Investigations on Biomimetic PLA Scaffold Fabricated by Fused Deposition Modelling

A Thesis Submitted in Fulfillment of the Requirement for the Award of the Degree of

MASTER OF ENGINEERING

in Production Engineering

Submitted By

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DEDICATED TO MY LOVING FAMILY

CERTIFICATE

I, Daljeet Singh, hereby declare that the thesis entitled "Investigations on Biomimetic PLA Scaffold Fabricated by Fused Deposition Modelling" is submitted in partial fulfilment of the requirements for the awards of the degree of Masters of Engineering in Production Engineering at Thapar Institute of Engineering and Technology, Patiala, is authentic record of my own research work carried out by me under the supervision of Dr. Vivek Jain, Associate Professor and Dr. Dheeraj Gupta, Associate Professor, Department of Mechanical Engineering, Thapar Institute of Engineering and Technology, Patiala. No part of the matter embodied in this report has been submitted to any other university or institute for the award of any degree.

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ABSTRACT

Now-a-days polylactic acid (PLA) is widely used in orthopedics surgeries as implants material due to well mechanical characterization and biomedical properties. But the polylactic acid implants suffer from slow degradation rate when it used in real life example. In this research, fabricate the PLA specimens using additive manufacturing technique and assess the mechanical characterization and degradation behavior of polylactic acid with different parameters. The three different process parameters (infill percentage, layer thickness and infill pattern) set on three levels for fabricating the various specimens. Simulated body fluid (SBF) used for in vitro study of the experiment. Immersed specimens in simulated body fluid for 7,14 and 28 days. To check the mechanical characterization of these samples on Universal Testing Machine before and after immersion in SBF solution. The amount of apatite formation on the surface of different parts show the biological behavior of different process parameters. The infilling percentage and infilling patterns greatly affect the formation on the surface of workpiece due to their high surface area to volume ratio. High infill percentage results the maximum mechanical strength and low degradation rate. On the other hands, the workpiece with low infill percentage results gives the minimum mechanical strength and higher degradation rate. The apatite formation is also high in case of low infill percentage. The study concludes the positive effect of process parameters on degradation rate and biocompatible behavior of polylactic acid implants.

Key Words: Polylactic acid, Additive manufacturing, Simulated body fluid, Degradation.

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INTRODUCTION

1.1 BIOMATERIALS

The definition of biomaterials by American institute of health as “any material or combination of material, it may synthetic or natural in nature, which can use for any time period in human body, which can replace the tissue or function of the body to enhance the quality of life of any person”. In the history of artificial implants, the first material which was used to replace the cranial defects was gold by Egyptians and Romans. During the period of 1900s, placenta was used as a biomaterial. The polymethyl methacrylate (PMMA) was first polymer which was accepted. The Williams Dictionary defined biocompatibility as ‘‘ability of the material to perform host response in the specific situation’’. This definition was unhelpful at first glance, it represented a quantum leap forward at the time of its introduction. Behind this definition, the prevailing view was that successful materials played largely inert roles in the human body. A long list of the properties for successful biomaterials: nontoxic, non-immunogenic, non-carcinogenic. The definition required that the materials not only provide some function. It also recognized that the interface created by introduction of the material will elicit a biological response.

There are basically three categories of the biomaterials based on the tissue reactions.

1.1 Bio Tolerant Materials: Which can separate from bone tissue by layer of fibrous tissue.

1.2 Bioactive Materials: The material has the property of construct chemical bonds with other bone tissue. The collagen phase of the adjacent bone is deposited on the implant surface.

1.3 Bioinert Materials: Under this category, it is possible to have direct contact with the adjacent bone tissue. No chemical reactions will occur between the implant material and bone tissues.

Materials are asked to play more sophisticated, customizing and optimizing the tissue interface to sure the good long term clinical outputs. We can change the surface of the material to control nonspecific protein absorption, add more appetite sequences to enhance native protein or cell interactions, provide a three-dimensional structure to matrix formation [1].

In addition to the tissue response, some other factors are also important in the adaptation of the biomaterial. Some factors like, which material used, applied load during function, and age play the most important role. These days' development of the advance technologies to develop new biocompatible materials which will capable to support new specifications and applications.

The world market of implant materials is estimated of 150–200 U$ billion in 2012 including all diagnostic and therapeutic required equipment. The US is the largest market following by japan and Germany. The growth of united states market share is 9 % per year being the most leading market in the world. The largest market for implants material based on the products is orthopaedic biomaterials and followed by cardiovascular and drug delivery materials. The dental implant market is around 1 U$ billion.

1.2 TYPES OF BIOMATERIALS

A biomaterial or implants are defined as any material that is used to exchange or reconstruction function to a body tissue and permanent in contact with body fluids. The implant materials should biocompatible. It should not elicit ineffective response from the body. It should be non-toxic. There are some basic requirements of biomaterial, which are eliminate the many engineering materials that are available on earth. Secondly, biomaterial should have higher mechanical properties to serve as replacement of human body tissues. A biomaterial should have easily machined into different shapes and structure with low cost and easily available.

Most of the biocompatible materials are come from similar family. Generally, implant materials can divide into following categories.

- Metals
- Polymers
- Ceramics and Composites

1.2.1 Metals

As per different categories, the metals are widely used in high load bearing requirements. In the previous decade most of the common orthopaedic surgeries used to implantation of metal implants. Applications of the metallic implants is very wide, from simple wire and screw to total joint replacement such as hips joint, knees joint, shoulders, ankles etc.

There are some basic metals that are using in the metallic implants like stainless steels, pure titanium and its alloy, gold alloys, silver products, platinum and nickel-titanium alloys. Following tables shows the principle applications of different metallic implants [2].

Table 1.1 Applications of different metals.

S. No	Metal	Principle Applications
1	316L stainless steel	Fracture fixations, surgical instruments
2	Pure titanium and its alloys	Bone replacements, fixations, dental implants
3	Nickel and titanium alloys	Bone plates, stunts and orthopaedic wires
4	Magnesium	Bio gradable plates and screw
5	Gold alloys	Dental restorations
6	Silver products	Antibacterial agents

1.2.2 Polymers

There is various type of polymers are used in biomedical field. The application range of these polymers vary from facial treatments to liver and heart valves and from dentistry to total knee and hip joints. Following table shows the applications of different polymers [2].

Table 1.2 Applications of different polymers.

S. No	Polymers	Principle Applications
1	Polyethylene	Joint replacement
2	Polypropylene	Sutures
3	PET	Vascular prosthesis
4	PTFE	Soft-tissue augmentation, drug delivery
5	Polyesters	Vascular prostheses, drug delivery systems
6	PLA	Fixation screws
7	PVC	Tubing
8	PMMA	Dental restorations, lenses, joint replacement

1.2.3 Glass and Ceramics

Some glasses and ceramics are mostly used in orthopaedic surgeries due to their good biocompatibility and mechanical properties. These materials are very hard and can resist high load capacity inside the human body. In advance era, the fabrication and machining of these materials are cost effective and easily available. The applications of these materials are directly depending upon the properties of these particular material. Applications of ceramics and glasses in the human body is vary according to position of implants. Following table shows the applications of the biomaterial ceramics and glass materials [2].

Table 1.3 Applications of different type of glass and ceramics.

S. No	Glass and Ceramics	Principle Applications
1	Alumina	Joint replacements and dental implants
2	Zirconia	Joint replacement
3	Calcium phosphate	Bone repair and surface coating
4	Bioactive glass	Bone replacements
5	Porcelain	Dental restorations
6	Carbons	Heart valves and dental implants

1.3 POLYMER SCAFFOLD IN TISSUE ENGINEERING

There are various conventional and non-conventional techniques to fabricate the porous material or polymer scaffold for tissue engineering. The purpose of this project was on the use of polymer scaffold in bone tissue engineering or biomedical field. The aim of the bone tissue engineering to enhance the role of traditional bone repair methods by using bio gradable polymer scaffold to increase the mechanism of human body to repair the bone fracture. The polymer scaffold should be design to match the mechanical and structural properties of the target tissue. The polymer scaffold with similar properties to native bone has many advantages. Porosity, structure of pores, interconnectivity of pores and mechanical characterisation of the scaffold affected the effectiveness of polymer scaffolds. From survey, has been found that the interconnectivity and porosity throughout the scaffold required to promote the cell culture, loading and migration of cells, tissue growth and flow of body fluid. The traditional fabrication techniques have drawback of poor permeability and interconnectivity between the porous. The development of non-conventional methods to fabricate scaffold lead to control pore morphology.

1.4 TRADITIONAL TISSUE REPAIR METHODS

Tissue repair is an ultimate method of surgery since ancient time. It can classify into two foams 1) Tissue grafting and organ transplantation and 2) alloplastic and synthetic material replacement. When the bone tissue is unable to resist the load of body, it may due to some injuries or may be some other reasons, the tissue replaced with the artificial material used to fill the bone injury. The defect bone tissue taken from other part of body and it may high risk of infection, high rate of treatment. The other option is take the bone tissue from other human, but it may cause major infection and disease transmission.

1.5 TISSUE ENGINEERING

Tissue engineering introduced in the year 1990 to address the drawbacks of tissue grafting and alloplastic tissue repair techniques [3]. The technique of transplant the cells, genes and proteins through the porous structure known as a scaffold. Tissue engineering aims for regenerating a load bearing tissue, scaffold should resist the mechanical function to aid biological delivery. The fabrication of porous scaffold can help in understanding the role of the scaffold structural parameters on mechanical characteristics and efficiency. Fabrication techniques used to fabricate the scaffold has a major role in morphology of the scaffold. There are number of methods to fabricate the scaffold, each one has its advantages and disadvantages.

Table 1.4 Different casting techniques with unique factors and applications.

S. No	Method	Polymers	Unique factors	Applications
1	Solvent casting	PLLA, PLGA	Porous scaffold control	Bone engineering
2	Ice particle leaching	PLLA, PLGA	Pore structure control	Tissue engineering
3	Gas foaming	PLLA, PDLLA	Porosity control	Drug delivery
4	Solvent evaporation	PLGA, PLAGA	High density cell culture	Bone repair

1.6 TRADITIONAL SCAFFOLD CASTING METHODS

1.6.1 Solvent Casting

Solvent casting is a method that involves dispersing mineral or organic particles in a polymer solution. Casting or freeze-drying is then performed as a dispersion process in order to produce porous scaffold [4]. This basic method has been used to fabricate scaffolds using various polymers but while the approach is relatively simple, there are disadvantages such as the use of toxic solvents and residual solvent that remains in the scaffold and it may harmful for human body [5].

1.6.2 Gas Foaming

Gas foaming is a method used under high pressure. Under this process the salt particles involve in melt polymer gel paste and casting with a Teflon mould for evaporation. After that the simple water added in the solution for gas foaming. The co_2 produced from the solution and make it porous. Now freeze the sample to produce final scaffold. This technique is sufficient for high degree of porosity in the final scaffold and the pore size in range of 100 microns. This method results the scaffold with low mechanical strength and poor porous structure.

1.6.3 Melt Processing

This method introduced for use of more than one polymer mixed together and exposed to temperature above the melting point of the two polymers. In this method, both polymers are continuous in the structure and allow for the interconnected pores network if one of the them can be leached from the scaffold using a solvent. The process of melting of polymer involves the use of NaCl and a polymer as well as the use of an extruder. If the use of extruder provides the very well blended, homogenous mixture. It may lead the salt particles breakdown during mixing, which results in small pore sizes. The way produced by the polymer network are usually too small for most tissue engineering applications [6].

1.7 FABRICATION OF SCAFFOLDS WITH ADDITIVE MANUFACTURING

The process 'Additive Manufacturing' adds material in layer formation or pattern to fabricate the required shape and size instead of material removing. On the bases of design and fabrication process, several areas are to use it such as prototyping, aerospace, military, buildings and cars 3D printing, home applications and biomedical engineering. The 3D Printing technique uses software which slices the 3D model into various layers. Each layer performed on the build platform by the printer, once the first layer is completed, the platform goes downward according to layer thickness or equal to layer thickness and another layer is fabricating above the previous layer. Typical manufacturing technique also known as 'Subtractive Manufacturing' because this process for removing material from a preformed block. Processes like Cutting and milling are subtractive manufacturing. This kind of process produce lot of waste material because the material in chips format generally cannot used for any purpose. 3D Printing technique can decrease such kind of waste because in 3D printing the material is placed only in required location, the rest will be left as empty space.

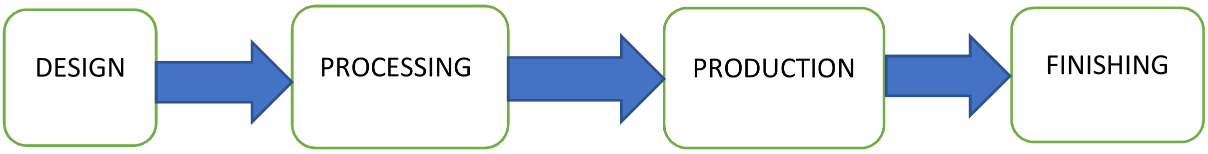


Figure 1.1 Flow diagram of the additive manufacturing.

From last few years, scientist and engineers has been done huge research to use 3D printing technique to fabricate artificial body parts and organs. This technique helps to create the artificial implants for patient's body same as the fabrication of plastic and metal parts. Another application of 3D printing in biomedical field is creating body parts out of metal or other material to replace the damaged and lost limbs. With the help of advancement of 3D printing, now the doctor firstly scans the patient's body and bone structure. The design engineers help to recreate the artificial part in the laboratory. It helps to meet the exact requirements of patient.

1.8 DIFFERENT TYPES OF 3D PRINTING

1.8.1 Stereo Lithography

Stereo lithography is a process in which an ultraviolet laser used to fabricate parts layer by layer. The resin is placed on the machine bed and laser beam strike on it and melt it. For each layer, the laser strikes on the surface of the resin. The high voltage laser light cures and solidify the pattern as per design traced on the resin and helps to join with lower layer. Once the pattern traced, the elevator platform descends by a distance equal to the layer thickness of a single layer, the layer thickness is generally varying from 0.05 mm to 0.15 mm. Once the single layer is completed the resin blade moves over the section of the part to fill fresh material. Again, this process is repeated until the final output created.

A complete part is fabricated by this process. Once the required part is created, immersed it in chemical bath to remove the support material and excessive resin and cured it in oven for some time below the glass transection temperature of the material. Supports are generated automatically as per process parameters setting during the design of 3D Computer Aided models for machining, it can be manipulated manually. Supports can remove from the finished product manually to decrease the process cost [7].

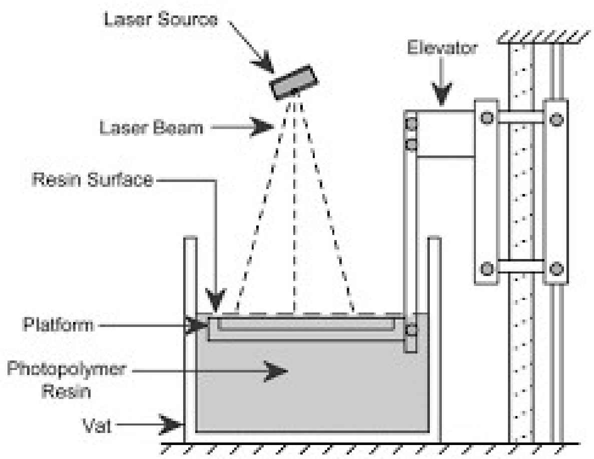


Figure 1.2 Stereo-lithography process. (Jain, 2013)

1.8.2 Selective Laser Sintering

The Selective laser sintering technique is another kind of 3D printing technology. The form of input material is the major difference between stereolithographic and selective laser sintering. The small powder form particles used as input material. The highly powered carbon dioxide laser used to perform the manufacturing operation. The highly powered laser scanned the cross section and melt the material. The fabricated process of the part generally based on the 3D design of the part. The movement in X- axis and Y- axis are controlled by laser head and the movement in Z- axis controlled by machine bed which is directly relative to the layer thickness of the required part. The density of final part is based upon the peak power of laser instead of laser time. Pre-heat the powder in chamber below its melting temperature, so it can help to raise the maximum temperature in minimum time. This technique can use for various materials, those are available like polymer, nylon, metals and alloys.

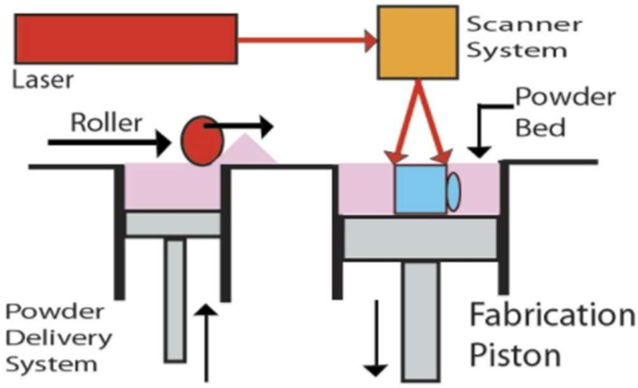


Figure 1.3 Selective laser sintering process [8].

1.8.3 Fused Deposition Modelling

The fused deposition modelling is widely used in production application of each sector. It is generally based on additive principle of manufacturing. The input material is basically used in the form of plastic filament or metal wire. As per showing in the figure, extrusion nozzle converts the material from solid form to liquid. All operations of FDM machine are numerically controlled. The process parameters are decided manually in the software package. The X- axis and Y- axis movements are controlled by extrusion nozzle and Z- directions are controlled by machine bed. The movements are controlled by Stepper motors or servo motors.

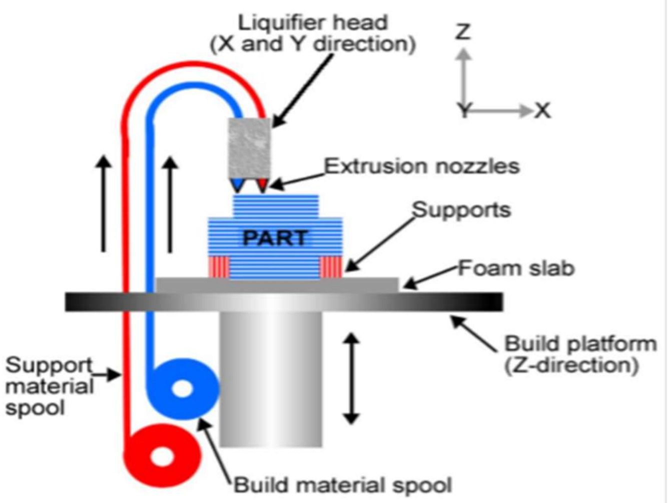


Figure 1.4 Fused deposition modelling machine [8].

1.9 PROCESS PARAMETERS FOR ADDITIVE MANUFACTURING

The process parameters play vital role in the properties of fabricated sample in 3D printing. From different researches observed that the different process parameter changes the almost properties of the created work piece. Some parameters are playing major role and some are playing minor role in the characterisation of 3D printed samples. Following are the main process parameters which affect the properties of material after fabrication. These parameters values show in software only.

1.9.1 Infill Percentage

The infill percentage of any work piece play a major role in mechanical characterisation. In 3D printing, the term "infill" means the structure inside the object that required to print. It is varying from minimum requirement to 100 percentage infilling. The work piece with 100 percentage infilling shows dense structure. The infill percentage of the design selected in the designed software. Infill percentage influence print weight, build time, usage material in fabrication, strength of part, and sometimes decorative properties. The sample with 100 % in filling has great mechanical strength and other properties instead of 5, 10, 20 %.

