanical properties of scaffold	• In scaffolds, mechanical strength is a serious difficulty that is met by 3D printing with new groundbreaking materials. • The mechanical strength of a 3D printed scaffold can be increased further utilizing post-processing techniques, depending on the requirements. • The part's strength is strengthened by reducing layer thickness.
08	Bone grafting	• A bone graft is a better strategy for bone repair and formation, utilizing data gathered with the use of CT and MRI scans. • When AM technologies are used properly, bone graft procedures become more convenient.
09	Biocompatibility of material printing	• 3D printing is a key technology that encompasses a wide range of bioceramics, biopolymers and biocomposites. • These materials are good for cell attachment and growth, as well as bone tissue regeneration.

		• Biodegradable polymer composite scaffolds can meet bone tissue engineering requirements. • PLA is a biocompatible substance that is employed in copolymers with other polymers for bone regeneration, such as poly—caprolactone and polyglycolic acid.
10	Treatment scopes	• Bone tissue engineering employs scaffold materials and patient - derived cells to improve the treatment of bone deformities. • Successful insertion of bone cells into the patient's body improves treatment. • Plantation of living cells and tissues is made simple with 3D printing.

TABLE 1.6.1: Uses of 3DP in Different Sectors of Bone Tissue Engineering.

1.7 Aim of the study:

3D printing technology improves overall the capacity to create individualized implants for patients. The use of 3DP technology in bone tissue engineering is currently proving to be effective. The main focus of this review is to discuss the 3D Printing's uses in bone tissue engineering, how 3D Printing (3DP) technology is being used in the orthopedics to help with regeneration of bone and linking 3DP's materials and structural design with the biological reaction of the host tissue after implantation.

Chapter 2

Materials and Methods

2.1 Essential Elements of Bone Tissue Engineering:

2.1.1 Cells:

Cells are the main components of living organisms and are distinctive to each body part. These cells play an important role the body maintain structure and perform certain special functions. Cells perform a variety of functions and give the bodily component its shape. 3D printing is used to help the targeted cell growth meet the requirements (Haleem et al., 2020).

Cells can be obtained from a variety of sources, such as- postnatal and adult stem cells, embryonic stem cells, etc. Isolating cells from tissue samples, modifying them, and reintroducing them into the host has been a typical strategy in engineered bone tissue regeneration. Fresh aspirated bone marrow, osteoblasts, purified and expanded bone marrow mesenchymal stem cells in culture and cells genetically engineered to express osteogenic factors such as stem or progenitor cells- Stems derived from adipose, umbilical cord blood or embryonic stem cells have all been studied for bone regeneration. (Kausar & Naga Kishore, 2013)

2.1.2 Growth Factors:

In bone tissue engineering, growth factors such as- bone morphogenic proteins, vascular endothelial growth factor (VEGF), fibroblast growth factor etc. are also necessary. The adverse effect of a 3D printed scaffold is reduced because of these. (Haleem et al., 2020) A colored 3D printer that has the multi-injector capabilities could theoretically allow the 3D structure control of incorporation of bio-factors and molecules within the scaffold. The distribution of bio-factors and chemicals has been proven to influence the success of regeneration by modifying cell signaling in the damaged region (Kausar & Naga Kishore, 2013).

2.1.3 Bone Scaffolds:

Scaffolds are essentially applied to construct a mechanically and physically stable structure like bones in the human body. It has a great potential to repair organs with appropriate function in medical field. It helps the cell to bind and migrate while keeping biochemical and other biological features. It also transports nutrients and metabolic wastes for cell growth and gives necessary support for the damaged site. This scaffold is manufactured using 3D printing

technology to adapt to the specific shape and size requirements of each specific customer. It facilitates in the effective treatments of disease/fracture by dealing with challenges of tissue regeneration and growth for medicinal applications, mainly to increase patient survival (Haleem et al., 2020).

2.2 Schematic Representation of These Elements for Typical Bone Tissue Engineering:

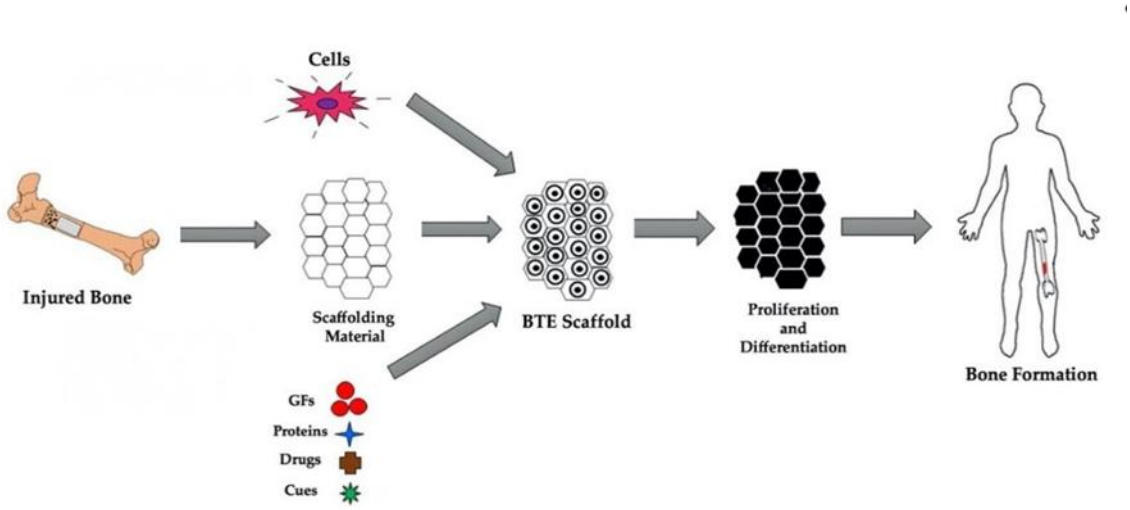


Figure 2.2.1: Schematic Representation of These Elements for Typical Bone Tissue Engineering (Dixon & Gomillion, 2022)

2.3 Stages Used in 3DP for Bone Tissue Engineering:

3DP combined with bone tissue engineering offers a remarkable therapeutic option for orthopedic patients. It uses a process similar to normal bone regeneration to create scaffolds in the desired shape and size (Haleem et al., 2020). Its manufacturing allows for the addition of drugs or proteins and cells into the scaffolding process, giving rise to a very highly advanced structure that resembles bone. As a result, additive manufacturing techniques are employed to create biological bone scaffolds (Ji et al., 2018). A figure is given below which depicts the many stages involved for the bone tissue engineering using 3DP-

