Design and Development of a 3D Bio-Printer

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Thesis Supervisor:

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AUGUST 2023

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Copyright Statement

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ABSTRACT

3D Bio-Printing is an advanced technology to fabricate scaffolds. Using this technique, the scaffolds of various tissues can be printed. A 3D Bio-Printer was designed for fabricating complex functional living tissues using bioink. Bioink is basically a blend of living cells along with biochemical materials including growth factors and support components and then printed. The factors to be considered during the development of a 3D Bio-Printer are compactness (To be placed in a laminar flow unit), Printing Resolution (Fabrication with high fidelity), Degree of freedom (Need multiple axes to print 3D construct), printing Speed (High speed required) and Process Biocompatibility. The aim of this research was to develop a syringe-based 3D Bio-Printer which may print Biomaterials and cells by considering these factors. The developed 3D Bio-Printer was then tested by printing a highly viscous hydrogel as proof-of-concept where gelatin was used to simulate the fluid properties of bioink. The syringe-based 3D Bio-Printer demonstrated a high level of printing resolution and stable prototype, and so has a potential for use in 3D cell culture after further in vitro testing.

Keywords: *Additive Manufacturing; Rapid Prototyping; Tissue Regeneration; Biofabrication; 3D Bio-Printing; Scaffold Printing; Bioinks; Drop on Demand (DOD) based Bio-Printing; Extrusion based Bio-Printing; Syringe based 3D Bio-Printer*

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CHAPTER 1: INTRODUCTION

Humans are vulnerable to tissue damage and ageing, but the body's natural ability to repair cells is frequently insufficient to handle this strain. Traditional approaches to treating these illnesses rely on tissue or organ transplantation, which is similarly reliant on the availability of donors, which may be limited, and has the risk of graft rejection owing to an immune response [1]. Tissue engineering (TE) has been offered as a viable technique of saving lives and improving living conditions. Tissue engineering is a concept that attempts to replace damaged tissue with functioning alternatives by merging technological and biological concepts [2]. 3D Bio-Printing, a novel tissue manufacturing method, has promise for bridging the gap between organ shortages and transplantation demands [3]. 3D Bio-Printing is an efficient technology for creating biologically functional tissues in vitro [4]. Rapid prototyping (RP) technology is used in organ printing to print cells, biomaterials, and biomaterials including cells one at a time or in combination, layer by layer, resulting in 3D tissue-like structures [5], [6]. Bio-Printing, rather than only providing scaffold support, enables for exact spatial placement of the cells themselves [7]-[12]. 3D Bio-Printing techniques have varying printing resolutions ranging from 10 to 10,000 mm, which is higher than alternative assembly methods like moulding or the use of porous scaffolds [13]-[16].

CHAPTER 2: LITERATURE REVIEW

According to the experimental setting, 3D Bio-Printing enables users to vary factors including the choice of biomaterial, cell type, and 3D design [17]. Researchers that are interested in the uses of 3D bioprinting in regenerative medicine have also been curious in the proliferation and growth of stem cells in 3D in vitro systems produced by Bio-Printers [18]. The capacity to "print" cells, tissues, and organs on demand owing to three-dimensional Bio-Printing has transformed the medical industry. Because of the advancement of in vitro research methods, which have in some instances supplanted the usage of animal models, this technique might have a big impact on biomedical research [19]. When paired with advancements in tissue engineering, these findings have the potential to help in the treatment of a variety of veterinary illnesses [20]. In contrast to 2D in vitro systems in which all cells are exposed to the same dose, drug resistance is visible in 3D in-vitro drug screening systems because of the solubility of chemical component gradients [21]. Furthermore, Bio-Printing technology has opened the way for the development of replacements that can be used to replace damaged tissues, as well as the development of physiological illness models to better understand the situation and build an intelligent, effective treatment strategy. [22]-[24].