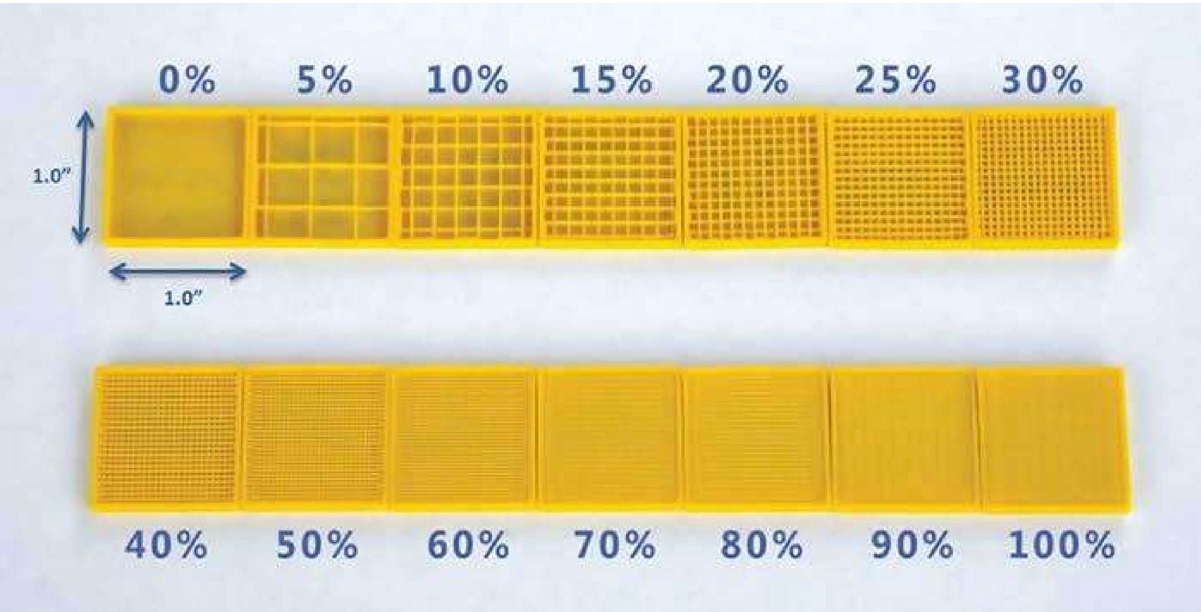


Figure 1.5 Different infill percentage

1.9.2 Infill Pattern

The infill pattern is the way of filling the inside portion of the work piece. The mechanical properties of any work piece directly depend upon the infill pattern. When using any infill pattern, it will decide the mechanical characterisation of that particular work piece. Now-a-days, various type of infill patterns options is available in 3D printers, each have some advantages and limitations between strength of the material, build time or print time and required material for fabrication. The simplify 3D software provides six different types of patterns with some of most popular. Designer can select any one of the infill pattern and fabricate the work piece. Followings are the most common uses infill patterns are.

1. Rectilinear
2. Grid
3. Triangular
4. Wiggle
5. Fast honeycomb
6. Full honeycomb

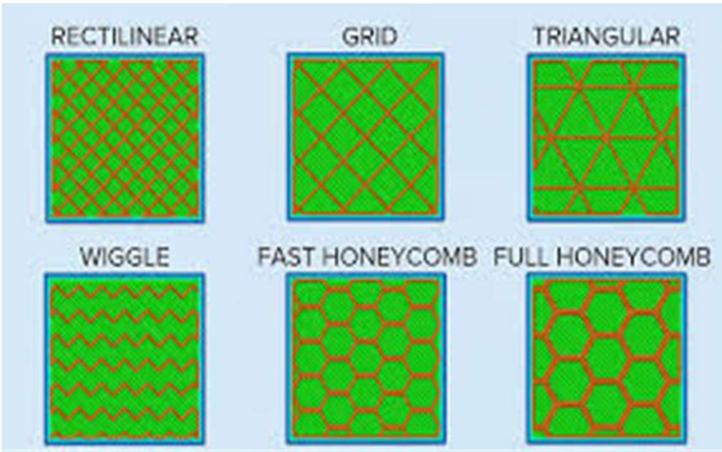


Figure 1.6 Different infill patterns.

1.9.3 Layer Thickness

The layer thickness in 3D printing can describe as the height of individual successive layer. The 3D printing works on the principle of layer formation, extruder moves on the bed of machine and fabricate a single layer as per design of the sample. The thickness of the single layer is controlled by the servo motor speed, which is connected with printing machine and control according to the design of the work piece. When the single layer is fabricated, the extruder repeats the same process and until the final layer or completion of the job. The build time and surface roughness directly depend upon the layer thickness [9].

1.9.4 Layer Width

The layer width in the 3D printing to measure the width of single layer. The width of single layer can decide in the software. The layer height or layer thickness and layer width are co-relative to each other. During fabrication of parts, layer width and layer thickness are controlled by the servo motor as per directions given in the software file.

1.9.5 Printing Speed

The printing speed generally can classify into 3 stages like high speed, medium speed and low speed. The low speed is around 40 mm/s to 50 mm/s and high build time, medium infill speed is generally from 80mm/s to 100mm/s. The third and last stage is high infill speed up to 150mm/s. the build time will increase with decrease in the infill speed. A small part with low quality can print in less than 10 minutes. But if the part is big and with high quality settings it could take time.

1.9.6 Build Orientation

Build orientation plays an important role in the properties of the build part. Fabricate the part with different directions of the orientations may result the significant change in the print quality. For example, if designer fabricate the two cylinders with different orientation, the dimensions of the cylinders are 10 mm outside diameter and 6 mm inside diameter with 20 mm height. The printing of first part with its centre axis vertical and the printer would construct series of concentric circle layered on the top of other. The result will be smooth outer surface. On the other side, the second part printed with its centre axis horizontally, the part print as the series of rectangles layered on the top of last one. The surface of cylinder that is touch the platform will be flat. There will be lot of difference between the characterisation of both parts.

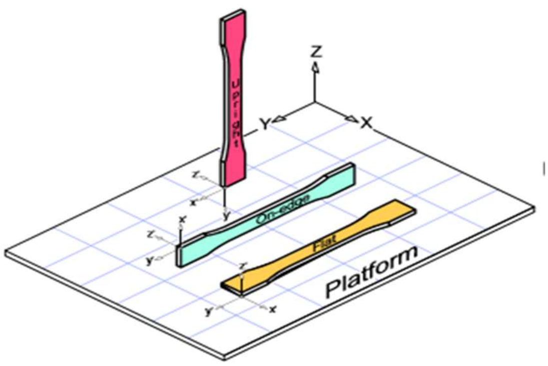


Figure 1.7 Different build orientation [10].

1.9.7 Raster Angle

Raster angle is the direction of layer fabrication with respect to platform axis. It gives significant result on the characterisation of the part with different raster angle. It varies from 0 degree to 90 degrees. The following diagram shows the term raster angle.

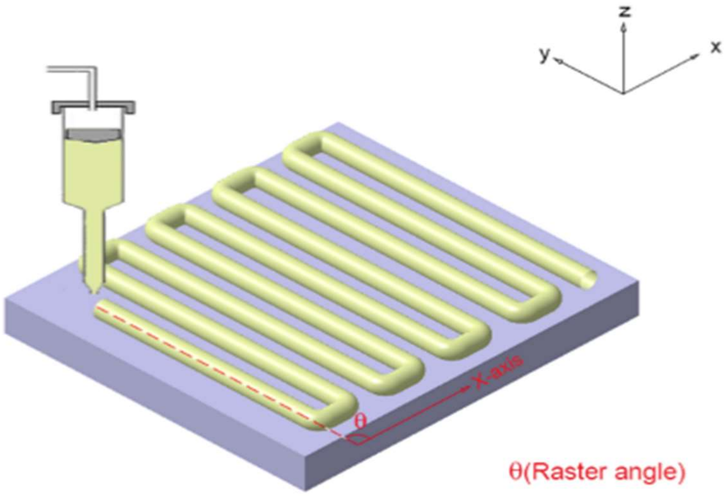


Figure 1.8 Raster angle [9].

1.9.8 Temperature

The temperature is a main parameter in 3D printing, because melting temperature, glass transition temperature and bed temperature are different for different materials. For example, the glass melting temperature of poly lactic acid is near to 180^{o} c and glass transition temperature near about 60^{0} c. On the other hands, the glass transition temperature of ABS near to 105^{0} c**.**

1.10 PROPERTIES OF PLA POLYMER

PLA is a biocompatible polymer. This material having some unusual properties due to which polymer is mainly suited for some biomedical applications. The polymer scaffold can float into the water because of their low densities. The strength of the PLA polymer is lower than the metallic implants and decreasing with decreasing density of material. There is no side effect of PLA polymer in human body. The properties of the polymer scaffold are affected after changing the process parameters of fabrication techniques.

1.10.1 Mechanical Properties

When polymer scaffolds are compared to dense polymer structure, the scaffold behave differently in testing due to their internal structure. Conventional testing techniques such as compression test and tensile test are used to check the meaningful results which show the stress strain diagram. The polymer scaffold behaves different with different process parameters and internal structure of the scaffold. Followings are the different mechanical properties of the dense PLA polymer [11].

Table 1.5 Mechanical properties of PLA

S. No	Properties	Range
1	Tensile strength	50-60 MPa
2	Elongation at break	6%
3	Young's modules	3.5 GPa
4	Flexural strength	80 MPa
5	Shear module	2.4 GPa
6	Flexural module	4.0 GPa
7	Density	1.25 g/cm^2

1.10.2 Thermal Properties

Poly lactic acid is a biodegradable and bioactive polymer which is produce from renewable energy sources such as cassava roots, chips or stretch and sugarcane. In 2010, Poly lactic acid had the second biggest consumption volume of any bioplastic of the world. Followings are some thermal properties of the PLA polymer [11].

Table 1.6 Thermal properties of PLA

S. NO	Properties	Range
1	Glass transition temperature	60 - 65 ^{o}c
2	Melting temperature	173-178 ^{o}c
3	Crystallinity temperature	37 ^{o}c
4	Heat resistance temperature	110 ^{o}c

1.10.3 Biocompatibility

Biocompatibility is a degree to which implants material result in a tissue engineering. There are several biomedical materials are available such as stainless steel, titanium and its alloys. If a material has less negative tissue reactions occurs, it means the material has more biocompatible. For example, Gold is the most biocompatible metal with compare to others but it is too soft and ductile, no doubt it is expansive with huge biomedical properties. The biocompatibility of any metal or material depends upon the weight relative to its mechanical properties. In other words, can say that there is always some amount of compromise, because there is no pure or perfect metal for this purpose. In case of metals, the corrosion resistance test is the main way to check the biocompatibility of any metal. Because the corrosion rete of any metal can decide the response of reaction. Implant material should have corrosion resistant. Adjacent tissues may discolour and allergic in patient's body due to release of elements [12].

1.11 MECHANICAL PROPERTIES OF HUMAN BODY TISSUES

The human body has different kind of bones relative to their properties. Some bones have great mechanical strength and some other have very low. The following table shows the different properties of the different bones.

Table 1.7 Mechanical properties of different bones.

S. No	Bones	Compressive Strength (Mpa)	Tensile Strength (Mpa)	Young's Modulus (Gpa)
1	Cancellous Bone	4-12	N/A	0.02-0.5
2	Cortical Bone	130-180	60-120	3-30
3	Cartilage	N/A	3.7-10.5	0.7- 15.3 (MPA)
4	Ligament	N/A	13-46	0.065-0.541
5	Tendon	N/A	24-112	0.143-2.31

1.12 APPLICATIONS OF POLYMER SCAFFOLDS IN BIOMEDICAL

Polymer scaffold is widely used in biomedical engineering. In the previous decades it was only used in the form of medical devices. The foremost requirement of the biomedical implants is; the choice of the material is adequacy by the human body. A material which is used for implant manufacturing should have some properties in order to long life usage inside the body without any side effects. Some polymeric material is widely used for medical purpose in these days like tissue engineering, artificial organ and implants, bone repair screws and dentistry etc. A polymeric material can be considered as 'biocompatible material', if its insertion into the body does not provoke an adverse reaction. A thrombus is formed very fast when polymers contact with blood cells [13].

The followings are the main applications of the polymer related biomaterials.

1.12.1 Tissues Scaffolding

Biomaterials play a vital role in tissues scaffolding, working as three-dimensional support structure for regeneration of cells. Polymers are most popular biomaterial in tissue growing due to their mechanical similarity with bone structure. Most polymers can break into natural products. But these biomaterials should have similar chemical properties like tissues engineering [13].

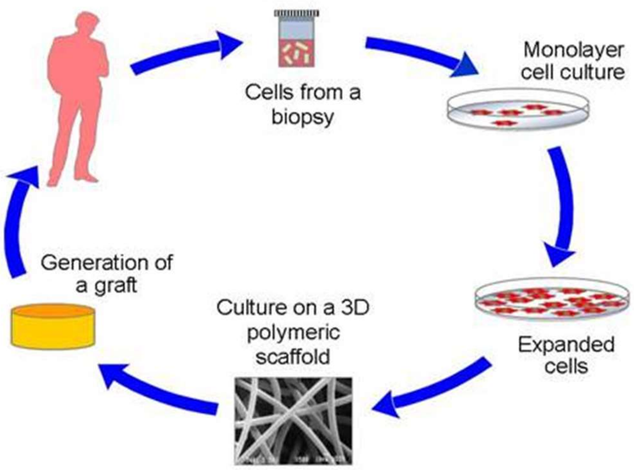


Figure 1.9 Regeneration of tissue engineering.

1.12.2 Implantation of Medical Devices

Biodegradable polymers are mostly used as a biodegradable and bioresorbable layer on stents to control the drugs release [14]. Biodegradable polymer is also candidate material for biodegradable stents due to their suitable properties to control drug release and good mechanical characteristics to prevent stents from deforming or fracturing [15]. The bone repair implants fabricate from degradable materials have advantages over non-degradable materials. The major advantage of usage of degradable implants, no need of surgery to remove the implants after recovery. Now-a-days most of the bone fixation screws, pins and plates are fabricating from degradable materials.

1.12.3 Joint Prostheses

Joints are necessary inside the body parts for movement. Hip joint, knee joint, and elbow are some examples of body joints. From last few decades, the artificial Hip joint and Knee Joint Replacement is most commonly in human beings. The following figure shows the typical design of total Hip joint replacement. The acetabular part and femoral part fixed with each other. The shaft of the femoral stem is specially tapered. The Knee Joint Replacement is also a more complicated geometry [2].

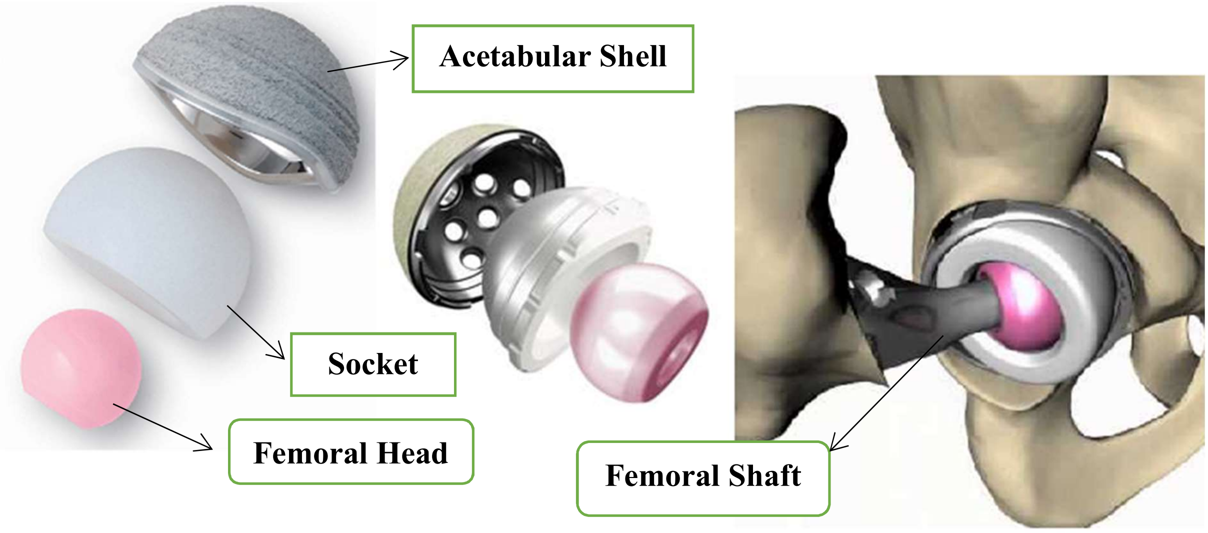


Figure 1.10 Components of artificial polymer hip joint.

1.12.4 Dentistry

A vast variety of materials like polymer, metals used in the dentistry like cavity filling, fluting, crown and bridge, prosthetic, orthodontic and periodontal treatment of teeth. The choice of dentistry material is pivot on its properties such as mechanical and biomedical properties of tooth structure.

Figure 1.11 Polymer Dentistry.

1.12.5 Bone Repair

Bone is a structural compound consists of collagen fibres with hydroxy apatite crystals [16]. Bone also space some other constituents like bone cells and blood vessels. Bone fractures are behaving towards in contrasting kind of ways and it can classify into two types such as internal fixation and external fixation. The external fixations are used when no need of surgery or can say that without any opening. On the other side internal fixation required surgery for recovery of the fracture [17].

1.12.6 Drug Delivery System

The bio-degradable polymers are useful for drug delivery. There are some drawbacks of the oldest methods of drug delivery like injections or tablets. The most prominent way to drug delivery is that the drug is hold in a polymer membrane or matrix and diffuses out into the tissues implantation. In some cases, the dissolution of the polymer contributes to the release mechanism. In these days' biodegradable polymers like PLA are widely used for drug delivery system [18].

CHAPTER 2

LITERATURE REVIEW

2.1 GENERAL

This chapter represent the reviews of researchers including history, effect of process parameters on different properties of PLA, Degradation behaviour of implants, in-vitro studies of different metallic and non-metallic implants under the different conditions. Various researchers have been worked on the properties of implant materials. There are few papers out of them, try to elaborate their research work and helps us to find the research gap.

2.2 LITERATURE REVIEW

Lopesa et al. [19] Bio absorbable polymers are the alternative material for biomedical applications, with the good biocompatibility, such as polylactide acid, poly glycolic acid and poly caprolactone are most commonly used biodegradable polymers used for fixations and supports material in human surgeries. PLA is most significant biopolymer used in medical field among the others. Various biomedical implants prepared from different PLA types. PLA overcome the disadvantages of the metallic fixation used in the several bone applications. Now-a-days, the biodegradable materials are replacing metallic implants for the fixation of fractured tissues. The fixation or implants material required optimum strength. PLA is the good example of a ‘‘biomaterial’’ with multiple applications.

