# Studies on Additive Manufacturing of Hard and Soft Scaffold for Tissue Engineering Applications

Md. Shahid Ansari

Under guidance of

Dr. S.Suryakumar & Dr. Subha Narayan Rath

A Dissertation Submitted to

Indian Institute of Technology Hyderabad

In Partial Fulfillment of the Requirements for

The Degree of Master of Technology

भारतीय प्रौद्योगिकी संस्थान हैदराबाद

Indian Institute of Technology Hyderabad

Department of Mechanical and Aerospace Engineering

June, 2015

## Declaration

I declare that this written submission represents my ideas in my own words, and where other's ideas or words have been included, I have adequately cited and referenced the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be a cause for disciplinary action by the Institute and can also evoke penal action from the sources that have thus not been properly cited, or from whom proper permission has not been taken when needed.

Shahid

(Signature)

MD. SHAHID ANSARI

(Student Name)

ME13M1023

(Roll No.)

# Approval Sheet

This thesis entitled Study on Additive Manufacturing of hard and soft scaffold for tissue engineering applications by Md. Shahid Ansari is approved for the degree of Master of Technology from IIT Hyderabad.

Prof. N.V.Reddy
Department of Mechanical Engineering
IIT Hyderabad
Examiner

Dr. S Surya Kumar
Department of Mechanical Engineering
IIT Hyderabad
Adviser

Dr. Subha Narayan Rath
Department of Biomedical Engineering
IIT Hyderabad
Co-Adviser

20.6.2015

Dr. Bharat Bhooshan Panigrahi
Department of Materials Science and Engineering
IIT Hyderabad
Chairman

# Acknowledgements

With pleasure I express my sincere gratitude, regards and thanks to my thesis advisor, **Dr. S. Suryakumar and Dr. Subha Narayan Rath** for their excellent guidance, invaluable suggestions and continuous encouragement at all the stages of my research work.

A special thanks goes to PhD scholar **Pathan Mosim Amin** (Chemistry Dept.), **D.E. Sindhuja** (Biomedical Dept.) and M.Tech. scholar and **Atul Kumar Maurya** (Material Science and Engineering Dept.) for providing me support in my project work. I would like to thank **Raj Kiran** and **Ramesh** for helping me in the Work shop.

It was a great pleasure to me as a part of Department of Mechanical, IIT Hyderabad and I would like to thank all the staff members and my friends for helping me in all stages of my work and making the great place to work in.

Above all, I extend my deepest gratitude to my parents for their love, affection, encouragement and support.

Dedicated to

*My Parents*

*&*

*My Friends*

# Abstract

Tissue engineering is a field that allows us to look into the future for regenerative medicine. It may become possible to regenerate or replace damaged tissues with laboratory-grown parts such as bone, cartilage, blood vessels and skin using this technology. It essentially tries to create friendly and conducive environment for controlled growth of cells with the help of connective tissues and collagenous scaffolds. Additive Manufacturing method with its ability to add material at desired location to make a component is best suited for creating such a scheme for cell culture. It has a unique ability to precisely control of the matrix architecture like size, shape, interconnectivity, branching, geometry and orientation. Due to these reasons, Additive Manufacturing is increasingly becoming a powerful tool for the realization of tissue engineering applications.

For good cell growth, structure need to be porous enough at the same time strong as well to guide the cell flow and bear the load respectively. The current work develops an idea to export a 3D CAD model to a Fused Deposition Modeler (FDM) to produce scaffolds of biocompatible materials like Polylactic acid (PLA) & Hydrogel (Sodium Alginate + Calcium Chloride) with varying pore structures. A study on the feasibility and methodology of fabricating biocompatible materials for tissue engineering has been done. Effect of different printing parameters has been studied, which is of interest in Tissue Engineering technology. Furthermore analysis and experimental feasibility of the process exploring various properties of the scaffold is done. *In vitro* studies for a span of one day showed non-toxic, bio-compatible behavior and healthy cell growth for a sample scaffold fabricated by this method.

# Nomenclature

| | | |
|---|---|---|
| AM | - | Additive manufacturing |
| CAM | - | Computer aided manufacturing |
| CAD | - | Computer aided design |
| FDM | - | Fused deposition modelling |
| PLA | - | Poly lactic acid |
| TE | - | Tissue engineering |
| TEC | - | Tissue engineering construct |
| SFF | - | Solid freeform fabrication |
| RP | - | Rapid prototyping |
| SLA | - | Steriolithography |
| SLS | - | Selective laser sintering |
| 3DP | - | 3 dimensional printing |
| PCL | - | Polycaprolactone |
| PEGT | - | Poly (ethylene-glycol) terephthalate |
| PBT | - | Poly butylene terephthalate |
| CaP | - | Calcium phosphate |
| PEG | - | Poly ethylene glycol |
| TIPS | - | Thermal induced phase separation |
| EL | - | Ethylene glycol |
| SEM | - | Scanning electron microscopy |
| TPU | - | Theroplastic polyurethane |
| SDS | - | Sodium n-dodecyl sulfonate |
| DOD | - | drop-on-demand |
| NaAlg | - | Sodium alginate |
| $CaCl_2$ | - | Calcium chloride |
| RW | - | Road width |
| FG | - | Fill gap |
| ST | - | Slice thickness |
| LG | - | Layer gap |
| ADHSC | - | Adipose derived human stem cells |
| PBS | - | Phosphate buffer solution |

# List of figures

# Contents

# Chapter 1

# Introduction

## 1.1 Tissue Engineering

Originally, tissue engineering was defined from a very broad and general perspective as the application of the principles and methods of engineering and life sciences toward the fundamental understanding of structure function relationships and the development of biological substitutes to restore, maintain, or improve functions [1]. The most common concept underlying tissue engineering is to combine a scaffold/matrix, living cells and/or biologically active molecules to form a tissue engineering construct (TEC) to promote the repair and regeneration of tissues (Figure – 1.1).

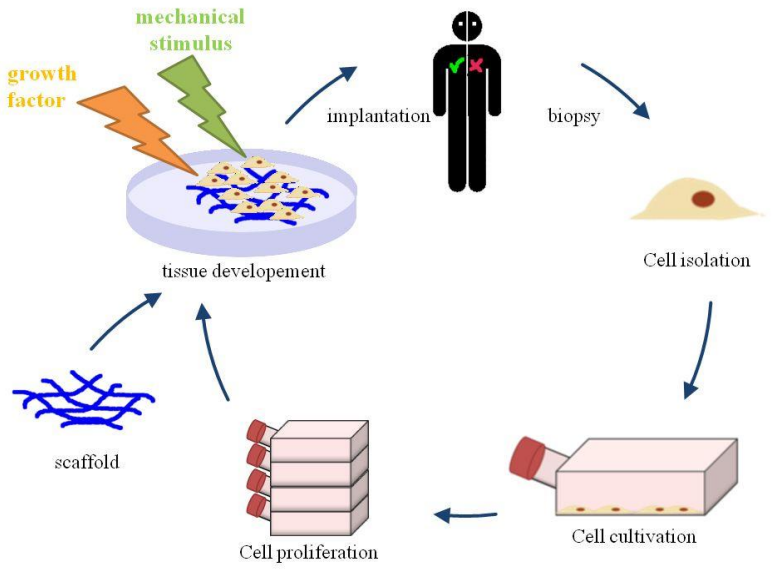



Figure – 1.1: Basic principle of Tissue Engineering [2]

The scaffold is expected to support cell colonization, migration, growth and differentiation and to guide the development of the required tissue or to act as a drug delivery device. Scaffolds are of great importance for tissue engineering because they enable the fabrication of functional living implants out of cells obtained from cell culture. As the scaffolds for tissue engineering will be implanted in the human body, the scaffold materials should be non-antigenic, non-carcinogenic, non-toxic and non-teratogenic and possess high cell/tissue biocompatibility so that they will not trigger any adverse cellular reactions after implantation. Besides material issues, the macro- and microstructural properties of the scaffold are also very important. In general, the scaffolds require individual external shape and well defined internal structure with interconnected porosity to host most cell types. From a biological point of view, the designed matrix should serve functions, including:

- an immobilization site for transplanted cells;
- formation of a protective space to prevent soft tissue prolapse into the wound bed and allow healing with differentiated tissue;
- directing migration or growth of cells via surface properties of the scaffold;
- directing migration or growth of cells via the release of soluble molecules such as growth factors, hormones and/or cytokines.

## 1.2 Additive Manufacturing

Research on the manufacturing of porous scaffold structures for tissue engineering has been carried out for more than three decades. Conventional techniques include solvent casting, fiber bonding and membrane lamination etc. Solid freeform fabrication (SFF) and rapid prototyping (RP) were applied in the 1990s to fabricate complex shaped scaffolds. Unlike conventional machining, which involves constant removal of materials, AM is able to build scaffolds by selectively adding materials, layer by layer, as specified by a computer program. Each layer represents the shape of the cross section of the CAD model at a specific level.

Today, additive manufacturing is viewed as a high-potential fabrication technology for the generation of scaffold technology platforms. In addition, one of the potential benefits offered by this technology is the ability to create parts with highly reproducible architecture and

compositional variation across the entire matrix owing to its computer controlled fabrication. The science in the field is still young, and different approaches and strategies are under experimental investigation. It is by no means clear what defines ideal scaffold/cell even for a specific tissue type.

## 1.3 Motivation

Improving tissues and organ disease is a major challenge within the field of regenerative medicines. In this technology people induced the regeneration by using a cell free porous scaffold which replaces the injured tissue. The special and selected biocompatible materials used are characterized by the dynamic behavior with the ability to respond and communicate to surrounding native tissue. Tailored made materials and structures can be developed by simple mix and match strategy.

Millions of patients are admitted to the hospitals in serious conditions having organ failure and tissue loss. Surgical procedure are performed all over the world to treat millions of patients annually who experience organ failure or tissue loss. Although these approaches are beneficial for saving many lives, still they are imperfect for a permanent cure. According to a survey by U.S. Department of Health & Human Services in the year 2006, approximately 29,000 donors organs were available for more than 95,000 patients in need. Although the number of organ donors is on the rise recently, additions to the transplant waiting list have increased more rapidly, resulting in shortage of donor organs. In some cases, patients are more likely to die while waiting for donor organ. Thus, there is always an unresolved issue of organ failure patients even with current therapies such as organ transplantation, reconstructive surgery, or by using mechanical devices such as kidney dialyzers or prosthetic hip joints.

The concept of tissue engineering came in to address this ongoing need of organ shortage. Langer and Vacanti (1993) first introduced the term tissue engineering as the application of principles and methods of engineering and life sciences to restore, maintain, or improve tissue functions. For last two decades researchers are searching the best way to have better results which will take the idea of tissue engineering to the next level which include fabrication of

laboratory generated organ implants. Few tissues like skin, cartilage and bone are in advanced stage of tissue engineering field because of their potential need and relative ease of application.

## 1.4 Problem definition

The main objective of this work is to leverage the unique advantages of Additive Manufacturing for Tissue Engineering applications. These applications can be broadly classified into two groups viz., hard scaffolds and soft scaffolds. The former use thermoplastics like PLA and the latter liquid solutions like Sodium Alginate. While FDM type extrusion based systems are available for hard scaffolds, liquid jetting of solutions is more challenging to achieve as the method of jetting should not involve direct contact with any thermal or electrical medium that may damage the cell growth. The control of the position, material deposition rate, filling path also assume importance. Hence, the aim of the current study is to establish the methodology for successful fabrication of hard and soft scaffolds for tissue engineering applications using open source software and hardware followed by in virto testing to check its feasibility.

## 1.5 Organization of the report

- Chapter 1 provide the background to tissue engineering, a brief overview of current need of tissue engineering, and defines aims and hypothesis of this research.

- Chapter 2 provides a detailed literature review of the relevant topics, leading to the formulation and strategies adopted in this research.

- Chapter 3 contents details about the machine or equipment setup as well as a brief details about the software used to fabricate the matrix architecture.

- Chapter 4 & 5 documents the individual experiments which is performed to study the submitted manuscript.

- Chapter 6 rounds up the thesis with the final conclusions and recommendations for future research in the topic.

# Chapter 2

# Literature Survey

## 2.1 AM for Tissue Engineering

The generation of tissue patterns involves precise special organizations of cells in the micrometer scale. For example, functional nervous systems or blood vessels form only when constituting cells are oriented in specific geometries [3,4]. In recent years, micro-fabrication technologies have been progressively used to fabricate tools for controlling cell organization and function *in vitro*. However, reports of cell patterning on biodegradable materials suitable for tissue engineering applications remain very limited.

Several AM have been developed in recent decades. The elaboration of different polymer and ceramic scaffolds with different geometries has been reported. It was found that various AM technology like Stereolithography (SLA), Solid laser sintering (SLS), 3D Printing (3DP), Fused deposition modeling (FDM) as well as indirect SFF is used by various researchers to achieve the desire structure of scaffold showing the properties like tissues/cells [5]. The nozzle-deposition-based techniques, particularly the approach consisting in a dispensing system integrated with pumping technology and a CAD/CAM tool. This is a versatile technique that allows the building of 3-D structures and complex geometry models with precise control and reproducibility, using a large variety of materials [6].

**Langer et al.** first studied and introduced scaffold based tissue engineering concepts which involve the use of combinations of viable cells, biomolecules and a structural scaffold combined into a construct to promote the repair and regeneration of tissues. The construct is intended to

support cell migration, growth and differentiation and guide tissue development and organization into a mature and healthy state.

Reviewing the literature on fabricated scaffolds reveals that numerous degradable polymers such as polycaprolactone, polylactic acid (PLA), polyglycolic acid, polypropylene, polyethylene glycol terephthalate (PEGT), polybutylene terephthalate (PBT), chitosan and their copolymers have been used to fabricate 3-D scaffolds. Table – 2.1 shows a summarized review about the details of the materials used by different researcher groups and setup used for fabrication by them.

Table – 2.1: List of materials used for scaffold fabrication

| Sl. No. | Material used | Calciumcomponent | Method | Reference |
|---|---|---|---|---|
| 1 | Poly (Ɛ-caprolactone) (PCL) | - | Extruder | [12] |
| 2 | Poly (L-lactic acid) (PLLA) | Tricalcium phosphate (TCP) | Refrigeration chamber | [8] |
| 3 | Polypropylene (PP) | Tricalcium phosphate (TCP) | Extruder | [5] |
| 4 | Poly(ethyleneglycol)-terephthalate(PGET) & Poly (butylene terephthalate (PBT) | - | Syringe (mechanical load) | [16] |
| 5 | Polycaprolactone (PCL) | Calcium phosphate (CaP) | - | [9] |
| 6 | Bone Morphogenic Protein (BMP) | Calcium phosphate cement (CPC) | Negative mould | [3] |
| 7 | Polycaprolactone (PCL) | Phosphat buffer saline | Syringe (Air pressure) | [11] |
| 8 | Poly (butylene terephthalate (PBT) | - | Extruder | [13] |
| 9 | Polycaprolactone (PCL) | Tricalcium phosphate (TCP) | Extruder | [17] |

**Hutmacher et al.** [5] studied the architecture, structural mechanics, surface properties, degradation products and composition of biological components and the changes of these factors with time *in vitro* and/or in vivo culture. They explored two methods of incorporating cells into

scaffolds (i) seeding of cells onto the surface of the scaffold following fabrication and (ii) the incorporation of cells into the scaffold fabrication process (Figure – 2.1).