2.1 Bioinks

Bioink is one of the most important prerequisites for 3D bioprinting. It is composed of cells, biomaterials, and other necessary elements [3]. The main goal of current Bio-Printing research is to provide a range of biomaterial formulations with traits including biocompatibility, bioprintability, mechanical stability, and shape fidelity. There are several biomaterials available for 3D bioprinted tissue engineering applications, both synthetic and natural. Bioinks have been created using gelatine [25], [26], alginate [27], [28], collagen [29], and silk protein [30]-[32]. Despite these benefits and conveniences, 3D Bio-Printing still faces several difficulties, including maintaining the functional integrity of the printed tissue and maintaining the tissue's vascularization, exchange of gases and nutrients, biocompatibility and biodegradability of the substrate material. Despite being the most biocompatible, these materials' stringent crosslinking requirements to maintain structural shape [33] inability to support cell proliferation and differentiation [33], [34] and low mechanical integrity [35] have piqued researchers' interest in learning more about the biomaterials. To strengthen the mechanical integrity of the printed

construct and to thwart biodegradation, bioprinting applications have included synthetic polymers including gelatine methacrylate (GelMA) and poly (ethylene glycol) diacrylate (PEGDA) [36]. Research teams developed a method known as hybrid printing, which required printing both natural and synthetic materials at the same time, and they were successful in doing so [37]. This method has the potential to eliminate the drawbacks associated with using either synthetic or natural materials as distinct bioinks [38]. Few publications have been published that demonstrate the usage of different combinations of purely natural materials as bioink. To examine the kinetics of the printed constructions' deterioration, a bioink made of alginate, gelatine, and collagen was also employed. Despite the fact that the printed constructions outperformed the trio according to the data, the material's use for 3D Bio-Printing does not appear promising due to its poor capacity to control degradation[34]. Maintaining the ideal homogeneous pH of the material and the ideal printing temperature is of the highest importance in Bio-Printing since any variation from the ideal circumstances dramatically alters the viability of cells. As a result, natural materials including alginate, gelatine, and collagen have their pH adjusted to a healthy pH range of 7.4 [26], [27], [39]. The main goal of temperature optimization for bioinks is to alter their rheological properties. The appropriate temperature to maintain during printing relies on the material's storage and loss modulus. For instance, collagen's storage modulus begins to rise at a particular temperature over 15 °C [33] which means that, beyond that temperature, the collagen becomes more viscous and blocks the nozzle. The second example is gelatin, which has a storage modulus that drops at a specific temperature and then becomes less viscous and readily flows out the nozzle. Materials are printed at a lower temperature than room temperature because structural stability relies on the printing temperature, even if a rapid change in temperature does not dramatically impair cellular viability [26], [27]. To generate a construct that is both mechanically sturdy and compatible with cells, it is necessary to build a scaffold out of a combination of harder and softer elements.

2.2 Scaffold based and Scaffold-free Bioinks

It is feasible to use both scaffold-based technique and scaffold-free techniques for bioprinting. In the scaffold-based approach, the biomaterial matrix produces a layer for cellular deposition. This matrix may be any kind of 3D structure, including nanofibers, films, hydrogels, or any combination of these. For cells to proliferate and reproduce, it is essential to keep in mind that the 3D structure should closely mirror the actual ECM environment. The cell or tissue aggregates that are directly deposited during scaffold-free Bio-Printing include spheroids, honeycomb, cylinders, etc. The

more cells there are in the smallest 3D print mould, the more likely it is that ECM deposition will begin automatically [40]. The tissue spheroids are placed into pipettes and extruded onto printing moulds to complete the process. As the tissue develops and the mould is ultimately destroyed, the cells release their own ECM and create a network. In contrast to being utilised in any way, the mould just serves as a framework for support. With the help of this method, cells may be freed from biomaterial that prevents them from interacting and limits their growth. Cellular self-organization promotes ECM synthesis and keeps tissues functioning [41].

2.3 Different strategies for 3D Bio-Printing

Depending on its principle, 3D bioprinting is divided into several types [43]. To produce the necessary tissue, any one of these techniques alone or in combination is employed. The most popular techniques for Bio-Printing are inkjet-based 3D Bio-Printing [44], laser-based 3D Bio-Printing [45] 3D Bio-Printing based on extrusion [46] and stereolithographic based 3D Bio-Printing [47]. These are the most crucial methods for arranging and stacking bioactive substances [48].

