

EXPLOTTING THE FEASIBILITY STUDY OF TRADITIONAL VERSUS 3D BIOSCAFFOLD SYNTHESIS PROCESS

Submitted in partial fulfilment of the requirements for the award of Bachelor of
Engineering Degree in **Biomedical Engineering**

by

RAMACHANDRAN K S (38240027)

HARISH T S (38240043)



**DEPARTMENT OF BIOMEDICAL ENGINEERING
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

Accredited with Grade "A" by NAAC

JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119

MAY - 2022



SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited "A" Grade by NAAC | 12B Status by UGC | Approved by AICTE

www.sathyabama.ac.in

DEPARTMENT OF BIOMEDICAL ENGINEERING

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **K.S.RAMACHANDRAN (38240027)** and **T.S.HARISH (38240043)** who carried out the project entitled "**EXPLOTTING THE FEASIBILITY STUDY OF TRADITIONAL VERSUS 3D BIOSCAFFOLD SYNTHESIS PROCESS**" under our supervision from **September 2021 to March 2022.**

Dr. ANIMA NANDA, FNRS, FAB, FIAS

Guide

Dr. T. SUDHAKAR, M.Sc., Ph.D.,

Head of the Department

Submitted for Viva-voce Examination held on 02.05.2022

Internal Examiner

Dr. BETHANNEY JANNAEY J

External Examiner

DECLARATION

We **RAMACHANDRAN K S (38240027)** and **HARISH T S (38240043)** hereby declare that the Project Report entitled “**EXPLOTTING THE FEASIBILITY STUDY OF TRADITIONAL VERSUS 3D BIOSCAFFOLD SYNTHESIS PROCESS**” done by us under the guidance of **Dr. ANIMA NANDA**, FNRS, FAB, FIAS, Professor and Coordinator-IQAC, Department of Biomedical Engineering is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biomedical Engineering.

DATE: 23-04-2022

PLACE:CHENNAI



SIGNATURE OF THE CANDIDATE

ACKNOWLEDGEMENT

We are pleased to acknowledge our sincere thanks to the **Board of Management of SATHYABAMA** for their kind encouragement in doing this project and for completing it successfully. We are grateful to them.

We would like to express our sincere and deep sense of gratitude to our Project Guide **Dr. ANIMA NANDA, FNRS, FAB, FIAS**, Professor and Coordinator-IQAC, Department of Biomedical Engineering for her valuable guidance, suggestions, and constant encouragement that paved the way for the successful completion of our project work.

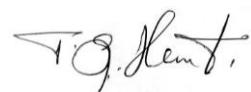
We convey our sincere thanks to **Dr. T. Sudhakar, M.Sc., Ph.D.**, Head of the Department, Department of Biomedical Engineering for providing us the necessary support and details at the right time during the progressive reviews.

We wish to express our thanks to all **Teaching and Non-teaching staff members** of the Department of Biomedical Engineering, who were helpful in many ways for the completion of the project.

Last but not the least, we would like to express our sincere thanks to the **Almighty** and heart felt gratitude to our **Beloved Parents**, for their constant support in financially, morally and physically in all our progress. Without their blessings and support the project would not have completed in a great success.



K.S. RAMACHANDRAN



T.S. HARISH

CONTENTS	TABLE OF CONTENTS	PAGE NO
	TITLE	
	ABSTRACT	iii
	LIST OF ABBREVIATIONS	iv
	LIST OF FIGURES	v
CHAPTER 1	INTRODUCTION	01
1.1	INTRODUCTION	01
1.2	STRUCTURE AND COMPOSITION OF THE BONE	01
1.2.1	CORTICAL BONE	02
1.2.2	CANCELLOUS BONE	02
1.3	FORMATION OF BONE	04
1.3.1	OSSIFICATION OF THE ENDOCHONDRAL BONE	04
1.3.2	OSSIFICATION OF INTRAMEMBRANOUS BONE	05
1.4	REMODELING OF THE BONE	05
1.5	BIOMECHANICS	06
1.6	BIOMATERIALS USED FOR TISSUE SCAFFOLDING	07
1.6.1	COLLAGEN	08
1.6.2	CHITOSAN	08
1.6.3	FIBRIN	08
1.6.4	GELATIN	09
1.6.5	CASEIN	
CHAPTER 2	LITERATURE SURVEY	10
2.1	SURVEY OF PREVIOUS WORKS	10
CHAPTER 3	AIM AND SCOPE	14
3.1	AIM	14

3.2	SCOPE	14
CHAPTER 4	MATERIALS AND METHODS USED	16
4.1	MATERIALS	16
4.2	FREEZE CASTING	
4.3	FUSED DEPOSITION METHOD	17
4.4	LIGHT MICROSCOPE TECHNOLOGY	18
4.5	MECHANICAL STIMULATION	18
4.6	SAMPLE PREPARATION	
CHAPTER 5	RESULTS AND DISCUSSION	20
5.1	DESIGN CONSIDERATION	20
5.1.1	SCAFFOLD DESIGN	20
5.1.2	SIMULATION DESIGN	22
5.1.3	SIMULATION CALCULATION	23
5.1.4	OPTICAL MICROSCOPIC IMAGE	23
CHAPTER 6	SUMMARY AND CONCLUSIONS	27
6.1	SUMMARY	27
6.2	CONCLUSIONS	27
	REFERENCES	28

ABSTRACT

Tissue engineering helps to regenerate, restore and improve the damaged tissues. Bio-scaffolds need to satisfy few criteria to allow osseointegration, osseointegration, osseointegration and osseointegration. A growing number of Traditional and three-dimensional (3D)-printing processes have been applied to tissue engineering. This project presents a feasibility study of traditional versus 3D-printing technologies for tissue-engineering applications, with particular focus on the development of a traditional versus computer-aided scaffold design system; Anything external, inserted into the body can cause inflammations and infections. Some study shows that the Bio-scaffold stimulates bone repair, the rate of calcification, and the formation & mineralization of bone callus, accelerating the process of new bone formation on the fracture region. Our aim is to exploiting the feasibility study of synthesis a bio-scaffold using traditional versus 3d printed scaffold.

LIST OF ABBREVIATIONS

ABBREVIATION	EXPANSION
BMPs	BONE MORPHOGENETIC PROTEINS
FDM	FUSED DEPOSITION MODELLING
FFF	FUSED FILAMENT FABRICATION
FGF	FIBROBLAST GROWTH FACTORS
HA	HYDROXYAPATITE
IGFs	INSULIN-LIKE GROWTH FACTORS
MNPs	MAGNETIC NANOPARTICLES
MSCs	MESENCHYMAL STEM CELLS
PGS	POLYGLYCEROL SEBACATE
PLA	PROPRIETARY LABORATORY ANALYSES
SGD	SKELETAL GENE DATABASE
TCP	TRANSMISSION CONTROL PROTOCOL
TE	TISSUE ENGINEERING
TGFs	TUMOUR GROWTH FACTORS

LIST OF FIGURES

FIGURE NO	NAME OF THE FIGURE	PAGE NO
1.1	THE STRUCTURE OF COMPACT AND CANCELLOUS BONE	01
1.2	THE CELLULAR STRUCTURE OF BONE	02
1.3	BONE REMODELLING PROCESS	06
1.4	THE STRESS APPLIED ON BONE(A) COMPRESSIVE STRENGTH AND (B) TENSILE STRENGTH	07
5.1	FABRICATED 3D DESIGN WITH RADIUS OF PORE 1.1mm	20
5.2	FABRICATED 3D DESIGN WITH RADIUS OF PORE 0.5mm	20
5.3	FABRICATED 3D DESIGN WITH RADIUS OF PORE 2mm	21
5.4	SIMULATION OF 3D DESIGN UNDER STRESS CONDITION	22
5.5	SIMULATION OF 3D DESIGN UNDER ELASTIC STRAIN CONDITION	22
5.6	SIMULATION OF 3D DESIGN UNDER DEFORMATION CONDITION	23
5.7	RIGHT SIDE VIEW OF TRADITIONAL POROUS SCAFFOLD	24
5.8	TOP SIDE VIEW OF TRADITIONAL POROUS SCAFFOLD	25
5.9	FRONT SIDE VIEW OF TRADITIONAL POROUS SCAFFOLD	25

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Bone is a dynamic, specialized connective tissue made up of cells, a dense intercellular material, and numerous blood vessels. It supports the body mechanically and allows for movement. Furthermore, bone contributes to the body's mineral homeostasis and participates in energy metabolism via endocrine regulation. They allow neighboring muscles to move freely and serve as tendons' attachment points. Bone, as a unique organ, has the potential to continuously reconstruct skeletal volume throughout life. Certain substances, such as bone morphogenetic proteins (BMPs), insulin-like growth factors (IGFs), fibroblast growth factors (FGFs), and retinoids, are required for optimal bone development. Growth hormone, thyroid hormones, androgen, and estrogen regulate these parameters.