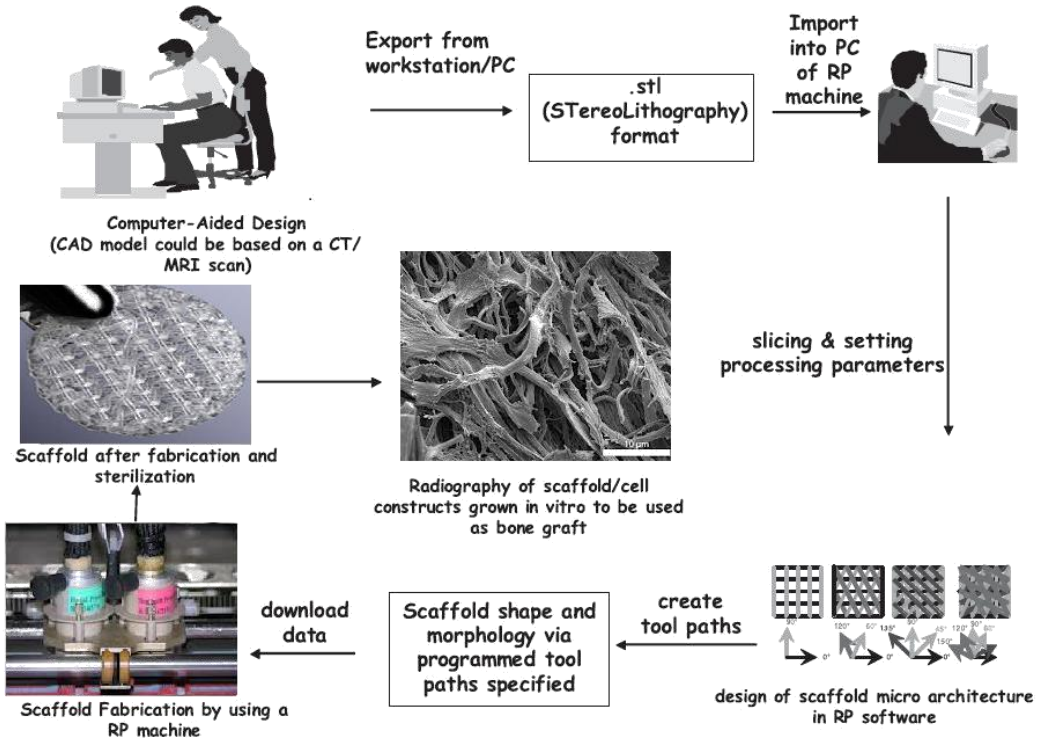



Figure – 2.1: Flow chart of Tissue Engineering [5]

Cell and tissue remodeling is important for achieving stable biomechanical conditions and vascularization at the host site. Hence, the 3D scaffold/tissue construct should maintain sufficient structural integrity during the *in vitro* and/or in vivo growth and remodeling process. The degree of remodeling depends on the tissue itself (e.g. skin 46 weeks, bone 46 months) and its host anatomy and physiology. Scaffold architecture has to allow for initial cell attachment and subsequent migration into and through the matrix, mass transfer of nutrients and metabolites and provision of sufficient space for development and later remodeling of organized tissue.

For the three applications described above, SFF scaffolds offer at least three advantages over current technologies for processing biomaterials:

- tailored macroscopic shapes;
- well defined microstructure, which may include multimodal pore size and distribution as well as directionally oriented pores and channels;

- incorporation of growth factors/cells during manufacture in order to provide controlled release of factors at specific sites.

The different techniques provide the tissue engineer with manufacturing processes that are capable of producing scaffolds from a range of biomaterials and to a range of architectures, morphologies and structures within the design tolerances and parameters based on the chosen tissue engineering strategy (road map).

## 2.2 Thermoplastic wire extrusion

PLA is a currently used biodegradable polymer that has been approved by the FDA for various biomedical applications. Though this polymer has been extensively studied, its use in the fabrication of RP scaffolds and specifically those elaborated through nozzle based systems has been limited and scarcely reported. At present, most of the reported PLA-based scaffolds fabricated by RP require the molecular modification of the PLA matrix, the use of temperature during printing or further processing of the structure by freeze-drying [7,8]. The RP tool used in the present study allows the fabrication of PLA 3-D structures without modifying the polymer structure with specific chemical groups.

**Serra et al.** [9] presented the study on high-resolution PLA-based composite scaffolds via 3-D printing technology in which they develop and characterize 3D scaffold that shows completely interconnected porosity, uniform distribution of particles with controlled and repetitive architecture. Bioactive calcium phosphate (CaP) glass are used that results in increased roughness and hydrophilicity of scaffold. They studied about the effect of adding polyethylene glycol (PEG) in PLA based blend for tissue engineering. According to their study addition of 5% PEG lowers the glass transition temperature from 59℃ to 40℃. Scaffold had average diameter of struts ~75μm, pore size ranged from 165 – 375 μm. The cell adhering to the surface of scaffold showed mostly rounded shapes and were sparsely spread on the surface.

Scaffold prepared by thermal induced phase separation (TIPS) along with super critical $CO_2$ drying technique from PLA-ethylene lactate (EL) solution performed and analyzed by **Salerno et al.** [10]. By using different percentage gelatin particles porosity of different ranges are achieved.

Further test and analysis were performed to check the feasibility and properties of the scaffold. Different size ranges of gelatin materials (100-200 μm, 200-400 μm, 400-600 μm) was sieved in homogeneous solution for controlling pores of the fabricated scaffolds. Morphological and structural properties were studied by using SEM analysis.

**Rezabeigi et al.** [11] tried to get the scaffold of PLA via nonsolvent induced phase separation technique. Although nonsolvent-involved technologies have been used for almost 50 years for the fabrication of membranes, the production of porous monoliths has only been studied in a limited way. They tried to get scaffold by liquid-liquid phase separation of PLA and Dichloromethane, The gels are allowed to age at room temperature for an additional 10-30% of their gelation time. The wet, aged gels are removed by breaking their glass vessels. Cubic specimens are carefully cut from the central portion of the gels, and immediately immersed in ~150 ml methanol. The porosity ranges from 40.7% to 90.8% and compressive modulus is reported in a range from 1.8 to 57 MPa.

**Mi et al.** [12] studied the properties of scaffolds made using compounded material of PLA and thermoplastic polyurethane (TPU) compounded in different percentage ratio by the help of twin-screw extruder. The scaffold fabricated had the porosities ranging from 49% to 79%, pore diameters from 115 to 252 μm, and pore densities from 1.4 x 105 to 3.9 x 105 /cm3. Cell culture experiments demonstrated the biocompatibility of the scaffolds with significance level ($p < 0.05$).

## 2.3 Liquid extrusion

High printing resolution and high cell viability testing of oxidized alginate as ink for bioprinting to hold homogeneous cell suspension were performed by **Jia et al.** [13]. They demonstrate that alginate-based bioink were not affect printability and structure integrity even after cell culture. **Shi et al.** [14] fabricate novel sodium alginate-based super porous hydrogel by grafting copolymerization with addition of sodium n-dodecyl sulfonate (SDS). SDS facilitates to form homogeneous and well-defined pore structure with higher swelling ration as well as swelling rate**. Morais et al.** [15] develops a hydrogel with highest degradation ah pH 7.4, about 80% weight loss, after 3 days showing with unique shear-thinning and viscoelastic behavior**. Herran**

**et al.** [16] experimentally studies the effect of materials properties and operating conditions on the formability of alginate microspheres and the microsphere size during wave based drop-on-demand (DOD)- based single nozzle jetting. Based on sodium alginate and calcium chloride concentrations, voltage rise/fall times, dwell and echo times, excitation voltage amplitudes, and frequency they studied the formability and size. It was observed that scaffold properties are very much sensitive to sodium alginate concentration and voltage dwell time. Size is independent of concentrations under investigated range but size increases with increase in dwell time and echo voltage amplitude.

**Wang et al.** [17] investigated application of microfluidic technology to get highly organized 3D alginate scaffold. The device they used is like a two channel jacket microencapsulator bubble formation equipment with a micropipette with inner diameter 45 μm and outer diameter 95 μm. 1.5% alginate with 1% Pluronic are used to make the blend. The scaffold was non-cytotoxic to chondrocytes; cells proliferated and functioned normally. Moreover, chondrocytes maintained their normal phenotypes within the scaffold.

In this chapter, various trials and methods adopted by researcher's shows there are verities of materials available which one can use in application of regenerative medicines. The review give an idea that PLA as well as NaAlg are one of the best and easily available biocompatible materials which we can use for tissue engineering applications and scaffold fabrication.

# Chapter 3

# Experimental setup

## 3.1 Material extrusion system

In the current study FDM machine is used to fabricate scaffold architecture for tissue engineering applications. FDM is now a crowded space with lot of manufacturers making AM machines based on that technology; 3D printing market are Stratasys, MakerBot, 3D systems, Ultimaker, ZCorp etc are some of the popular manufacturers of the same.

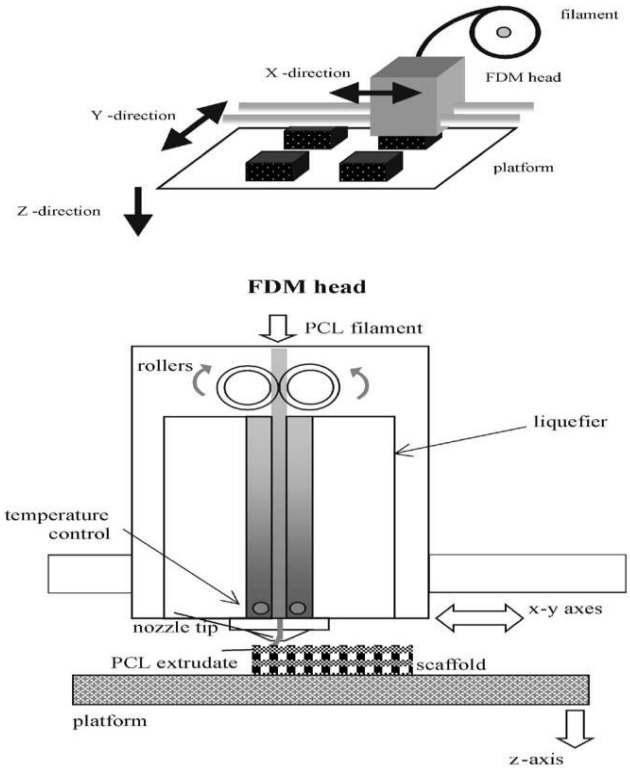



Figure – 3.1: Schematic diagram of FDM [4]

Although Stratasys was the first to introduce FDM technology, owing to its simplicity, this spectrum has seen a lot of activity on low end and make-it-yourself FDM machines. Figure – 3.1 shows the schematic diagram of FDM working principle and degree of freedom in X, Y and Z directions.

### 3.1.1 Thermoplastic wire extrusion

FDM employs the concept of melt extrusion to deposit a parallel series of material roads that forms a material layer. In FDM, filament material stock (generally thermoplastics) is fed and melted inside a heated liquefier head before being extruded through a nozzle with a small orifice (Figure – 3.2). The deposited material (individual roads) cools, solidifies, and bonds with the neighboring material. The bonding between the individual roads of the same layer and of neighboring layers is driven by the thermal energy of the semi-molten material and diffusion. For each deposited material layer, the direction of material deposition (i.e., laydown pattern) can be changed. By changing the direction of material deposition for consecutively deposited layers and the spacing between the material roads, scaffolds with highly uniform internal structures, controllable pore morphology and complete pore interconnectivity are obtained. In order to fabricate scaffold designs with overhanging features, removable supporting structures are deposited.

Figure – 3.2: Thermoplastic wire extrusion setup

Once the build process is completed, the FDM built part can be viewed as a laminate composite structure with anisotropic material properties. The mechanical properties of FDM parts are not only controlled by the build material, but also influenced by the selected fabrication parameters.

### 3.1.2 Liquid extrusion

In the current study a FDM machine (AlfaRod AR 1, Alfatek systems, Kolkata, India) with a special syringe arrangement is used for the fabrication of soft tissue hydrogels. The AlfaRod AR1 is based on open source design that adds ethernet, infra-red sensors and an advanced micro-controller to the 3D printer. This enables additional features such as auto-bed leveling, planarity compensation, orthogonal compensation and web-control. The AR1 is aimed at corporate clients and industries that want a professional-grade 3D printer for their prototyping and manufacturing needs. Figure – 3.3 shows the actual setup of machine for better visualization and understanding.


Figure –3.3: Liquid dispenser setup

The syringe arrangement is nothing but a setup in which the plunger of the syringe is controlled by the help of lead screw attached to a stepper motor. The syringe arrangement attached to the machine in place of the hot end (i.e. extruder) and liquid solution are filled in the syringe to perform the printing of scaffolds. Mainly the hydrogel scaffold fabricated are made by the fusion of NaAlg and $CaCl_2$ in an appropriate concentration in distilled water. As after interchanging the extruder setup with syringe parametric optimization for controlling the liquid print is required.

**Specification of machine:**

- Build volume : 200 x 200 x 200 mm
- Overall size : 500 x 500 x 450 mm
- Printing materials : Thermoplastic materials
- Build surface : glass bed
- Nozzle size : 0.5 mm
- Accuracy : 1000 micron
- Resolution : 12.5 micron
- Input file format : standard STL and OBJ format

## 3.2 Software for Slicing & Path planning

A 3D printer cannot cope directly with files from a CAD program. So, 3D or CAD files need to be processed before they become printable and this process is called as slicing and area filling. Hence, the slicing (software) is the first tool we use for 3D printing. A slicer commonly uses STL files to create the tool paths, usually in Gcode format. These files contain instructions for the 3D printer on where, when, and how fast to make movements. The slicer software slices the STL model into layers and print paths to create a 3D printable Gcode file. There are several slicing programs available, some of them being: 1) Kisslicer 2) Slic3r 3) Skeinforge 4) Repsnapper 5) Netfabb studio 15 6) Magics etc.