2.3.1 Drop-on-Demand (DOD) based Bio-Printing

Bio-ink is deposited in regulated-volume droplets at predefined locations in drop-on-demand based Bio-Printing. It may be used for a range of applications in a number of domains, including transplantation, high-throughput screening, cancer, and other areas, as a result of its unique switch of accumulation, high resolution, high precision, simplicity of usage, and flexibility [49]. Inkjet bioprinting (IBBP), acoustic bioprinting, and other methods fall under the category of droplet-based bioprinting. IBBP is a non-contact process that is similar to the usage of 2D desktop inkjet printers. IBBP may be divided into three different subcategories: thermal IBBP, electrostatic IBBP, and piezoelectric inkjet Bio-Printing [50]. In order to create sound waves over the Bioink (BI) compartment, piezoelectric actuators are employed in piezoelectric inkjet Bio-Printers [51]. The operating concept is briefly explained in the paragraphs that follow. Pressure waves propagate throughout the capillary as a consequence of a fast volume change brought on by the voltage applied to the piezoelectric actuator.

Fluid is forced out of the nozzle when a wave of positive pressure strikes it. When the kinetic energy conveyed outwardly exceeds the surface energy required to form a droplet, the droplet is evacuated [52]. Electrostatic inkjet Bio-Printers create voltage pulses by applying a pressure plate

and an electrode in order to create droplets. Thermal inkjet Bio-Printing generates heat in the BI compartment, which raises pressure. The manufacturing approach, which mainly focuses on producing ink droplets, has gained appeal since it is inexpensive, compatible with living things, and produces droplet bio-inks quickly [53].

Table 1: Summary of the key parameters for Inkjet based Bio-Printing

Name	**Advantages**	**Viscosity & Resolution**	**Cell Viability (%)**	**Price**	**Drawbacks**	**References**
Inkjet based 3D Bio-Printing	Fast process Readily available High Throughput High resolution	<15 mPa/s 50-100 um	>85%	Cheap	Lack of precision Low viscosity bioink need required. Nozzle clogging with dealing with bioinks with high cell densities. No uniform Droplet size. Chances of cross-contamination when multiple bioinks are used.	[12], [55]–[58]