1.2 STRUCTURE AND COMPOSITION OF THE BONE

The two types of normal human bone are (a) cortical bone and (b) trabecular bone. Compact bone is another name for cortical bone. The white, smooth, and solid appearance of the bone is due to the hard outer layer. Cortical bone accounts for 80% of bone mass in an adult skeleton, while trabecular bone accounts for 20%.

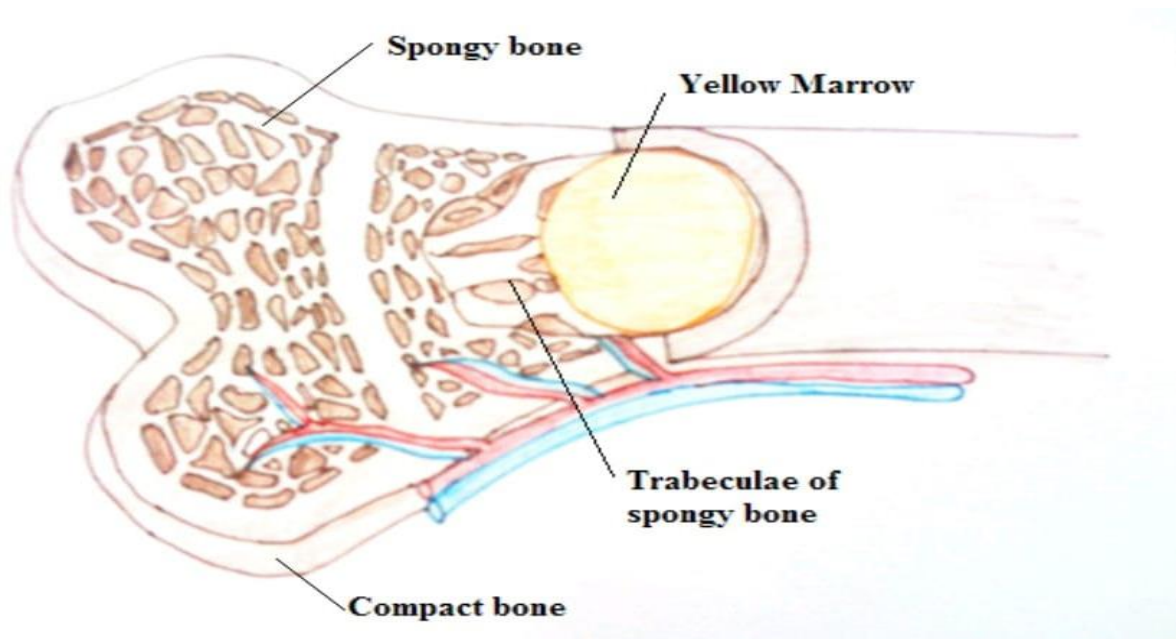


Fig 1.1: The structure of compact and cancellous bone

1.2.1 Cortical bone

Is made up of osteon, which are parallel cylindrical columns. Each column is made up of layers of osteoblasts and osteocytes that surround the Haversian canal, a central canal. Osteons are joined at right angles via volkmann's canals. All of these columns are metabolically active, and cell locations inside the osteon shift as bone is reabsorbed. Two surfaces cover the cortical bone. The periosteum is the outside surface, and the endosteum is the inner surface. The endosteum establishes the barrier between the cortical and trabecular bones (Livia Roseti et al 2017).

1.2.2 Cancellous bone

It also known as trabecular or spongy bone tissue, fills the inside of the bone (Livia Roseti et al 2017). It consists of a porous cell network. The endosteum is covered with osteoblast, which generates an uneven network of gaps (Livia Roseti 2017). Bone marrow and hematopoietic stem cells, which give rise to red blood cells, white blood cells, and platelets, occupy these areas (Livia Roseti 2017). Trabecular bone is made up of a network of rods and plate-like bone tissues that form an open-celled to a (Mauro 2017). Although trabecular bone only accounts for 20% of bone mass, it has ten times the surface area of compact bone.

Myeloid tissue is another name for bone marrow. The presence of bone marrow can be seen in any bone with cancellous tissue. All of the cancellous bones are filled with red marrow when a baby is born, but as the baby grows older, the red marrow is replaced with yellow or fatty marrow. Red marrow is abundant in the bone marrow of the ribs, femur, pelvic bones, and vertebrae in adults (Livia Roseti 2017).

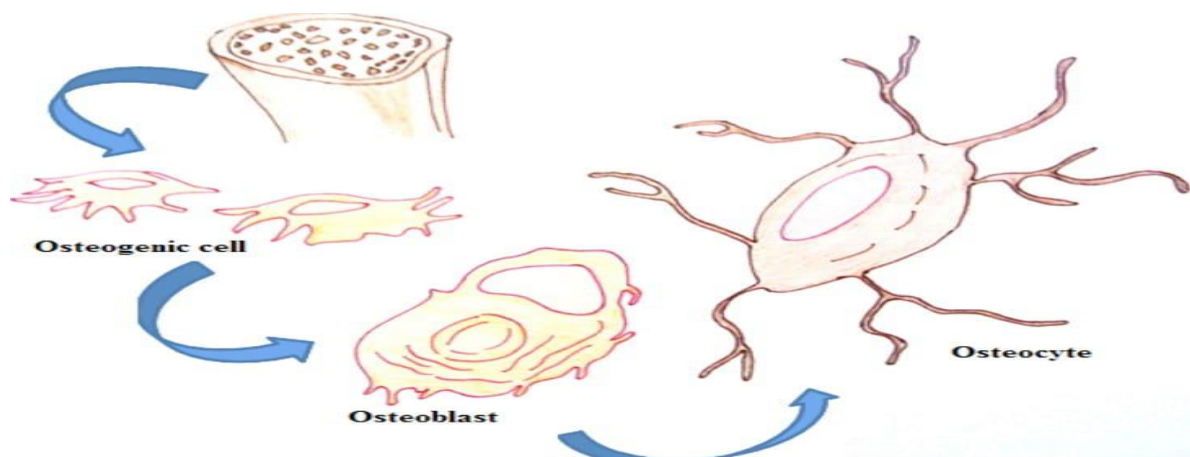


Fig 1.2: The cellular structure of bone

Three cellular components make up bone. These include osteoblasts, which are responsible for bone synthesis, osteocytes, which are responsible for bone maintenance, and osteoclasts, which are responsible for bone resorption. The many types of bone cells are depicted in Figure 1.2. Osteoblasts and Osteocytes are produced by the osteoprogenitor cell. Further differentiation of osteoprogenitor cells leads to the formation of macrophages and monocytes (Livia Roseti et al 2017). These cells' precursors can be found on the periosteum, endosteum, bone marrow, and peripheral blood. These unique cells have the ability to switch from bone deposition to bone removal and vice versa.

Bone-forming cells called osteoblasts are present on the surface of osteoid seams. Osteoid is a protein combination formed by it. The organic matrix of the bone is called osteoid, and it is made up of type I collagen as well as other components such proteoglycans, osteocalcin, and other phosphoproteins. By binding to calcium and collagen, osteonectin, a phosphoprotein, connects the two primary elements of bone matrix. The deposition of calcium phosphate crystals in the form of hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) converts osteoid into a hard bone matrix. Prostaglandin is an eicosanoid produced by osteoblasts that acts on the bone. It also generates alkaline phosphatase, an enzyme that affects bone mineralization.