Most of the 3D printing machine based on FDM principle needs similar type of software for the operation of the machine. Basically few common steps are followed to perform the fabrication process. First the person need to design the desired structure by the help of designing software (CAD, Solid Edge, Solid Works etc.). Once the designing process is done the file should be saved in standard format (STL, OBJ etc.). In the last and final step slicing software (Slic3r, KISSlicer, Cura etc.) are used to slice the structure with a predefined printing parameters for the printing operation.

The main influence on the final output of the print highly depends on the parameter which are fed in the slicing software Fig. As the slicing software provide the path planning of the nozzle while performing the fabrication. Therefore, proper optimization of parameters are need to be

done before starting the print. The following parameters plays an important role that are discussed below:

- **Fill patterns:** It is nothing but the different patterns that will be printed as infill in the structure. Different fill patterns option like linear, honeycomb, rectilinear, octagramspiral, hilbertcurve, archemedianchord are available in slicing software. With the help of different fill patterns different infill architecture are obtained which will give a specific kind of interconnected channels useful for tissue engineering applications.

- **Fill density:** It directly influence the porosity of scaffold structure. As porosity plays a major role in scaffold structure change in density will open different ways to achieve required scaffold structure with varying porosity and matrix properties that mimic human tissues. By varying the fill density one can get various mechanical as well as structural strength and property even by using same fill pattern.

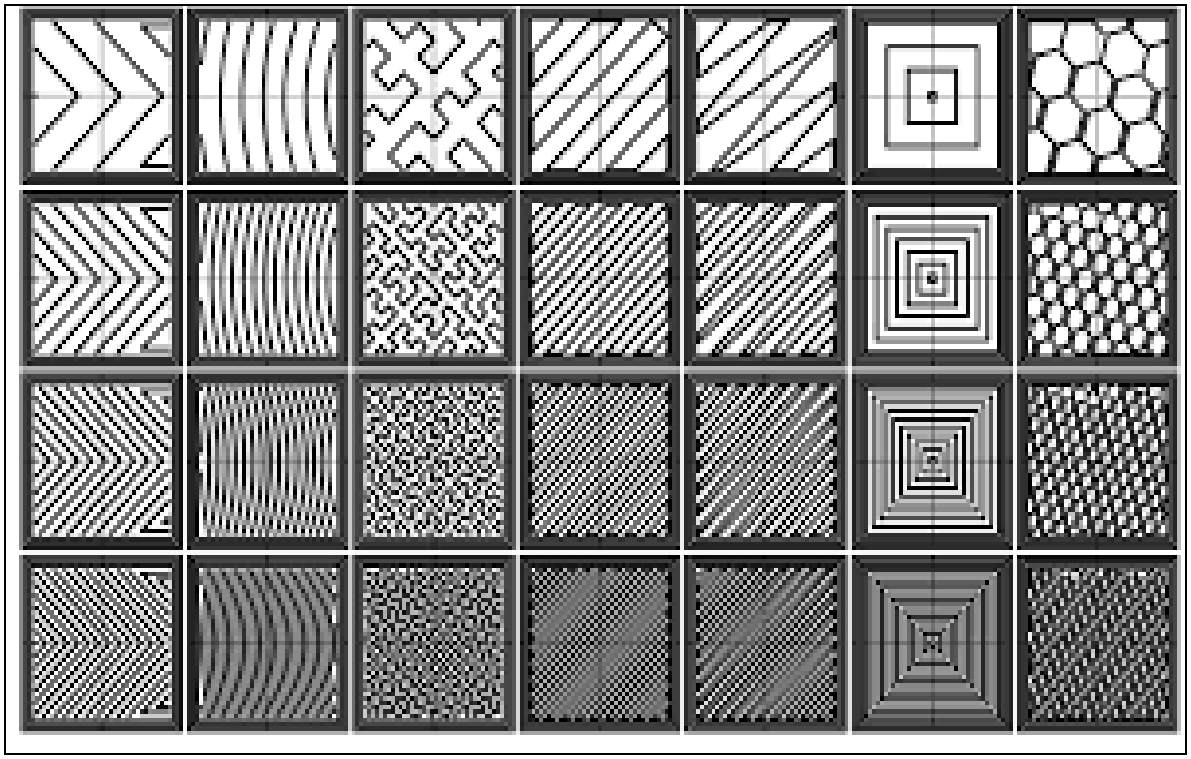


Figure –3.4: Different fill patterns (horizontal) and fill density (vertical)

- **Feed:** Optimize feed rate is required for better printing as if the feed rate is too slow the material will accumulate in the nozzle and thick layer of materials will be deposited which

damages the structure. On the other hand if the feed rate is too high then material will not come out from the nozzle and fine hair like structure will be deposited with very weak strength and loosely attached with other layers.

- **Extruder factor (E):** It is the amount of material need to be feed for a defined path or structure. It is clear that if we give low E then printed material will behave to be unevenly stretched and detached with each other. High E value will form globules of material and in some cases over burnt material will come out from nozzle.

Table – 3.1: Slic3r configuration settings

| | |
|---|---|
| (1) Layer and Perimeter | |
| (a) Layer height | 0.2 mm |
| (b) Perimeter | 0 |
| (c) Top layer | 0 |
| (d) Bottom layer | 0 |
| (2) Infill | |
| (a) Fill density | As per requirement |
| (b) Fill pattern | As per requirement |
| (c) Top/Bottom pattern | As per requirement |
| (d) Fill angle | 45 (default) |
| (3) Speed | |
| (a) Perimeter | 30 mm/s |
| (b) Infill | 60 mm/s |
| (c) Support material | 60 mm/s |
| (d) Bridges | 60 mm/s |
| (e) Gap fill | 20 mm/s |
| (f) Non-print quick move | 120 mm/s |
| (4) No skirt and brim | |
| (5) Filament diameter | 1.75 mm |
| (6) Extrusion multiplier | 1 |

## 3.3 Control Parameter

Control parameters play an important role in building a part. These are used to find the optimal parameters for a desired product. This can be achieved by varying the different parameters options available in the machine and the software. The processing parameters of filling each layer depend on the earlier inputs into the slicing software. These include the FDM head speed, the roller speed, the slice interval, and the direction of deposition within each layer. Each layer is made of ‘‘roads’’ deposited in the X and Y-directions, in a raster, a contour or a combination of both. The direction of deposition is known as the ‘‘raster angle’’ for raster filling and this can be specified for each layer between 0˚and 180˚with respect to the X-axis.

The road width (RW) is controlled both by the flow parameters at a set temperature above the melting temperature of the thermoplastic material and also by the fine size of nozzle tip used. The RW was targeted slightly above the inner diameter of the smallest nozzle tip in-use, for stable flow during extrusion.

The liquefier temperature and the filament feed rate had the most direct influence on the material flow for the fabrication of porous models. The optimization of the processing parameters should primarily focus on both the liquefier temperature and the roller speed.

The structure of the 3D scaffolds designed and fabricated using the FDM method was highly similar to the honeycomb of a bee, with its regular array of identical pores, when viewed in the Z-direction of the fabrication process (Figure – 3.5(a)). The main difference lies in the shape of the pores: the bee’s honeycomb comprises of hexagonal pores surrounded by solid faces which nest together to fill a plane, but the FDM scaffold structure was built from neatly aligned filaments stacked in horizontal layers and comprised of pores surrounded by solid struts.

The SEM micrographs were used to measure the RW (in mm) values besides studying the scaffold morphology. Other parameters like fill gap (FG) (in mm) and slice thickness (ST) (in mm) were measured and compared with the set values. Measurement of a fourth dimension, a layer gap (LG), was also included. LG was defined as the edge-to-edge distance between layers

of filaments of the same alignment. This served to indicate the amount of vertical space as compared with FG for the horizontal space between adjacent filaments. ST and LG values were measured from the cross-section views of scaffold specimens, which showed the stacked layers. RW and FG values were measured from the layer views, which showed the lay-down pattern. The dimensions RW, FG, ST and LG are illustrated schematically in (Figure – 3.5). All dimensions were measured with respect to the scale bar on each SEM micrograph.

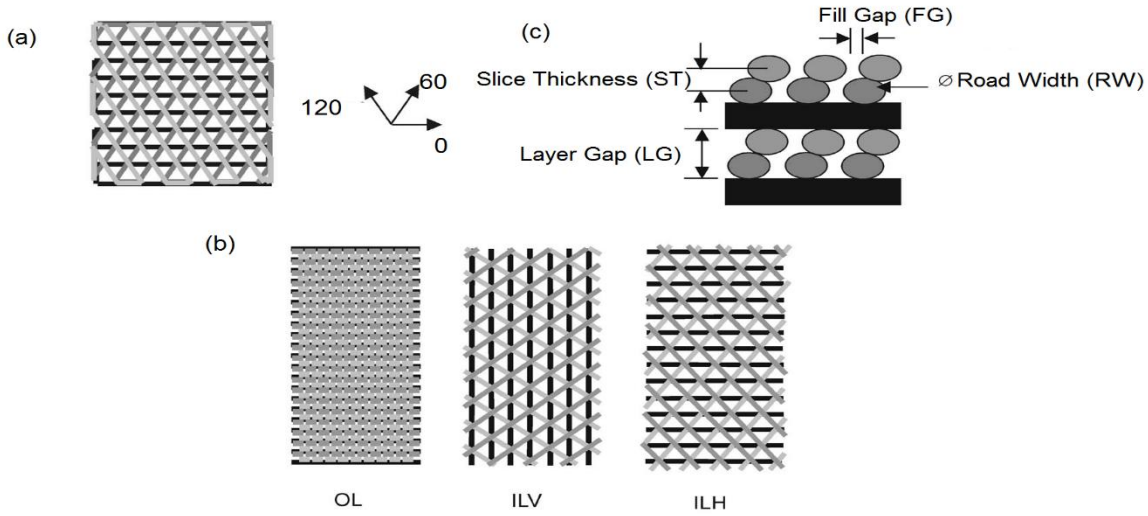



Figure –3.5: (a) Lay-down pattern of 0/60/120 ˚ forming triangular honeycomb pores viewed in the Z direction of the FDM build process . (b) Alignment of filaments in scaffold specimens with a 0/60/120˚ lay-down pattern. In the out-of layer (OL) orientation, the filaments are aligned in the XY-plane. In both in-layer-vertical (ILV) and in-layer-horizontal (ILH) orientations, the filaments are aligned in the XZ-plane and YZ-plane, respectively. (c) Cross-section viewed in the XZ plane of the FDM build process. Symbols are denoted as RW: road width, FG: fill gap, ST: slice thickness, LG: layer gap. [4]

When the scaffolds were viewed in the X or Y direction of the fabrication process, the pores seen from the cross-sections revealed a network of interconnected channels of high regularity. Hence, the FDM scaffolds not only had honeycomb-like patterns but in a 3D sense, the structure was that of an open-pore cellular solid with the pores interconnecting through adjacent faces.

By increasing the number of layers with different raster angles and arranging the sequence of raster directions in a multilayered scaffold, it was possible to create a porous 3D scaffold with various degrees of channel dimensions. The use of a finer nozzle tip (<0.010 inch in diameter)

allowed the fabrication of scaffolds with bigger channel size than using the wider nozzle tip (>0.015 inch in diameter) under similar settings of FG and ST.

Scaffolds fabricated using the fine nozzle tip had smaller filaments of circular cross-section and a higher surface area to volume ratio than those fabricated using a wider nozzle tip. Insufficient adhesion between adjacent layers, delamination of layers was observed in some scaffold specimens printed by finer tip. The ST could be reduced for the scaffolds of fine nozzle to improve layer-to-layer adhesion but this would also reduce the scaffold porosity. Further reduction in the nozzle tip size would allow fabrication of finer structures.

The FG value played an important role in imparting the porosity for each scaffold model. To maximize porosity, the FG could be widened between adjacent raster roads. However, this value could not be increased indefinitely as slacking would occur when there was insufficient strength for an extruded filament to bridge a wide gap. It was essential that every layer be well deposited as the preceding layer served as the only foundation for the next layer of deposition.

# Chapter 4

# Fabrication of Soft Scaffold

In this study, sodium alginate (NaAlg) hydrogel that can be utilized for large-dimension tissue fabrication with its fast gelation property was studied regarding material-specific printing technique and printing parameters using a single nozzle bioprinting system developed by the Alfatek. A NaAlg solution was prepared, and calcium chloride ($CaCl_2$) solution was used as cross-linker for the gelation. The materials were loaded in syringe in the bioprinting system in which the injection speed can be independently controlled. The ultimate aim of the study is to get a 3-D alginate structure fabricated through layer-by-layer printing. The feasibility of sodium alginate hydrogel free-form formation by alternate printing of alginate solution and sodium chloride solution was confirmed in the available bioprinting system. By varying the nozzle moving speed and the injection speed, various pattern widths could be achieved.

## 4.1 Identification of suitable control parameters

Printing any structure by the use of additive manufacturing technology strongly depends on the parameters of layers to be printed one over other in a series. Therefore, it is the most important and necessary need to optimize all the parameters before printing. In the current study effect of different parameters were studied and value of each parameter is optimized to get desired result.

### 4.1.1 Calibration of speed and flow-rate

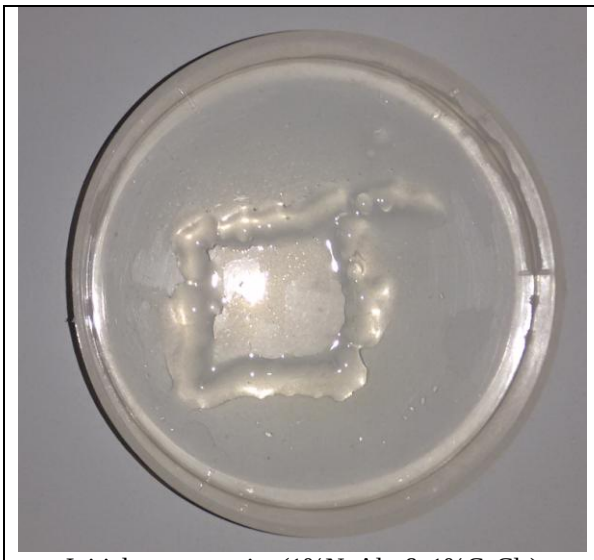
Flow is the amount of material coming out of the extruder or syringe needle (0.5 mm diameter) to print the required structure. As one need to be precise on the control of flow of droplets so that

one can have the desired structure, it is important to calibrate the flow value for getting perfect result. Speed is the factor controls the motion of nozzle while printing any structure. One should have right combination of flow and speed to achieve the desired architecture.