Chac’on et al. [20] This paper represents the build orientation and layer thickness effect the mechanical characterisation of fused deposition modelling. The layer thickness considers at 4 levels (0.06, 0.12, 0.18, 0.24) and the build orientation consider as 3 levels (Flat, on edge and upwards). Upright parts show the inter-layer failure due to lower strength. Besides this, on-edge and flat parts display the trans-layer failure with the good mechanical properties. The results exhibit the brittle fracture behaviour for upright orientation and on the other side the ductile fracture behaviour for the on-edge and flat orientations. The layer height effects the mechanical characteristics with different manners, the part with minimum layer thickness, shows the maximum tensile strength and minimum flexural strength. On the other hands the ductility is directly relative to the layer thickness.

Motoparti et al. [21] Fused deposition machining used build the parts to varied in build parameters and compressive properties studied. Three process parameters build directions, raster angle and air gap change up to three levels and design the full factorial design of experiment to discover the effects on results. The raster angle and build direction are the two most significant factors in result difference. The compression test shows that the horizontal-build direction has 14-40% higher strength in comparison to vertical. The compressive strength of vertical-build specimens is 12-30% higher with 0° and 90° raster angle with comparison to 45° and -45° raster angle. The difference in compressive strength was very small for horizontal-build specimens with different raster angle.

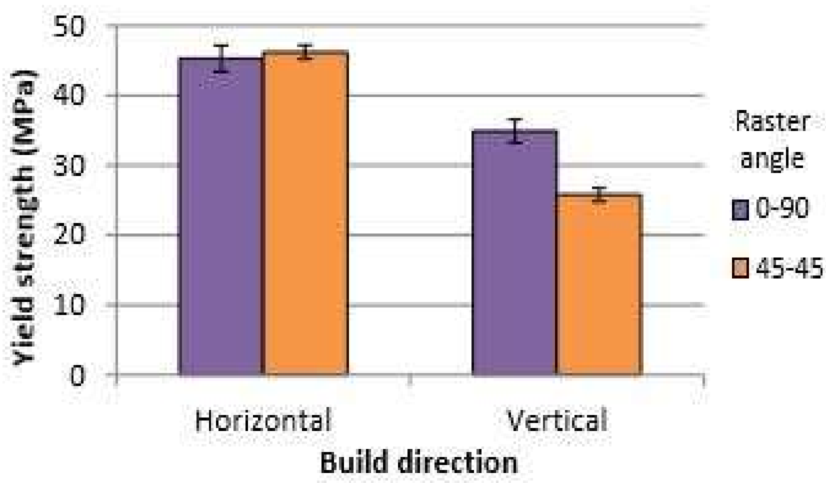


Figure 2.1 Effect of raster angle for horizontal and vertical build.

Basavaraj et al. [22] The three process parameters changed up to 3 levels to check the significant change on mechanical strength of the parts. All three parameters set on three levels. The ultimate strength test conduct to measure the effect of process parameters. The results show the best tensile strength with lower layer thickness, higher orientation angle and shell thickness. The dimensional accuracy is maximum with lower layer thickness and medium shell thickness at higher angle of orientation. For manufacturing time maximum layer thickness, lower degrees' orientation angle and lowest shell thickness gives best results. Instead of all three, the layer height was the most affected parameter on the response characteristics. Because the lower layer thickness or layer height gives good bonding strength and gives better axial loading capability.

Matter [23] The author tries to explore the effect of infill percentage, layer height and infill pattern on the mechanical characterisation of Poly Lactone Acid. The three process parameters set up to five levels. The results show that the infill percentage gives significant effects on strength of the PLA parts. If infill percentage changes from 10 to 100 percentage, the strength of the parts increase from 11 to 46 MPa. The layer height or layer thickness vary from 0.10 mm to 0.30 mm. Increase in layer thickness changes the value of strength up to 21 %. The third parameter, linear infill pattern gives maximum results with compare to other five.

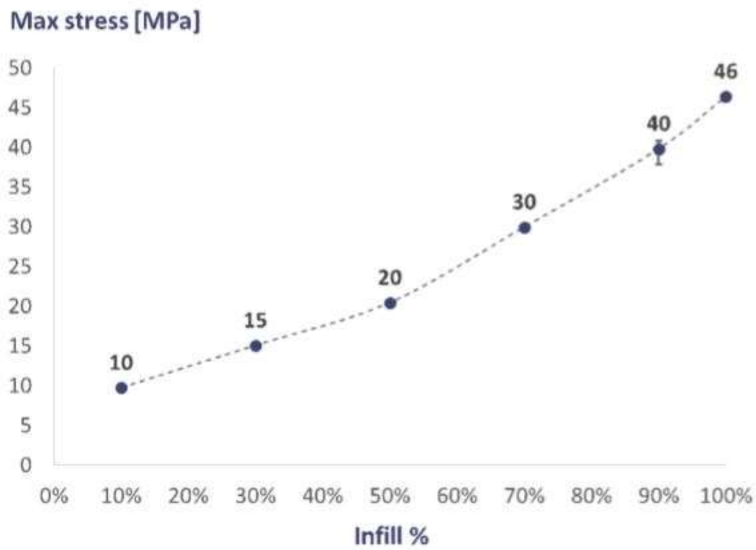


Figure 2.2 Effect of infill percentage on tensile strength

Valentina et al. [24] Polymeric waste is becoming a big issue for environment day by day. The use of alternative bio degradable polymer is a possible strategy to overcome this problem in future. Among of the them, PLA is a best bio degradable polymer due to its biocompatibility and mechanical properties. The control of degradation rate of polymer is major concern of this research. The PH value of solution directly affect the degradation rate of PLA. In this research, bio and eco-friendly nanofiller fumaric acid is used to control the degradation rate of the polymer. The increase and decrease rate or time scale of bio degradability with soma additives and control the rate of hydrolysis rate.

Lopesa et al. [25] Some bio absorbable polymers are identified for biomedical applications, since the polymers are biodegraded by simple hydrolysis. Few polymers are highly biocompatible, such as PLA, PGA, and PCL, and their copolymers are commonly used as fixation devices. Among the biomaterials used in tissue engineering, the PLA has significant attention. PLA is at present one of the most promising biodegradable polymers for biomedical field. It has very good mechanical profile, thermal and biological properties, like biocompatibility and biodegradability. PLA generally produced from naturally organic acid which can produced by fermentation. The purpose of the study was to explore the synthesis of PLA in laboratory to characterize it according to the biomedical needs.

Dann da Silva [26] PLA is a naturally degraded biomaterial, over the time it well-tolerated and safe degradation products, which are discharge from the body. The degradation of PLA polymer is affected by various parameters. For in-vitro study, the temperature of the solution directly effects the degradation rate of polymer. It enhances the degradation speed with four-time high speed at 37^0 c instead of 25^0 c.

Yachouh et al. [27] PEEK material is widely used in various biomedical fields. These implants are substitute of metal and ceramic based tissue engineering in a cranial, orthopaedic and spine surgeries. Author try to compact the changes in the shape and the surface of the PEEK implants. A small implant is described in vitro and in vivo studies, to examine the structure shape and surface changes in implants. The 3D printed implants will not possess the same surface properties like designed ones. The laboratory studies confirmed that new methodology are needed.

Park et al. [28] The author tries to express the biomedical applications of 3D porous scaffold in tissue engineering. The fabrication of polycaprolactone (PCL) polymer part with additive manufacturing with different density. The fabricated parts immersed in simulated body fluid for different days and check the biomimetic surface coating of hydroxyapatite on the surface of parts. The biological properties of the PCL polymer examine by using the WST-1 assays of alkaline phosphate and gene generation of D1 mouse stem cells. The biomimetic scaffold shows the favourable apatite formation and cell proliferation. It shows that the PCL scaffold is promising material in biomedical applications.

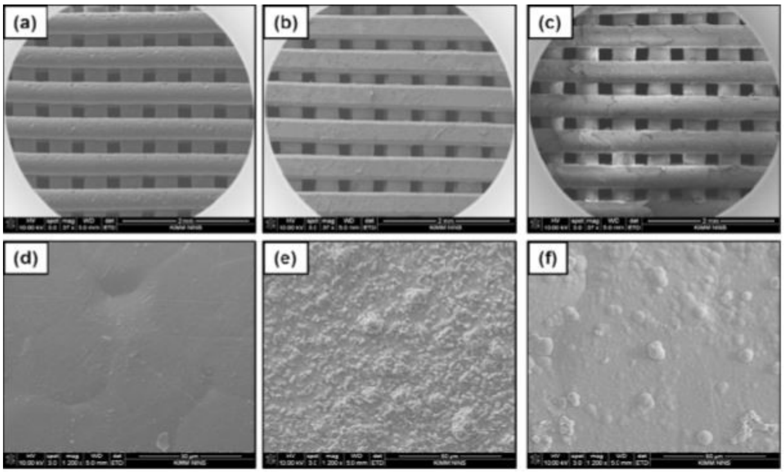


Figure 2.3 SEM of PCL scaffold, (a,d) SEM image of PCL without formation, (b,e) mineralized in SBF for 3 days and (c,f) for 7 days.

Rodrigues et al. [29] The purpose of this study to examine the effect of laser cutting on PLA scaffold. The manufacturing of the part with two methods, 3D printing of polymer scaffold and laser cutting of polymer scaffold from 3D printing parts. There is no significant effect of laser cutting on the degradation behaviour and inter connectivity of the PLA scaffolds. The mechanical characterisation of PLA scaffold is directly affected by laser cutting. The compressive module and stress at yield were decreased by laser cutting. After immersion in PBS solution for 8 weeks of both parts, there is no significant change in molecular weight and mechanical strength.

Zhao et al. [30] Poly lactone acid implants may suffer from low degradation rate and osteoconductive reaction. To minimize this problem, author tries to mix magnesium particles with poly lactone acid to increase the degradation rate and biocompatibility of the PLA implants. PLA were fabricated with solvent casting technique with 2% and 5% of magnesium particles. The fabricated parts immersed in simulated body fluid for different days to examine the in vitro study of degradation rate. The results show that the degradation rate increased with increasing the percentage of magnesium particles. The cell culture revealed good biocompatibility of magnesium and PLA composite.

Giuliana Gorrasi [31] This tries to focused on a detailed analysis of the hydrolysis process of different three grades of poly lactone acid with different morphology with different processing conditions. The three kinds of samples with 15%, 4% and 2% lactide are prepared. The hydrolysis process was examined in distilled water at 58^0 c. No change in Ph value up to 10 days from starting the test but after 20 days of hydrolysis, all samples reached same value of Ph. The results show that the sample with higher lactide value, present the high weight loss. So, it is observed that the lactide value play an important role in degradation.

Choy et al. [32] The author tries to express the importance of the degradation rate of PLA for biomedical applications. The minimum degradation rate of PLA polymer reduces its applications in special cases, where required fast degradation rate like fixation device and drug delivery. The main example of fixation device is a bone fixation screw (absorbable). If the degradation of bone screw is low, the lifespan of the screw would outlive the healing period of the wound site, hindering wound repair. The use of additive lauric acid can increase the degradation rate and also decrease strength of the polymer within 2 weeks.

Alexis et al. [33] The author tries to express the factors that affect degradation rate of poly lactic acid. To examine the effect of important factors on degradation rate, various test was conducted. Composition directly affect the degradation rate, with increasing the content of lactide, will accelerate the degradation rate. Low degradation rate will be with higher molecular weight. The size of the matrix is directly propositional to the degradation rate, the sample with large size will degrade first. The PH value of the solution for in vitro study is directly proportional to the degradation rate. High degradation rate will be at PH 5 and slow down when the PH value increase to 9.24 or more.

Kai et al. [34] The author tries to express the effects of temperature and relative humidity on the degradation rate of poly lactic acid. The both process parameters change up to three levels. Set the temperature of solution at 28, 40 and 55^0 c and relative humidity adjust at 10, 50 and 100 %. The tensile test conduct to examine the significant effects of both factors. The results show that the degradation rate of poly lactic acid accelerate by the increase in temperature and relative humidity.

Ying Li [35] The author tries to express the effect of mechanical loads on the degradation rate of the aliphatic bio gradable polyester. The experiment shows that the mechanical load can influence the degradation rate of aliphatic polyester. The mechanical load can cause the more cavities on the surface of parts, so that the water molecular easy to diffuse it into inner parts and leads to faster degradation rate.

Lee et al. [36] To examine the enzyme biodegradation, total three types of enzymes are selected, lipase, esterase, and alcalase. Degradation time was diagnosing under particular enzyme conditions. The enzyme degradation has an impact on thickness and width of PLA.

The degree of crystallinity of the PLA grow in first 21 days and then decreased. Alcalase was more effective than lipase and esterase in degradation. Degradation was found in the forms of create cracks on the surface and making the fibre rough. There is no significant change in weight and tensile strength. The propose of the mechanism of enzymatic degradation of PLA, can helpful to control the waste in the textile industry.

Hong et al. [37] The purpose of this research to examine the degradation rate of pure PLA and distiller's dries grain with soluble (DDGS) composite. The 20 % value of DDGS added in pure poly lactic acid to check the degradation rate. The results show that the cracks and voids on the surface of parts due to degradation. The weight loss of the composite after 24 weeks was 10.5 % and on the other side the weight loss of pure poly lactic acid was only 0.1 % during the equal time period.

Lebo et al. [38] The purpose of this to examine the effect of temperature and PH value on the degradation rate of pure poly lactic acid. The degradation rate of pure PLA examined by tracking the thickness of PLA brushes. The solution temperature and PH values give significant change in the degradation rate of the samples. The results show that the degradation rate increasing with increase in PH value up to optimum limit and the increase in temperature also accelerate the degradation rate. If the temperature is around 25^0 c, the degradation time was around 400 hours. But if the temperature increased with 12^0 c or at 37^0 c the degradation time reduce by 4 times the last one.

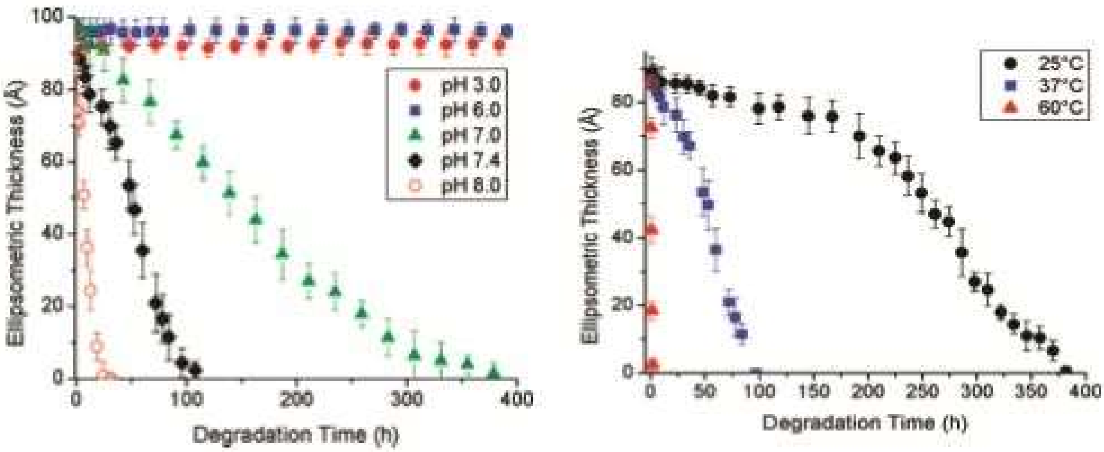


Figure 2.4 effect of pH value and temperature on degradation rate of PLA

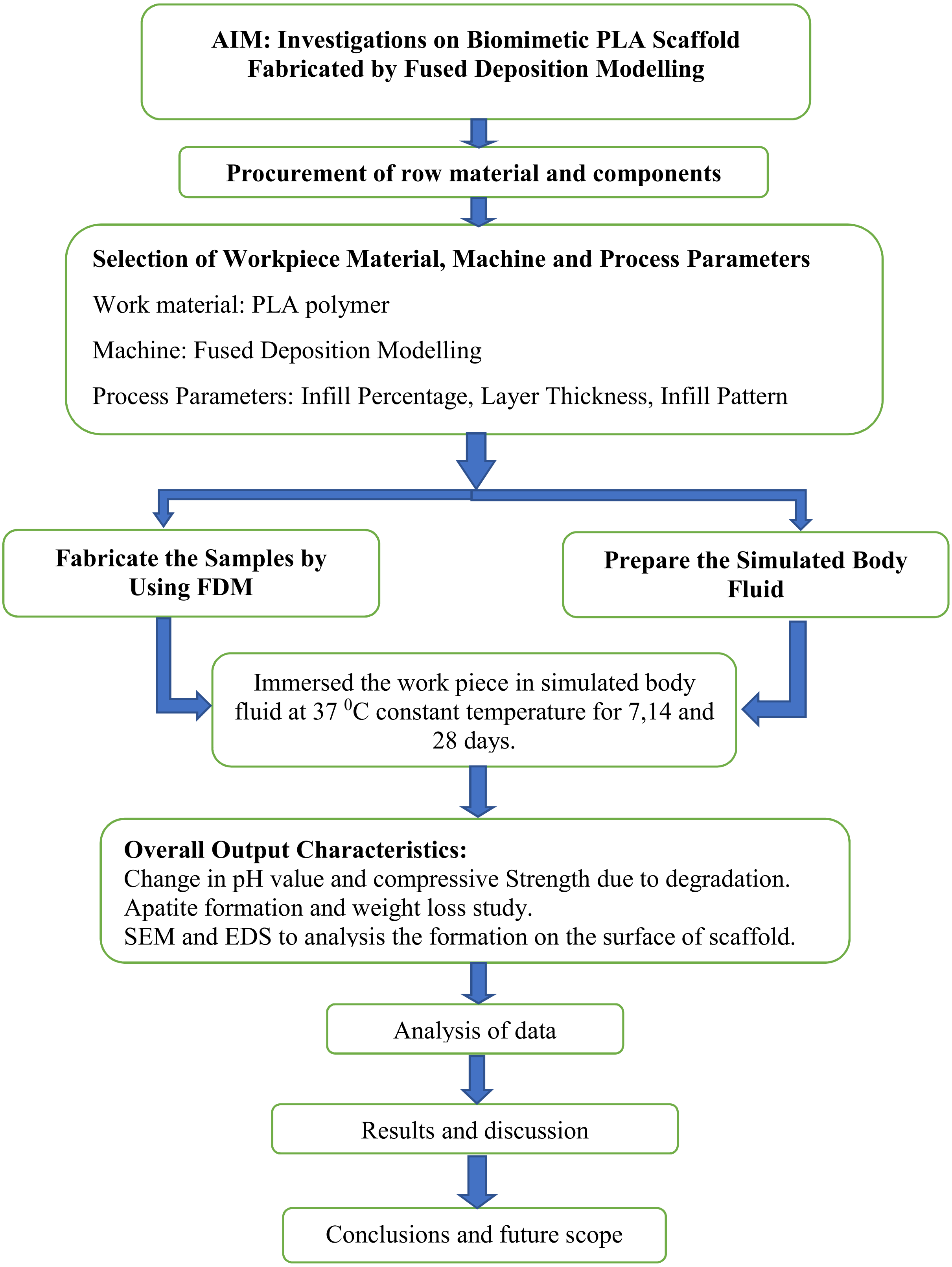
2.3 PROBLEM FORMULATION

From the previous literature survey, it has been founded that the fabrication of polymer scaffold with traditional or conventional methods was widely popular in previous decades. From past few years, number of researchers are working with non-conventional techniques. The additive manufacturing technique becoming most popular in biomedical field. Various types of polymer are used in this area with the help of additive manufacturing. PLA polymer is one of the best biocompatible polymer and widely used for biomedical applications. The major drawback of PLA scaffold, it is suffering from low degradation rate. So, patient required one more surgery after recovery to remove implants. This study generally focused on the degradation rate of the PLA scaffold and characterise it. The fabrication of polymer scaffold with different process parameters may affect the degradation rate and mechanical characterization of the scaffold.