The dormant osteoblast gives rise to osteocytes (Mauro Petretta 2017). Osteoblasts move into bone matrix and become stuck and encircled by it, resulting in the development of osteocytes (Mauro Petretta 2017). Lacunae are the empty spaces occupied by these cells. Osteocytes communicate with osteoblasts and other osteocytes by extending their microfilaments. Osteocytes communicate with other cells via gap junctions and canaliculi in the bone matrix.

Osteoclasts are involved in bone resorption, which is the breakdown of bone followed by the formation of new bone by the osteoblast. Osteoclasts are multinucleated big cells that form resorption pits on the surface of bones. How ships lacunae, an osteoclast enzyme, causes these pits to form. These lacunae are formed by reabsorbed bone tissue from the surrounding area (Mauro Petretta 2017). Hormones train the osteoclast, which controls bone degradation. Osteoclasts, like macrophages, are endowed with phagocytic mechanisms. (Mauro and colleagues, 2017). Calcium homeostasis is dependent on bone resorption.

The main minerals found in bone are calcium and phosphorus. The skeleton stores nearly all of the calcium in the body as hydroxyapatite crystals and some non-crystalline phosphates and carbonates (Arash khojasteh 2017). Collagen, water, and other organic material such as proteins, lipids, and polysaccharides account for half of the bone mass, while collagen, water, and other organic material such as proteins, lipids, and polysaccharides account for the rest.

1.3 FORMATION OF THE BONE

1.3.1 Ossification of the Endochondral Bone

Endochondral ossification is a process that happens throughout the fetal development of the skeletal system and results in the formation of bone tissue (Macro Tatullo 2017, Bice Conti 2018). Condensation of cells occurs during bone formation, resulting in the creation of chondrocytes, the major cartilage cell type (Ida Genta and R Mofed 2018). Cell division and the secretion of extracellular matrix occur in these chondrocytes. Interstitial growth is the term for this process. Type II collagen and the proteoglycan aggrecan, as well as other transcription factors, are abundant in this matrix (D Sharifi 2018). The cartilage expands as a result of chondrocyte proliferation and matrix formation. Chondrocytes in cartilage's center stop proliferating and expand, resulting in hypertrophy. This alters the genetic message.

The first site of ossification occurs in the middle of diaphysis of shaft and this forms the primary center of ossification. The perichondrium developed by the chondrocytes becomes the periosteum. The periosteum has a layer of osteoprogenitor cells (Undifferentiated cells) which later turns into osteoblasts. To support the new bone, osteoblast secrete osteoid (oppositional growth) leading to the formation of bone collar.

Alkaline phosphatase, an enzyme necessary for mineral deposition, is secreted by chondrocytes in the major center of ossification. Calcification of the matrix occurs as a result of this, and osteoprogenitor cells are prompted to secrete osteoid on the calcified matrix. The trabecula of bone is formed by this. The bone marrow is formed when osteoclasts tear down the spongy bone (Macro Tatullo 2017, Bice Conti 2018, Ida Genta and R Mofed 2018).

At the moment of birth, a secondary ossification center forms in the epiphysis of long bones in mammals. Periosteal buds, like the major ossification center transport blood vessels and mesenchyme. The cartilage between the primary and secondary ossification centers that continues to create new cartilage is known as the epiphyseal plate. This cartilage is replaced by bone, which causes the length of the bone to expand. The growth of an individual continues until the cartilage in the plate is replaced by bone.

1.3.2 Ossification of the Intramembranous Membrane

Intramembranous osteogenesis, in combination with direct differentiation of mesenchymal stem cells, results in the development of osteoblasts, which then replace the connective tissue membrane sheet with bone tissue. Flat bones result as a result of this (Bice Conti 2018, S Vahabi et al 2008).

Several substances or pathways control both intramembranous and endochondral ossification (Bice Conti 2018, S Vahabi et al 2008). Vascularization is a common process in both the ossification and vascularization processes (Francesco Benazzo 2018, Ida Genta and D Sharifi, 2018, A Mashhadi 2018). During the transfer of mesenchymal stem cells, intramembranous ossification causes vascularization. The hypertrophic chondrocytes that act as a template for bone development produce angiogenic growth factors during endochondral ossification to replace cartilage with bone and bone marrow. The invasion of blood vessels begins as a result of this (S Latour and Agathe Gremare 2018). During bone formation, angiogenesis is critical for secreting growth factors, which govern proliferation, differentiation, and bone formation and resorption (Razavi 2017).

1.4 REMODELING OF THE BONE

The disorganised structure of newly generated bone is characterised by a heavily mineralized matrix including collagen fibrils. This bone goes through a regulated remodelling process, as seen in Figure 1.3. Remodeling is a continuous process in which new bone is formed and old bone is replaced. In adult human cortical and trabecular bone, this process occurs at a rate of around 3% and 26% every year, respectively (Yang Xia 2018). For an adult, about 10% of their skeletal mass is rebuilt every year (Wilson Wang 2017). Adult bones are constantly remodelled,

allowing the body to adapt to mechanical stress, mend minor bone injuries, and maintain bone health.

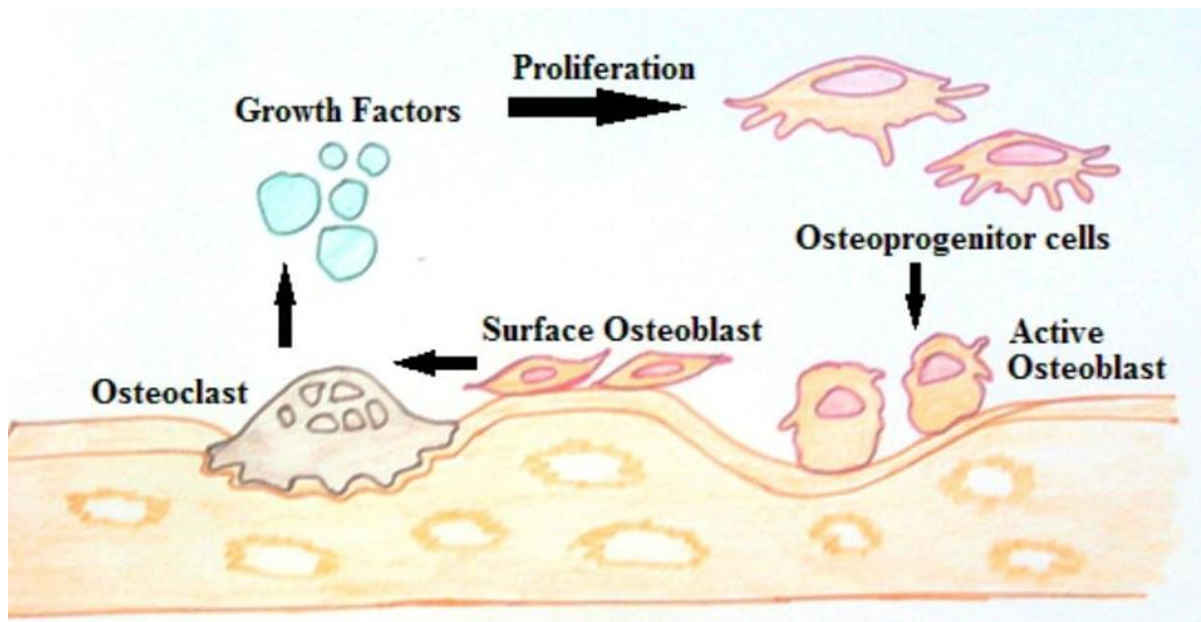


Fig 1.3: Illustrates the bone remodeling process

Researchers have discovered that the activity of osteoclasts (bone resorption) and osteoblasts (bone production) must be coordinated in order to preserve bone health, and that this coordinated process is mediated by tumour growth factor (TGF-) (Yang Wu et al 2017). The lamellar structure of remodelled bone is well-organized, and each layer has a distinct orientation of thick collagen fibres. In cortical bone, 3 to 7 m thick lamellae are stacked on a flat surface or as concentric rings surrounding blood vessels, forming a structure resembling a Harversian system or simply called secondary osteon. In certain lesser species, such as rats, remodelling does not occur on a regular basis (Peng Zhao 2018). The alignment of lamellae parallel to physiological stress aids in maintaining the bone's anisotropic biomechanical nature (Haoyang MI, Chegchen, Jianzhong Fu 2018).