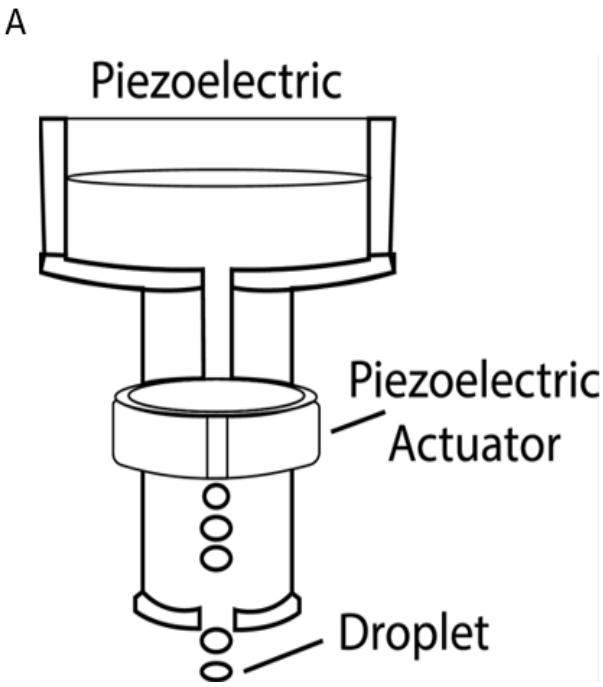

Figure 1: Schematic diagram of Piezoelectric IBBP

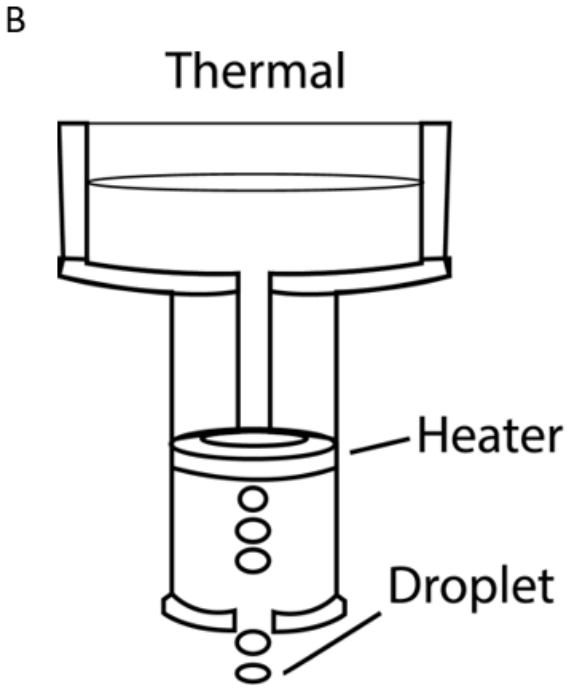

Figure 2: Schematic diagram of Thermal IBBP

2.3.2 Laser Assisted Bio-Printing

In order to swiftly deposit cell solutions with high cell concentrations, a variety of approaches are utilised with laser-assisted Bio-Printing, including laser-guided direct writing (LGDW), laser-induced forwards transfer (LIFT), and modified laser-induced forwards transfer (modified-LIFT) procedures[60]. One of the LAB Bio-Printing methods, laser-induced forwards transfer (LIFT), has been successfully utilised to print DNA or organ cells in the past [61]. Recently, laser-aided Bio-Printers have been created to more precisely apply biological designs to surfaces and print biomaterials [62]. The contributor in LAB has a "ribbon" frame with a layer of BI at the bottom and an energy-absorbing layer (EAL) made of Ti or Au on top. The beam of laser is focused on energy absorbing layer and the printing is done.

Due to the properties of the "ribbon" cell coating, it is difficult to push cells precisely, and operating the laser system is more difficult than printing with nozzles. However, it takes some time for the "ribbon" structure of each kind of cell or hydrogel to form, particularly when many cell types are used or other materials are co-accumulated. It is not yet understood what harm the laser irradiation does to cells. A major problem related to LAB is cell viability due to excessive heat [63]-[65].

Table 2**:** Summary of the key parameters for Laser Assisted Bio-Printing:

	Advantages	**Viscosity & Resolution**	**Cell Viability (%)**	**Price**	**Drawbacks**	**References**
Laser Assisted Bio-Printing	Highest resolution High cell viability High precision Only few Bioinks can be printed Bioink remains free	<300 mPa/s 20 um	>95%	Expensive	Time consuming	[51], [66]–[69]