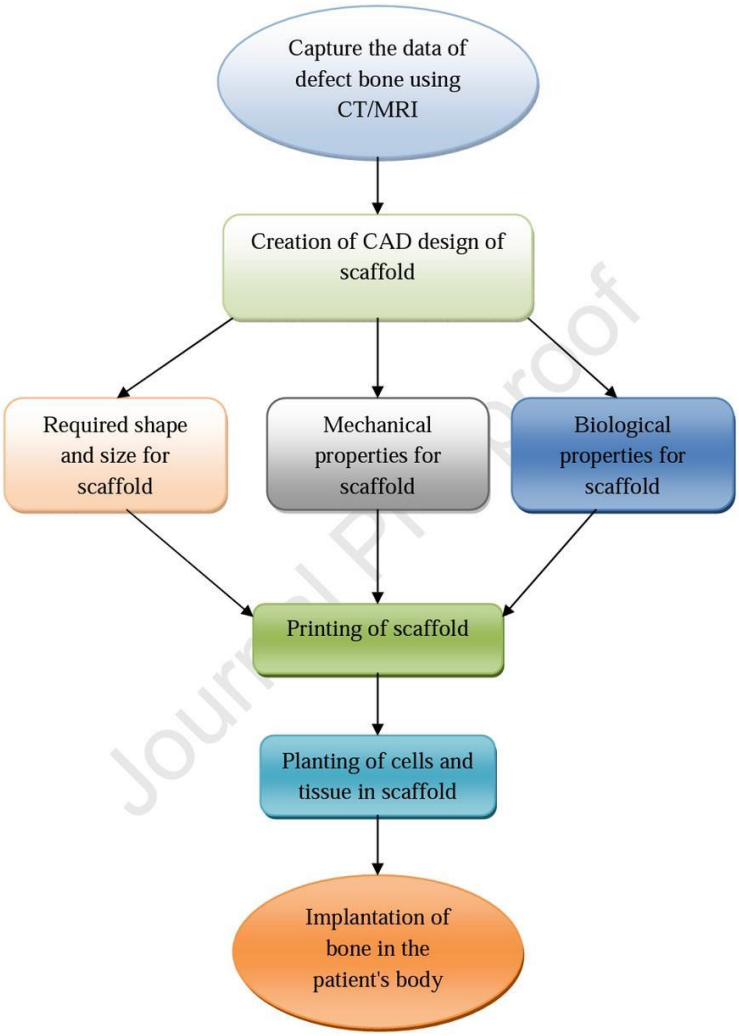


Figure 2.3.1: Stages Used in 3DP for Bone Tissue Engineering (Haleem et al., 2020).

The initial stage in bone tissue engineering is to use CT/MRI to collect the data of a patient's damaged bone. Various software, such as 3D doctors, 3D slicer, mimics, magics and others are used to translate this data into 3D CAD format and develop a perfect scaffold design. To enhance the accuracy of the patient result post-surgery, exact size, shape, biological and mechanical characteristics are required during scaffold production. The digital CAD data is then exported in STL format for 3D printing and printed with the needed material. After the printing is done, scaffold are inserted with tissues and cells. Lastly, it is implanted in the patient's body (Haleem et al., 2020).

2.4 Different 3DP Processes Involved Along with Their Materials Used:

2.4.1 SLA (Stereolithography)/ VAT Polymerization:

Stereolithography or SLA was the first commercial procedure for the 3D printing process (Bandyopadhyay et al., 2020). SLA is the technique for the photopolymerization molding. Here the beam detects the path's motion of the template in order to solidify and form a photosensitive material by applying the mechanism of photopolymerization of photosensitive resin with a definite intensity and wavelength. Then, a layer-by-layer scanning is done by finishing the movement of the lift table. (Ji et al., 2018) A photopolymer resin is hardened and formed on a build platform with the help of Ultra-Violet (UV) light in SLA. Depending on the part design, the UV beam moves one layer at a time. Digital Light Processing (DLP) or Continuous Digital Light Processing (cDLP) was used to improve the hardness of each layer soon afterwards. The part which is to be printed is submerged in a liquid containing monomers, so it has a smoother surface compared to the other 3DP methods involving polymers. In the photopolymer resin, by establishing a homogeneous suspension of powder containing ceramic which serves the purpose of a binder and after that removing the binder and sintering process, this process is utilized to print a part of the ceramic. (Bandyopadhyay et al., 2020)

The high resolution of SLA is its main advantage. To design more precise parts, Micro-stereolithography (MSLA) is used now-a-days as it has the ability to achieve a 20 μm precision. Lee et al. completed Polypropylene fumarate (PPF) with polylactic acid microspheres (PLGA) loaded with bone morphogenetic protein (BMP2) and MSLA technology to build interconnected highly porous scaffolds. During in vitro culture, growth factors are gradually added, and osteoblasts develop well, offering a way for fabricating bone scaffolds. The lack of biocompatible materials is SLA's primary drawback, but researchers are exploring with innovative composites (Ji et al., 2018).

2.4.2 FDM (Fused deposition modeling)/ Materials Extrusion:

Because of its simplicity and convenience of use, materials extrusion, also known as "fused deposition modeling (FDM)," is one of the most prominent AM (Additive Manufacturing) or 3DP processes (Bandyopadhyay et al., 2020). FDM uses thermoplastic filaments such as wax and molten metal as raw materials, and uses pinch rollers and screw feed mechanisms to feed the raw materials into the die. After that, computer control is used to heat it. In accordance with the model's layered processing path, the nozzles are repeatedly pressed to the printing platform and then arranged with alternate layers to finish the model's building. The structure of the

model depends on the nozzle diameter, deposition rate, layer thickness, and deposition angle. etc. (Ji et al., 2018)

This method is versatile and easy to manage, and it has a wide range of applications. An FDM machine has a lower resolution (layer's thickness of 50–250 μm) compared to other techniques, but it is simple to use, customize and maintain with low-cost equipment and filament material. In latest years, researchers are experimenting with nanomaterials and composites to produce amazing quality of the print, besides using ceramics, collagen, PLGA, and printing consumables. Li et al. found that PCL-TCP (PolyCaproLactoneTriCalciumPhosphate) scaffolds can better stimulate bone formation and distribution by comparing the effects of titanium alloys as spinal cages with FDM molded PCL-TCP scaffolds .FDM technology was applied by Zein et al. to create degradable porous scaffolds utilizing filamentous polycaprolactone (PCL). FDM molding has several flaws, such as it requires heat during the molding process, which results in the breakdown of polymers, biological macromolecules are deactivated and unfavorable to the biological component. Longer hardening period, difficulty in forming the porous structure of prosthetic implant and the flat model's surface make cell attachment challenging. (Ji et al., 2018)

2.4.3 Material jetting (MJ):

In the 1990s, MJ was first used to create a full-color visual prototype. MJ creates 3D objects by depositing build materials droplets precisely. It can print multi-material objects with many nozzles and has the most accurate printing technologies (up to 16 mm). It typically produces high quality prints with a fast and smooth surface finish. The main factor is the liquid resin's rheology (viscosity and shear thinning). A liquid resin (usually a photopolymer) is poured into the area of interest and cured using UV light placed near the printhead. The build platform moves up and down when each layer is complete and repeats this process until the entire part is printed (Park et al., 2022).