1.5 BIOMECHANICS

The mechanical properties of bone are influenced by its deformation grade, which is a viscous-elastic material. Collagen in the matrix gives the bone flexibility and the ability to support compressive pressures (Paul F Fagan et al 2017, Stephen J Ferguson 2017). Because of its anisotropic structure, bone behaviour varies depending on the load applied. In general, bone tissue can withstand more load in the longitudinal direction than the bone surface. The load sustained before failure or

the point of failure is referred to as bone strength. Compression, tension, curvature, shear, and torsion are all forces that the skeletal system is susceptible.

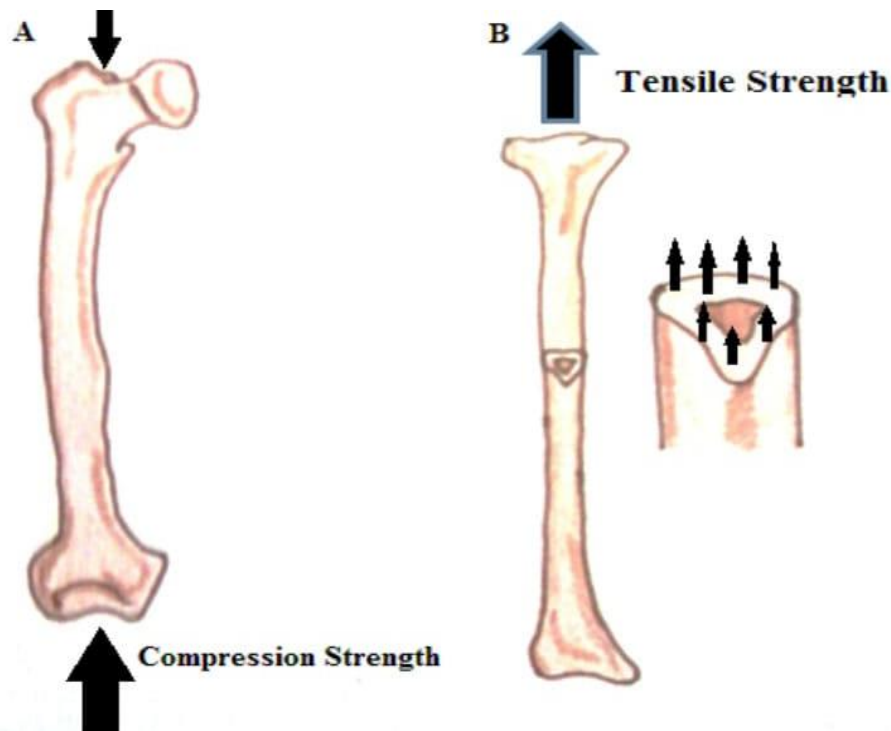


Fig 1.4: The stress applied on bone (A) compressive strength and (B) Tensile strength

The force applied to the edges of the same bone at the same time is known as compressive strength. Muscles, gravity, weight support, or external stress applied along the length of the bone all contribute to this force. Bone growth and development require high compressive strengths. Compressive strength produces stress, which aids in the deposition of bone formation. Fracture happens when compressive strength and load are exerted beyond the limit.

Tensile strength refers to the force exerted on the surface of the bone. It pulls or extends the bone, causing it to expand or compress. Tensile strength is usually obtained from muscle. In children, tensile strength can cause ligament avulsion, most commonly in the ankle, resulting in an ankle sprain.

1.6 BIOMATERIALS USED FOR TISSUE SCAFFOLDING

Collagen, chitosan, fibrin, gelatin, alginate, elastin casein, and other natural or biologically based materials, in combination with the scaffold, demonstrate higher

biocompatibility, regenerative qualities such as osteo induction, osteo conduction, and osteointegration, and better biodegradability.

1.6.1 Collagen

Collagen is the most common protein in the human body, and it gives the body structure and stability (Mojtaba Ansari 2019). It has a variety of qualities, including high mechanical strength, good crosslinking agent, high biocompatibility, outstanding biodegradability, and low antigenicity as the principal organic ingredient of ECM (Vaclav Babuska and vlastimil Kulda 2019). It has been widely employed in tissue engineering grafts because to these features.

In artificial skin products, for example, bi layered collagen gels seeded with fibroblast are employed (Liang Zhao 2018). Collagen sponges and collagen membranes are used to repair lengthy bone fractures and to regenerate periodontal tissue (Michael Dweir 2018). Despite its many uses, collagen has processing and sterilizing restrictions, as well as problems controlling the pace of disintegration (YU Guo et al 2019).

1.6.2 Chitosan

Chitosan (poly-1,4 D-glucosamine) is a common linear polymer found in crustacean shells, fungal cell walls, and insect cuticles. Because of its positive charge, mild gelation capability, and film-forming property, it has been used in a variety of biological applications, including wound dressing. It has an adhesive property when blood coagulation is boosted (Miriam Filippi et al 2020, H Bherei et al 2018). When chitosan is combined with alginate, its absorbency is increased. It has a variety of uses in tissue engineering because of its antibacterial properties, low cost, and wide scale availability (Mansoor and Mabruk 2020). Because of its positive charge in acidic conditions, it is employed in transdermal drug delivery and works as a mucoadhesive (Yang Wu et al 2018).

1.6.3 Fibrin

Fibrin is made from fibrinogen, a precursor component found in blood plasma. After an injury, it develops a natural wound healing matrix (D Vashae et al 2017). Fibrin scaffolds have cell adhesion domains and can have different capabilities depending on their concentration. Fibrin has been widely employed as a biological scaffold for

bone, adipose tissue, cardiac tissue, liver, ophthalmic tissue, nerve tissue, tendons, skin, and ligament regeneration in tissue engineering. For many years, fibrin glue has been frequently utilized as a surgical hemostatic agent. Fibrin degrades quickly, limiting its use in shape-specific scaffolds, and it is also susceptible to covalent alteration, which can alter its characteristics (petra et al 2019).

1.6.4 Gelatin

Gelatin, a collagen derivative, is flexible under particular variables such as pH, temperature, and solvent, allowing the material to adapt to diverse confirmations (C dutta et al 2017). Because of its biocompatibility and absence of antigenicity, it has been widely employed in the manufacture of monodisperse colloidal particles (Madhuri Dey 2017). Gelatin supports cell adhesion, motility, and proliferation when utilized in scaffolds (K Dutta et al 2017). When applied in vivo, gelatin is completely resorbable (C Dutta et al 2017). Gelatin can be combined with other biomaterials such as chitosan, HA, and TCP to create a tissue engineering composite.

1.6.5 Casein

Casein, a phosphoprotein, accounts for around 80% of the protein in milk. Because casein is high in proline and lacks disulfide linkages, the protein contains fewer secondary and tertiary structures. Casein is widely used in the production of polymers, culinary additives, adhesives, protective coatings, binders, and fabrics due to its hydrophobicity (Sai Praneeth 2021). Casein has recently been employed in tissue engineering due to its low cost, ease of availability, biocompatibility, non-toxicity, and high stability. Casein is used as a drug carrier molecule in the form of microspheres, and it has been shown that casein has good progesterone loading characteristics (satyen kale et al 2021). Casein is manufactured with other inorganic materials and used as a composite in bone tissue engineering.

CHAPTER 2

LITERATURE SURVEY

The author outlines the research that has been done in this chapter. Relevant publications from several reasserts have been included in this chapter to demonstrate scientific subject expertise, particularly scaffolds and traditional approaches. This chapter also discusses era research and the statistics employed at the time.

2.1 SURVEY OF PREVIOUS WORKS

Livia Roseti, Valentina Parisi (2017) Numerous scaffolds have been tested in the orthopedic field with the aim of improving cell viability, attachment, proliferation and homing, osteogenic differentiation, vascularization, host integration and load bearing. The main traits that characterize a scaffold suitable for bone regeneration concerning its biological requirements, structural features, composition, and types of fabrication are described in detail. Attention is then focused on conventional and Rapid Prototyping scaffold manufacturing techniques. A conclusion, it is highlighted that, despite its encouraging results, the clinical approach of Bone Tissue Engineering has not taken place on a large scale yet, due to the need of more depth studies, its high manufacturing costs and the difficulty to obtain regulatory approval.