2.4 OBJECTIVES

- To investigate and analyse the influence of the various process parameters of fused deposition modelling while developing a PLA scaffold
- To characterize the polymer scaffold for its biological assessment such as apatite formation & degradation rate.
- To characterize the polymer scaffold for its mechanical competences.

2.5 METHODOLOGY



CHAPTER 3

EXPERIMENTAL PROGRAM

3.1 GENERAL

Porous structure and polymer scaffold are now a day the focus of various research and development activities. There are number of institutes are working on polymer scaffold worldwide, some of them focusing on characterisation of polymer scaffold, properties, applications and fabrication methods. According to the applications of polymer implants, such methods to fabricate the porous structure, like laser beam machining, Fuse deposition machining, solvent casting, Conventional drilling, Powder compaction etc. In this chapter porous scaffolds are fabricated by additive manufacturing or 3D printing techniques. 3D printing technique is selected based on the applications which requires low density and uniformity of pores in the scaffold. The below mention steps are followed to fabricate the specimens and for experimental results.

3.2 3D PRINTING

Additive manufacturing(AM), such as fused deposition modelling is mutating the printing world and education. Properties of the fabricated parts through conventional manufacturing are understood well enough to accurately forecast their deportment through analytical models. The Additive Manufacturing process parameters affects the properties of parts. AM parameters being necessary to forecast the part behaviour. In this work, the compressive strength test was conduct to check the effect of print parameters on the strength and degradation behaviour of the parts. Since 1980, additive manufacturing called as 3D printing. From that time period, the technology is undergone exploration of new materials and advanced applications [39]. Improvements in the form of print quality give permission to high-technologies industries to fabricate parts that were not possible with traditional machining [40]. The 3D printers are the commonly used AM machines. 3D printing uses an extruder to deposit the heated filament into the form of layers to fabricate the parts. The design of the layers is depending on the process parameters set by user. The mastery to understand how these processes are not common or complete. Proper research and lab testing are required to improve understanding of 3D printing to support this growing technology. The 3D printer, which is used in research is manufactured by Flash-forge Creator Pro with below mention specification. The fig. shows the 3D printer which was used to fabricate the specimen with different process parameters.

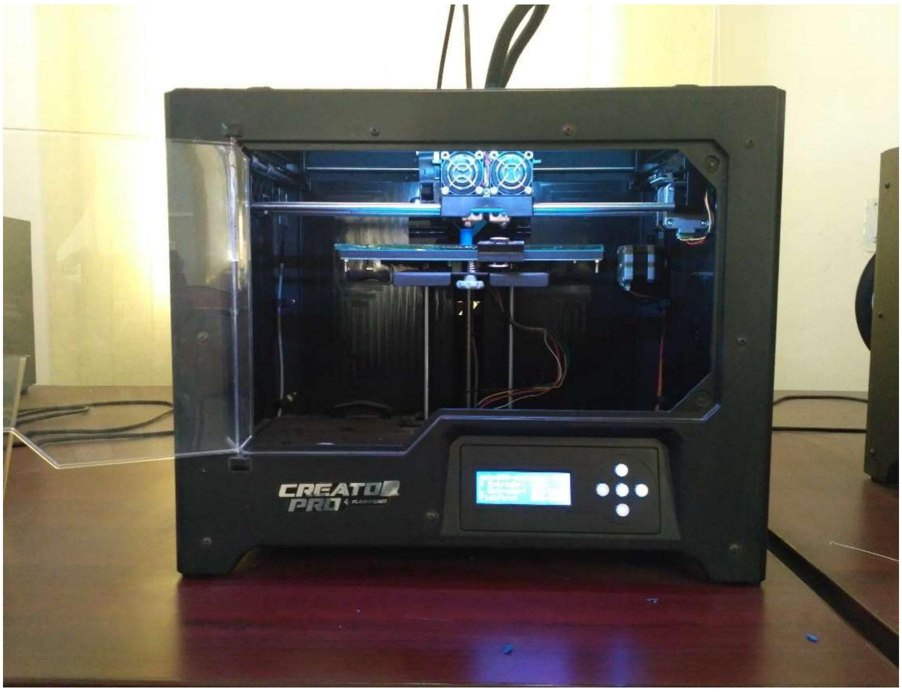


Figure 3.1 3D printer (RP Lab, TIET Patiala)

3.3 TECHNICAL SPECIFICATION OF 3D PRINTER

Table 3.1 Technical specification of 3D printer

Model	Flash forge
Nozzle Diameter	0.4 mm
Operating software	Simplify 3D
Supporting files	.stl
Print inputs	SD card
Input voltage	AC 100-240 v, 50-60Hz
Bed size	150 x 150
Support filament	ABS/PLA

3.4 SELECTION OF MATERIAL

In biomedical applications, there are various criteria for selecting the materials as biomaterials, including their properties such as material chemistry, molecular weight, solubility, shape and structure, hydrophilicity, water absorption, and degradation or erosion rate [41].

PLA is a synthetic polymer that has been found be nontoxic and is approved by the United States Food and Drug Administration as excipients and as carriers in different pharmaceutical formulations, foods, and cosmetics. It has been gaining lot of attention in the field of bioengineering due to it is biocompatibility, biodegradable, and good mechanical properties. It is also approved by the FDA for some human clinical applications such as implantable devices. This is widely used in the orthopaedic surgeries as bone fixation screws and pins [42]. Therefore, PLA is being selected as a work material to the present study.

3.5 PROPERTIES OF POLY LACTIC ACID

Table 3.2 Properties of poly lactic acid with unit values [42].

Property	Symbol	Value	Unit
Specific Density	ρ	1.24	G/Cm^3
Molecular Weight	M_w	190000	G/Mol
Tensile Strength	Σ	66	MPa
Compressive Strength	σ	93.7	MPa
Elongation at Break	E	56	%
Glass Transition Temperature	T_g	55	^0c
Melting Temperature	T_m	150	^0c
Melt Enthalpy	ΔH_m	93.1	j/g

3.6 DESIGN OF POLYMER SCAFFOLD

To fabricate the specimens in 3D printing, the first step to design the specimen in any design software (PRO-E). The dimensions of the specimen are as per American society for testing and materials (ASTM D695). As per standard the height of the specimen should be double to the diameter of the specimen. For optimum outputs, the dimensions for cylindrical specimen was decided as 12.7 mm diameter and 25.4 mm height. The design of the specimen as per dimensions in Pro-E software is prepared. The below mention figure shows the design of cylinder in Pro-E software. H represent the height and D represent the diameter of the specimen.

Figure 3.2 3D design of specimen

3.7 FABRICATION PROCESS OF POLYMER SCAFFOLD

To fabricate the physical part on 3D printing machine requires the following main steps. Firstly, to create the 3D model of the part in design software. Once the 3D model is designed, convert it into a stereo lithography (.stl) file format and saved in system. Once the .stl file is created, it processed by various programs to create the build file for final printing. This technique is also called slicers. Slicers choice the surface of designed model and break it into numbers of 2-dimensional layers. The slicer program builds the way and the nozzle follows that path to lay down of material. This entire process of converting .stl files is called slicing. The process Parameters command the control of print, such as layer thickness, print quality, infill percentage and orientation.

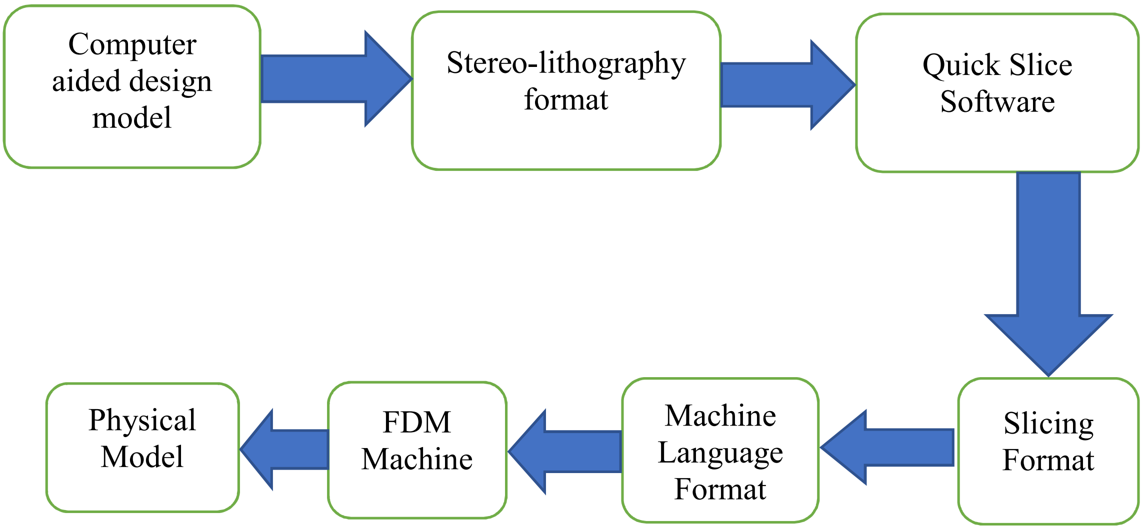


Figure 3.3 Flow diagram of 3D printing

Once the print file is generated, translate it into SD card of the printer. various printers are designed to print from 160°C to 250°C temperature. The printing temperature of ABS around 230°C, and PLA with a printing temperature of 210°C. One of the most significant parameter is glass transition temperature, which play vital role in the fabrication of different materials. There are some important points that should remember for accurate and good quality of printing [42].

1. The build platform must be levelled and depends on the printer, it may automatically or manually.
2. The machine should be on pre- heating before starting the fabrication. Printing will start once the temperature of nozzle reaches the maximum temperature as per parameters.
3. A servo motor control the speed of filament according to the process parameter settings and pushes the filament through the heated nozzle.
4. The extruder head is a combination of servo motor and nozzle. The movement of the head in the X axis and Y axis only, and the build platform moves up and down in the Z axis with the help of servo motor. All these three movements allow the extruded to walk behind the path until the final part is fabricated.

As per showing in the below figure, the part is printing inside the machine chamber. According to the machine configuration, the machine's extruder moves in X- axis and Y- axis directions only. The platform or machine bed moves up and down for movement in Z- axis only. All these movements are controlled by servo motors as per instructions. The movement of the table was as per the specification of the part. Whenever the part is fabricating, the table goes down. The movement of the table in Z-direction as per the later thickness. After completing the one layer, the table goes down equal to layer thickness. The machine also shows the percentage of completed work on screen of the machine.

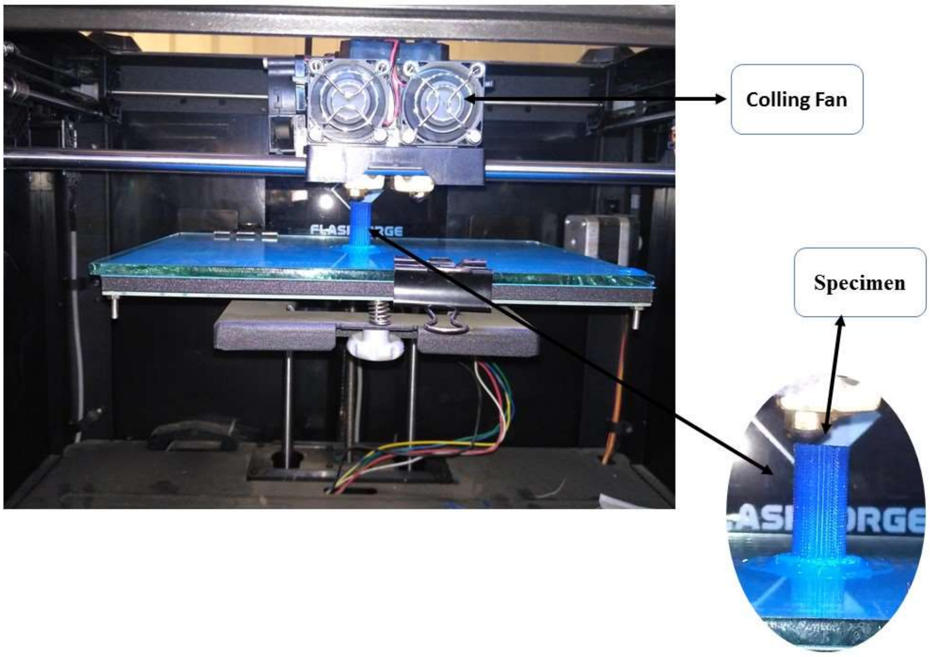


Figure 3.4 Fabrication of Scaffold

3.8 DESIGN OF EXPERIMENT

There are various process parameters effect the properties of the fabricated parts. As per previous literature, change the three most significant process parameters that highly effect the properties of the fabricated parts. The layer thickness, infill density and infill pattern changes as per requirements up to three levels, remaining parameters are constant. Table 3.3 shows the different process parameters and the values of below mentioned process parameters are generally based on literature review and specifications of available equipment. The temperature was selected at 230º c and the printing speed was 2500 mm/ minute. The movement of X/Y axis was 4200 mm/ minute and Z axis was 1200 mm/ minute.

Table 3.3 Values of process parameters

S. No.	Process Parameter	Level	Value
1	Infill Density	1	60%
		2	80%
		3	100%
2	Layer Thickness	1	0.15 (MM)
		2	0.25 (MM)
		3	0.35 (MM)
3	Infill Pattern	1	Rectilinear
		2	Triangular
		3	Honeycomb

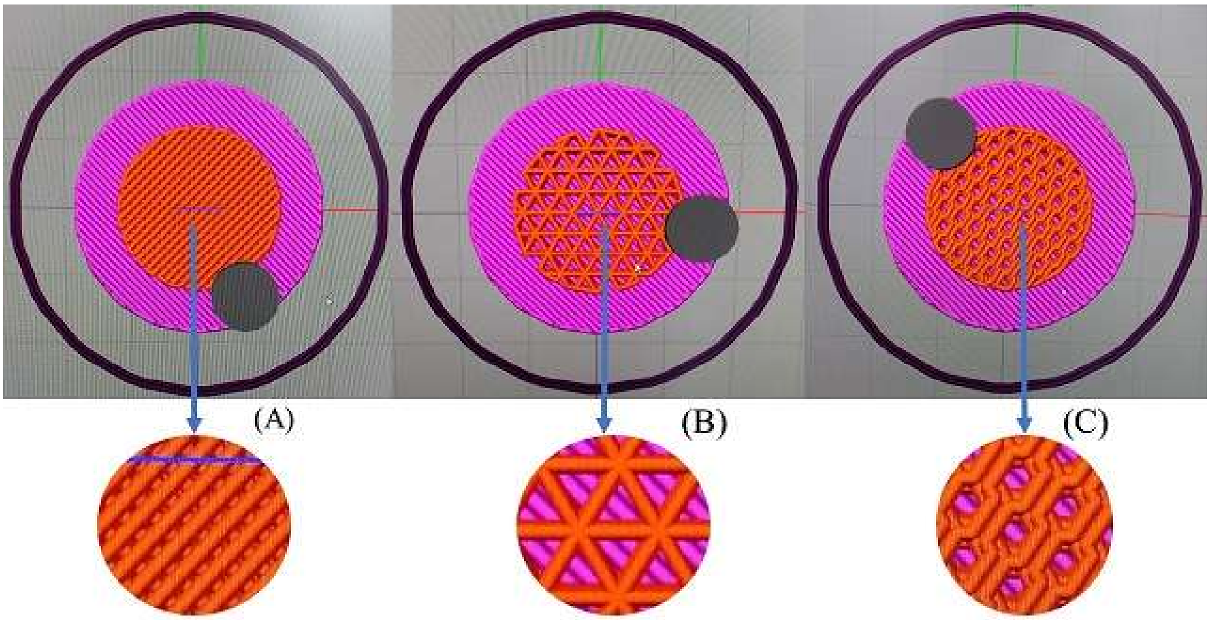


Figure 3.5 Different infill pattern, a) triangular, b) rectilinear, c) honeycomb.

Select the mentioned process parameters up to three levels and prepare the design of experiment in Mini Tab software to check the optimum effect of individual parameter on the properties of fabricated scaffold. The L9 approach is selected to prepare the DOE (Design of experiment). As per L9 approach, nine number of samples was prepared for testing. The process parameters for each part, as per given table.

Table 3.4 L9 Design Approach

S. No.	A	B	C	Infill Percentage	Layer Thickness	Infill Pattern
1	1	1	1	60%	150	Triangular
2	1	2	2	60%	250	Rectilinear
3	1	3	3	60%	350	Honeycomb
4	2	1	2	80%	150	Rectilinear
5	2	2	3	80%	250	Honeycomb
6	2	3	1	80%	350	Triangular
7	3	1	3	100%	150	Honeycomb
8	3	2	1	100%	250	Triangular
9	3	3	2	100%	350	Rectilinear

As per L9 approach, fabrication of nine number of samples with three selected process parameters up to three levels will show the effect of individual. For example, sample number 1 with 60% infill density, 0.150 mm layer thickness and triangular infill pattern. followings number of samples are different process parameters with last one. So, L9 approach is preferred to find the appreciate results. Select these process parameters in software (Simplify 3D) as per sample number and print the part one by one. The principle of 3D printing, to print the part have discussed earlier.

3.9 SIMULATED BODY FLUID

Simulated body fluid is an artificial fluid in which ion concentration almost equivalent to human blood plasma. The SBF has ability of apatite to form on various biomedical materials. It is widely used for in vitro study of various biomedical research. It is basically a combination of various regents, behave like human blood plasma and in other words we can say that it is an artificial blood plasma to assess the bioactivity of various artificial material. The big advantage of this solution is, can save the life of animals that was used during the duration of animal experiments.

In 2003, conventional simulated body fluid with distil recipe was submitted to the technical committee ISO/TC150 of international organisation for standardization to in vitro measurements and ability of implants materials and is being discussed by committee. Following table shows the ion concentration of SBF with compare to human blood plasma [43].

Table 3.5 Comparison of ion concentration of human blood plasma and SBF.

Ion concentration (mM)			
S. No.	Regents	Blood Plasma	SBF
1	Na^+	142.0	142.0
2	K^+	5.0	5.0
3	Mg^2	1.5	1.5
4	Ca^{2+}	2.5	2.5
5	Cl^-	103.0	147.8
6	HCO^-_3	27	4.2
7	HPO^{2-}_4	1.0	1.0
8	SO^{2-}_4	0.5	0.5
9	pH	7.2-7.4	7.4

3.10 PREPARATION OF SIMULATED BODY FLUID

The preparation of simulated body fluid is not such difficult, make sure the prepared simulated body fluid should have transparent and no formation on the surface of preparing bottle. If see any precipitation occur, terminate the process, wash out all the apparatus safely and again restart the process [44].

Prepared the simulated body fluid in the research laboratory of Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala, as per the following steps.

1. Take a 1000 ml plastic beaker and washed it with distilled water and then dry to remove the moisture or water content. Alternative of water, can use sprit to clean the beaker.