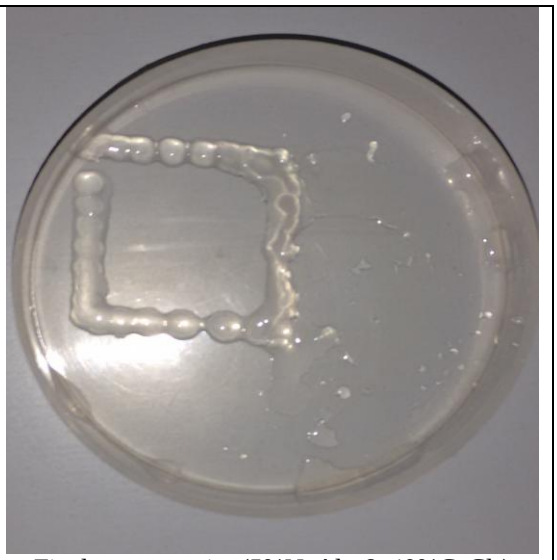
Both parameters are calibrated with respect to each other keeping one of them to be fixed or constant. It means if one wants to calibrate flow value then different value of flow are tried at the same speed. Same procedure is followed for calculating proper speed also. Different observations conclude that 72 extruder value will extrude 1 ml of liquid which is equivalent to 1000 $mm^3$ volume of liquid coming out from the syringe needle. When counted in droplet 1000 $mm^3$ gives us approximately 117 droplets with needle and 21 droplets without needle. This gives 1 extruder value equivalent to 13.89 $mm^3$ of liquid.

### 4.1.2 Effect of solution concentration on feature resolution

Initially 1% (wt/vol) concentration solution of both $CaCl_2$ (fused) and NaAlg (powder) were prepared in distilled water and used for the calibration of the process parameter for getting desirable result. After having some trials it is observed that the strength of hydrogel after the fusion of solution is too weak. Therefore, the concentration of both the solution are gradually increased and manually observed for the best combination of the concentration of solution. 2%, 3%, 4%, and 5% wt/vol solution of NaAlg were prepared and tested with 2%, 5%, 10% 20%, and 40% wt/vol solution of $CaCl_2$. $CaCl_2$ salt is easily soluble in water were as NaAlg take some extra time and effort to be dissolved in water. Manual stirring is done to dissolve the material completely in water. A combination of 5% NaAlg and 40% $CaCl_2$ gives better result observed manually. After formation of hydrogel it was observed that the strength as well as bond between the solutions are much stronger than the earlier one. Figure – 4.1(a) and Figure – 4.1(b) shows the print with initial and final solution respectively.



Initial concentration(1%NaAlg & 1%$CaCl_2$)



Final concentration(5%NaAlg & 40%$CaCl_2$)

Figure – 4.1: Print with different concentrations

### 4.1.3 Effect of solution mixing direction

It is also equally important to decide which solution should be better to keep over the bed to get a better result of a fabrication. As in the present case $CaCl_2$ solution is less viscous in compare with NaAlg solution. One can use anyone of the solution to be kept on the bed. Let take $CaCl_2$ to be the solution kept as bed, in this case as it is less viscous in nature while printing the entire structure shifts with the jerk caused by the motion of the bed as well as the hydrogel formed after reaction is allowed to float over the solution. In other case where NaAlg solution (i.e. more viscous) is kept on the bed, high viscosity of solution hold the structure and not allow the structure to move along with the jerk and motion of the bed. In this case one limitation is that the highly viscous liquid not allow the printed liquid to penetrate and solidify properly. According to the observation made by different trials, it is better to keep more viscous solution on the bed and take less viscous solution in the syringe.

## 4.2 Experimental Trials

Different trials were performed to test the feasibility of printing different structure and to study effect of parameter while performing operation with the experimental setup. Firstly single droplet were printed followed by uniform linear fabrication after that fill patterns for soft scaffold fabrication were tried. The observation in every trials of fabrication were discussed in the subsections.

### 4.2.1 Single droplet deposition

To have the control on the position of the machine it is required to print droplets in different positions. Combination of feed and extruded value are made to get a fine droplet of solution and by the help of G – code, syringe is allowed to print droplet in different positions. Feed ranges from 1 – 1000 mm/min is used keeping the extruder value to be 1 (~14 $mm^3$) which is equivalent to 1mm of wire extrusion for each trials (Fig. – 4.2). For better understanding and to cross check the result further trials with feed rate ranges from 1 – 10000 mm/min is used to print 3 (~42 $mm^3$) of extruded material (Fig. – 4.3).

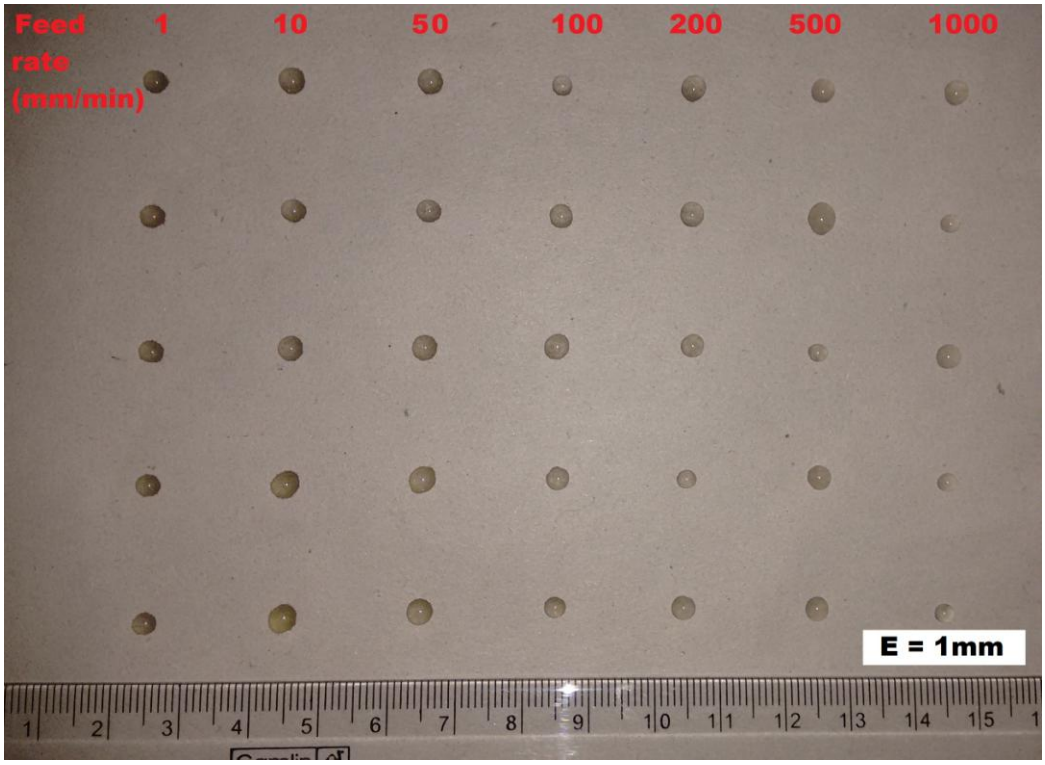



Figure – 4.2: Trials for fine droplet at different plunger feed rate of with E=1(~14 $mm^3$) ~ 1mm of wire movement in thermoplastic extrusion system

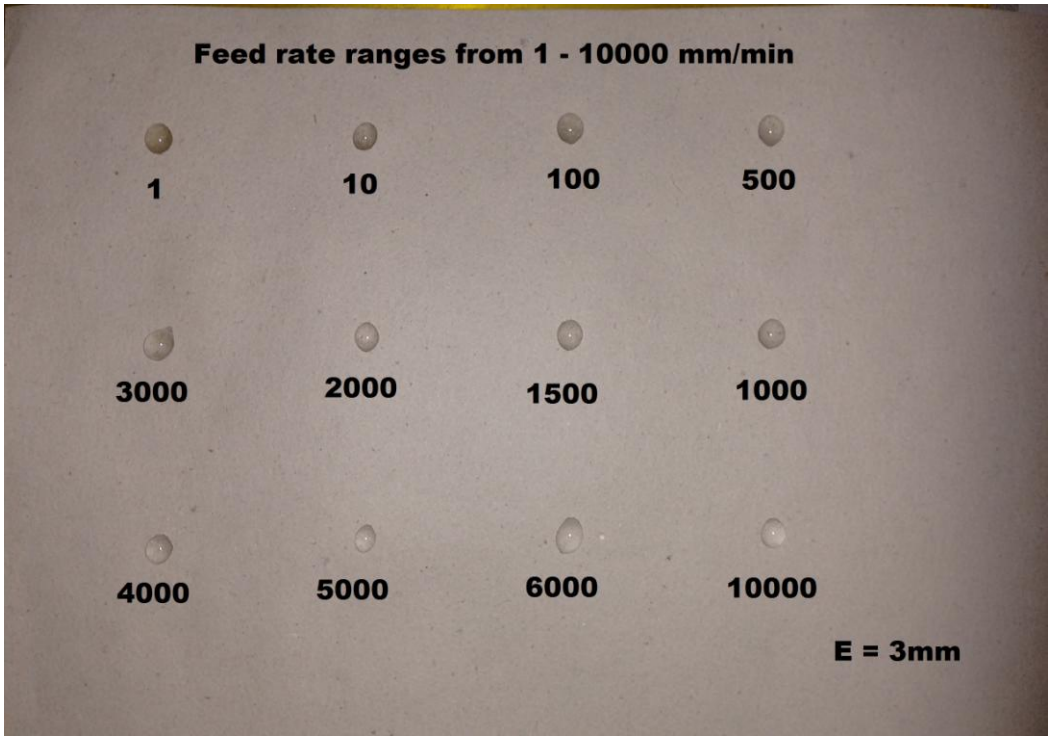



Figure – 4.3: Trials for E = 3 (~ 42 $mm^3$) material with varying plunger feed rate ~ 3mm of wire movement in thermoplastic extrusion system

The final result conclude that in case of droplet printing extruder value is independent of the feed rate as same amount of liquid or droplets are coming out from the nozzle. Feed rate only varies the rate of material deposition it never effects the amount of material extruded.

### 4.2.2 Uniform linear deposition

Getting a line by printing is not similar to the process to print droplets at different position. In this process additional parameter i.e. nozzle height (z) also plays an important role as well as effect of feed rate can be observed while fabricating a straight line. While printing liquid with the help of syringe. If the nozzle height is too low then material will either solidify on the nozzle tip or the nozzle tip will move the entire structure along with its movement (Figure – 4.4(a)). On the other hand if the tip height is high then the resulting structure will be non-continuous and distorted (Figure – 4.4(b)) and perfect clearance between nozzle tip and top layer of liquid kept in the bed will give better result (Figure – 4.4(c)). To print an area of 4 $mm^2$ approximately 0.1 ml material is required. If the value of extruded material is kept constant while increasing the

length of print the printer distribute the same amount of material over the length (Figure – 4.4(d)).

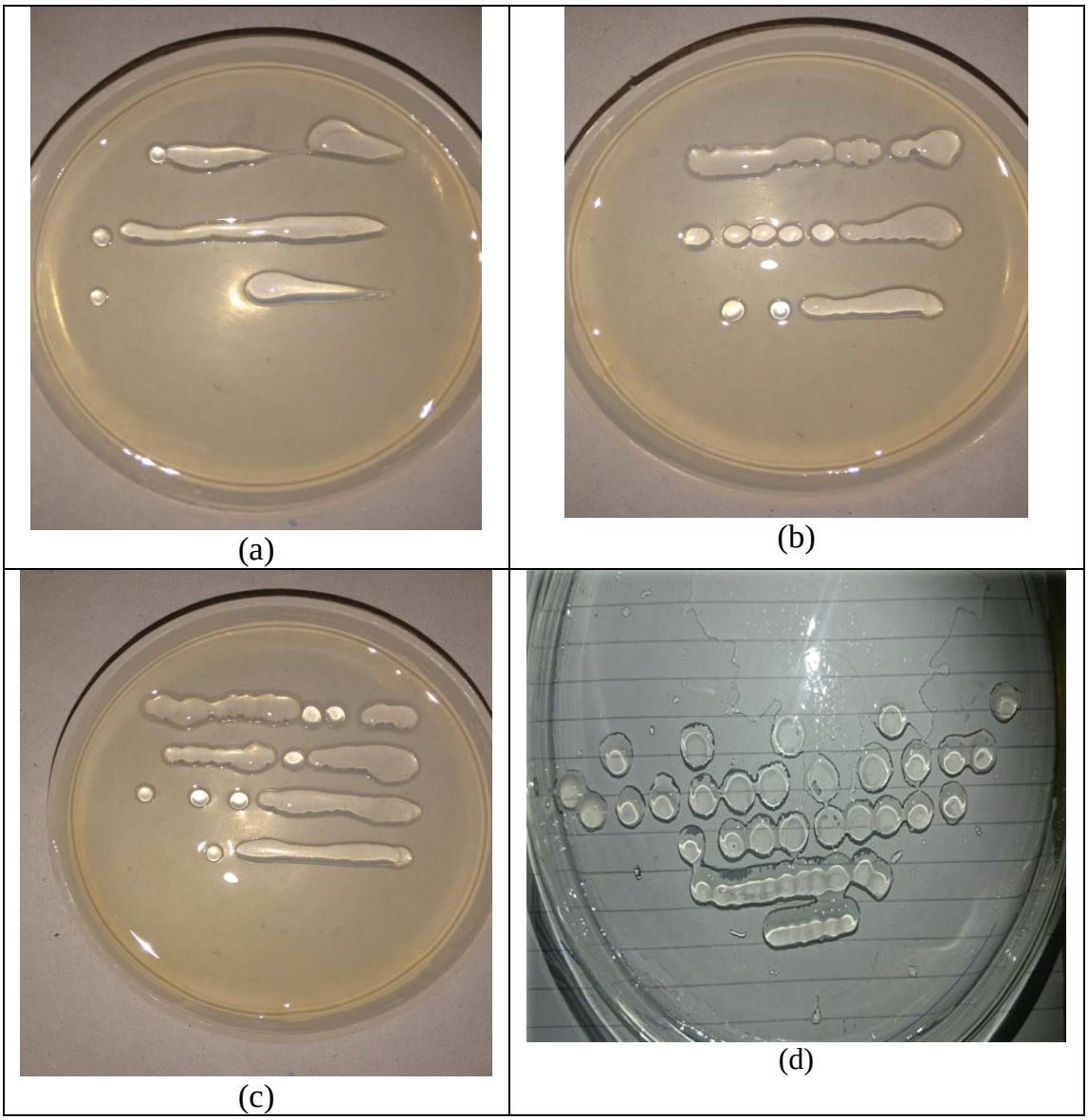
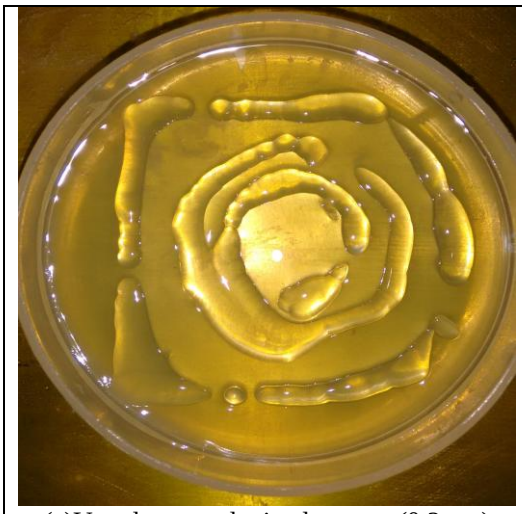



Figure – 4.4: Fabrication of line E = 10 (~140 $mm^3$), print direction left – right, (a) feed rate 500, 1000, and 2000 from top to bottom resp., z = 0.2mm. (b) ) feed rate 500, 1000, and 2000 from top to bottom resp., z = 2mm. (c) feed rate 200, 500, 1000, and 2000 from top to bottom resp., z = 5mm. (d) E = 6(~84$mm^3$), feed rate 1000, length 3, 6, 8, 10, and 12 from bottom to top.