Gordian Born Mansoor Chaaban and Arnaud Scherberich (2020) despite considerable advances in microsurgical techniques over the past decades, bone tissue remains a challenging arena to obtain a satisfying functional and structural restoration after damage. Through the production of substituting materials mimicking the physical and biological properties of the healthy tissue engineering. Cross - linking and other strategies of chemical modification enable the designer to control the lifetime of the implant, with important impact in the applications where scaffolds are supposed to deliver a specific function over a precise period of time before being replaced by newly formed tissue.

Rossella Dorati, Antonella DeTrizio (2018) A great deal of research is ongoing in the area of tissue engineering (TE) for bone regeneration. A possible improvement in restoring damaged tissues involves the loading of drugs such as proteins, genes, growth factors, antibiotics, and anti-inflammatory drugs into scaffolds for tissue

regeneration. This mini-review is focused on the combination of the local delivery of antibiotic agents with bone regenerative therapy for the treatment of a severe bone infection such as osteomyelitis. The review includes a brief explanation of scaffolds for bone regeneration including scaffolds characteristics and types, a focus on severe bone infections and a literature review of local antibiotic delivery by the combination of scaffolds and drug-delivery systems

Sai Praneeth Karimireddy and Satyen (2021) proposed a new algorithm (SCAFFOLD) which uses control variates (variance reduction) to correct for the 'client-drift' in its local updates. We prove that SCAFFOLD requires significantly fewer communication rounds and is not affected by data heterogeneity or client sampling. Our work studied the impact of heterogeneity on the performance of optimization methods for federated learning. Our careful theoretical analysis showed that FEDAVG can be severely hampered by gradient dissimilarity, and can be even slower than SGD.

Vlastimil Kulda and Vaclav Babuska (2019) the main objective is to restore and improve the function of bone tissue by scaffolds, providing a suitable environment for tissue regeneration and repair. Strategies and materials used in oral regenerative therapies correspond to techniques generally used in bone tissue engineering. Bone tissue engineering is a rapidly developing branch, and researchers are focusing on developing and improving new materials to imitate the biological environment of the body as authentically as possible.

Maryam Rezai Rad, Pooja Joshi (2017) a comprehensive electronic search was conducted according to the PRISMA guidelines in NCBI PMC and PubMed from January 2001 to December 2017 limited to English language publications with available full texts. In vivo studies in relation to "bone healing," "bone regeneration," and at least one of the following items were investigated: allograft, β -tricalcium phosphate, deproteinized bovine bone mineral, hydroxyapatite tricalcium phosphate, nanohydroxyapatite, and composite scaffolds. A total of 1252 articles were reviewed, and 46 articles completely fulfilled the inclusion criteria of this study. The highest bone regeneration has been achieved when combination of all three elements, given scaffolds, mesenchymal stem cells, and growth factors, were used. Among studies being reported in this review, bone marrow mesenchymal stem cells are the most

studied mesenchymal stem cells, β -tricalcium phosphate is the most frequently used scaffold, and platelet-rich plasma is the most commonly used growth factor.

Surena Vahabi, N Amirizadeh, MA Shokrgozar (2018) Mesenchymal stem cells were aspirated from iliac crest bone marrow after extracting the first, second and third premolars and the first molar in five mature hybrid dogs. The cells were cultured and their osteogenic differentiation potential was evaluated after the third cell passage using Alizarin red staining in experimental conditions. The empty cavity demonstrated more bone formation (60.80%) than the HA/TCP (44.93%) and Bio-Oss (40.60%) ($p < 0.05$) groups. However, the difference from the HA/TCP+ MSCs group was not significant (46.38%) ($p > 0.05$).

Punyavee Kerativitayanan, Marco Tatullo (2017) Synthesis and fabrication of porous and elastomeric nanocomposite scaffolds from biodegradable polyglycerol sebacate (PGS) and osteo inductive nano silicates is reported. Nano silicates are mineral-based two-dimensional (2D) nanomaterials with high surface area which reinforced PGS network. Overall, the combination of elasticity and tailorable stiffness, tunable degradation profiles, and the osteo inductive capability of the scaffolds offer a promising approach for bone tissue engineering.

Yu Guo, Michael D Weir, Mark A Reynolds (2018) Novel strategies utilizing magnetic nanoparticles (MNPs) and magnetic fields are being developed to enhance bone tissue engineering efficacy. This article first reviewed cutting-edge research on the osteogenic enhancements via magnetic fields and MNPs. Then the current developments in magnetic strategies to improve the cells, scaffolds and growth factor deliveries were describe potential clinical applications of magnetic nanoparticles and scaffolds with magnetic fields and stem cells include dental, craniofacial and orthopedic treatments with substantially increased bone repair and regeneration efficacy.

Mojtaba Ansari (2019) this review paper is intended to sum up and discuss the most relevant studies performed in the field of bone biology and bone regeneration approaches. Therefore, the bone tissue regeneration was investigated by synthetic substitutes, scaffolds incorporating active molecules, nanomedicine, cell-based products, biomimetic fibrous and nonfibrous substitutes, biomaterial-based three-dimensional (3D) cell-printing substitutes, bioactive porous polymer/inorganic

composites. This review investigated the bone physiology, different strategies for bone repair and interface studies. The published results provide insightful information for development of new biomaterial-based products. However, there is a vital necessity to investigate the results of all clinical trials. There are several bone repair strategies including bone graft replacements, implantable materials and scaffolds, optimized 3D structure and favoured surface properties. Bio-active constituents are necessitated while the bone defects become larger.

CHAPTER 3

AIM AND SCOPE

3.1 AIM

1. To Synthesize Bio scaffold using traditional and 3D printed process.
2. To check the stability of the sample.
3. To check the feasibility or biocompatibility and self life of the sample.

Scaffolds, which are usually constructed of polymeric biomaterials, provide structural support for cell adhesion and tissue formation. Tissue engineering aids in the regeneration, restoration, and enhancement of damaged tissues. To allow osseointegration, osseointegration, and osseointegration, bio-scaffolds must meet a few conditions. Tissue engineering uses a growing number of traditional and three-dimensional (3D) printing technologies. This research compares the viability of traditional versus 3D printing methods for tissue engineering applications, with a focus on the creation of a traditional versus computer-aided scaffold design system. Anything external, inserted into the body can cause inflammations and infections. Our aim is to exploiting the feasibility study of synthesis a bio-scaffold using traditional versus 3d printed scaffold. The main target of Bio Scaffolding is to simulate natural behaviour.

3.2 SCOPE

The developing field of Tissue engineering (TE) aims to regenerate damaged tissues by combining cells from the body with highly porous scaffold biomaterials which acts a template for Tissue regeneration, to guide the growth of new tissue. Some study shows that the bio-scaffold stimulates bone repair, the rate of calcification and the formation & mineralization of bone callus etc.

Scaffolds are materials that have been created to contribute to the development of new functioning tissues for medicinal reasons by causing desired cellular interactions. Cells are frequently 'seeded' into these structures capable of supporting the creation of three-dimensional tissue. Tissue engineering's major goal is to restore and improve tissue function by constructing porous three-dimensional scaffolds and seeding them with cells and growth factors. The most straightforward strategy is to

deliver the proper signal molecules (tissue-inducing chemicals) to the right location. 3D scaffolds are commonly used for drug delivery, investigation of cell behaviour and material studies in the field of tissue engineering. Three-dimensional scaffolds are porous, biocompatible, and biodegradable materials that provide appropriate microenvironments, such as mechanical support, physical simulation, and biochemical simulation, for optimal cell development and function. 3D scaffolds' porosity and pore size have a direct impact on their functionality in biological applications. For tissue vascularization and development of new tissues, open porosity and linked networks are required for cell nutrition, proliferation, and migration. A porous surface also aids mechanical interaction between the scaffolds and surrounding tissue, which improves the implant's mechanical stability. 11 Furthermore, the pores' network structure aids in guiding and stimulating the development of new tissue.

CHAPTER 4

MATERIALS AND METHODS USED

4.1 MATERIAL

Hydroxyapatite, Polypropylene, NaCl, MilliQ or deionised water.