2. Take 500-700 ml distilled water in the plastic beaker and cover it with transparent glass.

3. Put the beaker on magnetic stirrer plate and dissolve the weigh regent one by one as per given in the table. Make sure that add another regent when last one dissolve properly.

4. Adjust the temperature of the prepared solution at 37.5^0 c +_0.5^0 c. Check the pH value of the solution and adjust it around 7.4. To adjust the pH value can add 1N- HCL as acid and TRIS as base. After getting required pH value, pH meter is removed from the solution and add water up to 1000 ml [44].

5. The total volume of the water should be 1000 ml if use the quantity as per regent table.

6. Transfer the solution from beaker to plastic bottle and stored it in cold room, below 10^0 c temperature. Make sure the solution should be transparent, if see some substances in solution during storage. Please don't use this solution and prepared it again.

Table 3.6 Quantity of different regents in SBF.

S. No.	Regents	Amount
1	NaCl	7.996 g
2	$NaHCO_3$	0.350 g
3	$K_2HPO_4\ 3H_2O$	0.228 g
4	KCl	0.224 g
5	$MgCl_2\ 6H_2O$	0.305 g
6	1M-HCL	40 ML
7	$CaCl_2$	0.278 g
8	Na_2SO_4	0.071 g
9	$(CH_2OH)_3\ CNH_2$	6.057 g

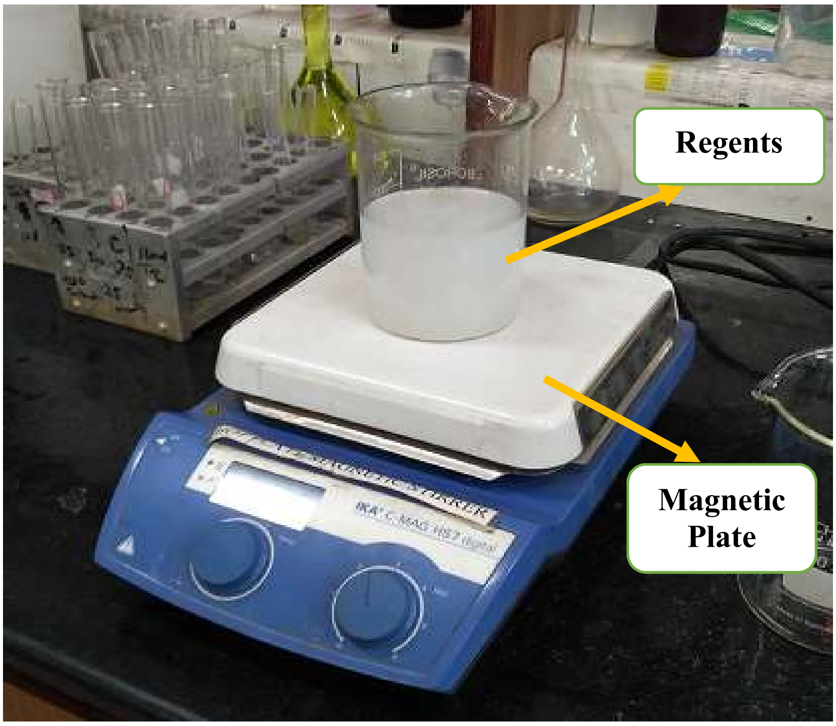


Figure 3.6 Preparation of simulated body fluid on hot plate magnetic stirrer. (TIET)

3.11 PRESERVATION OF SPECIMENS IN SIMULATED BODY FLUID

After preparing the simulated body fluid and fabricate the parts of poly lactic acid scaffold, preserve these parts in simulated body fluid for biological assessment and mechanical characterisation. There should be zero moisture content in the parts, so heat up all parts at 50^0 c for an hour and take the weight of each sample with mg weigh machine. Fill the SBF solution in 150 ml plastic bottles and labelled these bottles with label strip from L1 to L9 as per the number of design of experiment. Putt all the samples in filled bottles as per serial number and tight the lid.

The temperature of the solution should maintain within the range equal to normal human body temperature. So, the temperature controller incubator used to store the bottles. The temperature of the incubator adjusts at 37.5^0 c and revolution per minute around 130 RPM. Fixed all the bottles inside the incubator, close the door properly and switch ON the incubator.

Note: The inside temperature of incubator should be close to 37.5^0 c.

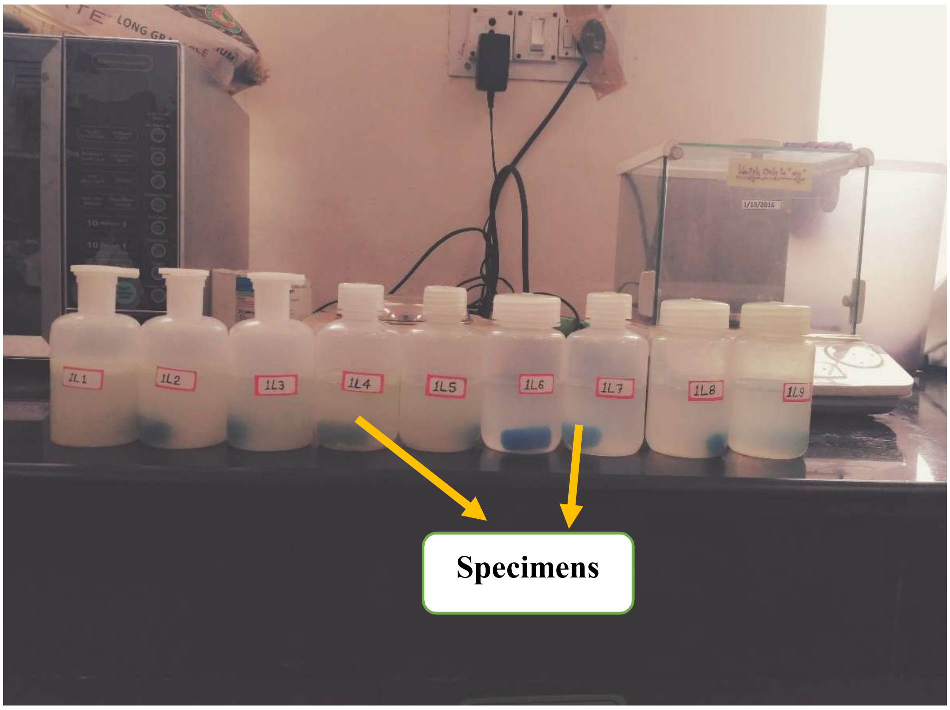


Figure 3.7 Different specimens in SBF solution (Research Lab 2, TIET)

Figure 3.8 Specimen placed in incubator (Research Lab 2, TIET)

3.12 SPECIMENS CHARACTERISATION

3.12.1 Porosity

To check the porosity of each sample, the Archimedes principle volume of each samples need to measured. It is a best and easiest way to find out the porosity of each sample. The Archimedes principle indicates that when a body is immersed in a fluid which is filled in glass tube, to check the porosity or volume of samples, using the cylindrical measuring glass.

To check the porosity of parts, firstly measure the volume of each parts and fill the cylindrical glass vessel up to a fix level with water. After that, fabricated part is immersed in the fluid. When the part will pour inside the fluid, the level of fluid on glass vessel will increase definitely, that is the volume of part.

The porosity of each sample can be defined with mathematical calculations:

Volume of the dense sample = $\mathbf{V_d}$

Volume of fabricated sample = $\mathbf{V_f}$

Volume of voids = $\mathbf{V_v = Vd - V_f}$

Percentage of porosity = $\mathbf{V_v / V_d \ X\ 100}$

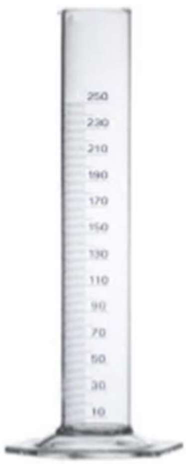


Figure 3.9 Measuring cylindrical glass

3.12.2 pH Value Test

Test the pH value of any substance tells us, it is acidic or base. The pure tap water or unpolluted tap water normally close to 7 pH value. The pH of the SBF solution directly affect the degradation rate of the polymer scaffold. The higher acid nature of the solution or lower pH value enhance the degradation rate of the scaffold. The technique to check the pH value of the SBF solution after particular time period is as per following steps:

1. Firstly, need to calibrate the probe and meter for accurate results. Wash the probe of the meter with pure water and dry it with tissue paper.
2. Take the optimum amount of solution and dip the probe of pH meter inside it.
3. The probe sits for some time so that the value can stabilize.
4. After some time, the meter comes to equilibrium state, and shows the final value.
5. The pH meter can provide the reading on scale up to 0-14. The value of pure water should close to 7.

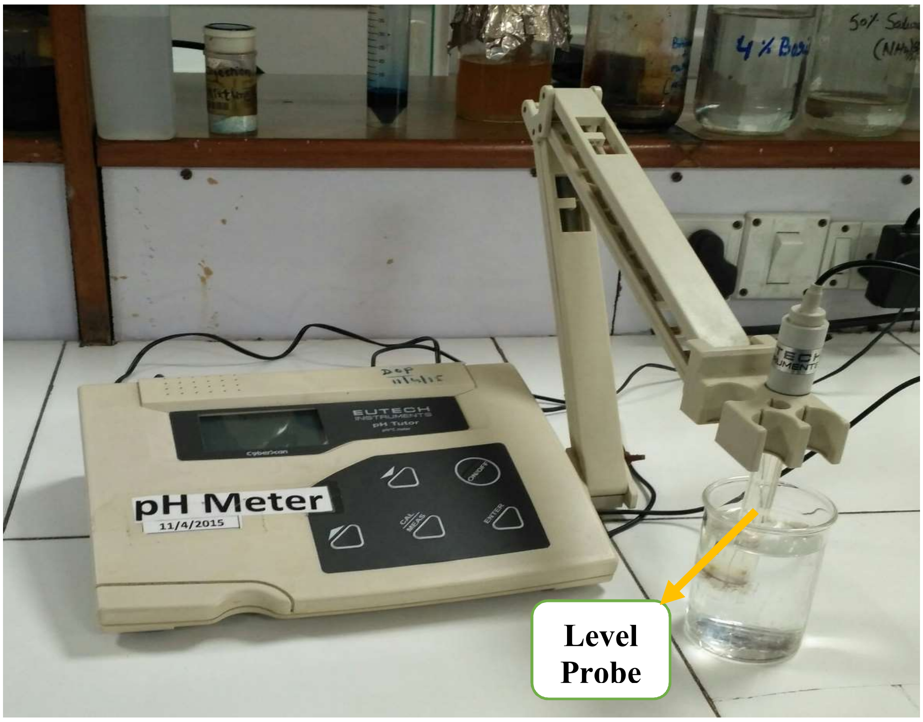


Figure 3.10 pH meter (Research Lab 1, TIET)

3.12.4 Degradation Rate

Weight loss study is best way to check the degradation rate of each sample. Dry the specimen at 50^0 c in oven for an hour to remove the moisture from it. Check the weight of each specimen after drying with weigh machine and consider this weight as initial weight. Now immersed these specimens in SBF for 14, 28 days' time period. After that, take out these specimens gently from SBF at particular day and washed it with distilled water for 4-5 times. To remove the water content, dry these specimens again in oven for one hour at 50^0 c. Remove all specimen from oven after one hour and take the weight of each specimen at weigh machine, consider this weight as final weight. Compare the initial weight and final weight to calculate the degraded weight of each specimen.

Weight loss (W_D) = Final weight (W_F) – Initial weight (W_I)

Weight loss % = W_D/ W_I X 100

Figure 3.11 Weigh machine (Research Lab 2, TIET)

3.13 COMPRESSIVE STRENGTH

The construction of universal testing machine can divide in to three parts:

- Hydraulic system
- Loading frame
- Electrical system

The working principle of universal testing machine is control panel based. It consists of displacement type piston pump with free plunger, which help to maintain the high pressure. The pump is electrical motor operated and fixed at the bottom of the tank. During loading condition, the return valve is closed and the oil delivered to the cylinder through control valve. The pressure starts developing and when the specimen breaks, the value of load controlled by dyno meter. The ON and OFF switches are available on the control panel to start and stop the machine.

Table 3.7 Technical specification of UTM.

Model	H50KS
Measuring Capacity	50 kN
Measuring Range	0.2 – 100 %
Resolution of Piston Movement	0.1 µm
Overall Dimensions	1100 x 405
Test Speed	0.001 – 500 mm/min

3.13.1 Process for Compressive Strength Testing

The size of the specimens is as per ASTM-D695. The polymer based cylindrical samples with dimensions such as 12.7 mm diameter and 25.4 mm height selected for compression testing. All the compression test was conducted with universal testing machine having 50 KN capacity. The total nine number of samples were tested in each result. The load which is applied should always gradually in control manner in increment of 0.5 KN by servo-controlled panel. The speed of the piston movement is selected 1mm/ minute. To compare the results for each specimen, calculate the compressive strength of each specimen individually. The highest peak in the graph consider as the maximum load bearing capacity of that specimen. Calculate the area of each specimen with Vernier.

$$\textbf{Maximum Compressive Strength (Mpa)} = \frac{\textbf{Ultimate Compression Load}}{\textbf{Area of Cross Section}}$$

Figure 3.12 Universal Testing Machine (MED, IIT Mandi)

3.14 APATITE FORMATION AND MICRO STRUCTURE

Measuring microscope used to check the micro structure of the of the specimens and apatite formation or calcium phosphate formation on the surface of immersed specimens for different time periods. The microscopic test shows the effect of process parameter on the micro structure and apatite layer formation for different time periods. Figure 3.13 represent the microscope that is manufactured by Nikon with four different magnification lenses, they are varying from 10X/0.10 with high magnification to 10X/0.1 with low magnification images.

Figure 3.13 Microscope (Research Lab 2, TIET)

Scanning Electron Microscope (SEM) is used for inspecting topographies of the specimen at very high magnification. SEM magnification can go more than the 300000 X. During the SEM examination the electrons are focused on the mark of the specimen, resulting to shift the energy toward the spot. This technology widely used to examine the cracks and fractures on the surface, formation, bond failure and non-visible defects on the sample. Figure 3.14 represent the SEM that is generally electron-based microscope which can produce an image of part by scanning it. The electrons scanned the part in a raster pattern, and the place of the beam is bond with the detected signal to produce an image. With the help of SEM, can detect the surface of samples, physical defects, formation, cell uniformity inside the part, the results will discuss in next chapter.

Fig 3.14. Scanning electron microscope (SAI LAB, TIET)

The Energy Dispersive Spectroscopy (EDS) technique perceive x-rays release from the sample in the course of shelling by electrons to identify the elemental mixture of the volume. It can examine as small as 1 µm or less then it. The electrons are discharge from the sample's surface. The proceed electron positions are fill up by electrons from a higher state, and an x-ray are discharge to balance the energy difference. When x-rays strike the detector, it produces a charge pulse that is relative to the energy of the x-ray. The pulses are rerouting to the voltage pulses by a charge-sensitive pre-amplifier. The energy, resolved from the voltage quantification, for all incident X-rays are sent to a computer for data assessment. The different spectrum of X-ray energy is assessing to find out the elemental composition at various places of the sampled surface.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 GENERAL

In this chapter, discussed about the biological and mechanical characterisation of the polymer scaffold, such as porosity, pH value, apatite formation on scaffold surface, degradation rate and compressive strength. L9 approach is applied for design of experiment and find the most effective parameter. Table 4.1 shows the build time of each specimen. As per discussed in last chapter (Experimentation), the three process parameters changed up to 3 three appropriate levels. Put these parametric values in simplify 3D software to fabricate different specimens. Each specimen has different build time due to different process parameters. The sample with maximum layer thickness and minimum density take very less time in fabrication. On another side, the sample with minimum layer thickness and maximum infill density takes maximum time in fabrication.

Table 4.1 Build time of the specimens with different parameters.

S. No.	Infill Percentage	Layer Thickness	Infill Pattern	Build time (Mint)
1	60%	150	Triangular	42
2	60%	250	Rectilinear	24
3	60%	350	Honeycomb	17
4	80%	150	Rectilinear	44
5	80%	250	Honeycomb	27
6	80%	350	Triangular	20
7	100%	150	Honeycomb	44
8	100%	250	Triangular	27
9	100%	350	Rectilinear	20

4.2 POROSITY

To measure the porosity of the specimens, Archimedes principal technique have been discussed in previous chapter.

To find the porosity of each specimen with Archimedes principle, firstly calculate the volume of each specimen with mathematical calculation. Measure the diameter (at three different places) and height of each specimen with digital Vernier calliper up to three decimal fractions. Table 4.2 represent the dimensions and calculated volume of each specimen. The volume shows in the table 4.2 is dense volume of each specimen.

Table 4.2 Dense volume of each specimen

S. No	D1(mm)	D2(mm)	D3(mm)	Mean D(mm)	Height(mm)	Volume (cm^3)
1	11.40	11.65	11.70	11.58	25.70	2.71
2	11.55	11.45	11.60	11.53	25.65	2.67
3	11.40	11.45	11.60	11.48	25.60	2.65
4	11.45	11.57	11.60	11.54	25.45	2.66
5	11.40	11.45	11.50	11.45	25..60	2.63
6	11.53	11.62	11.55	11.56	25.70	2.70
7	11.50	11.55	11.50	11.51	25.45	2.64
8	12.00	11.90	11.50	11.80	25.55	2.79
9	11.70	11.65	11.66	11.67	25.70	2.74

The actual volume of the material used to fabricate the specimens can find out with the help of Archimedes principle. After immersing the specimen in water tube, the volume of water is increased equal to the amount of material used to fabricate the specimen. 1 ML increase in water level is equal to 1Cm^3 volume of the specimen.

Mathematical calculation for porosity:

Volume of the dense sample = $\mathbf{V_d}$

Volume of fabricated sample = $\mathbf{V_f}$

Volume of voids = $\mathbf{V_v = Vd - V_f}$ (Eq. 4.1)

Percentage of porosity = $\mathbf{V_v / V_d \ X\ 100}$ (Eq. 4.2)

Table 4.3 represent the honeycomb pattern with 100 % infilling (Specimen No. 7) shows the 25 % porosity level. No doubt it should be close to dense structure but as per micro structure of the honeycomb pattern the extruder nozzle is unable to fill the micro gap. As per specification of 3D printer, the diameter of the nozzle is close to 0.4 mm. so it is unable to fill the micro gaps.

Table 4.3 Porosity level of different specimens.