In order to achieve a printed structure, the polymer must fluidize and flow through a narrow nozzle and exhibit shear thinning properties in order to regain its modulus immediately after leaving the nozzle. Furthermore, the viscous liquid polymer must allow it to drop on demand. As a result, the photopolymers are pre-heated to temperatures between 30 and 60 degrees Celsius. To efficiently change a printing substance from a continuous liquid to minute droplets, other parameters such as liquid density and surface tension must be really well. (Park et al., 2022)

2.4.4 Binder Jetting:

Binder jetting is a method invented in 1993 by Massachusetts Institute of Technology researchers. Here, depending on the CAD file, the rollers apply a homogeneous thin layer of ceramic, metal, or polymer powder from the powder bed to the surface plate accompanied by the deposition of liquid binder from the head of the inkjet. Then this binder works as a glue and the printed item has a temporary strength that is provided by the binder. After that, further heating of the powder bed is carried out at the binder's moderate temperature to get considerable tensile strength for the metallic and ceramic parts allowing powder removal without harming the part which is again carried by binder removal and sintering. It has high tolerance and considered more appropriate for the ceramics because it is the ideal method for printing complex geometry. Due to nonuniform contraction, the sintering step can produce cracking. The most important variables in ensuring good component quality are optimizing the size of the binder droplet and composition. This technique is not applicable for larger parts. (Bandyopadhyay et al., 2020)

2.4.5 Powder Bed Fusion:

It was first developed at the University of Texas located in Austin using the same like process of binder jetting, in which a focused laser beam is used instead of an inkjet head or an electron beam for sintering or melting the powder. Sintering is utilized largely for the polymers, while melting or electronic beam techniques are employed for metal products. It can be used to print complex designs using powder which is unused as a support material, or to print support structures to withstand the thermal stresses caused by the processes of high-temperature.. To achieve the appropriate physical and mechanical qualities, factors such as laser power, scanning speed, and thickness of the layer thickness must be controlled. (Bandyopadhyay et al., 2020)

2.4.6 Sheet Lamination:

Sheet laminating is a common laminating molding technique used for polymer composites by binding a sheet of material to a 3D element. Instead of welding, a thin layer of material and adhesive is used in the manufacturing process of laminated products that can be used to print polymer composites. To melt and glue the sheets together, heat and pressure are combined in this printing technique. When the layer-by-layer connection is complete, the current material is cropped to create a layer designed for the 3D model. Material management is simple here, making it ideal for high-volume manufacturing. Unfortunately, due to the constant sheet

thickness, the resolution is inadequate, and the mechanical qualities are regulated by the adhesive strength. Moreover, post-processing is needed for the heat treatment and to polish the surface for improving interlayer bonding. Impossible Objects, one of the successful startups, has succeeded in 3D printing objects using composite materials (such as carbon fiber sheet and glass fiber sheet) in Second Life. In this process, the sheets are glued, each sheet is coated with polymer powder, and finally the sheets are glued together (Park et al., 2022).

2.4.7 DED (Directed energy deposition):

It uses a targeted high-energy laser beam to melt or sinter metal or ceramic powder deposited from the coaxial nozzles pointed at the surface plate. Because no powder bed is utilized, DED can readily print multi-material structures with a compositional gradient to customize component qualities in numerous directions. Scan speed, laser power etc. are some of the parameters that need to be tuned for diverse materials depending on their thermal conductivity and laser absorptivity much as they are for powder bed fusion. Part resolution and tolerances are inadequate in DED (layer thicknesses of 250–500 μm). Structures with variable compositions, surface modification on existing components and high-value repair are all common uses for DED. (Bandyopadhyay et al., 2020)

2.4.8 Bioprinting:

It is the 3D printing of structures which uses both acellular material and living cell so that tissues are replaced and restore their function. It works on the same principle like 3DP technique; bioink is deposited, tissue or cell is packed in biopolymeric material, layer by layer, to create three-dimensional body structures from a three-dimensional computer model. To assure the survival of cells while providing acceptable printing resolution, bioprinting takes substantial process improvement in addition to maintaining a sterile environment. In order to provide cells with sufficient nutrients for cell growth and reproduction, the combination of cells with the appropriate scaffolding medium such as collagen, nanocellulose, etc. must be used to produce function-specific bioinks. Then, 3D structure is made to anneal the polymeric substance by using UV light in order to bond one polymer chain to another. Some heat-sensitive polymers for bioprinting operations have been applied throughout the years, with PNIPAm PNIPAm Poly(N-isopropylacrylamide) being the most widely utilized polymer. Its biomedical grafts are mostly made by the irradiation of electron beam and utilized in engineering cell-layers. Nanocellulose can be used in two ways: (1)) As a bio ink, nanocellulose functions as a scaffolding medium for living cells introduced before printing, and (2) As a matrix used in

the first stage of cell-attachment, in which growth factors and other acellular components are introduced into the nanocellulose matrix (Bandyopadhyay et al., 2020).