4.2 FREEZE CASTING

To attain the traditional method, we are using Freeze casting (ice templating, Freeze-casting, also known as ice-templating or freeze alignment. Is a process that uses the highly anisotropic solidification behaviour of a solvent (usually water) in a well-dispersed slurry to generate a directionally porous ceramic in a controlled manner. Ice crystals will nucleate on one side of an aqueous slurry and grow along the temperature gradient if it is exposed to a directional temperature gradient. As the ice crystals form within the slurry, they rearrange the suspended ceramic particles, thereby templating the ceramic. Once the solidification process is complete, the frozen, templated ceramic is placed in a freeze-dryer to remove any remaining ice crystals. The resulting green body has anisotropic macropores in the form of sublimated ice crystals, as well as micropores between ceramic particles in the walls. This structure is frequently sintered to strengthen the porous material and consolidate the particle walls. The porosity left by the sublimation of solvent crystals is typically between 2–200 μm .

For this method materials that we used

- Sodium chloride.
- Polypropylene.
- Alpha tricalcium Phosphate.

Freeze casting is unique in its ability to produce aligned pore structures. Because such shapes are common in nature, freeze casting has become a valuable method for fabricating biomimetic structures. The ability of freeze casting to transport fluids through aligned pores has led to its use in biomedical applications such as bone scaffold materials. In freeze cast structures, the alignment of pores also results in extremely high heat resistance in the direction perpendicular to the aligned pores. The use of spinning methods to freeze cast aligned porous fibres is a possible

method for producing high-performance insulating garments. Three-dimensional pore architectures that can be fine-tuned during solidification. Freeze casting can be used to create materials with application-specific microstructures, but the templating process is extremely difficult, and the fundamental principles are only partially understood.

4.3 FUSED DEPOSITION MODELLING

Fused deposition modelling (FDM) is a popular additive manufacturing technique for modelling, prototyping, and production. Fused Deposition Modelling (FDM) creates three-dimensional things directly from three-dimensional CAD data. Fused deposition modelling (FDM) is a technique for depositing hot plastic filaments in a specified pattern using the melt extrusion process. FDM uses a printhead that can move in both the X and Y axes over a build platform, similar to 3DP. Most industrial grade thermoplastics can be used in FDM 3D printing to make sturdy and durable products. It is widely used for prototypes, final products and concept models. The FDM process begins with the import of a model's STL file into a pre-processing programme. This model is aligned and mathematically split into horizontal layers with thicknesses ranging from 0.127 mm to 0.254 mm. A support structure is created where needed, based on the parts position and geometry. The data is downloaded to the FDM machine after it has been reviewed and toolpaths have been generated. The system draws the model one layer at a time in the X, Y, and Z axes. This procedure is similar to how melted glue beads are extruded from a hot glue gun. The thermoplastic modelling material is heated to a semi-liquid state and supplied into the temperature-controlled extrusion head. The head precisely extrudes and guides the material onto a fixtureless base in ultrathin layers. A plastic 3D model is created by laminating the hardened material to the previous layer one strand at a time. Once the part is completed the support columns are removed and the surface is finished. Pre-processing, production, and post-processing are the three phases involved in producing parts in FDM. PRE-PROCESSING, the printer software separates the layers in the 3D CAD file. The software then turns the data into machine code for each slice, which sets the machine's tool paths. Stratasys Inc. owns the trademarks for fused deposition modelling and its abbreviation FDM. Members of the RepRap project invented the exact same term, fused filament fabrication (FFF), to give a language that would be legally unrestricted in its use. It's a brand-new design.

4.4 LIGHT MICROSCOPE TECHNOLOGY

The optical microscope, often known as a light microscope, is a type of microscope that uses light to magnify objects. It is a type of microscope that produces enlarged images of small objects by using visible light and a lens system. The object is put on a stage and seen directly via one or two microscope eyepieces.

Both eyepieces of a high-power microscope generally show the same image, but a stereo microscope uses slightly distinct images to create a 3-D effects. The sample can be lighted in a number of different ways. Solid objects can be lighted with light coming through (bright field) or around (dark field) the objective lens, while transparent objects can be lit from below. The use of polarised light to identify the crystal orientation of metallic objects is possible.

Increase picture contrast with phase-contrast imaging by highlighting small details with different refractive indices. A turret is frequently used to hold a variety of objective lenses with varying magnifications, allowing them to be rotated into position and zoomed in. Scanning electron microscopy, transmission electron microscopy, and scanning probe microscopy are non-visible light alternatives to optical microscopy that may attain significantly higher magnifications.

4.5 MECHANICAL SIMULATION

For this 3D simulation we are using ANSYS software. ANSYS is a finite-element modelling tool that may be used to solve a wide range of mechanical problems numerically. Static/dynamic, structural analysis, heat transmission, and fluid problems, as well as acoustic and electromagnetic challenges, are among these issues. Ansys gives you the power to deploy technology at a scale that's appropriate for the size of the problem. Depending on what's appropriate and available, run it on a wide variety of computing resources. Finally, you'll be able to deploy the technology among your company's users. Temperature distribution data is provided via real-time modelling and data within your 3D model via Ansys Discovery as you change models. Change the input qualities or the materials. Ansys aids in the optimization of design to a large extent. It's essentially a solver that assists us in simulating loading situations on the component's 3D model.

4.6 SAMPLE PREPARATION

Material used: Hydroxyapatite, Polypropylene, NaCl and Double distilled water

First prepare the polymeric solution

- 40ml of double distilled water is taken.
- 1.5g of Hydroxyapatite and 1.5g of polypropylene is mixed with the above solution and heated for 1.5 hour.
- And the solution poured in Petri dish after that sprinkle the NaCl salt over that let it in room temperature for 30minutes and repeat the step for about 3-5times.
- After 24 hours remove the sample from petri dish and start the leaching process.
- For leaching put the sample into distilled water for 1 hour.
- After 1-hour porous scaffold were obtained.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 DESIGN CONSIDERATION

For 3D method we gone through Fused Deposition modelling, the further results are done by using Ansys software to get the simulation result (Surena Vahabi, N Amirizadeh, MA Shokrgozar (2018).