S. No	Initial Volume (V_d) (cm^3)	Volume with 5 ml water(cm^3)	Actual Volume (V_f) (cm^3)	Voids Volume (V_v) (cm^3)	Porosity (%)
1	2.71	6.48	1.48	1.23	45.38 %
2	2.67	6.62	1.62	1.05	39.32 %
3	2.65	6.42	1.42	1.23	46.41 %
4	2.66	6.95	1.95	0.71	26.69 %
5	2.63	6.70	1.70	0.93	35.36 %
6	2.70	6.95	1.95	0.75	27.77 %
7	2.64	6.98	1.98	0.66	25 %
8	2.79	7.40	2.40	0.39	14.77 %
9	2.74	7.55	2.55	0.19	6.93 %

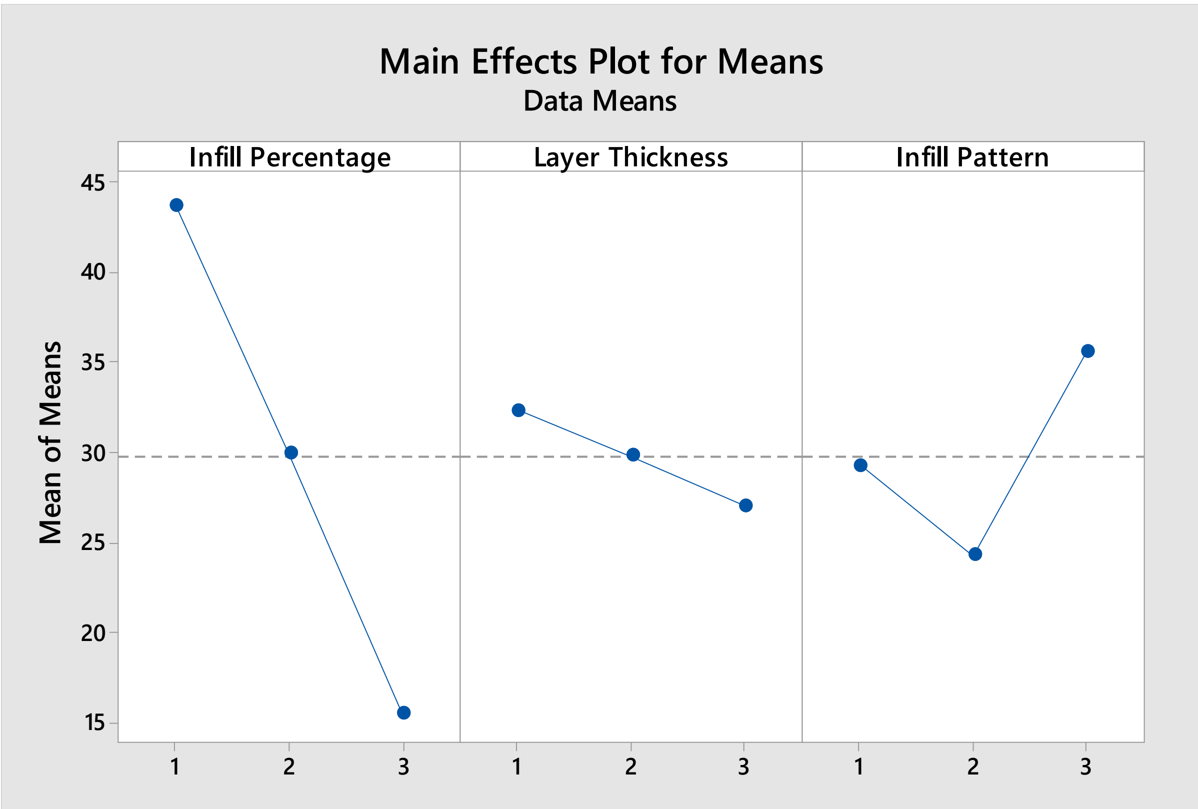
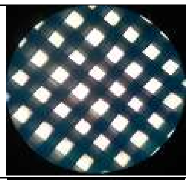
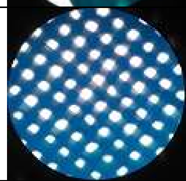
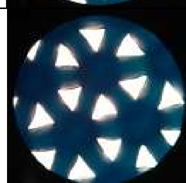
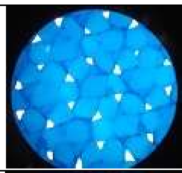
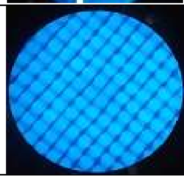


Figure 4.1 Means effect of process parameters on porosity level

Figure 4.1 shows the means effect of process parameters on porosity level of different specimens. The Results shows that the 60% infill density, 0.150 mm layer thickness, and honeycomb infill pattern give maximum porosity level. On another side, 100 % infilling, 0.350 mm layer thickness, and rectilinear infill pattern have minimum porosity level. The results show that the infill percentage have greater effect on porosity level rather then layer thickness and infill pattern. Table 4.4 shows the micro structure of each specimens with different infill percentage and infill patterns.

Table 4.4 Porosity percentage and micro structure with different parameters.

S. No.	A	B	C	Porosity %	Microstructure of specimens
1	60%	150	Triangular	45.38 %	
2	60%	250	Rectilinear	39.32 %	
3	60%	350	Honeycomb	46.41 %	
4	80%	150	Rectilinear	26.69 %	
5	80%	250	Honeycomb	35.36 %	
6	80%	350	Triangular	27.77 %	
7	100%	150	Honeycomb	25 %	

8	100%	250	Triangular	14.77 %	
9	100%	350	Rectilinear	6.93 %	

4.3 pH VALUE

During the in-vitro study of PLA scaffold shows the pH value of the solution changes due to the immersion of pure poly lactic specimens. The pH value of the solution decreases significantly with compare to the control value (Table 4.5). This may the cause of polymer degradation as the by-product of poly lactic acid (PLA) generate an acidic environment. The results show that the pH value of solution continuously decreases with immersion time period. Figure 4.2 A bar chart illustrate the comparison between pH value with respect to days (i.e, 7 days, 14, 21, 28 days). The rate of the pH value is directly related to the porosity of the scaffolds. The higher the porosity more will be degradation rate owing to more exposed surface area. The solution with high porous specimen, shows highly acidic nature. Although, the effect of decreased pH value on a bone tissue is not in the scope of the present study & hence didn't evaluate here. But, this may be taken as a future direction for further study.

Table 4.5 pH value of solution with different time period.

S. No	Control value	After 7 days	After 14 days	After 21 days	After 28 days
1	7.4	6.16	5.25	5.08	5.03
2	7.4	6.10	5.35	5.22	5.15
3	7.4	6.18	5.30	5.06	5.01
4	7.4	6.35	5.80	5.42	5.25
5	7.4	6.25	5.40	5.25	5.11
6	7.4	6.40	5.95	5.39	5.28
7	7.4	6.37	5.84	5.48	5.23
8	7.4	6.48	6.08	5.88	5.64
9	7.4	6.73	6.12	5.92	5.72

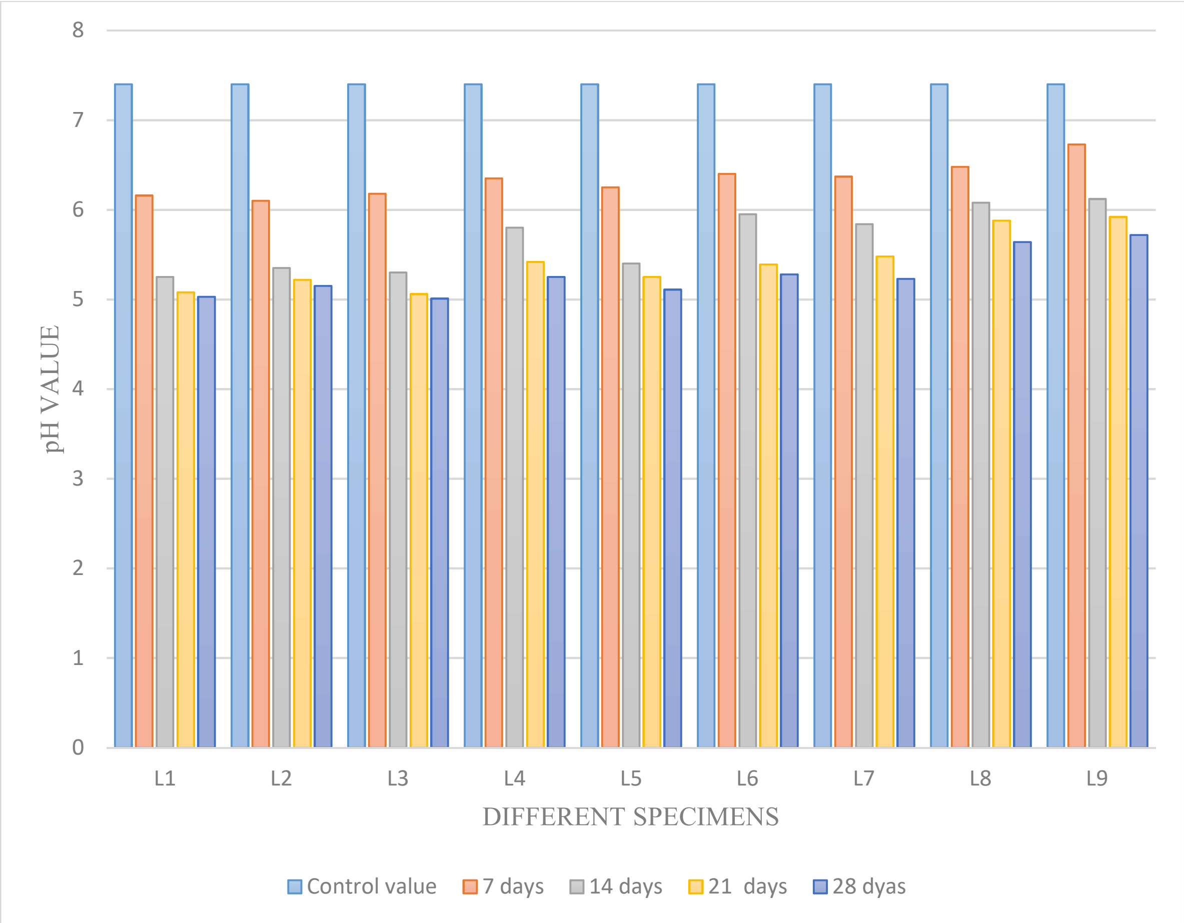


Figure 4.2 pH values of different specimens at different days

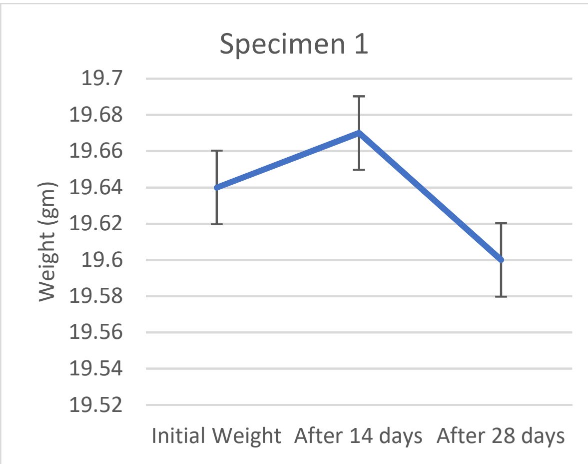
4.4 DEGRADATION RATE

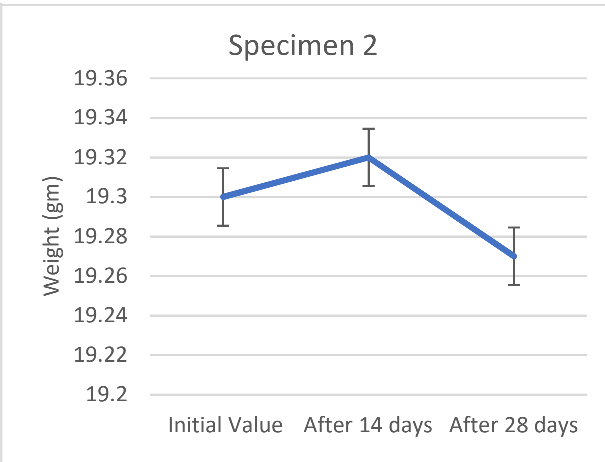
The change in weight of specimen with different time period represent the degradation rate of different specimens. Dry the specimen at 50^0 c for one hour to remove the moisture and then take the weight of each specimen as initial weight. Repeat the same process after immersion of work piece in SBF solution for 14 days and 28 days to check the weight difference.

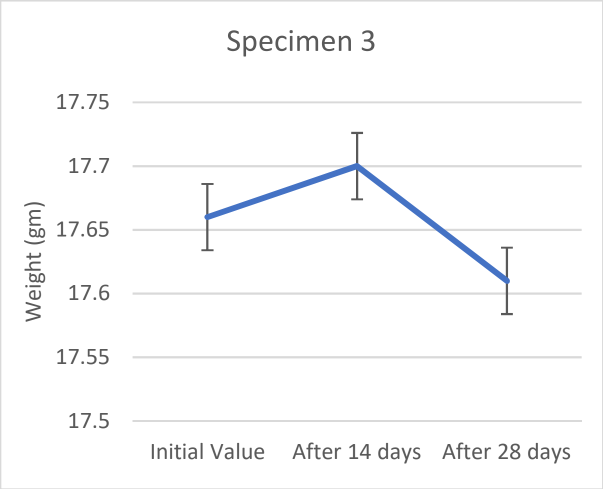
Table 4.6 Change in weight of the specimen with respect to 14 and 28 days.

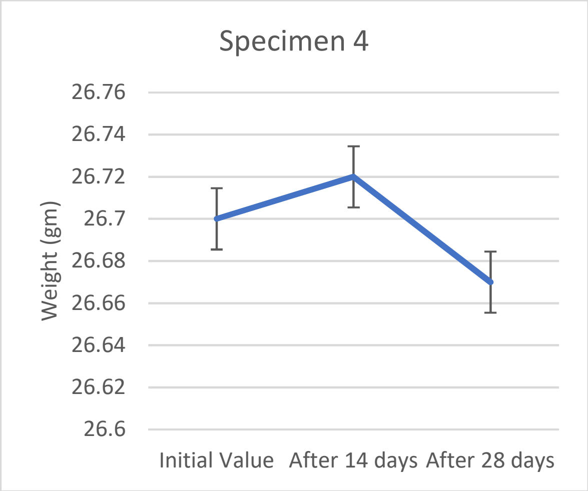
S. No	Initial Weight(mg)	Weight after 14 days (mg)	Weight after 28 days (mg)
1	1964	1967	1960
2	1930	1932	1927
3	1766	1770	1761
4	2670	2672	2667
5	2193	2196	2189
6	2611	2613	2608
7	2641	2643	2638
8	3100	3102	3098
9	2936	2937	2935

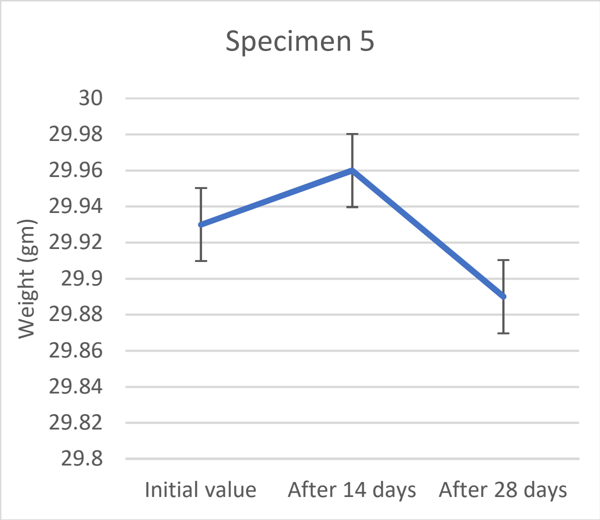
Table 4.6 represent the change in weight of each specimen after different time period. The results show that the weight of each specimen slightly increase after 14 days. It is expected that apatite formation on the surface of specimens may be the dominating phenomenon at the start, hence increase in weight. Although, it is difficult to say that this phenomenon will be for this much of days as the studies not done on daily basis. More insight is required on daily basis for exact duration of apatite formation. After 28 days, weight of each specimen is less. It may be anticipated that degradation rate is now the governing phenomenon here. The specimen is more likely to degrade at a higher speed with the passage of time. Figure 4.3 represent the loss of weight in individual specimen.

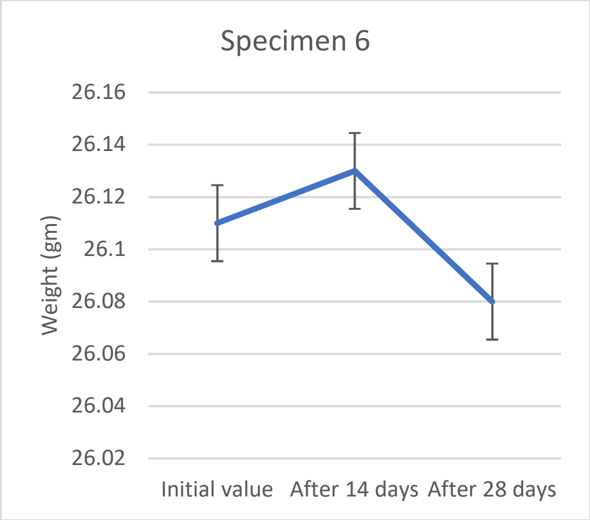


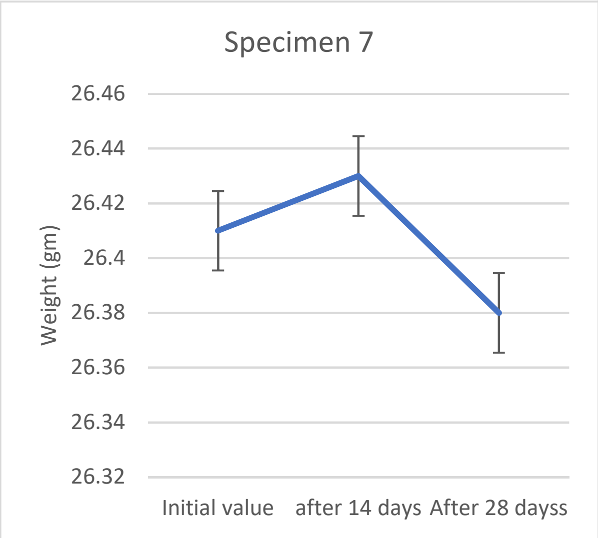


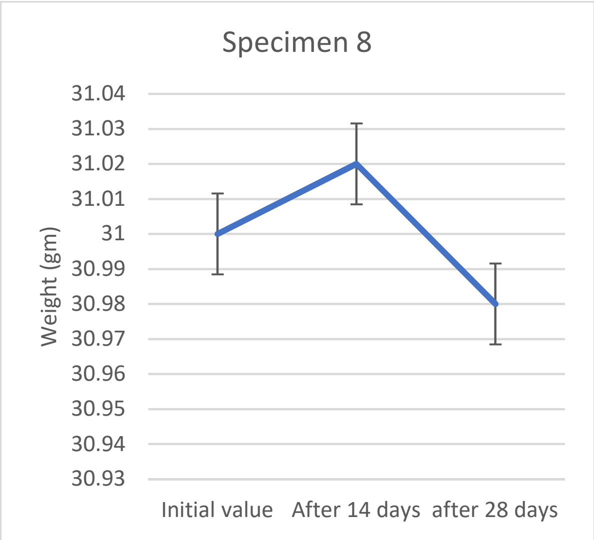

Specimen 3
17.75
17.7
17.65
17.6
17.55
17.5
Weight (gm)
Initial Value
After 14 days
After 28 days


Specimen 4
26.76
26.74
26.72
26.7
26.68
26.66
26.64
26.62
26.6
Weight (gm)
Initial Value
After 14 days
After 28 days


Specimen 5
30
29.98
29.96
29.94
29.92
29.9
29.88
29.86
29.84
29.82
29.8
Weight (gm)
Initial value
After 14 days
After 28 days


Specimen 6
26.16
26.14
26.12
26.1
26.08
26.06
26.04
26.02
Weight (gm)
Initial value
After 14 days
After 28 days


Specimen 7
26.46
26.44
26.42
26.4
26.38
26.36
26.34
26.32
Weight (gm)
Initial value
after 14 days
After 28 dayss


Specimen 8
31.04
31.03
31.02
31.01
31
30.99
30.98
30.97
30.96
30.95
30.94
30.93
Weight (gm)
Initial value
After 14 days
after 28 days

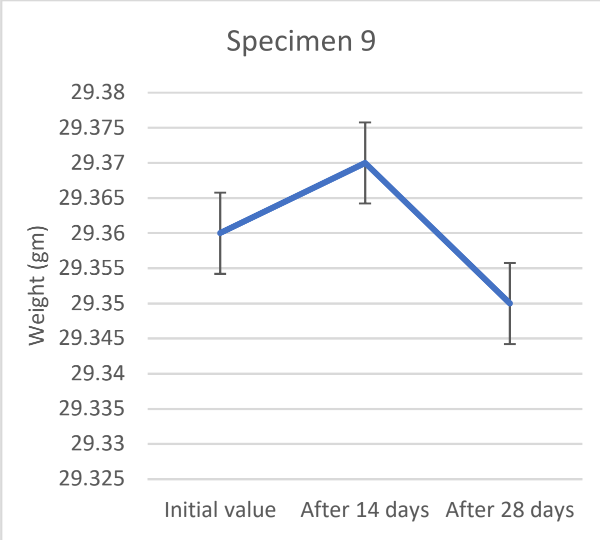


Figure 4.3 Weight loss or degradation rate of each specimen with respect to time.