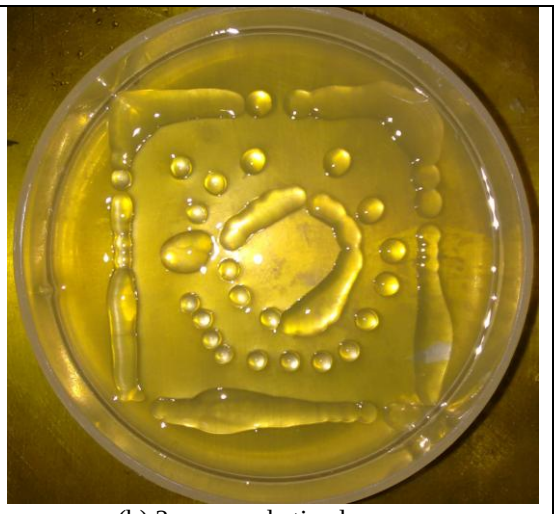
### 4.2.3 Fill pattern cation of soft scaffold

The direction or way choose by a designer or slicing software to print the pattern is known as the path. While handling liquid for printing print path shows a significant effect over the print

structure. Figure – 4.5 shows the effect of path while printing concentric hexagon in a square and circle in a hexagon.



(a)Very low nozzle tip clearance (0.2mm)

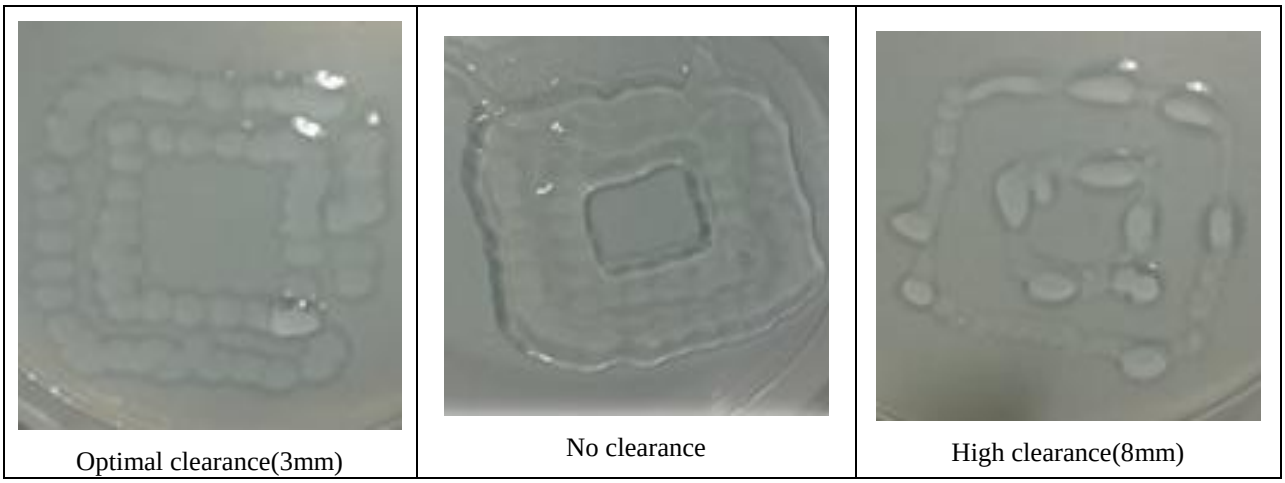


(b) 2mm nozzle tip clearance

Figure – 4.5: Fill pattern cation of soft scaffold printed from bottom left anticlockwise direction with E = 6 (~84mm$^3$) to print 30mm length.

If the path is circular the slicer software break it first into small lines. After slicing the circular path is nothing but the integration of small line. This makes printing of circular path tougher than the straight path. It can also be observed that even though complication is more in circular path the printing of circular path is better with more accuracy. As from the figure it clear that while dealing with liquid the printer head fails to print fine edges. If a person want to print hexagonal structure with small dimension it will not print perfect edges of the structure and distorted structure will be a result in place of desired one. Even in case of turn at very low angle nearly less than $30^0$ makes a globule of liquid material over the bed and losses the fine edge property of the structure.

The gap between two adjacent paths also plays important role as if the gap is too low then the road will overlap and form globule on the other side if the gap is too high then the adjacent road will detach with each other as shown in Figure – 4.6. Therefore there should be an optimal clearance (approx. – 2 or 3 mm) between two adjacent roads.



Optimal clearance(3mm) | No clearance | High clearance(8mm)

Figure – 4.6: Printing of adjacent path with different clearance printed from bottom left anticlockwise direction with E = 6 (~84mm$^3$) to print 30mm length.

All the observation shows to achieve complexity in structure while dealing with liquid is still more complicated and challenging than dealing with semisolids. Adjacent fill road clearance, height of nozzle tip, feed-rate of extruder, extruded volume of material, and solution concentration all are the parameters effecting fabrication process.

### 4.2.4 Single layer deposition

Every slicing software give a collection of fill pattern like rectilinear, line, honeycomb, hilbertcurve etc. which a designer can choose to infill the structure. Different fill pattern give specific porosity to the structure.

Various trials are performed to print different fill patterns as shown in fig. – 4.7. After trials of different fill pattern it was observed that printer is capable of printing all the complex structure given as an input command to print. In some cases where the clearance is not appropriate the droplets coming out from the nozzle tip merge together and forms larger globules which damages the expected fill pattern (Figure – 4.7(a) and 4.7(b)). When the parameters are optimized perfectly designer can have a fine structure with porosity (Figure – 4.7 (c) – (f)).

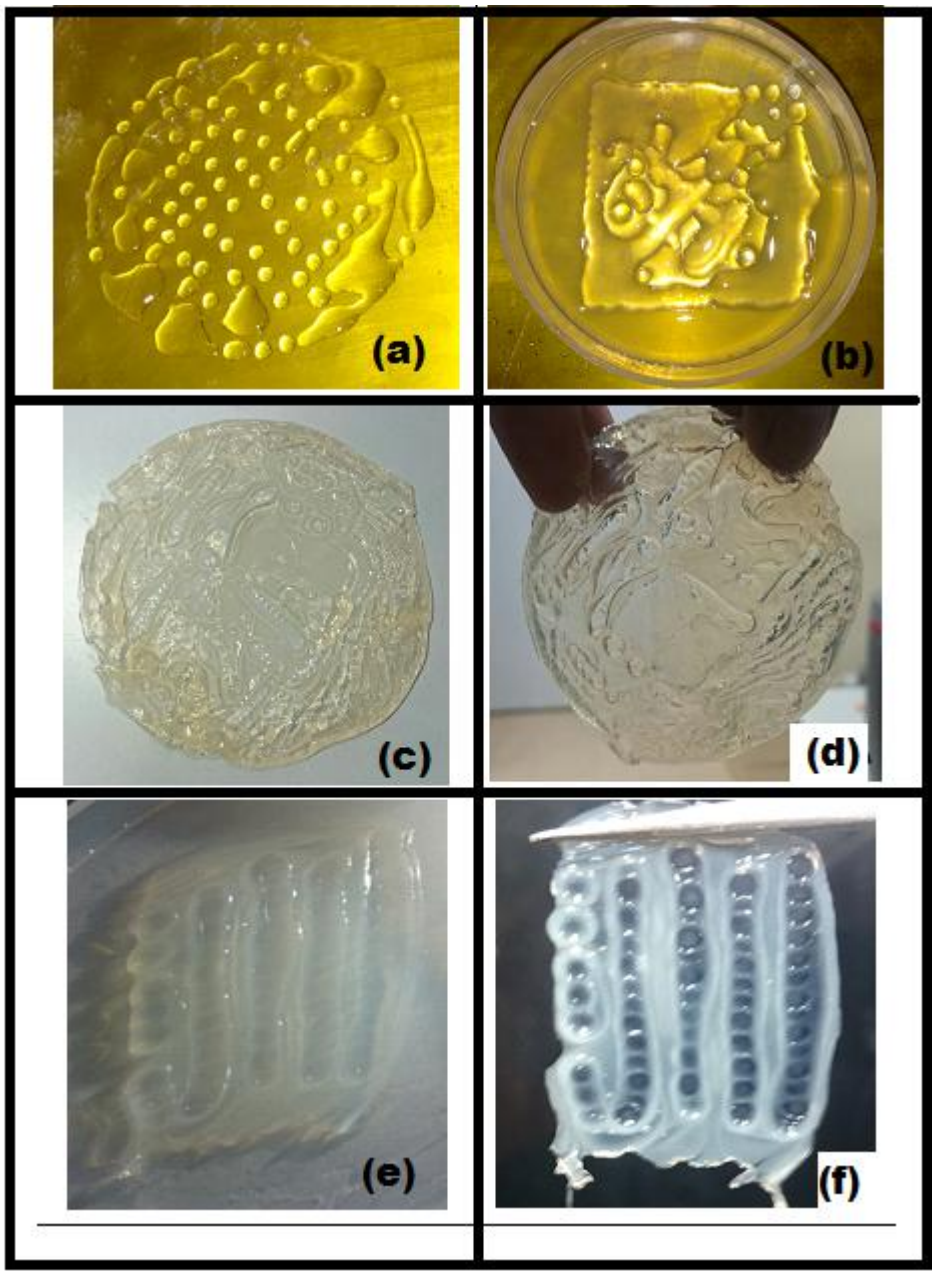



Figure – 4.7: Single layer deposition, direction with E = 6 (~84mm$^3$) to print 30mm length.

### 4.2.5 Multiple layer deposition

After getting the idea for single layer taking the calibration to the next level multiple layer deposition of scaffold are tried and observed. In this case after printing a single layer it is required to increase the height of nozzle tip as well as increase in the level of base liquid. Figure – 4.8 shows the results of multiple layer deposition of scaffold.

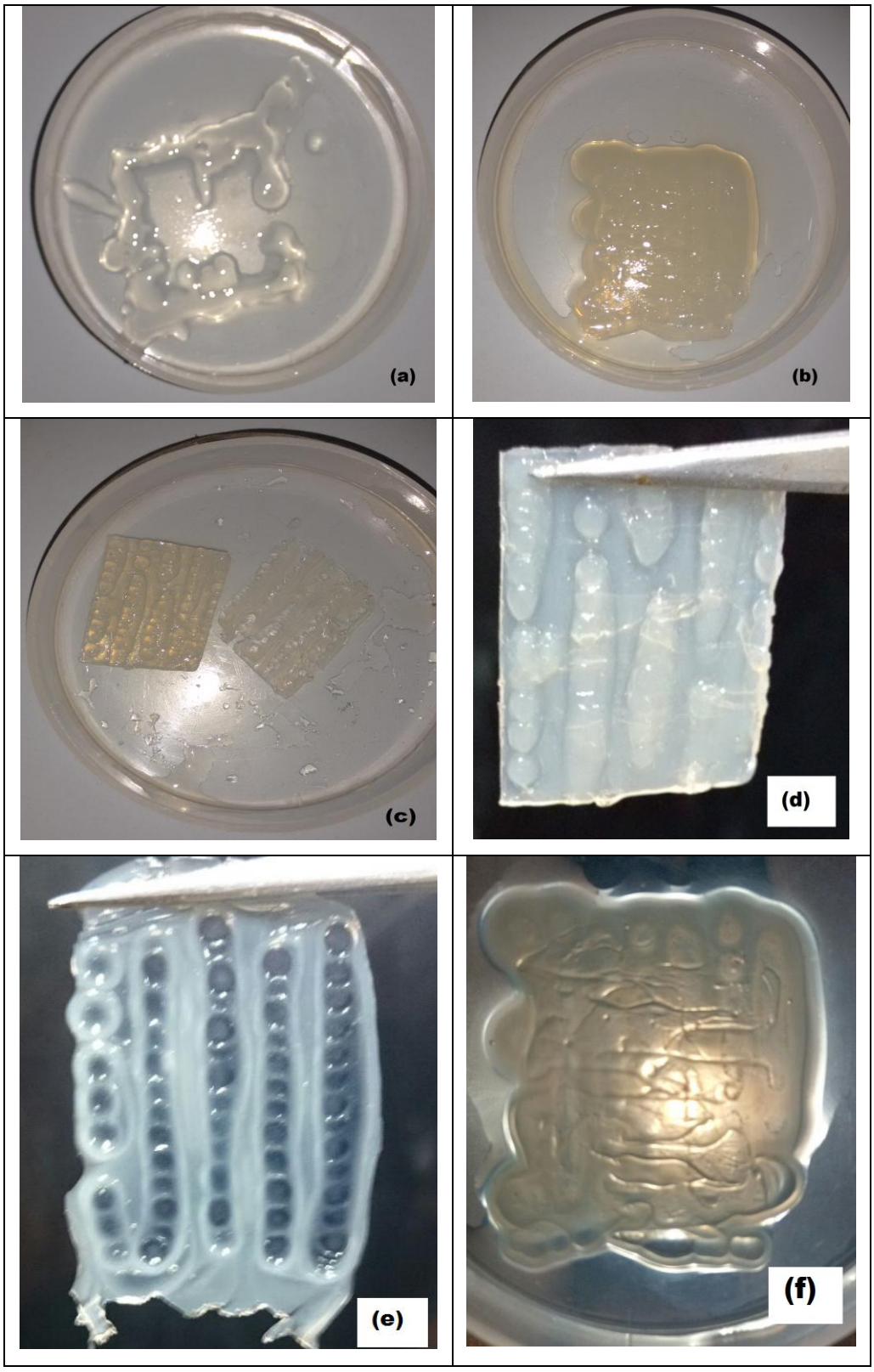



Fig. – 4.8: Multiple layer deposition, direction with E = 6 (~84mm$^3$) to print 30mm length.