2.4.9 Schematic Representation of 3DP Processes:

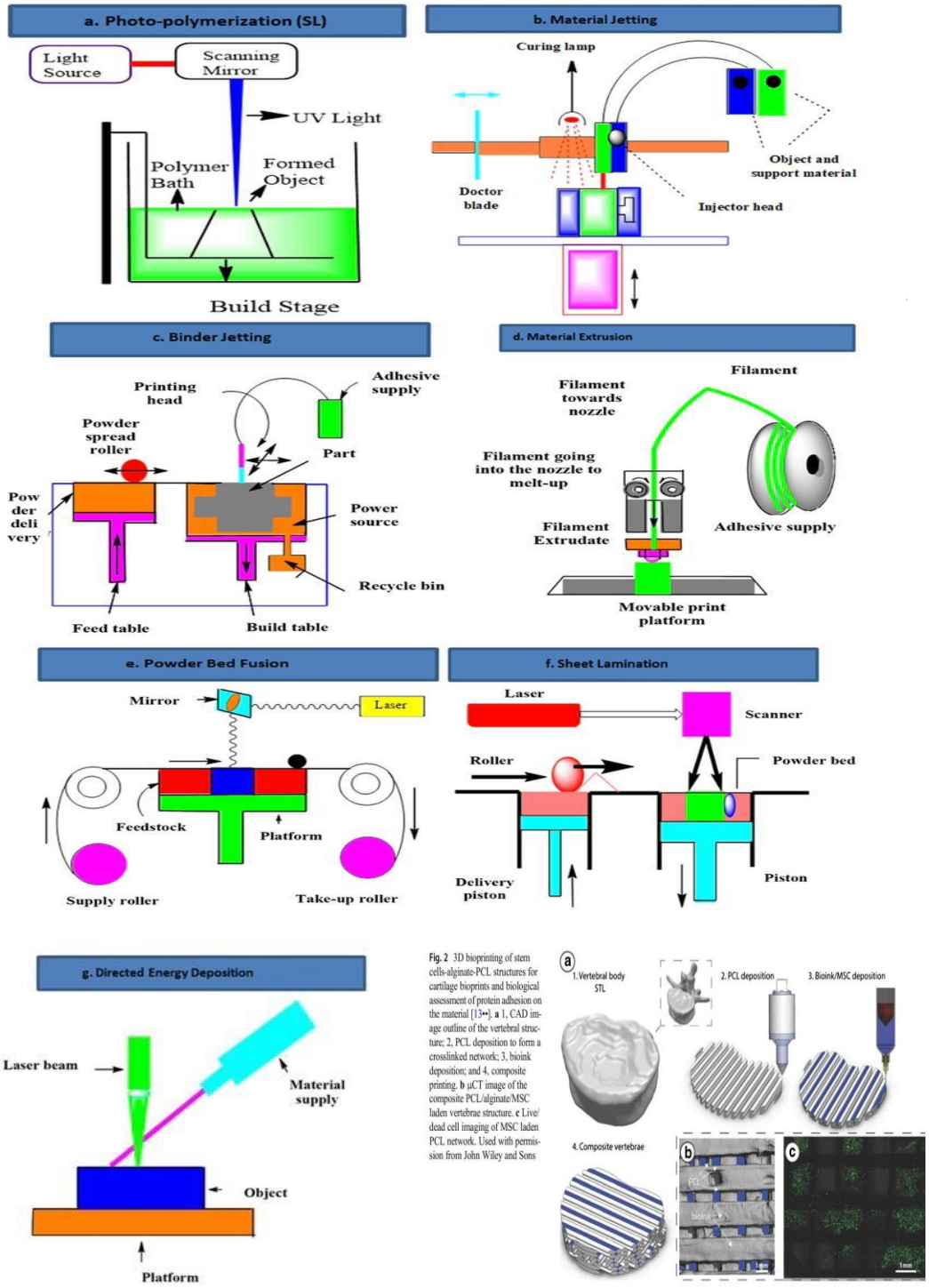


Figure 2.4.8.1: Schematic Representation of 3DP Processes (Bandyopadhyay et al., 2020; Poomathi et al., 2020)

2.4.10 Comparison Between the 3DP Processes:

Processes	Principle	Printing materials	Advantages	Disadvantages
1. SLA (Stereolithography)/VAT polymerization	UV-induced curing where liquid polymer is solidified selectively at curing temperature and uncovered monomers stays at fluid (Bogdan & Michorczyk, 2020; Park et al., 2022)	Photopolymers, ceramics (Bogdan & Michorczyk, 2020)	-High resolution (6–140 μm)(Park et al., 2022) - Suitable to the most photopolymers (Poomathi et al., 2020)	-Only applicable to heat sensitive polymers (Jariwala et al., 2015) -Unreactive monomer and photoinitiation source could be toxic(Bittner et al., 2018) -Limited mechanical strength (Bittner et al., 2018) -Limited resolution by light source and expensive (Bittner et al., 2018)
2. FDM (Fused deposition modeling)/ Materials extrusion	Filament passes through the heated print nozzle, melts and deposited layer by layer on the surface (Bittner et al., 2018)	Polymers, metals, ceramics, composites (Bandyopadhyay et al., 2020)	-Simple, cheap and fast (Jariwala et al., 2015) -Higher mechanical strength of the scaffold (Jariwala et al., 2015)	-Restricted materials which needs filament of thermoplastic polymers (Jariwala et al., 2015) -Lower resolution (Jariwala et al., 2015)

			-No platform needed (Jariwala et al., 2015)	-Cannot insert biomolecules or cells into scaffolds (Jariwala et al., 2015) - Ineffective to resemble geometries (Jariwala et al., 2015)
3. Material jetting	Drop on demand material deposited with solidification by UV light (Park et al., 2022)	Polymers, multi-materials (Bandyopadhyay et al., 2020)	-High resolution (up to 16 μm) (Park et al., 2022) -Multi-material Capabilities (Park et al., 2022) - Homogeneous properties (Park et al., 2022)	-Expensive (Park et al., 2022) -Unsuitable for structural properties (Park et al., 2022)
4. Binder jetting	A print head sprays photopolymer and at the same time, a support structure is printed and 3D shape is built from successive layers (Poomathi et al., 2020)	Metals, ceramics, polymers (Bandyopadhyay et al., 2020)	-High accuracy (Poomathi et al., 2020) - Flexibility of materials and design (Park et al., 2022)	-Rough surface (Park et al., 2022) -Can produce cracking (Bandyopadhyay et al., 2020)

5. Powder bed fusion	A thin layer of powder is applied to the fabrication stage, and the print head next sprays liquid binding agents onto the powder particles. After that, the platform is lowered and the process is repeated.(Poomathi et al., 2020)	Metals, polymers, ceramics (Bandyopadhyay et al., 2020)	-Higher mass production (Park et al., 2022) -Design flexibility (Park et al., 2022) -No support structure needed (Poomathi et al., 2020)	-Expensive (Park et al., 2022) - Rough surface (Park et al., 2022) -Can produce cracking (Bittner et al., 2018) -Excess powder needs to be removed during post processing (Poomathi et al., 2020)
6. Sheet laminati on	The building materials form a layer-by-layer sheets to form the object (Park et al., 2022; Poomathi et al., 2020) A high focused energy of beam is utilized to melt the materials by sintering process (Bandyopadhyay et al., 2020)	Metals, papers, composites (Bandyopadhyay et al., 2020)	-Easy handling (Park et al., 2022) - Fast process (Park et al., 2022)	-Low resolution of parts (Park et al., 2022) -Uneven finishes (Park et al., 2022) -Lower mechanical properties (Park et al., 2022)
7. Directed energy depositi	A high focused energy of beam is utilized to melt the materials by	Metals, ceramics, composites (Bandyopad	-Easy surface modification (Bandyopadhyay et al., 2020)	-Lower resolution of parts (Bandyopadhyay et al., 2020)

on (DED)	sintering process (Bandyopadhyay et al., 2020)	hyay et al., 2020)	-Tuneable composition properties (Bandyopadhyay et al., 2020)	-Lower mechanical strength (Bandyopadhyay et al., 2020)
8. Bioprinting	Deposition of bioink occurs, a cell or tissue enclosed in biopolymeric material to create a 3D layer-by-layer structures which is then annealed by UV light (Bandyopadhyay et al., 2020)	Polymers (Bandyopadhyay et al., 2020)	-High printing resolution -Sterile environment (Bandyopadhyay et al., 2020)	-Biocompatibility -Lack of suitable printing materials (Mao et al., 2020)

Table 2.4.9.1: Comparison Between the 3DP Processes (Bandyopadhyay et al., 2020; Bittner et al., 2018; Bogdan & Michorczyk, 2020; Jariwala et al., 2015; Mao et al., 2020; Park et al., 2022; Poomathi et al., 2020)

2.5 Materials: Role of biomaterials, composites & supramolecular materials in bone scaffolds

2.5.1 Ceramics:

A number of bioactive ceramics, such as- hydroxyapatite (HAP), beta tricalcium phosphate (β-TCP), bioglass (BG) and alumina ceramic have been shown to exhibit bone regeneration properties. These have a high affinity for the tissues and cells in the body and can interact with them. Some important ceramics that are used as the biomaterials for bone regeneration process are discussed below (Lacerda, 2018).