	from shear-stress Pinting speed in between medium-fast					

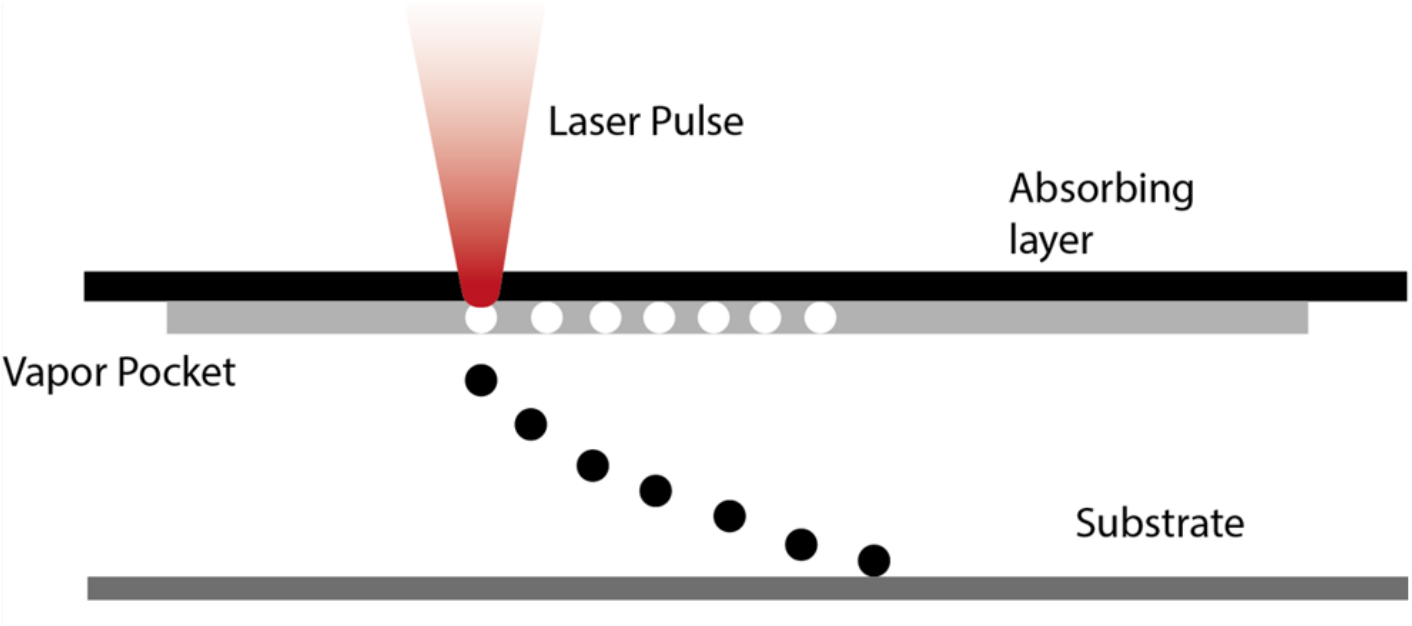

Figure 3: Schematic diagram of LAB

2.3.3 Stereolithography based Bio-Printing

Under precisely regulated light, the photocuring-based stereolithography (SL) approach hardens light sensitive polymers to form tissue structures [42]. A UV laser and a focused mirror array, which emit a beam onto the surface of a liquid, photocurable resin, are the main components of the process. The concentrated beam interacts with the BI material to polymerise it in a predefined pattern for each layer of deposition as the laser scans a 2D pattern point-by-point. The most recent unpolymerized-ink component must be moved up or down and away from the laser source to be put on the printing pedestal for the subsequent stratum [70]. The curing time and thickness of the polymerised surface are controlled by the main kinetic parameters of the curing activity, such as

the light beam amplitude, scanning speed, and exposure time, both of which are crucial for the correct functioning of the produced components [71]. The depth of polymerisation may also be managed by adding UV absorbers and photo-initiators to the resin [72]. However, Wang et al. [36] contend that the upkeep and expansion of the built environment is hastening the development of the visible light SL option in Bio-Printing. The development of the model is being helped by the light-sensitive bio-inks since SL Bio-Printing is set up such that a certain light controls the output. This is because 3D framework designs printed using conventional techniques often contain weak, spongy channels. As a result, the bio-inks are created one plane at a time [73]. The ability of SL to produce complicated products with really high-quality interior architecture is another well-known feature.

Disadvantages of SL includes high cost, slow printing speed and limited choice of biomaterial that are available for SL based 3D Bio-Printing

Table 3: Summary of the key parameters for Stereolithography based Bio-Printing:

Name	**Advantages**	**Viscosity & Resolution**	**Cell Viability (%)**	**Price**	**Drawbacks**	**References**
Stereolithography based Bio-Printing	High accuracy Rapid printing speed	No limitation 100 um	>90%	Not too expensive	Extremely intricate procedure Complex processing after printing	[75] [76], [77]