While printing multiple layers one over other floating of liquid is observed (Figure – 4.8(a)). The porosity of first layer acts like a mound and allows the second layer liquid material to be solidify over it that results in packing of all porosity obtained by deposition of first layer (Figure -4.8(b)-(e)). Inside the structure porosity was observed shown in Figure – 4.8(f). Therefore it is difficult to print multiple layer by the use of single syringe arrangement. Further different possible arrangements may be applicable to get multiple layer. Use of double barrel syringe may give some help in this field.

The scaffold printed by the syringe arrangement shows desired porosity and interconnected channels for cell culture for single layer printed successfully. Still effort and special arrangement is required to fabricate multiple layer. It is tough to fabricate a sharp edge while dealing with liquid in comparison to solid. Globule formation of droplet after getting merge to each other was observed during fabrication of scaffold. Addition of gelatin, nano-particles can be done to enhance cell adhesion property as well as viscosity of the liquid for better printing of scaffolds.

# Chapter 5

# Fabrication of Hard Scaffold

## 5.1 Biocompatible 3D printable materials

Literature of RP fabricated scaffolds reveals numerous polymers that have been used for 3D scaffold printing. In particular, PLA is a currently used biodegradable polymer that has been approved by the FDA for various biomedical applications. PLA is also one of them that can be used for tissue printing application. Though this polymer has been extensively studied, its use in the fabrication of RP scaffolds and specifically those elaborated through nozzle based systems has been limited and scarcely reported. At present, most of the reported PLA-based scaffolds fabricated by RP require the molecular modification of the PLA matrix, the use of temperature during printing or further processing of the structure.

The RP tool used in the present study allows the fabrication of PLA 3-D structures without modifying the polymer structure with specific chemical groups. In the present work FDM machine developed by AlfaTek, Kolkata, India is used with extruder arrangement for the fabrication of scaffold. Spool of PLA material in wire form of diameter 1.75 mm is also purchased from them. This work describes the fabrication of PLA-based 3-D scaffolds by RP. The structures obtained were characterized in terms of their processing effect, final architecture, mechanical behavior, surface properties and biological response.

## 5.2 Optimal process parameter of PLA

For every material optimization of parameters are the most important work to perform before printing. As PLA is brittle in comparison with ABS like material. The optimal printing temperature for PLA is observed in the range of 190 – 220 $^{0}$C. It is important to be careful for the selection of printing temperature as if the printing temperature is low the material will stuck in the nozzle and will not come out of the nozzle tip. On the other hand if the temperature is too high then the material will be burnt and weak bonding will be observed in the resulting structure.

The feed rate of material should be sufficient enough to print the layer as every layer will act and represent the behavior of final structure printed. If the feed rate is too high the nozzle will move with high speed without depositing any material and with low feed rate the material accumulation will occur just outside the nozzle tip resulting in the failure of print.

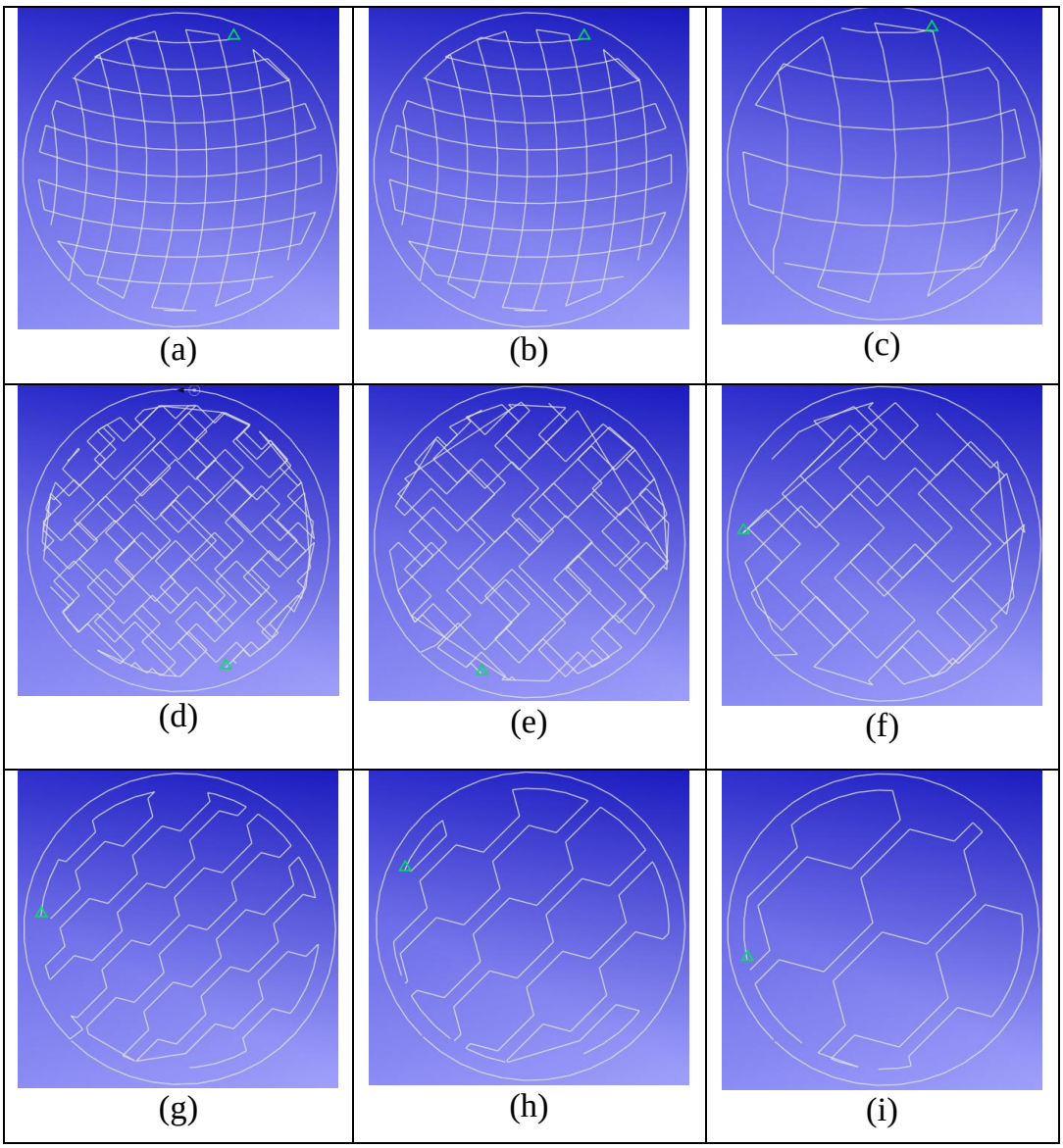
In every FDM machine the amount of material extruded by rollers to be melted in the hot end should be perfect enough to print as if more material is extruded then thick uneven road of material will be deposited resulting in the deposition of layer with high roughness. Smoothness is required in between the layer and adjacent road printed in same layer for better bonding and adhesion of material with each other. If the extruded material is less as compare to the required one then fine discontinuous lines will be printed which weakens the strength of the entire structure with undesired extra porosity.

## 5.3 Study of various feasible fill patterns

Different fill pattern are available in the slicing software which shows individual specific property with different interconnectivity and porosity. Fill pattern like honeycomb, lines, rectilinear, archemidianchord, octagramspiral, hilbertcurve, 3D honeycomb are available to be printed by the help of FDM machine or any 3D printing machine. A combination of varying fill density with different porosity is tried to get a better result for tissue engineering application. Figure – 5.1 shows the various combination of fill pattern and fill density.

It is observed that a variety of combination of fill pattern and fill density can be made and can be fabricated providing collection of complex structure for the application of scaffold fabrication

(Figure – 5.2). In the present work honeycomb and rectilinear fill pattern were used for the fabrication and investigation about the characteristics of the printed scaffold. Different researcher may use other combination according to their convenience. As the strength of honeycomb structure is more and the rectilinear structure shows better scaffold property these two fill patterns were used in the study. There were no restriction for choosing fill pattern to get porous structure researchers are free to choose any fill pattern even some can try to get new fill patterns also to get desired porosity at the same time interconnected channels with proper strength for better results.



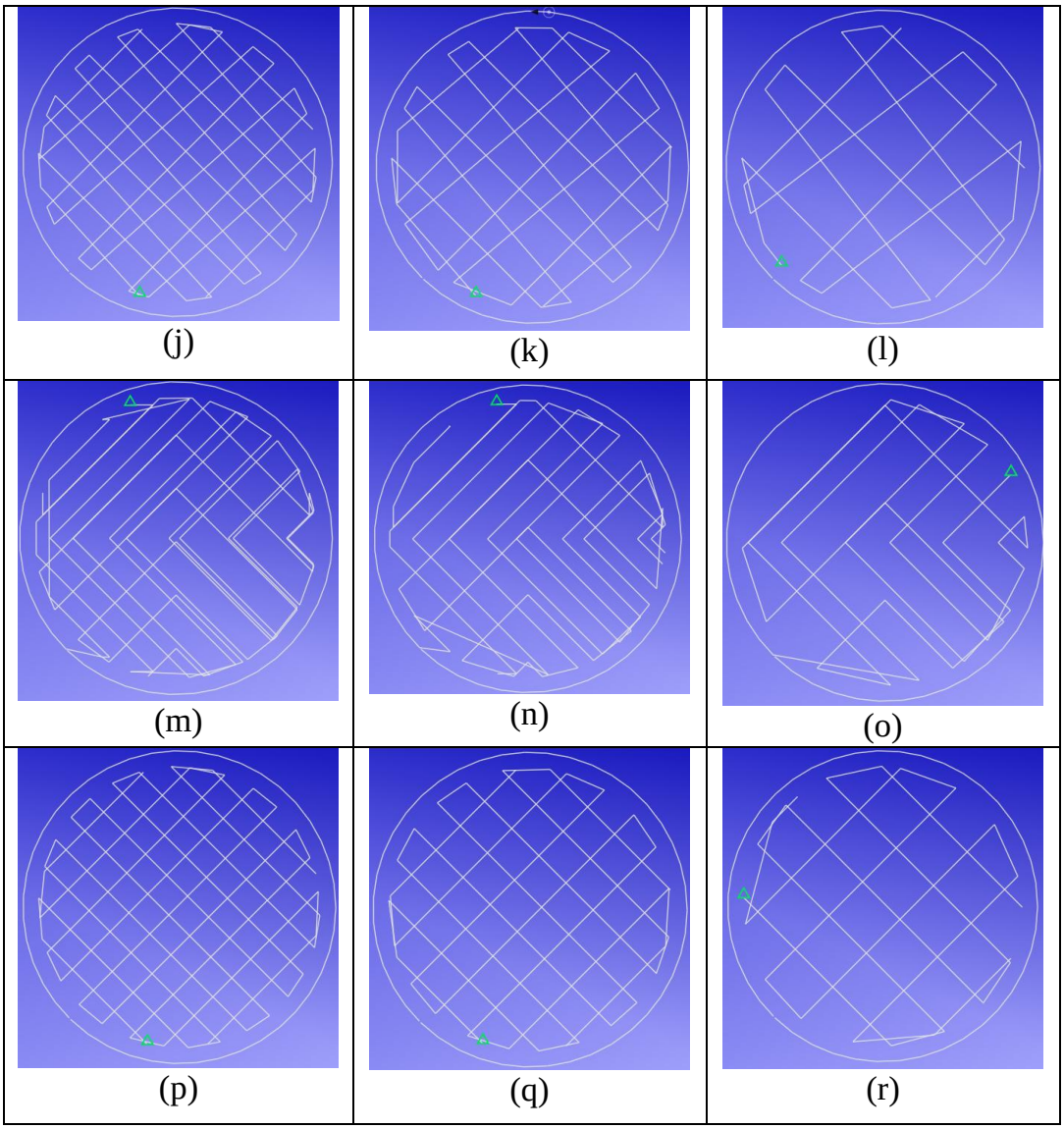



Figure – 5.1: Different fill pattern (archemedianchord (a)-(c), hilbertcurve (d)-(f), honeycomb (g)-(i), linear (j)-(l), octagramspiral (m)-(o), and rectilinear (p)-(r) with varying fill density (45%, 35%, and 25% from left to right)

A variety of samples were prepared to test the feasibility and physical appearance of different fill patterns. It was observed that different fill patterns have its own specific advantage. Honeycomb structure shows better strength whereas hilbertcurve pattern gives better interconnectivity. Linear and rectilinear gives better symmetry of channels and RW. Octagramspiral and concentric pattern gives axial channels throughout the structure.

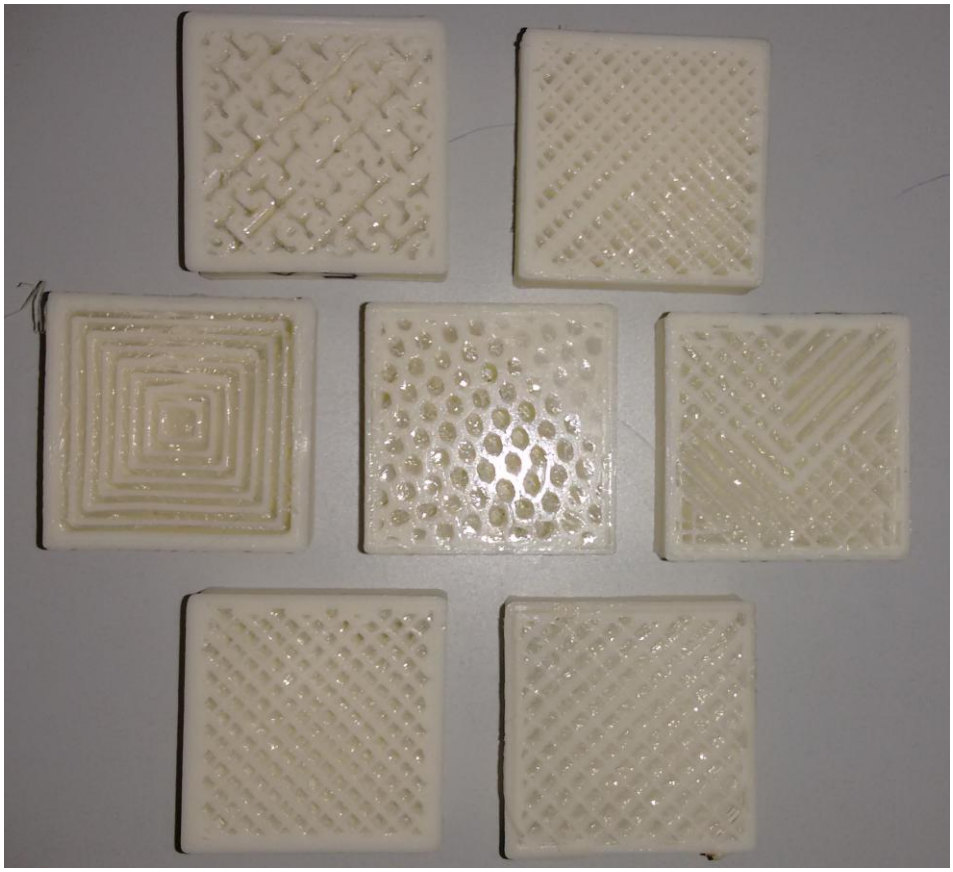


Fig. – 5.2: Fabricated fill pattern

## 5.4 Trials with variable fill density

Human bone is mot uniform throughout the cross-section, the structure is more complicated than it appears from outside. Bone structure is a combination of different density structure, outer part is more dens, less porous and tough which is known as cortex and the inner part is soft, highly dense and less dens which is known as medulla. To achieve this type of structure it is required to merge two different structure sliced code with each other so that one can have a combination of different density at a single layer and for the overall structure.