a. Calcium Phosphate Ceramics (CPCs):

Biomaterials based on calcium orthophosphate are applied in a variety of purposes throughout the body and in all parts of the skeleton. The chemical resemblance of calcium phosphate ceramics (CPCs) to the human bone's mineral components is the major factor for their application as bone substitute materials. Few others inorganic nanoparticles (NPs) and ceramics were shown to be less biocompatible than CPCs. They were successful to exhibit bone growth in vivo and for the transportation of bone marrow stromal cells (BMSCs) to the defected site in order to induce bone formation. They also have good biodegradability and the capability to make RNA and proteins from a gene for bone, as well as the ability to enhance the production of the phases of mineral-like of the bones and deliver medicines. CaP bioceramics degradation products (such as calcium and phosphate ions) do not cause undesirable calcium or phosphate levels following ingestion and are naturally destroyed.(Lacerda, 2018)

b. β-Tricalcium Phosphate (β-TCP): By melting calcium-deficient apatite with a Ca/P molar ratio of 1.5: 1, or by performing a solid-state reaction in dry air at a high temperature usually greater than 1000°C, **β**-TCP can be made. **β**-TCP has a high biocompatibility, biodegradability and biological properties and it can promote bone regeneration and proliferation of stem cell. Because **β**-TCP is similar to the mineral phase of natural bone, it was among one of the first scaffolds utilized as a basis for bone regeneration in vivo. Once the **β**-TCP scaffold was put next to bone, mixtures of proteins of the bone matrix developed immediately on the scaffold's surface of CaP and insertion of soft tissue was not needed. But, the limited mechanical properties and extreme fragility of **β**-TCP scaffolds make them unsuitable for clinical applications such as repairing heavy bones. (Lacerda, 2018)

c. Hydroxyapatite (HAP): HAP can be made from natural or synthetic sources and is used to stimulate bone formation with good structural properties. HAP makes up about 65 percent of the weight of bone tissue, making it an essential component of natural bone. HAP has a high level of biocompatibility with skin, muscle tissue, and bone. Also, scaffolds made from it have a high mechanical strength. HAP scaffolds with the cells that make new bones can provide better bone growth and repair than HAP alone. Also, a foam of HAP which can be in injectable form can provide great benefits to less troublesome surgery because of good molding properties of the formulation.(Lacerda, 2018)

d. Bio-Glass (BG): BGs are silica-based glasses which have an irregular shape with randomly distributed atoms and when they are hydrolyzed, they release ions into physiological solutions. They have been shown to enhance growth of the osteoblast and secretion of collagen through their bioactive properties. Besides, they can boost gene expression and synthesis of osteocalcin for the bone tissue engineering. The formation of a hydroxy carbonate apatite (HCA) layer on the surface of BG scaffolds was found to greatly boost osteoblast activity. Protein and growth factors were also recruited by the HCA layer, which aided in the production of new bone in vivo. Unfortunately, in aqueous conditions, the irregular structure and breakdown characteristic of BGs might reduce their mechanical strength and fracture resistance over time. they are inadequate for the bone regeneration of the bones that carry most of the load as for the mechanical strength of many BG scaffolds is in the range of 40–60 MPa,.(Lacerda, 2018)

2.5.2 Metals:

Copper (Cu), Magnesium (Mg) etc. metals are crucial for bone restructuring, metabolic activity, and regeneration, are found in very small amounts in the human body. They have excellent mechanical properties which allows them to be used in the musculo-skeletal implants. (Lacerda, 2018)

a. Magnesium (Mg):

Mg, for example, has high resistance to fracture and tensile strength which is similar to our bones. Under physiological conditions, magnesium and magnesium alloys can totally corrode, alleviating the necessity for the second surgical process so that after bone healing the implant is removed. In humans, Mg implants were used to treat fractures in the musculoskeletal system. The full breakdown of Mg implants was observed in vivo trials from a timeframe of three weeks to one year, depending on the shape, location and size of the implants in the body.(Lacerda, 2018)

b. Copper (Cu): Copper ions have been integrated into functional scaffolds in recent years, and the Cu scaffolds releases Cu ions in a controlled manner, enhancing new blood vessel formation and antibacterial properties. It caused bactericidal effects by causing membrane damage without causing mutations or DNA damage. Cu2+ ions aid wound healing in vivo by increasing the production of and a number of bone-specific proteins and vascular endothelial growth factor (VEGF).(Lacerda, 2018)

2.5.3 Natural Polymers:

Natural polymers have biologically active and biodegradable properties. The strength of biodegradable polymers to promote growth of the tissues and restructure before being resorbed by the body is the main advantage of employing them in bone tissue engineering. Natural polymers include collagen, fibrin, chitosan, hyaluronic acid (HA), and alginate.(Lacerda, 2018)

a. Collagen:

Collagen is the major component of the extracellular matrix and highly biocompatible with cells in our body. It makes up 89 percent of the organic part of the bone and 32 percent of its volume. It has caught the interest of numerous researchers due to its abundance and strong cell signaling properties. Collagen has been the most widely utilized cell scaffolds currently, and has shown to be safe for a considerable time in clinical trials. Sponges of collagen with associated pores can support cells penetrate and receive nutrients and oxygen. Also, it has other benefits, such as blood clotting activity and the ability to easily make scaffolds of various geometries. The disadvantage of using collagen as scaffolds is that it degrades rapidly and has low mechanical strength.(Lacerda, 2018)

b. Silk Fibroin (SF):

Silk is a protein polymer fiber produced from the full transformation of arthropods such as silkworms, scorpions, spiders, mites etc. It is suitable as a bone tissue engineering scaffold for the treatment of bone loss. Silk fibers have been used to support a variety of cell types, including stem cells, osteoblasts etc. Silk's good processability allows it to be fashioned into a variety of shapes with tune-able physical qualities. Because of its excellent biocompatibility, great mechanical properties, controllable degradation rate etc. SF, which is mostly derived from silkworms, is an excellent alternative for bone tissue engineering. The breakdown substances of SF, peptides or amino acids, were reported to be absorbed in vivo or in vitro with minimal damage to neuronal cells. SF scaffolds are shown to be more effective at enhancing cell metabolism and bone regeneration than PLGA scaffolds. Again, hydrogels made from SF were

also used as the carriers to administer growth factors like VEGF and Bone Morphogenic Protein-2 (BMP-2) in abnormal or unreachable cavities in a less invasive way.(Lacerda, 2018)

c. Chitosan:

The deacetylated derivative of chitin which makes a linear polysaccharide is known as chitosan. They are mostly found in crab shells. The primary component of extracellular matrix in bone and cartilage is glycosaminoglycan which has structural similarity to chitosan and helps osteoblast cells attach and grow. It has a number of unique properties, as- natural antibacterial properties, a low level of foreign body response, and a great hydrophilicity which makes it suitable as a material of bone scaffold.(Lacerda, 2018)

d. Hyaluronic Acid (HA):