4.5 COMPRESSIVE STRENGTH

The compressive strength was determined using a universal testing machine at room temperature and the calculations were done. The load was applied on the cross section of the specimens. The cross-head speed of 0.5 mm min. The test carried out up to maximum load bearing capacity with permanent deformation of the scaffold. The maximum load considers as the highest peak value of load graph and calculate the compressive strength with respect to surface area of the specimen. Figure 4.4 represent the universal testing machine during compression testing.

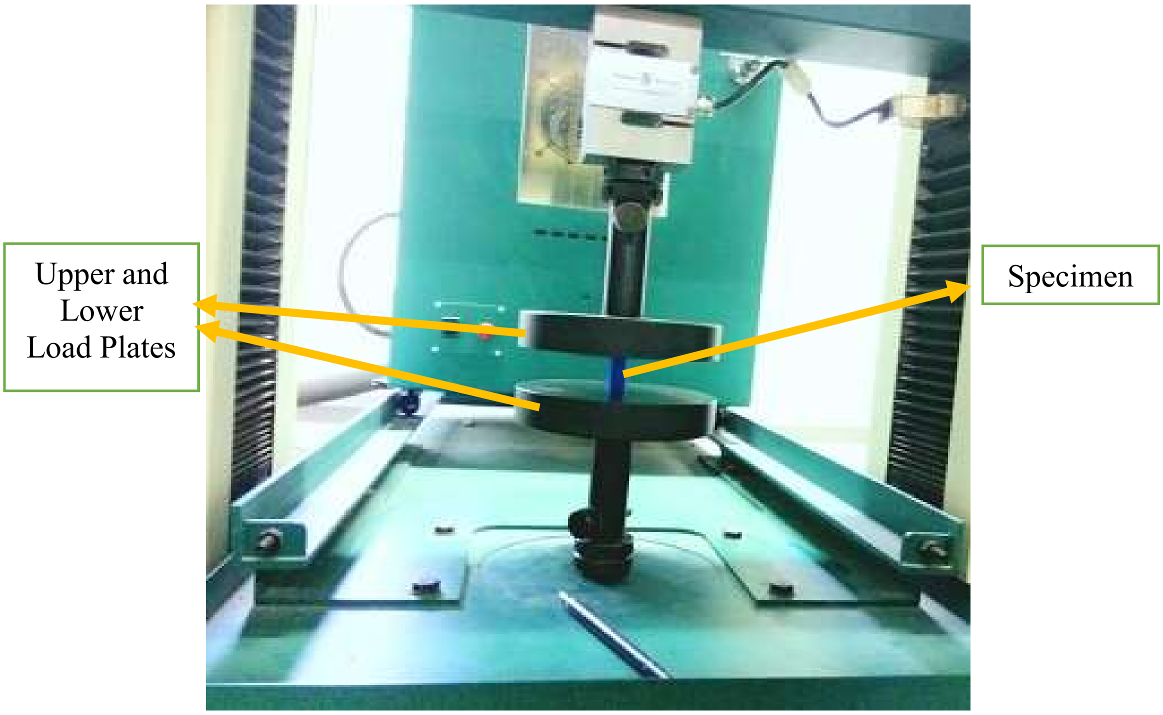


Figure 4.4 Specimen under loading condition (MED, IIT Mandi)

Table 4.7 DOE approach and compressive strength with respect to process parameters.

S. No.	Process Parameters			Ultimate Strength (Mpa)
	Infill %	Layer Thickness(µm)	Infill Pattern	
1	60%	150	Triangular	45.4
2	60%	250	Rectilinear	25.9
3	60%	350	Honeycomb	35.2
4	80%	150	Rectilinear	52.1
5	80%	250	Honeycomb	31.6
6	80%	350	Triangular	61.8
7	100%	150	Honeycomb	63.4
8	100%	250	Triangular	87.9
9	100%	350	Rectilinear	76.1

Table 4.7 results show the ultimate strength of different specimens with respect to process parameters. All three process parameters directly affect the strength of the scaffold. Taguchi's technique used to find out the deviation between the desired value and the experimental value.

This includes three different ways to examine the relation, such as nominal is better, larger is better, smaller is better. The maximum compressive strength indicates of improved performance. This technique used to find out the significant parameters and contribution of these factors on compressive strength.

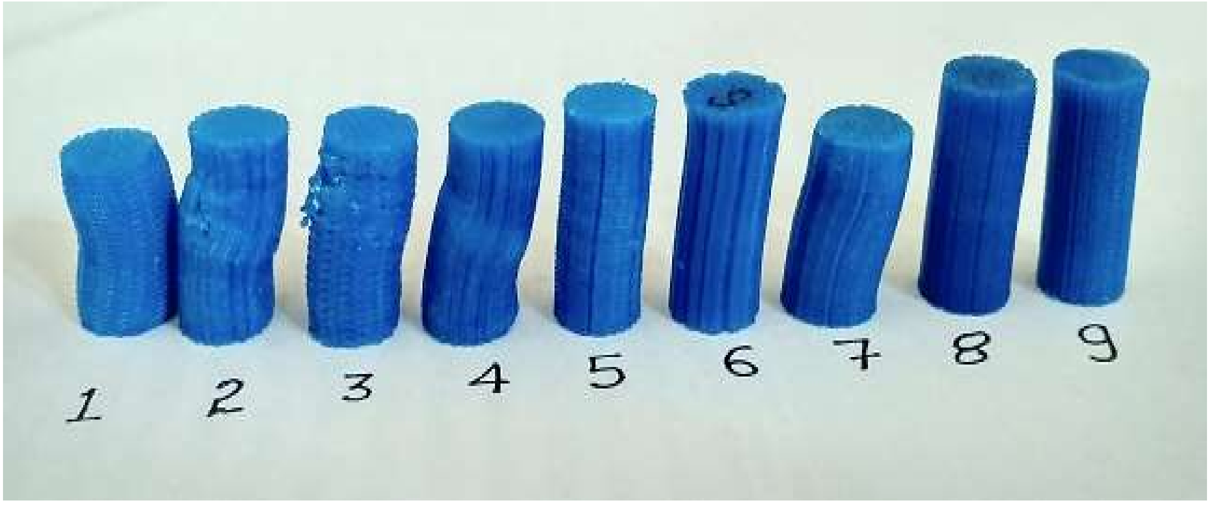


Figure 4.5 Specimens after compressive test

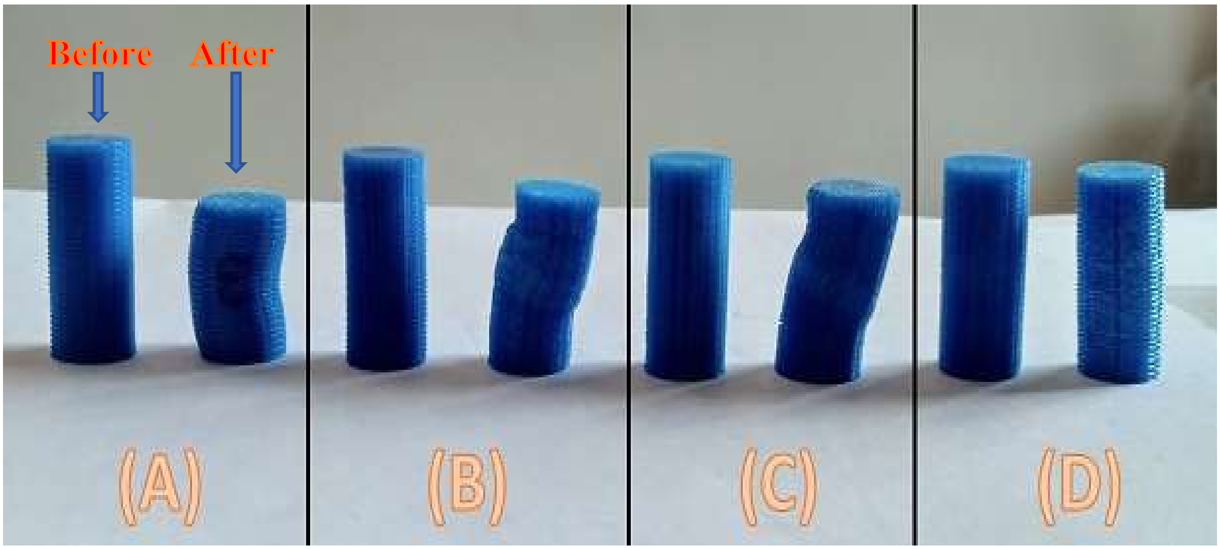


Figure 4.6 Specimens before and after testing (28 days), A) represent the specimen with maximum porosity, b) and c) are medium and D) with maximum porosity level

The figure 4.7 shows the key effect plots for ultimate compressive strength. As per the means value better value for compressive strength obtained by greater value of means. On the bases of analysis, for maximum compressive strength the boosted process parameters are 100 % infill percentage, 0.35 mm layer thickness and triangular infill pattern.

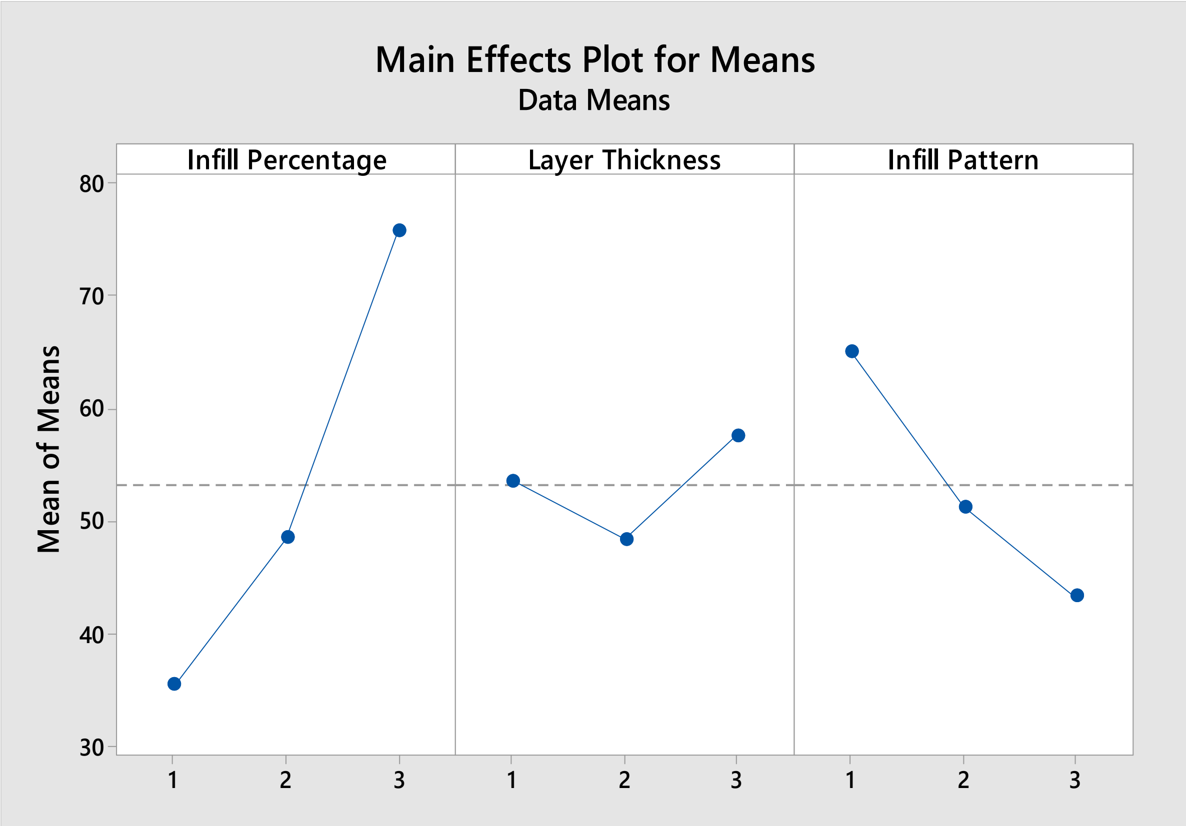


Figure 4.7 means effect value for compressive strength.

No doubt the infill percentage directly affect the strength of the specimen because dense structure shows maximum strength with compare to porous structure. Maximum layer thickness gives maximum strength because with increased layer thickness, few layers are required for given height, therefore number of layer bonds was reduced and strength increased.

Table 4.8 shows the value of compressive strength with respect to time. The results at zero day means the test conduct on different specimens at initial stage or without immersing in simulated body fluid. These compression test values consider as control value to compare the results with different days. The value of compressive strength decreased in all specimens after 14 days. It means the degradation of the scaffolds has been started.

Table 4.8 Different values of compressive strength at various days

S. No.	After 0 days (Mpa)	After 14 days (Mpa)	Decrease (%) after 14 days	After 28 days (Mpa)	Decreases (%) after 28 days
1	45.4	41.7	8.14 %	35.3	22.1 %
2	25.9	23.8	8.10 %	21.2	19.2 %
3	35.2	31.2	12.8 %	27.4	22.2 %
4	52.1	47.8	8.25 %	45.3	13.1 %
5	31.6	28.3	10.4 %	25.9	18.1 %
6	61.8	58.3	5.66 %	52.4	15.3 %
7	63.4	58.9	7.09 %	55.1	13.2 %
8	87.9	84.38	4.00 %	80.9	8.1 %
9	76.1	73.81	3.002 %	71.5	6.1 %

The second set of specimens carried out from SBF solution after 28 days and repeat the same process for testing. The compressive strength of all the specimens highly decreased after 28 days with compare to 14 days. After few days the compressive strength of the specimens start decreasing due to the reason of degradation of the scaffold. The figure 4.7 represent the value of compressive strength at different days. Porosity level directly affects the compressive strength and degradation rate. The specimen with high porous structure having minimum compressive strength and maximum degradation rate. On the other hand, the specimen with highly dense structure having maximum compressive strength and very low degradation rate.

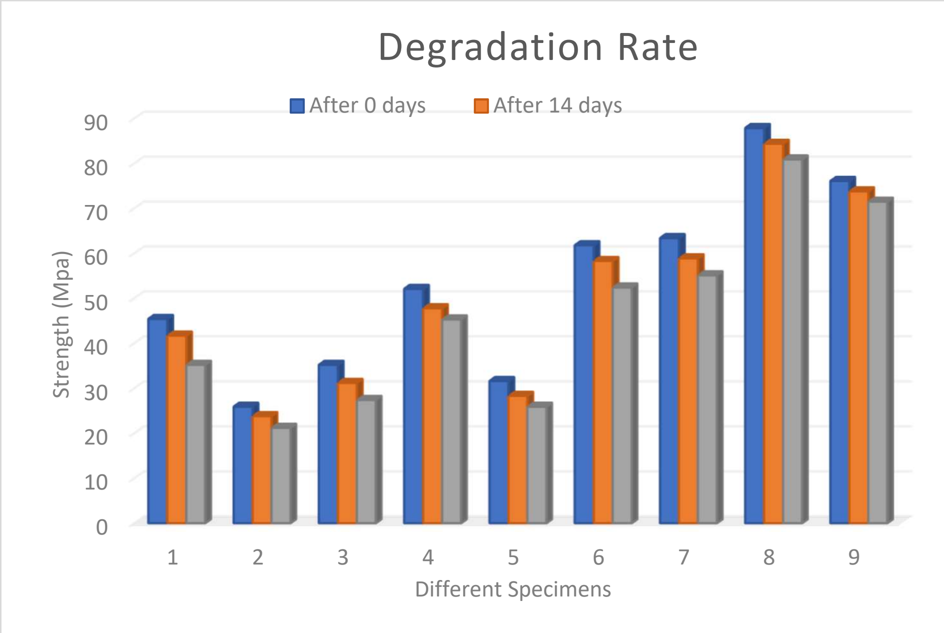


Figure 4.8 Value of compressive strength of different specimens at different days.

Figure 4.9 represent the relation between the porosity and decreases in compressive strength (degradation rate) after 28 days. The change in compressive strength is directly related to the porosity level. The result shows that the highly dense structure have maximum decrease in compressive strength after particular time period. The higher the porosity more will be decrease in compressive strength owing to more exposed surface area.

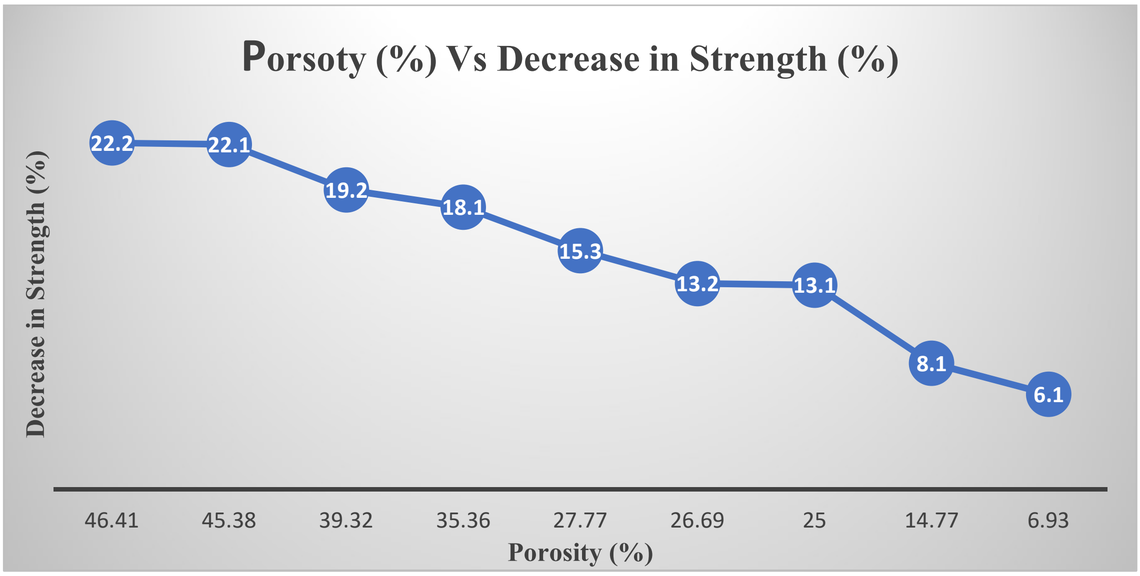


Figure 4.9 Effect of porosity on decreases in compressive strength

4.6 MORPHOLOGICAL BEHAVIOUR AND APATITE AVAILABILITY ON PLA SCAFFOLD

The microscope images of the top view of the different specimens are showing in the figure 4.10. It shows the well define geometry and pore distribution of the scaffold. From A to I clearly shows the different porosity level and infill patterns. The figure A shows the triangular infill pattern with 60 % infill density and figure B and C with shows the same pattern with 80 and 100 % infill density. The figure D represent the honeycomb infill pattern with 60 % infill density and figure E and F show the same pattern with 80 and 100 % infilling density. The parameters setting for figure C and F was 100 % infilling, but it shows the pores. It may the reason of extruder nozzle diameter, because the nozzle diameter was 0.4 mm so it was unable to fill the small porous during fabrication. Figure G, H and I show the rectilinear infill pattern with 60,80 and 100 % infilling respectively. The Specimen with 100 % infilling and rectilinear infill pattern shows very small amount of porosity with compare to others.