Figure shows the feasibility of design allowed to combine different fill pattern in a single structure. In figure – 5.3 combination of rectilinear fill pattern with different fill density (45%, 35%, and 25%) as well as different fill pattern hilbertcurve (fig. – 5.3(a)-(c)), honeycomb (fig. – 5.3(d)-(e)), rectilinear (fig. – 5.3(g)-(i)) respectively. After generating different combinations it is clear that one can choose any one of them as per application it is going to be used.

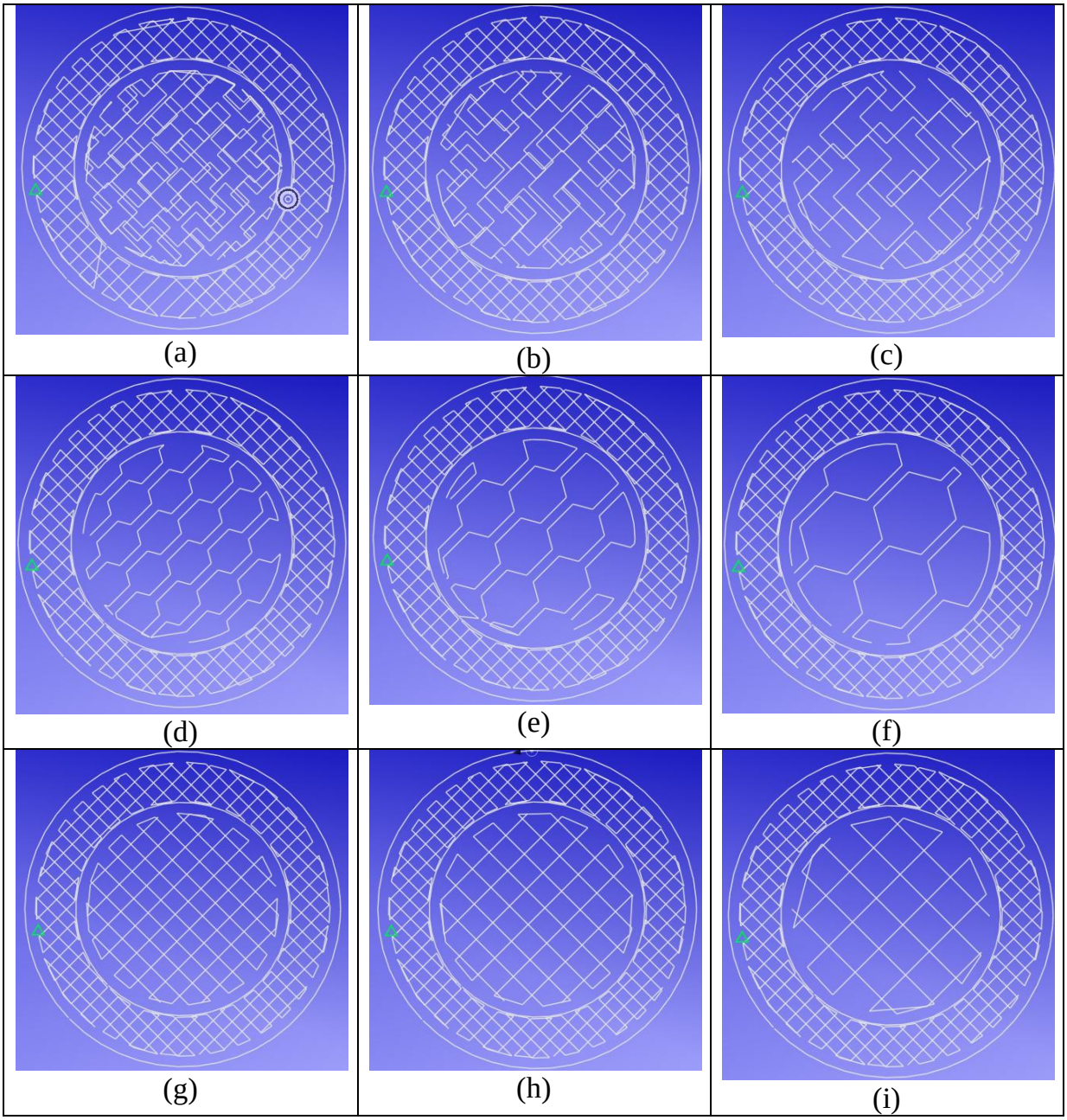
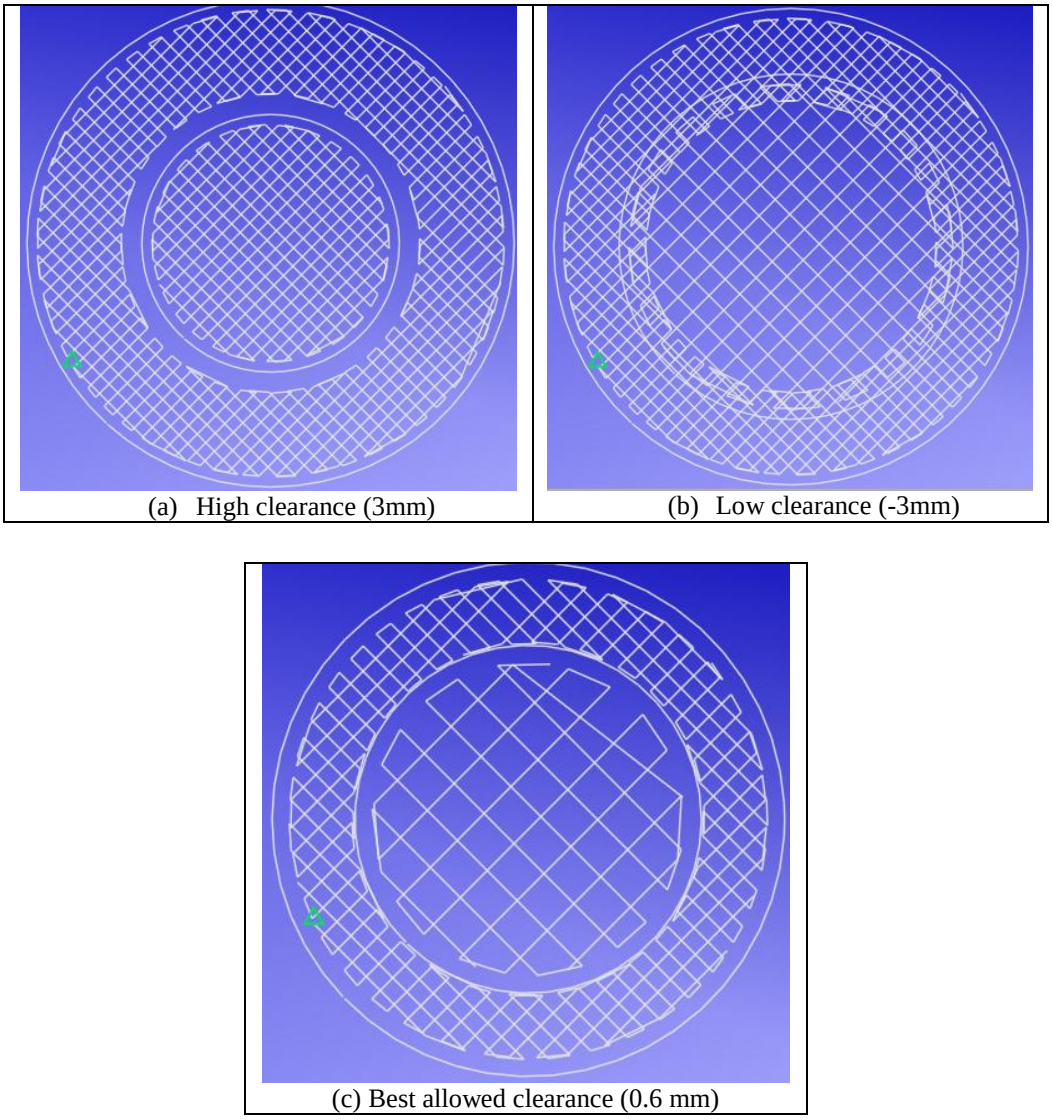



Figure – 5.3: Combination of variable fill pattern (rectilinear – hilbertcurve (a)-(c), rectilinear – honeycomb (d)-(f), and rectilinear-rectilinear (g)-(i)) with variable fill density in single print structure (65% (outer) and 25%, 35%, and 45% (inner) from right-left)

### 5.4.1 Effect of allowance between the fill patterns

When we are dealing with different fill pattern in a single structure in a single layer it is most important to care about the clearance between the intersecting surfaces as shown in Figure – 5.3. If the clearance is too high the two layers will detach from each other (Figure – 5.4(a)) and if the clearance is too low the overlapping of layer will cause uneven surface and uncomfortable environment for printing layer over layer (Figure – 5.4(b)). After the study and trials it was observed that the clearance considered by the slicing software is the best one i.e. approx. 0.6 mm (Figure – 5.4(c)).



(a) High clearance (3mm)

(b) Low clearance (-3mm)

(c) Best allowed clearance (0.6 mm)

Figure – 5.4: Allowance between fill patterns

## 5.5 *In vitro* cell culture test

For testing the feasibility of scaffold in biological environment *in vitro* cell culture test were performed. As the aim of the thesis is to achieve a scaffold structure feasible for tissue engineering it is important to study about the properties of printed scaffold *in vitro* environment. The test results will give the biocompatibility factor of the fabricated scaffold.

PLA scaffold of cylindrical in structure are prepared with diameter of 16 mm for cell culture with two different fill pattern rectilinear and honeycomb as show in Figure – 5.5 with 30% density i.e. 70% porosity. All the solid layer values from the top, bottom as well as wall surface (perimeter) is kept zero which means no solid surface were printed over the fill pattern. This is done just to allow the cells applied for tissue culture to go inside the interconnected structure and grow inside the entire structure on surface as well as core.

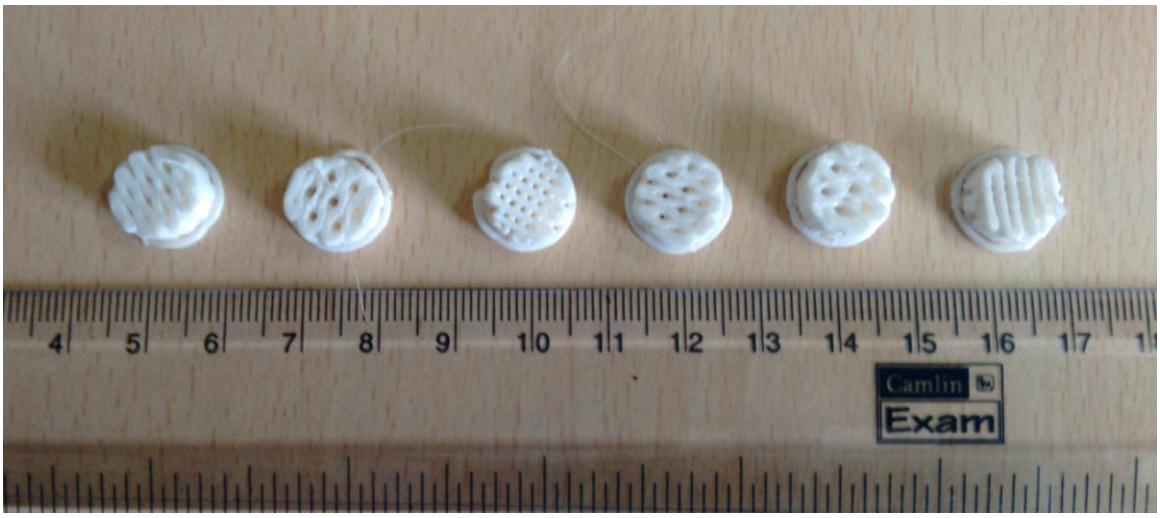



Figure – 5.5: Scaffolds for cell culture 16mm diameter honeycomb & rectilinear pattern

Human adipose derived stem cells (ADSCs) from the fat tissue abdomen of a human donor were isolated and cultured *in vitro*. These cells are used for seeding the cells into the scaffold. Before feeding cells scaffolds were sterilized by autoclave and washed thoroughly for four days in complete cultured medium. The scaffold were seeded with approximately 1 million ADSCs and

cultured for 1 day in $CO_2$ incubator at 37 $^0$C.Cells are allowed to grow only for one day and the samples were observed after completion of 1 day.

The medium was completely changed after 1 day. The scaffolds were washed with phosphate buffer saline (PBS) and incubated with 2 μg/ml of fluorescein diacetate (FDA) at 37 $^0$C temperature for 10 minutes. After that they were gently rinsed. The samples are observed under fluorescent microscope. Different passage are performed to take the reading of the tissue culture. The dye FDA is non-fluorescent; however, it is metabolized to a green fluorescence product by live cells. The viable cell cytoplasm were labeled as green on the other side non-viable cells nuclei were labeled red. Fig – 5.6 shows the result of the cell culture performed in 500 μm scale (Figure – 5.6(a)) and 100 μm scale (Figure – 5.6(b)).