HA plays an important role in wound healing since it is a significant component of the extracellular matrix of bone and found in the connective tissue. HA is FDA-approved for treating osteoarthritis and wound healing. Because of their similarity to tissues in terms of water structure, content and ability to contain bioactive chemicals, its hydrogels are a promising scaffold (e.g. cells and growth factors). Besides, HA hydrogel scaffolds provide a number of benefits, including strong cell adhesion, anti-inflammatory properties and intracellular signaling. The metabolic activity of tendon, cartilage, and the spine may be stimulated by sulfated HA. Mesenchymal stromal cells are brought by it to the damage site and promotes target tissue regeneration.(Lacerda, 2018)

e. Extracellular Matrix (ECM):

The ECM is mainly made up of collagens and proteoglycans which plays a key role in migration of cell, growth and transformation with the help of interactions between cells and the ECM. It has various kinds of both soluble and insoluble bioactive chemicals that allows ECM to give biochemical and structural support to surrounding cells without causing undesirable immune response of the host. Decellularization process which involves removing cellular components while preserving ECM components, is a feasible method for obtaining ECM scaffolds naturally. (Lacerda, 2018)

2.5.4 Synthetic Polymers :

In comparison to natural polymers, synthetic polymers have more controlled biodegradation rates, greater predictability of characteristics and larger batch-to-batch consistency. They may be manufactured and utilized to quickly fabricate scaffolds in a variety of shapes and sizes. Some of these are discussed below (Lacerda, 2018).

a. Polycaprolactone (PCL):

PCL is made by polymerizing 2-methylene-1,3-dioxepane with a free radical or ring-opening polymerizing -caprolactone with a free radical. It has a capacity to preserve its shape and mechanical properties after implantation, so it is much used in the fabrication of 3D scaffolds. Besides, it has a low melting temperature and melt viscosity which helps to make PCL scaffolds easily using 3D print. Osteoblast cells thrived on PCL scaffolds according to Gómez-Lizárraga. PCL breakdown products are rapidly eliminated by metabolic processes and do not cause any side effects. In vivo, it takes roughly two years or longer to completely degrade. It is hydrophobic, has weak cell adhesion ability and a slow degradation rate so to add a ceramic material or other materials with PCL is more preferable for tissue engineering applications.(Lacerda, 2018)

b. Poly (Glycolic Acid) (PGA)/Poly (L-Lactic Acid) (PLA)/Poly (Lactic-Co-Glycolic) Acid (PLGA):

The US FDA has approved PGA for clinical use because it is strongly crystalline polymer and hydrophilic in nature. The collagen sponge with PGA fibers was found to improve cell adherence to the scaffold in a study. Again, PLA is a biodegradable, biocompatible polymer with amazing mechanical properties and thermally stable material but it is decomposed in the absence of catalysts or enzymes when utilized in vivo, and through regular cell metabolism its by-products were eliminated. Its main disadvantage is that it has low cell affinity due to the lack of cell binding peptides and high hydrophobicity. It also has a slower degradation rate and its tiny metabolites create a local acidic environment, which might cause tissue response adversely. On the other hand, because of PLGA's favorable mechanical properties, biocompatibility, and controlled degradation it is greatly used. The crystallinity and content of each component can be adjusted to control PLGA degradation. Mesenchymal cells were found to adhere to and grow on pure PLGA scaffolds throughout time. But, it is not very much useful in clinical uses because it cannot mineralize a scaffold as it contains low ionic functional groups. So it is combined with other bioactive compounds like ceramics or bioglass.(Lacerda, 2018)

2.5.5 Composites:

Metals and polymers are used to make composites, which are made up of two or more different components. Composite scaffolds have recently attracted a lot of interest as a way to speed up the healing process of bone fractures. Controlling the amount of components in a composite

can simply change its mechanical properties. When coupled with ECM components like growth factors and collagen, biomaterials like synthetic polymers and CaP have dramatically improved regeneration potential.(Lacerda, 2018)

a. PCL-Based Composites:

PCL alone cannot stimulate bone healing process so ceramics or biological components must be included into PCL to induce bone regeneration in their scaffolds. PCL is commonly combined with natural bioactive ingredients (e.g. HA, ECM, collagen etc.) to increase cell adhesion, bioactivity, and bone regeneration of PCL-based scaffolds. The use of nHAP improved the mechanical characteristics of the PCL/nHAP composite film without affecting the composite's chemistry.(Lacerda, 2018)

b. Collagen-Apatite:

To resemble natural human bone in terms of its constituents, these scaffolds have been frequently used for bone tissue engineering. In vitro study shows that collagen-apatite composites have increased mechanical strength that support osteoblast adhesion and multiplication. In seven hospitals, seven clinical trial was performed by using collagen-apatite (Col-Ap) and β-TCP porous ceramics concerning the bone fractures at donor site, filling up scraped portion of bone tumor and after the reconstruction of the bone fracture. It showed that, Col-Ap scaffold had 71.4% efficacy whereas β-TCP scaffold had 0% efficacy. Despite of these advancements, there remains a great challenge to control the degradation rate accurately in Col-Ap scaffolds.(Lacerda, 2018)

2.5.6 Supramolecular Materials:

Noncovalent interactions such as hydrogen bond interactions, ionic interactions etc. are used to make supramolecular materials. High customization, sensitivity, and to construct design resembling to human body or functions are all important features of supramolecular biomaterials. Structural features can be replicated in the structure of supramolecular biomaterials. Supramolecular materials made from protein building units could resemble matrix components of the bone. Again, self-assembled supramolecular scaffolds proved to stimulate osteoblast growth and proliferation. The drawbacks of using these molecules include-complex steps in synthesis, expensive materials and low mechanical properties which limits their uses in bone tissue engineering.(Lacerda, 2018)

2.4.7 Summary of Biomaterials:

Biomaterials	Characteristics	Regeneration capability	Phase	Reference
Ceramics	Bioactivity, biodegradability, biocompatibility; low mechanical strength, low toughness	High	Clinical trials	[5, 7, 8]
Metals	Excellent mechanical properties, good machinability; poor integration	Medium	Clinical trials	[26, 33]
Polymers (natural)	Bioactivity biodegradability biocompatibility; low mechanical strength	Very high	Commercial	[33, 35]
Polymers (synthetic)	Tailorable biodegradation rates, adjustable mechanical properties, mass-produced; poor wettability, low cell adhesion	Low	Clinical trials	[25, 56]
Composites	Adjustable mechanical properties, good cell adhesion; uncontrolled degradation	High	Commercial	[63, 65]
Supramolecular materials	Modularity, mechanical tenability, responsiveness biomimicry; rigorous synthesis process, low scale and synthesis efficiency, high cost	High	Commercial	[72, 74]

Figure 2.4.7: Summary of Biomaterials (Lacerda, 2018)

Chapter 3

Clinical Studies and Successes

3.1 In Vitro and In Vivo Testing of 3DP Bone Scaffolds:

Klammert et al. repaired cranial defects by developing blush stone (di CaP dihydrate) and monetite (di CaP anhydrous) 3D scaffolds and ensuring that 3DP implants meet geometric requirements and are properly fitted. I proved that I could do it. These researchers used CAD files obtained by imaging the skulls of human corpses with accurate skull anomalies to create an anatomically shaped framework (Jariwala et al., 2015).