5.1.1 Scaffold Design

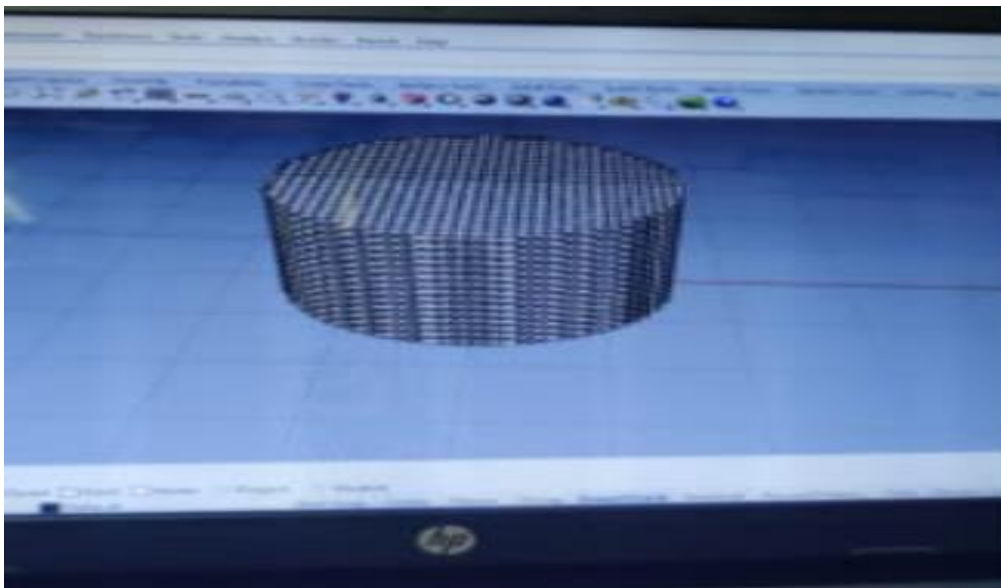


Figure 5.1 Fabricated 3D Design With Radius Of Pore 1.1mm

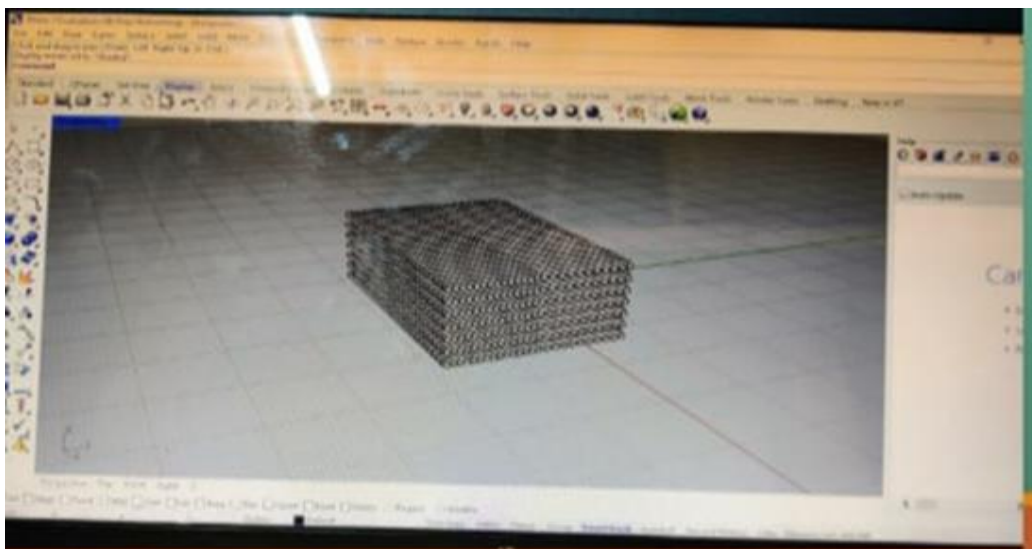


Figure 5.2 Fabricated 3D Design With Radius Of Pore 0.5mm

Fig shows the results of fabricated designs with different pore size and radius for PLA scaffold. The fabricated prototype of circle design made up of PLA with radius of pipe 0.5mm and size of pore 0.1mm, 0.35mm and size of pore 0.5mm.

Comments used in the rhino software to sketch this 3D scaffold design are

- Circle: centre, radius
- Ellipse: from centre
- Rectangle corner to corner
- Polylines
- Group
- Array
- Array linear
- Pipe
- Trim
- Maximize
- Shaded
- Rendered
- Wide frame
- And other comments are depending on the usage.

Further, scaffold made up of PLA with radius of pipe 0.2 mm and size of pore 2mm were analysed by using ANSYS software to study and compare its mechanical properties.



Figure 5.3 Fabricated 3D Design With Radius Of Pore 2mm

5.1.2 Simulation design

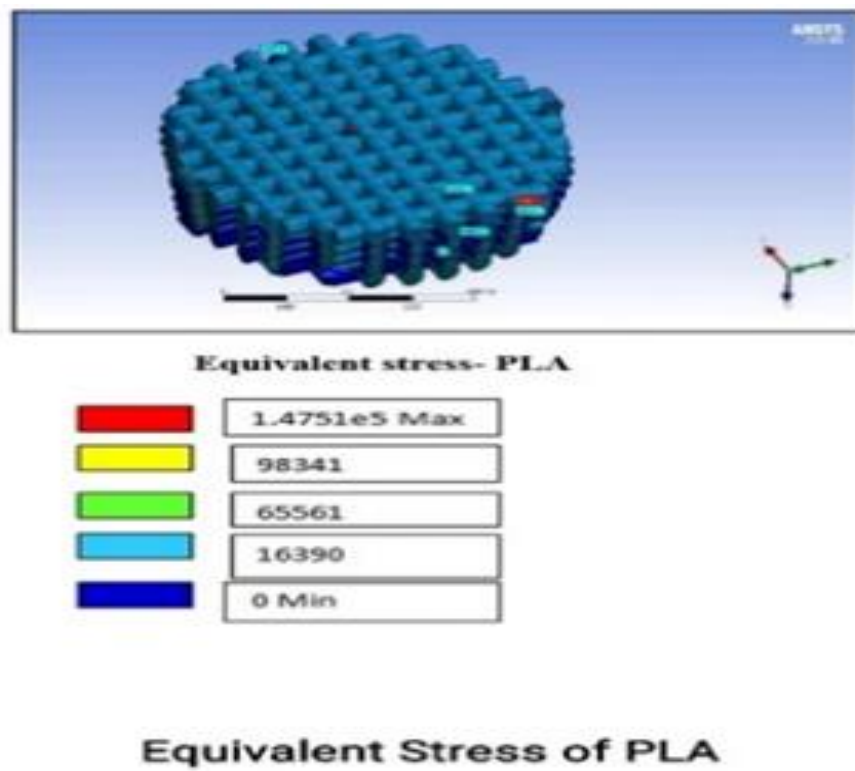


Figure 5.4 Simulation of 3D design under Stress condition

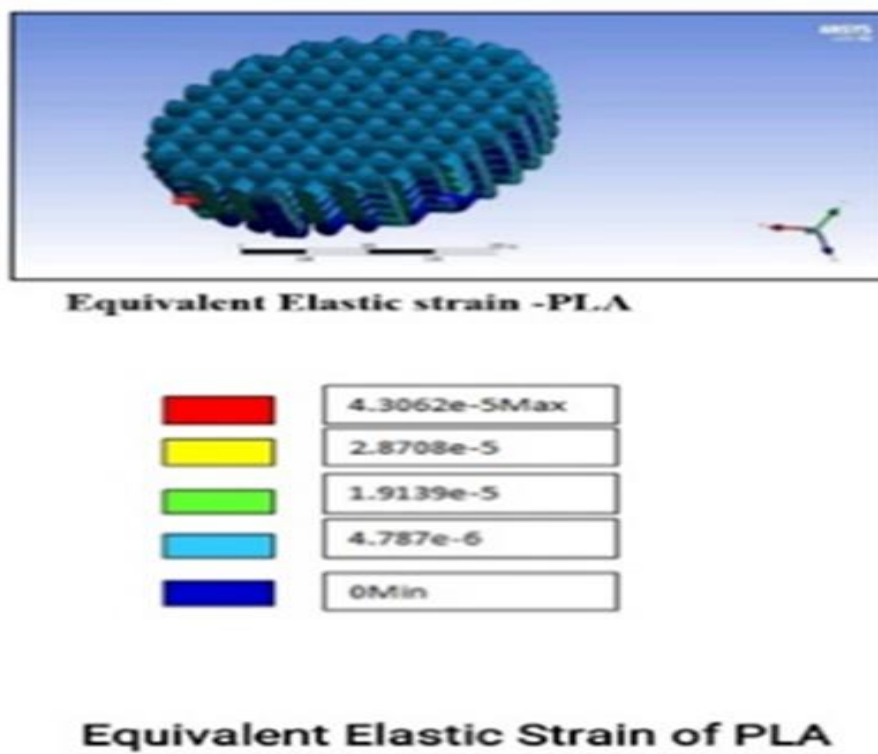


Figure 5.5 Simulation of 3D design under Elastic Strain condition

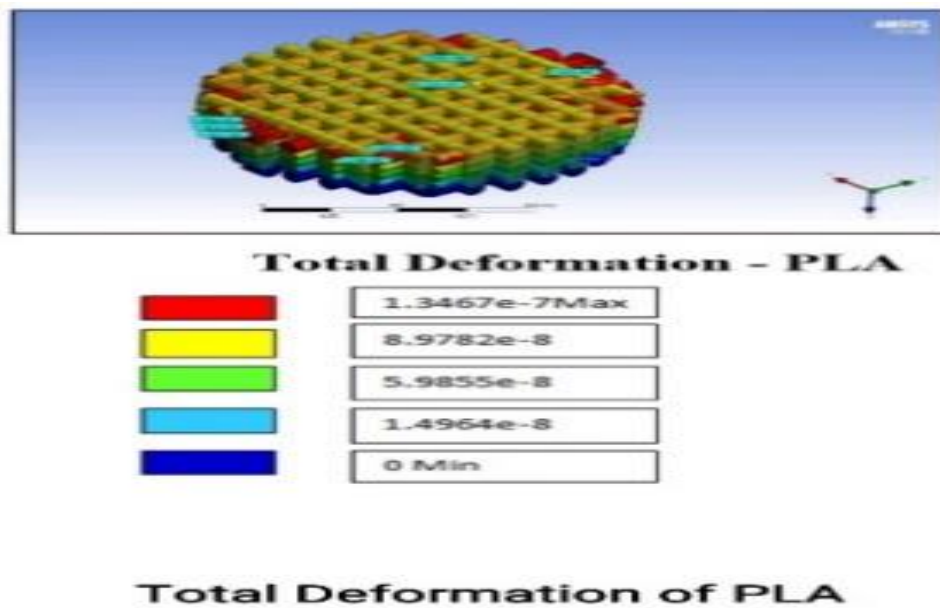


Figure 5.6 Simulation of 3D design under Deformation condition

The first step was static and dynamic structural model of the design, the option available in the Ansys software to study the mechanical properties of designed scaffold. The second step was to update the data in the engineering data fig. After that we were uploaded the model to the geometry column. The final step was to setup all the properties like total deformation, equivalent elastic strain, maximum shear elastic strain, equivalent stress, maximum shear stress. The simulation results were analysed and the best suitable material was selected for the fabrication of scaffold for bone tissue regeneration.