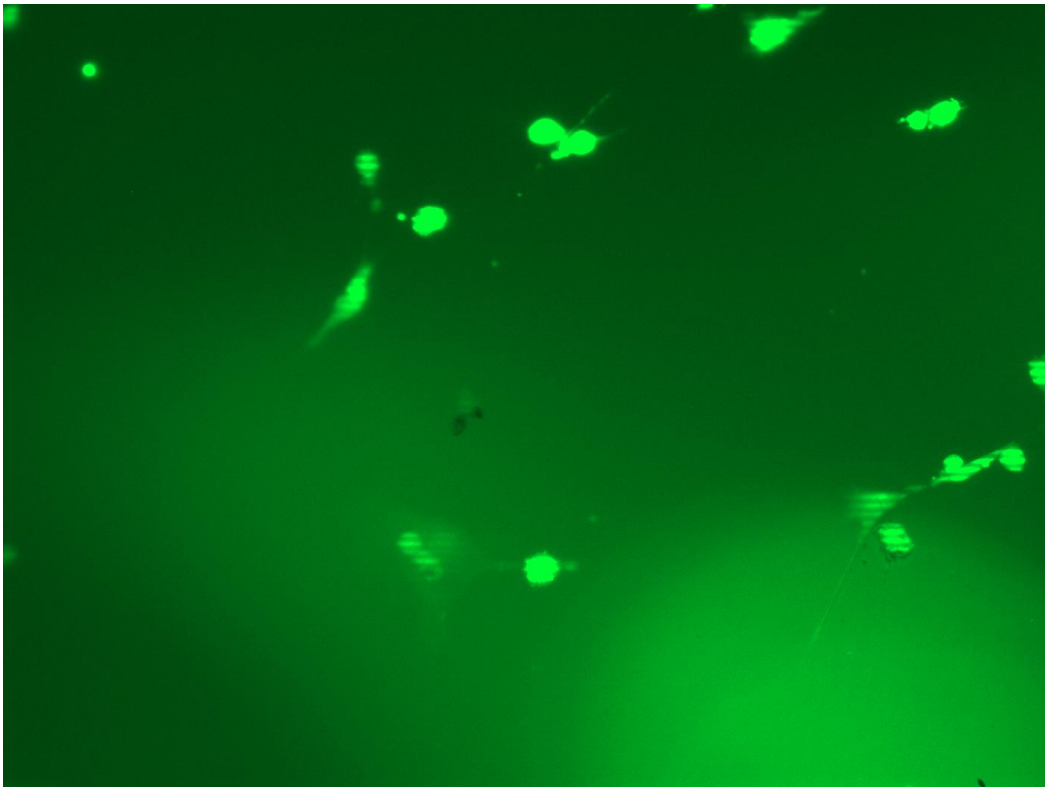


Fig. – 5.6 (a): Cell culture result (showing viable live cells)

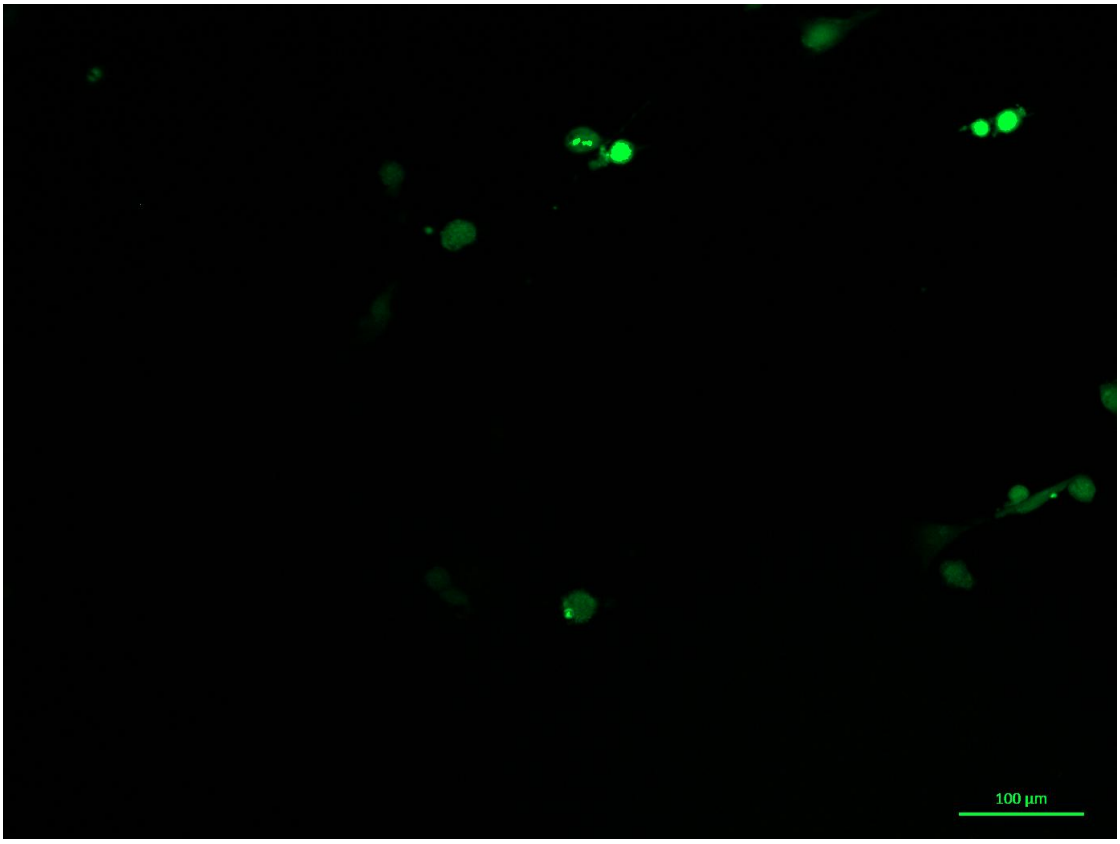



Fig. – 5.6(b): Cell culture result at higher magnification

After 1 day many cells are found to be alive may be because of the hydrophobic behavior of PLA. Rounded adhesion of cell over the scaffold structure were observed. The cells are not appeared to be spread. However, the cell are alive show the non-toxic nature of scaffold and its material used. No dead cells were observed, either no cells were dead or they are washed away.

The above studies shows the feasibility of material as well as machine parameter and arrangement to be capable of printing bio-compatible scaffold. The scaffold printed shows desired scaffold matrix with interconnected porosity and porous structure for cell culture. The live tissues shows the non-toxic behavior of scaffold material. By combining two g-codes of two different fill patterns it is possible to generate single matrix architecture with multiple fill pattern in a single layer. In vivo feasibility of the scaffold still needs to be done.

# Chapter 6

# Summary and Future scope

## 6.1 Summary

Doctors and surgeons have always been looking for better ways to describe, understand and diagnose the condition of individual patients. In many cases doctors and surgeons might like to have a physical model in front of them rather than have to look at images on a computer screen for better clarity and accuracy. AM have the ability to create such physically solid models of an individual directly from the 3D data output by the medical imaging system or different designing software. AM represent a range of technology that can fabricate 3D object in a single stage, from their CAD description.

Tissue engineering (TE) is a field that explore the potential future of regenerative medicine. The specific use of different biomaterials and cell isolation techniques has revolutionized the field of tissue engineering in fabricating tissue-like constructs in the laboratory. The required mechanical and physical properties can be customized and characterized with available biomaterials, whereas specific tissues might be fabricated with the application of specific cell types and growth factors. However, the matrix of the constructs affects the applied number of cells. Their survival *in vivo* crucially depends on the architecture. The diffusion of micronutrients can be possible for only few hundred micrometres. The success of printed part is strongly dependent on the structures having interconnected channels and well defined porous structure. Otherwise the central region gets frequently necrotic due to lack of nutrition.

Current attempts rely on the accurate architecture of the structure with fine and well defined interconnected channels for improving the growth factor in the graft. The thesis reports application of fused deposition modelling to fabricate a viable construct structure for tissue engineering and cell implants. This uses a special syringe arrangement for the fabrication of desired scaffold architecture relatively quickly. Optimization of the infill pattern of structure and improvement of growth factors in the scaffolds might eventually allow generation of different axially vascularized grafts for application in reconstructive surgery. This research project makes a promising approach for a study of printing parameters and feasibility of the process for further exploration of scaffolds for tissue engineering.

In this thesis research, a biomaterial polymer for tissue engineering approach was studied, in order to mimic characteristics of bone in spite of highly porous structure for effective tissue in-growth. A systematic approach was attempted to develop a scaffold with PLA and alginate hydrogel along with additives for proper differentiation of the tissue construct. The biomaterial needs to be biologically well compatible for viable bone in-growth. Therefore, the biomaterial with a specific shape and along with a hydrogel was studied for possible tissue engineering applications. The experiments conducted were focused on the development of a optimize parameters for different printing condition and circumstances.

The following conclusion were drawn from the experimental results:

- With the FDM arrangement used in the study it is possible to print with help of liquid as well as solid by using liquid and thermoplastic extruder arrangement respectively. The amount of material extruded plays important role for both soft and hard scaffold printing. It is possible to create different density in a same layer of a single structure.
- During fabrication of soft scaffold from liquid solution, solidification/fusion of materials need to be done instantaneously along with the motion of nozzle. This is influenced by material flow rate, bed to nozzle tip clearance, rate of fusion, and concentration of solution.
- In case of printing a droplet at defined position, feed rate doesn’t affect the extrusion process except the speed/time of fabrication. On the other hand, feed rate plays major role in fabrication of line or structure.

- Successful single layer fabrication of porous scaffold with required porous and interconnected channels demonstrated.
- Multiple layer deposition resulted in the earlier layer acting as mound for the latter. Hence, some intermediately steps may have to be explored to realize continuous layers.
- When liquid jetting through a syringe is used, the droplets merge into each other forming globules due to surface tension. Hence, an alternate mechanism may be needed if sharp edges are to be printed.
- Cell culture report shows the feasibility and biocompatibility of the material used. Live adhered cells show that the material used is non-toxic and hydrophilic in nature.

This study of fabricating soft and hard scaffold on different printing parameter to get desired matrix has the potential to be developed into a clinically viable tissue engineered construct.

## 6.2 Future Scope

The results in this research showed that using PLA and alginate hydrogel for hard and soft scaffold with suitable parameters can make a useful matrix architecture for tissue engineering application. However, for clinically useful application of this scaffold product, further evaluation of a number of aspects will need to be carried out, as recommended below:

- Mechanical properties of the scaffold must be studied and tested to check the strength in case of bone constructs.
- Bio-compatible Nano materials can be added to the materials for making composites and enhancing the *in vitro* property of scaffold.
- Nano materials can be used for encapsulation in case of soft scaffold fabrication technique which can directly be used as drug delivery for the patient.
- Feasibility of fabricating soft and hard scaffold are shown, separate setup which may help in printing both soft and hard scaffold together can be developed and studied.

# References


[1] Vacanti, J. P., & Langer, R. (1999). Tissue engineering: the design and fabrication of living replacement devices for surgical reconstruction and transplantation. The Lancet, 354, S32-S34.

[2] https://en.wikipedia.org/wiki/Tissue_engineering, date of access: 29 June 29, 2015.

[3] Dike, L. E., Chen, C. S., Mrksich, M., Tien, J., Whitesides, G. M., & Ingber, D. E. (1999). Geometric control of switching between growth, apoptosis, and differentiation during angiogenesis using micropatterned substrates. In Vitro Cellular & Developmental Biology-Animal, 35(8), 441-448.

[4] Hutmacher, D. W., Sittinger, M., & Risbud, M. V. (2004). Scaffold-based tissue engineering: rationale for computer-aided design and solid free-form fabrication systems. TRENDS in Biotechnology, 22(7), 354-362.

[5] Butscher, A., Bohner, M., Hofmann, S., Gauckler, L., & Müller, R. (2011). Structural and material approaches to bone tissue engineering in powder-based three-dimensional printing. Acta Biomaterialia, 7(3), 907-920.

[6] De Mulder, E. L., Buma, P., & Hannink, G. (2009). Anisotropic porous biodegradable scaffolds for musculoskeletal tissue engineering. Materials, 2(4), 1674-1696.

[7] Xiong, Z., Yan, Y., Wang, S., Zhang, R., & Zhang, C. (2002). Fabrication of porous scaffolds for bone tissue engineering via low-temperature deposition.Scripta Materialia, 46(11), 771-776.

[8] Serra, T., Planell, J. A., & Navarro, M. (2013). High-resolution PLA-based composite scaffolds via 3-D printing technology. Acta biomaterialia, 9(3), 5521-5530.

[9] Salerno, A., & Pascual, C. D. (2015). Bio-based polymers, supercritical fluids and tissue engineering. Process Biochemistry, 50(5), 826-838.

[10] Rezabeigi, E., Wood-Adams, P. M., & Drew, R. A. (2014). Production of porous polylactic acid monoliths via nonsolvent induced phase separation. Polymer, 55(26), 6743-6753.

[11] Jing, X., Mi, H. Y., Peng, J., Peng, X. F., & Turng, L. S. (2015). Electrospun aligned poly (propylene carbonate) microfibers with chitosan nanofibers as tissue engineering scaffolds. Carbohydrate polymers, 117, 941-949.

[12] Jia, J., Richards, D. J., Pollard, S., Tan, Y., Rodriguez, J., Visconti, R. P., & Mei, Y. (2014). Engineering alginate as bioink for bioprinting. Acta biomaterialia, 10(10), 4323-4331.

[13] Marras-Marquez, T., Peña, J., & Veiga-Ochoa, M. D. (2014). Agarose drug delivery systems upgraded by surfactants inclusion: Critical role of the pore architecture. Carbohydrate polymers, 103, 359-368.

[14] Grehan, L., Killion, J., Devery, S., Higginbotham, C., & Geever, L. (2014, November). Biological Evaluation and Characterisation of Novel Hydrogel Matrices as Scaffolds for Bone Tissue Engineering. In Applied Mechanics and Materials (Vol. 679, pp. 63-75).

[15] Herran, C. L., & Huang, Y. (2012). Alginate microsphere fabrication using bipolar wave-based drop-on-demand jetting. Journal of manufacturing processes, 14(2), 98-106.

[16] Wang, C. C., Yang, K. C., Lin, K. H., Liu, H. C., & Lin, F. H. (2011). A highly organized three-dimensional alginate scaffold for cartilage tissue engineering prepared by microfluidic technology. Biomaterials, 32(29), 7118-7126.

[17] Rath, S. N., Arkudas, A., Lam, C. X., Olkowski, R., Polykandroitis, E., Chróścicka, A., & Kneser, U. (2012). Development of a pre-vascularized 3D scaffold-hydrogel composite graft using an arterio-venous loop for tissue engineering applications. Journal of biomaterials applications, 27(3), 277-289.