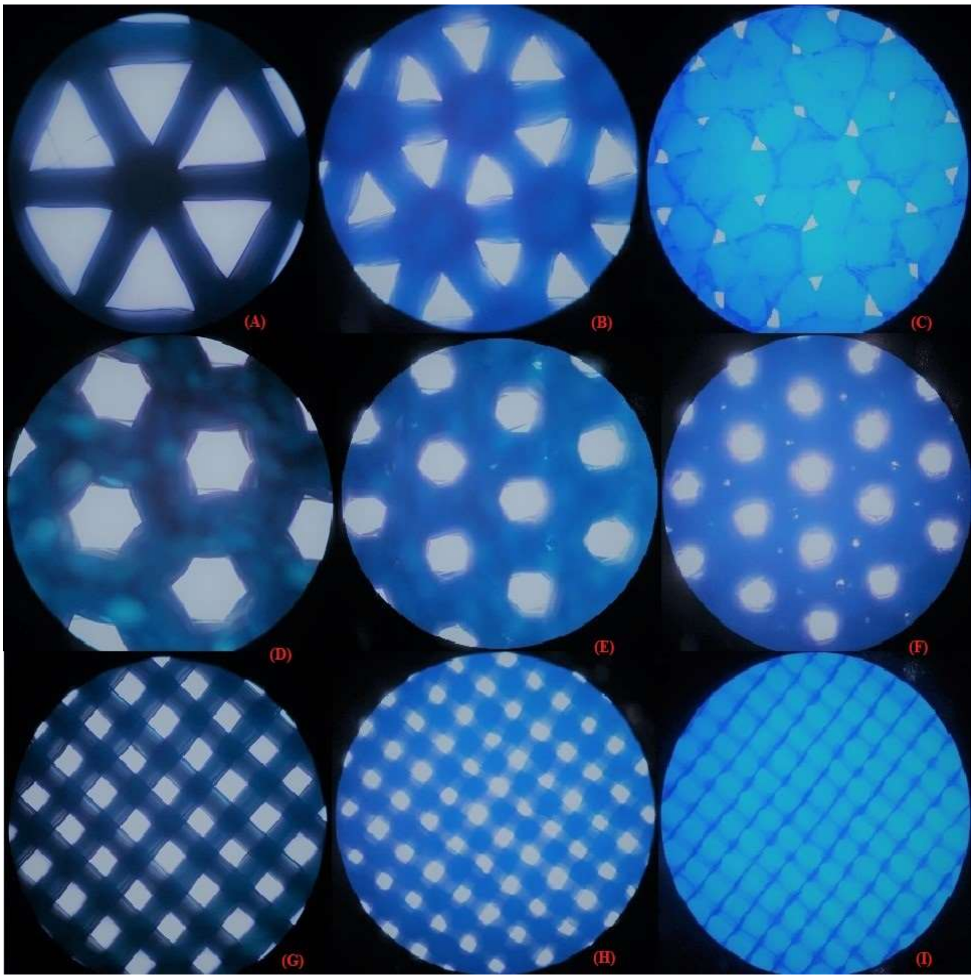


Figure 4.10 Pore morphology of different infill patterns and density

The apatite formation on the surface of the polymer scaffolds shows the biocompatible nature of the poly lactic acid scaffold. The results clearly show the high amount of formation on the surface of highly porous scaffold with compare to dense or low porous scaffold. The figure 4.11 shows the different amount of calcium phosphorous layer on the surface of polymer scaffold. The specimen with honeycomb pattern and 60 % infilling was maximum porosity level and it shows the high amount of formation on scaffold surface. The level of formation is continuously increased with increasing time periods. Its shows the high biocompatible nature of the scaffold.

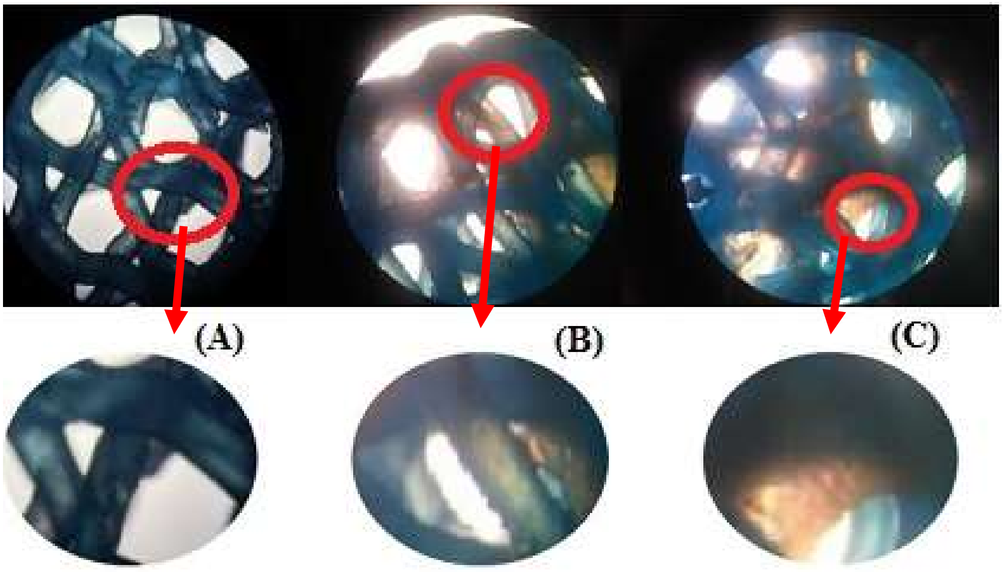


Figure 4.11 Apatite formation, (A) after 7 days, (B) after 14 days, (C) after 28 days

4.7 METALLURGICAL TEST

The high magnification SEM technique inspects the micro layer formation on the surface of specimen. Figure 4.12 shows the different level of formation on highly dense structure (Specimen No. 3) at different days. The results show that the no formation on highly dense structure after 7 days, small amount of formation after 7 days and higher amount of formation after 28 days with compare to 7 days, 14 and 28 days respectively.

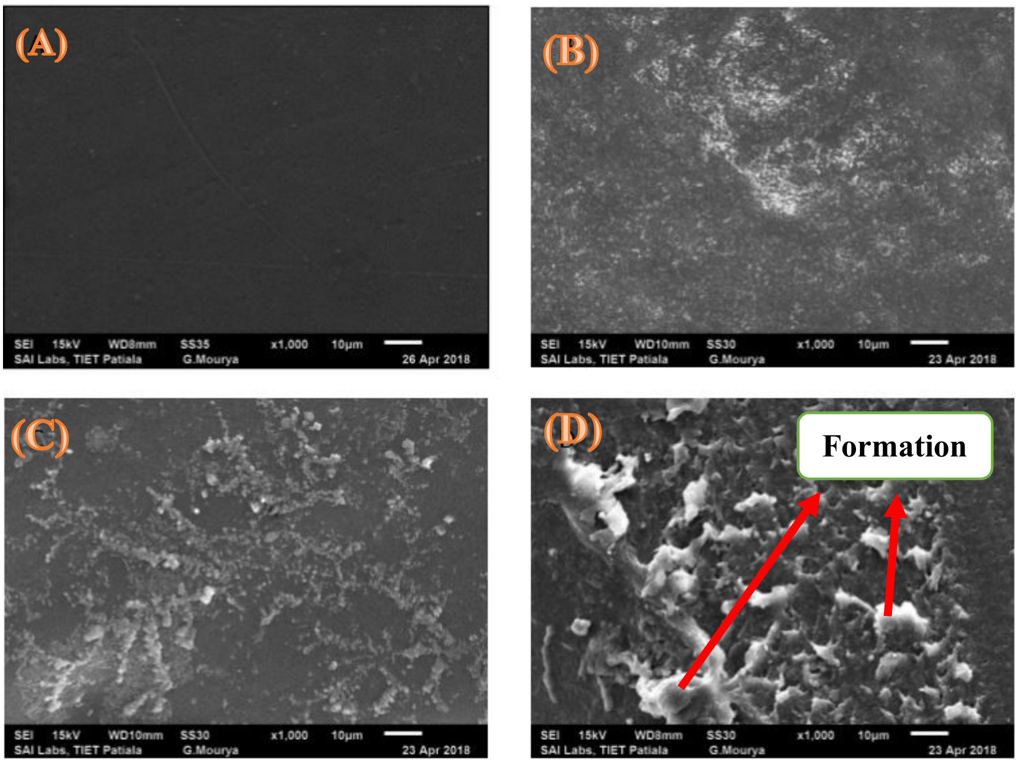


Figure 4.12 SEM image of PLA scaffold with the amount of formation at different days, A) at 0 days, B) at 7 days, C) at 14 days and D) at 28 days.

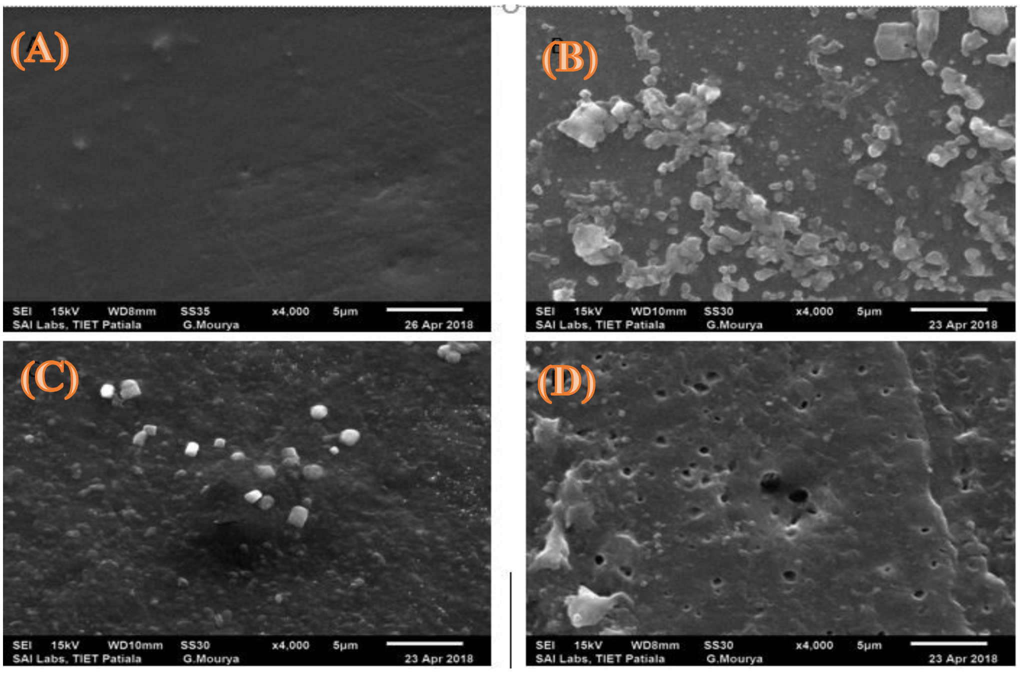


Figure 4.13 SEM images with the calcium formation on PLA scaffold with respect to porosity level, A) represent the control sample or dense sample, B) with high porosity level, C) with medium porosity level and D) with low porosity level or close to dense specimen.

Figure 4.13 represent the specimen with maximum porosity level shows the positive results with compare to dense specimen after 28 days. The porous structure helps the bone tissue to infiltration and growth with compare to dense structure. The tissue engineering scaffold is actually a porous structure which act as template for bone tissue formation. Highly porous structure helps to grow the bone cells and regeneration of bone for fast recovery.

The energy dispersive spectroscopy technique used to check the composition of the elements. Figure 4.14 and 4.15 represent the sum spectrum of entire elements of control Specimen and after immersion of 28 days. Table 4.9 show the relative amount of calcium on the surface of specimens. The weight percentage change with specimens and positions and also compares the atomic weight and weight percentage of different elements with control sample. The pure PLA consider as control specimen to check the elements composition and highly porous structure after 28 days shows the lesser amount of formation on the surface of specimens.

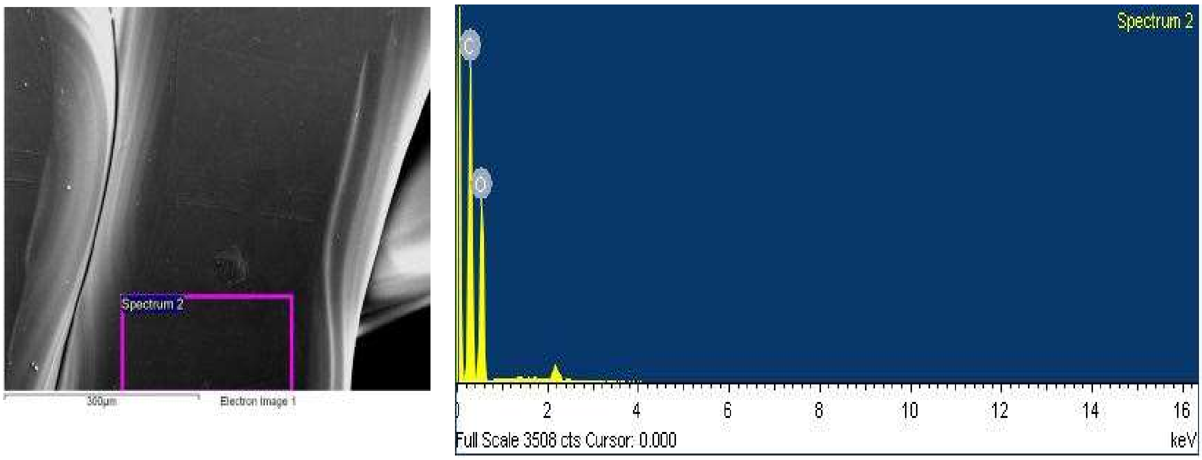


Figure 4.14 Selected surface and sum spectrum of entire elements of control Specimen

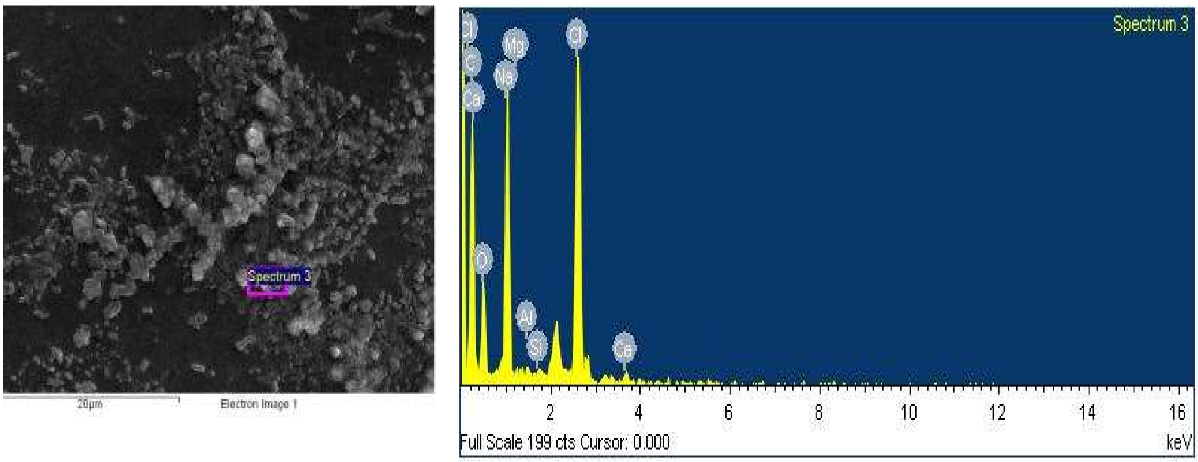


Figure 4.15 Selected surface and sum spectrum of entire elements of the specimen after 28 days immersion

Table 4.9 Shows the results of EDS for entire elements in weight and Atomic %.

Elements	Control Sample		After 28 days immersion	
	Weight %	Atomic %	Weight %	Atomic %
C	50.65	57.75	56.76	70.69
O	49.35	42.25	17.22	16.10
Na	-	-	9.71	6.32
Mg	-	-	0.02	0.01
Al	-	-	0.11	0.06
Si	-	-	0.15	0.08
Cl	-	-	15.45	6.52
Ca	**-**	**-**	**0.58**	**0.22**
Total	100	-	100	-

CHAPTER 5

CONCLUSIONS

6.1 GENERAL

The present study was to check the degradation rate of the polylactic acid scaffolds with in-vitro study. The three different process parameters such as infill percentage, layer thickness and infill pattern change up-to three different levels to examine the effects. The conclusions are given below:

6.2 CONCLUSION

1. Porosity is directly relative to infill percentage and infill pattern, there is no significant effect of layer thickness on porosity level. The honeycomb pattern with 100% filling shows the 25% porosity only due to geometry of pattern because nozzle of printer is unable to fill the micro gaps.
2. pH value of the solution represents the degradation of the different specimens. The pH value decreases with the time period. The solution with maximum porous specimen shows the maximum decreases in pH value, this is only due to surface area to volume ratio. No doubt the specimen with high porous structure have high surface area to volume ratio due to micro gaps.
3. Weight loss study represent the weight of specimens firstly increase and then decreases, it is only due to apatite formation weight and degradation rate of the specimen. Firstly, weight increase due to formation of calcium on the surface of specimen, but it was very less in amount and then decreases due to high degradation rate.
4. The process parameters give significant effects on compressive strength. The design of experiment represents the level of parameters for maximum required output. The highly dense structure and rectilinear infill pattern gives maximum strength. The maximum layer thickness gives optimum results because the it shows maximum bonding due to less individual gaps.
5. The change in compressive strength represent the degradation rate of the individual scaffold. Specimen number 1 shows the maximum degradation rate due to highly porous structure and specimen number 9 shows the low degradation rate due to highly dense structure. So, the results show that the degradation rate is directly based on the porosity of the specimen.

6. Metallurgical test shows the maximum formation on highly dense structure, it represents the maximum bone cell growth in porous structure.

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