5.1.3 Simulation Calculation

- The values are ranging from minimum 0N to maximum 27N

$$1\text{kg} = 9.8\text{N}$$

$$3.0\text{kg} = 29.4\text{N (Rat)}$$

- We took value which is equivalent to 27N

5.1.4 Optical Microscopic Image

Material used: Hydroxyapatite, Polypropylene, NaCl and Double distilled water

First prepare the polymeric solution

- 40ml of double distilled water is taken.
- 1.5g of Hydroxyapatite and 1.5g of polypropylene is mixed with the above solution and heated for 1.5 hour.
- And the solution poured in Petri dish after that sprinkle the NaCl salt over that let it in room temperature for 30minutes and repeat the step for about 3-5times.
- After 24 hours remove the sample from petri dish and start the leaching process.
- For leaching put the sample into distilled water for 1 hour.
- After 1-hour porous scaffold were obtained.

After two days the solution is filtered and the extract is obtained.0



Figure 5.7: Right side view of traditional porous scaffold



Figure 5.8: Top side view of traditional porous scaffold



Figure 5.9: Front side view of traditional porous scaffold

The three-dimensional porous scaffold is critical since it influences the mechanical properties, cellular fate, nutrient supply, vascularization and tissue ingrowth. The pore size is usually in the range of 100 - 1500 μ m. Even though the ideal size has

been estimated around 350 μ m, it is believed that larger pore sizes support deeper cell proliferation and tissue penetration. Concerning the pore structure, the geometry can be dictated by the fabrication process (for instance in the case of biological based scaffold), or Figure shows a synthesized scaffold. Most of these scaffolds are homogeneous porous media with isotropic transport properties. Considering the recent advances in fabrication techniques (eg. 3D-printing, stereolithography), it may be possible to produce controlled pore size scaffolds with hierarchical microstructures and oriented channels to guide the patterns of cell migration, fluid flow and diffusion through the construct. Finally, the nano-structural architecture of the scaffold should be considered since the substrate rigidity and rugosity is known to influence cell adhesion, differentiation and migration.

CHAPTER 6

SUMMARY AND CONCLUSIONS

6.1 SUMMARY

We go through research and reference papers, and studied about existing methodology of 3D Bio Scaffolding. Started to learn about Rhino software. Designed 3D bio scaffold model in Rhino software. Completed testing's like stress, strain , Deformation, We go through research and reference papers, and studied about existing methodology of Traditional Bio scaffolding by Freeze casting method. We attached Traditional Light Microscopic Image.

6.2 CONCLUSIONS

Shape is a big issue in the traditional approach, which means we utilise a beaker or conical flask for the process and only achieve the shape of the beaker or conical flask. We use 3D software to achieve our desired shape in the 3D approach. Because NaCl is used in the traditional manner, the porosity for the scaffold is uneven. Porous for the scaffold in the 3D approach are configurable according to the ratio we require. For any given result, the 3D technique is more viable in terms of output time. When compared to the 3D approach, the traditional method will take longer to get our results.

REFERENCES

- 1) Agathe Grémare, Vera Guduric, Reine Bareille, Valérie Heroguez, Simon Latour, Nicolas L'heureux, Jean-Christophe Fricain, Sylvain Catros, Characterization of printed PLA scaffolds for bone tissue engineering, 2018,106(4):887-894.
- 2) Livia Roseti, Valentina Parisi, Mauro Petretta, Carola Cavallo, Giovanna Desando, Isabella Bartolotti, Brunella Grigolo, Scaffold for bone tissue engineering State of the art and new perspectives, 2017, 8:1246-1262.
- 3) Miriam Filippi, Gordian Born Mansoor Chaaban and Arnaud Scherberich (2020). Natural Polymeric Scaffolds in BoneRegeneration, 2020, <https://doi.org/10.3389/fbioe.2020.00474>.
- 4) Mojtaba Ansari, Bone tissue regeneration: biology, strategies and interface studies, 2019, 8(4):223-237.
- 5) Mostafa Mabrouk, Hanan H Beherei (2020) Recent progress in the fabrication techniques of 3D scaffolds for tissue engineering, 2020, 110:110716.
- 6) Mostafa Yazdimamaghani, Mehdi Razavi, Daryoosh Vashaei, Porous magnesium-based scaffolds for tissue engineering, 2017, Volume 71, 1253-1266.
- 7) Paul F Egan, Stephen J Ferguson, Design of hierarchical three-dimensional printed scaffolds considering mechanical and biological factors for bone tissue engineering, 2017, 139(6): 061401.
- 8) Peng ZHAO, Haibing GU, Haoyang MI, Chengchen RAO, Jianzhong FU, Lihsheng TURNG (2018) Fabrication of scaffolds in tissue engineering, 2018, **13**, pages107–119
- 9) Petra Chocholata, Vlastimil Kulda and Vaclav Babuska (2019) Fabrication of Scaffolds for Bone-Tissue Regeneration, 2019, v. 12(4).
- 10) Punyavee Kerativitayanan, Marco Tatullo, Margarita Khariton, Pooja Joshi, Barbara Perniconi, Akhilesh K Gaharwar, Nanoengineered osteoinductive and elastomeric scaffolds for bone tissue engineering, 2017, 3, 4, 590–600.
- 11) Ranjna C Dutta, Madhuri Dey, Aroop K Dutta, Competent processing techniques for scaffolds in tissue engineering, 2017, 35(2):240-250.
- 12) Rossella Dorati, Antonella DeTrizio, Tiziana Modena, Bice Conti, Francesco Benazzo, Giulia Gastaldi, and Ida Genta, Biodegradable Scaffolds for Bone

- Regeneration Combined with Drug-Delivery Systems in Osteomyelitis Therapy, 2018, 10(4):96.
- 13) Sai Praneeth Karimireddy, Satyen Kale, Mehryar Mohri, SCAFFOLD: Stochastic Controlled Averaging for Federated Learning, 2021, 1910.06378.
 - 14) Sepanta Hosseinpour, Mitra Ghazizadeh Ahsaie, Maryam Rezai Rad, Mohammad taghi Baghani, Saeed Reza Motamedian, Arash Khojasteh, Application of selected scaffolds for bone tissue engineering: a systematic review, 2017, 21(2):109-129.
 - 15) Surena Vahabi, N Amirizadeh, MA Shokrgozar, R Mofeed, Abbas Mashhadi, M Aghaloo, D Sharifi, L Jabbareh, A comparison between the efficacy of Bio-Oss, hydroxyapatite tricalcium phosphate and combination of mesenchymal stem cells in inducing bone regeneration, 2018, 35(1):28-37.
 - 16) Yang Wu, Zuyong Wang, Jerry Ying Hsi Fuh, Yoke San Wong, Wilson Wang, Direct E-jet printing of three-dimensional fibrous scaffold for tendon tissue engineering, 2017, 616-627.
 - 17) Yang Xia, Jianfei Sun, Liang Zhao, Feimin Zhang, Xing-Jie Liang, Yu Guo, Michael D Weir, Mark A Reynolds, Ning Gu, Hockin HK Xu, Magnetic field and nano-scaffolds with stem cells to enhance bone regeneration, 2018, 183:151-170.
 - 18) Zahrasadat Paknejad, Maryam Rezai Rad, Saeed Reza Motamedian, Mohammad Jafar Eghbal, Polymeric scaffolds in tissue engineering, 2017, 1-9.