Tada et al. used the patient's three-dimensional CT data to create a realistic CAD model of the defect, which was then used to shape the HA-TCP artificial bone implant to repair the facial defect. They came to the conclusion that physically shaped models and implants benefited in the development of implant design and improved shaping in patients with complex abnormalities (Jariwala et al., 2015).

Temple et al. showed the effectiveness of 3DP polycaprolactone (PCL) scaffolds in the shape of human mandibular and maxillary bones with different pore sizes (Jariwala et al., 2015).

Grayson et al. An effectively constructed scaffold that has been decellularized, shaped like a human temporo-mandibular joint, fed with human mesenchymal stem cells, and transferred to a bioreactor system for transplant development prior to transplantation (Jariwala et al., 2015).

Seitz et al. used a water-soluble polymer binder (Schelofix) and sintering, evaluated the feasibility of manufacturing a 3DP-HA scaffold with complex internal design and high resolution. They showed that a cylindrical scaffold could be manufactured with a small tube size of 450 μm, allowing the implant to completely fuse with the bone. MT3T3-E1 osteoblasts also adhered, propagated and maintained their shape on the 3DP scaffold (Jariwala et al., 2015).

Castilhoetal. 23 produced a biphasic 3D-CaP (BCP) scaffold using a hydraulic curing reaction of HA-TCP powder containing phosphoric acid and post-treatment with a polylactic acid-co-

glycolic acid solution. They found that BCP composites promoted osteoblastic MG63 cell growth more than pure HA or TCP scaffolds (Jariwala et al., 2015).

Becker et al. 24 used dextrin as a binder solution to prepare 3DP-HA and TCP blocks, and also used bovine HA blocks with a central channel for intramuscular bone development in rat models. They discovered that HA and TCP blocks stimulated the development of new bone exhibiting in vivo biocompatibility (Jariwala et al., 2015).

Konopnicki et al. developed 3DP 50 percent PCL and 50 percent TCP composite scaffolds which was loaded with pig bone marrow progenitor cells and inserted them into mandibular defects in minipigs for eight weeks. They discovered that TCP / PCL scaffolds injected with that bone marrow cell allowed for good bone penetration in the scaffold. New blood vessel formation was also seen in the core of the construct and surrounding freshly produced bone (Jariwala et al., 2015).

Koski et al. Melt deposition modeling was used to produce the scaffold from a calcium phosphate and starch-based composite. The addition of calcium phosphate, PCL, and starch improved mechanical properties while improving biological performance. Later, these scaffolds were sought as a vehicle for the removal of chemopreventive agents (Bandyopadhyay et al., 2020).

Chapter 4

Discussion

Bone tissue engineering is a cutting-edge technique for effectively repairing bone deformities and tissue for transplantation. Biomaterials play a significant part in this process by supporting regenerating cells and tissue regeneration. Additive manufacturing is a revolutionary technology that employs several types of scanning, printing, and software assistance. It is used to create complicated shapes tailored scaffolds from photos in a shorter amount of time and at a lower cost. It is successfully employed in bone tissue engineering for repairing and regenerating bone. It has been created in a variety of techniques in orthopaedics for the cost-effective manufacture of customized human hard tissue. The development of substitute implantable bone for crucial applications is the focus of 3D printing. These are the most prevalent problems in orthopaedics and craniofacial surgery, such as bone loss due to trauma, infection and tumor removal. In the future, 3D printing will bring breakthrough innovations in bone tissue formation by inserting cells and bioactive chemicals directly into scaffolds of 3D biomaterials (Haleem et al., 2020).

In precise, this article presents a summary of the main 3D printing techniques ranging from traditional technique like SLA and powder fusion to modern technique like bioprinting, processes, materials to make a functional scaffold for bone tissue engineering and also how to induce bone regeneration using conductive materials. It is a very challenging task for developing an ideal scaffold with only one material. So, combination of materials are used to make it according to the specific patient with the customizable characteristics in order to lessen the unwanted side effects by resembling to the tissues like human. The properties of each materials are discussed in this article for constructing a new scaffold with enhanced structural support and good mechanical properties.

Some recent progresses that has been made in order to induce bone regeneration currently is discussed below-

4.1. By Using Conductive Materials:

They are termed as smart biomaterials in tissue engineering for constructing a 3D scaffold. These materials are frequently used as additions in 3D tissue scaffolding. Based on their composition, these conductive scaffolds can be divided into two types: polymer-based conductive

scaffolds and nanomaterial-based conductive scaffolds. Numerous studies have shown that conductive bone scaffolds can directly provide electrical and electromechanical signals to targeted cells, boosting cell proliferation and bone tissue regeneration in vitro as well as bone development in vivo. As a result, conductive scaffolds can be considered as a technique to promote tissue restoration while also reducing healing time caused by severe injuries. In addition, conductive scaffolds can alleviate one of the major challenges associated with current tissue transplantation by providing patients with more accessible options (Dixon & Gomillion, 2022).

More than 40 years ago, it was discovered that the conductivity of polyacetylene (PAc) increased by a factor of 1 million after oxidizing the polymer with iodine vapor. Charge carriers are generated in the polymer by the redox process, giving the polymer high conductivity. Besides, CPs (Conductive Polymers) have a greater chemical diversity than their inorganic analogues, allowing them to be more corrosion resistant and have greater conductivity and can be precisely controlled (Dixon & Gomillion, 2022).

Chemical or electrochemical synthesis are the two most common ways for producing conductive polymers. The key difference between these two synthesis procedures is the CP's final shape. A thick powder film or CP can be produced by chemical synthesis, but only a thin film can be produced by electrochemical synthesis. This makes chemical synthesis more cost-effective. Chemical synthesis creates a polymer with lesser conductivity than electrosynthesized polymers, according to research. Polypyrrole (PPy), polyaniline (PANI), and poly(3,4-ethylenedioxythiophene) (PEDOT) are three of the most investigated CP systems now available. PPy is a conjugate polymer which can be produced by reacting with other bioactive molecules and enhance the suitability to be used in scaffolds as it contains high electric conductibility. PANI is the second widely used material because it is cheap, can be easily produced and it can change from its electric to resistive condition. On the other hand, PEDOT is better than PPy because it is stable both chemically and thermally. These materials have been proven to promote adhesion and growth of cells in a variety of cell types for a number of tissue engineering applications, which is an important feature of a material's biocompatibility (Dixon & Gomillion, 2022).

Nanotubes, nanosheets, nanoparticles, and nanowires are among the many types of conductive nanomaterials. Gold, silver, copper, and aluminum are some of the most common conductive nanoparticles and nanowires. Carbon nanotubes (CNTs) and graphene nanosheets (graphene

nanosheets) have also been studied for bone regeneration. Conductive nanoparticles can be used as additives, thin films, and functional coatings on scaffolds to improve properties, but composite scaffolds did not. Incorporating conductive carbon-based nanomaterials such as graphene oxide (GO) into the scaffolds allows researchers to enhance the hydrophilic surface properties of commonly used synthetic polymers, thereby improving cell adhesion capabilities. The strength and flexibility of the scaffold and new blood vessel formation in the tissue of bone has proved to be increased by inserting CNTs to it (Dixon & Gomillion, 2022).

4.2 By Electrical & Mechanical Stimulation for Inducing Bone Regeneration:

Electrical stimulation (ES) has also been shown to influence cell orientation as well as cell adhesion to surfaces. ES to cells can be improved by conductive biomaterials, significantly increasing cell-scaffold interactions. Electrical and mechanical stimuli have been used in in vitro experimental environments and in vivo therapeutic recovery with positive consequences for bone regeneration. Electrical conductivity inside the 3D matrix of scaffolds has been proven to produce superior tissue response when compared to induced stimulation via various medium. Conductive fibers, particularly electrospun fibers, are now being investigated the most. This is owing in part to the simplicity with which scaffolds can be built and the resemblance to the ECM of bone in human body. Studies have shown that nanofiber scaffolds containing conductive nanoparticles of PANI and graphene may increase cell viability at high concentrations of PANI and graphene (up to 2% in PCL).Dipoles occur as a result of electrochemical processes in natural bone tissue in response to normal loading. Electroactive bone tissue adjusts structurally as a result of this. Mature bone cells, known as osteocytes, are important cells that are trapped in the matrix and remain after the development of new bone tissue to pick up these signals.These cells act as receivers by taking up electrochemical signals and transferring them as intracellular Ca2+ signals via their metabolic pathway.(Dixon & Gomillion, 2022)

Capacitive, inductive, and direct current ES are only some of the ways ES can be delivered to cells. Capacitive ES stimulates the target with an electric field, inductive ES stimulates the target with an electromagnetic field generated by the current flowing through the magnetic field, and direct current ES stimulates the target with an electromagnetic field generated by the current flowing through the magnetic field. The electric field and current flow through the target are delivered by ES. Capacitive ES causes Ca2+ influx in bone cells, whereas inductive ES causes Ca2+ release from intracellular reserve, which leads to osteoblast proliferation. The

combination of conductive scaffolds (PPy integrated into PCL) and direct current-ES has been shown to significantly improve mineral deposition from adipose stem cells compared to conductive scaffolds alone(Dixon & Gomillion, 2022).

Chapter 5

Conclusion

Although 3D printing methods have proved to be a great exploration in the biomedical industry especially in bone tissue engineering, the modeling methods and materials still have discrepancies, such as- lower mechanical strength, lower resolution, cracking, slow degradation of structures etc. Currently, the main drawback of the 3D printing method is its void formation between the layers during printing which can affect the mechanical strength of the scaffold. Some of the researches of biomaterials and methods have proved to be somewhat useful whereas others cause cracking. In this case, more varieties of materials are needed considering both the structure and function of the scaffold. Again, the bone has the capability to produce electrical stimulus caused by injury to treat the injury by supplying enough minerals to the site. This mechanism of treating injury of bone by itself has not been explored fully yet, but this field can possibly substitute artificial bone if utilized properly. Besides, it is also a serious difficulty to mimic the natural bone structure to make a porous scaffold. This difficulty needs more diverse branches of knowledge to develop a scaffold considering the right selection of biomaterials and growth factors.

References:

Bandyopadhyay, A., Mitra, I., & Bose, S. (2020). 3D Printing for Bone Regeneration. *Current Osteoporosis Reports*, *18*(5), 505–514. https://doi.org/10.1007/s11914-020-00606-2

Bittner, S. M., Guo, J. L., Melchiorri, A., & Mikos, A. G. (2018). Three-dimensional printing of multilayered tissue engineering scaffolds. *Materials Today*, *21*(8), 861–874. https://doi.org/10.1016/j.mattod.2018.02.006

Bogdan, E., & Michorczyk, P. (2020). 3d printing in heterogeneous catalysis—the state of the art. In *Materials* (Vol. 13, Issue 20, pp. 1–23). MDPI AG. https://doi.org/10.3390/ma13204534

Dixon, D. T., & Gomillion, C. T. (2022). *Conductive Scaffolds for Bone Tissue Engineering : Current State and Future Outlook*.

Haleem, A., Javaid, M., Khan, R. H., & Suman, R. (2020). 3D printing applications in bone tissue engineering. *Journal of Clinical Orthopaedics and Trauma*, *11*, S118–S124. https://doi.org/10.1016/j.jcot.2019.12.002

Jariwala, S. H., Lewis, G. S., Bushman, Z. J., Adair, J. H., & Donahue, H. J. (2015). 3D Printing of Personalized Artificial Bone Scaffolds. *3D Printing and Additive Manufacturing*, *2*(2), 56–64. https://doi.org/10.1089/3dp.2015.0001

Ji, K., Wang, Y., Wei, Q., Zhang, K., Jiang, A., Rao, Y., & Cai, X. (2018). Application of 3D printing technology in bone tissue engineering. *Bio-Design and Manufacturing*, *1*(3), 203–210. https://doi.org/10.1007/s42242-018-0021-2

Kausar, H., & Naga Kishore, R. (2013). Bone tissue engineering. In *International Journal of Pharmacy and Pharmaceutical Sciences* (Vol. 5, Issue 1). INC. https://doi.org/10.1016/b978-0-12-818422-6.00083-6

Lacerda, C. M. R. (2018). Biomaterials for bone tissue engineering. In *Bone Regeneration: Concepts, Clinical Aspects and Future Directions* (pp. 231–254). Nova Science Publishers, Inc. https://doi.org/10.1002/9783527603978.mst0481

Mao, H., Yang, L., Zhu, H., Wu, L., Ji, P., Yang, J., & Gu, Z. (2020). Recent advances and challenges in materials for 3D bioprinting. In *Progress in Natural Science: Materials*

International (Vol. 30, Issue 5, pp. 618–634). Elsevier B.V. https://doi.org/10.1016/j.pnsc.2020.09.015

Park, S., Shou, W., Makatura, L., Matusik, W., & Fu, K. (Kelvin). (2022). 3D printing of polymer composites: Materials, processes, and applications. *Matter*, *5*(1), 43–76. https://doi.org/10.1016/j.matt.2021.10.018

Poomathi, N., Singh, S., Prakash, C., Subramanian, A., Sahay, R., Cinappan, A., & Ramakrishna, S. (2020). 3D printing in tissue engineering: a state of the art review of technologies and biomaterials. *Rapid Prototyping Journal*, *26*(7), 1313–1334. https://doi.org/10.1108/RPJ-08-2018-0217

Wang, C., Huang, W., Zhou, Y., He, L., He, Z., Chen, Z., He, X., Tian, S., Liao, J., Lu, B., Wei, Y., & Wang, M. (2020). 3D printing of bone tissue engineering scaffolds. In *Bioactive Materials* (Vol. 5, Issue 1, pp. 82–91). KeAi Communications Co. https://doi.org/10.1016/j.bioactmat.2020.01.004