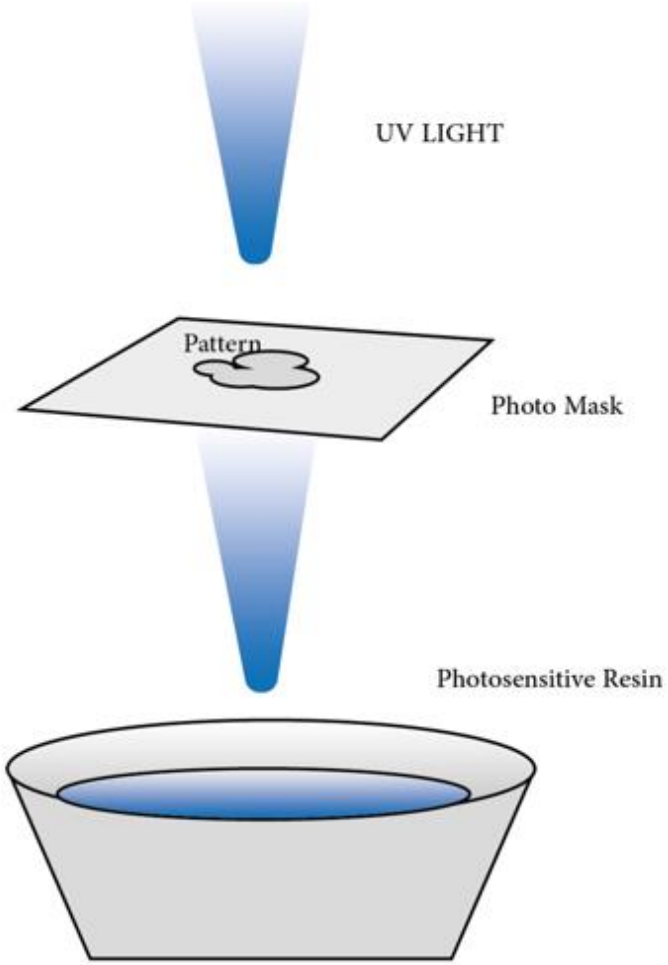

Figure 4: Schematic diagram of SL based Bio-Printing.

2.3.4 Extrusion based Bio-Printing.

Extrusion-based Bio-Printing (EBBP) is the single method that allows the developing of highly viscous materials and thick cell layers to create three-dimensional (3D) structures. Extrusion printing, also known as direct writing, has developed into one of the best, least expensive methods for quick development as a result of well-known open-source efforts [78]. On a wider scale, it may be similar to the use of inkjet Bio-Printing. Recently, there has been a big rise in the application of this Bio-Printing method for tissue engineering and bio-fabrication [79]. Two critical processes are applied to spread the produced bio-inks: air force and mechanical force. Compressed air is used to give gas force during extrusion in a valve-free or valve-based device [80]. An air pump that has been sterilised is joined to a BI-containing needle. Only bio-inks with shear-thinning powers can

support helical structure after extrusion due to shear stress caused by air extrusion. Even without valves, extrusion is a rather simple method. However, for high-precision efficiency, valve-based extrusion is prefered. This is one of the most cost-effective methods of printing using living-cell bio-ink [81].

Hand extrusion is required for particularly viscous bio-inks, such as synthetic and natural polymers. A common mechanical micro-extrusion technology that uses a piston and an electric motor is called piston-based extrusion. The motor rotates as a result of an electrical pulse, moving the piston forwards and forcing BI out of the outlet. Despite the need for more precise jet and nozzle setup, mechanical techniques provide greater resolution and enhanced printability for a broader range of biomaterials. On a robotic stage is a stage controller running an extrusion-based Bio-Printing dispenser system. The piston-propelled accumulation arrangement uses screw-driven mechanisms to regulate the overflow of the ink after applying bio-ink to a building material [82]-[83]. Various factor to be kept in mind during Extrusion Bio-Printing like how viscous and dense the material is, the rate at which extrusion occurred and its cross-linking of the biomaterial to achieve prints of high resolution [84]-[85].

Table 4: Summary of the key parameters for Extrusion based Bio-Printing:

Name	**Advantages**	**Viscosity & Resolution**	**Cell Viability (%)**	**Price**	**Drawbacks**	**References**
Extrusion based Bio-Printing	A higher density of cells may be produced using high viscosity bioink.	<6 x10^7 mPa/s 100 um	>45%	Not too expensive	Undue tension during printing may cause cell structure to be altered. Slow process	[86]–[93]

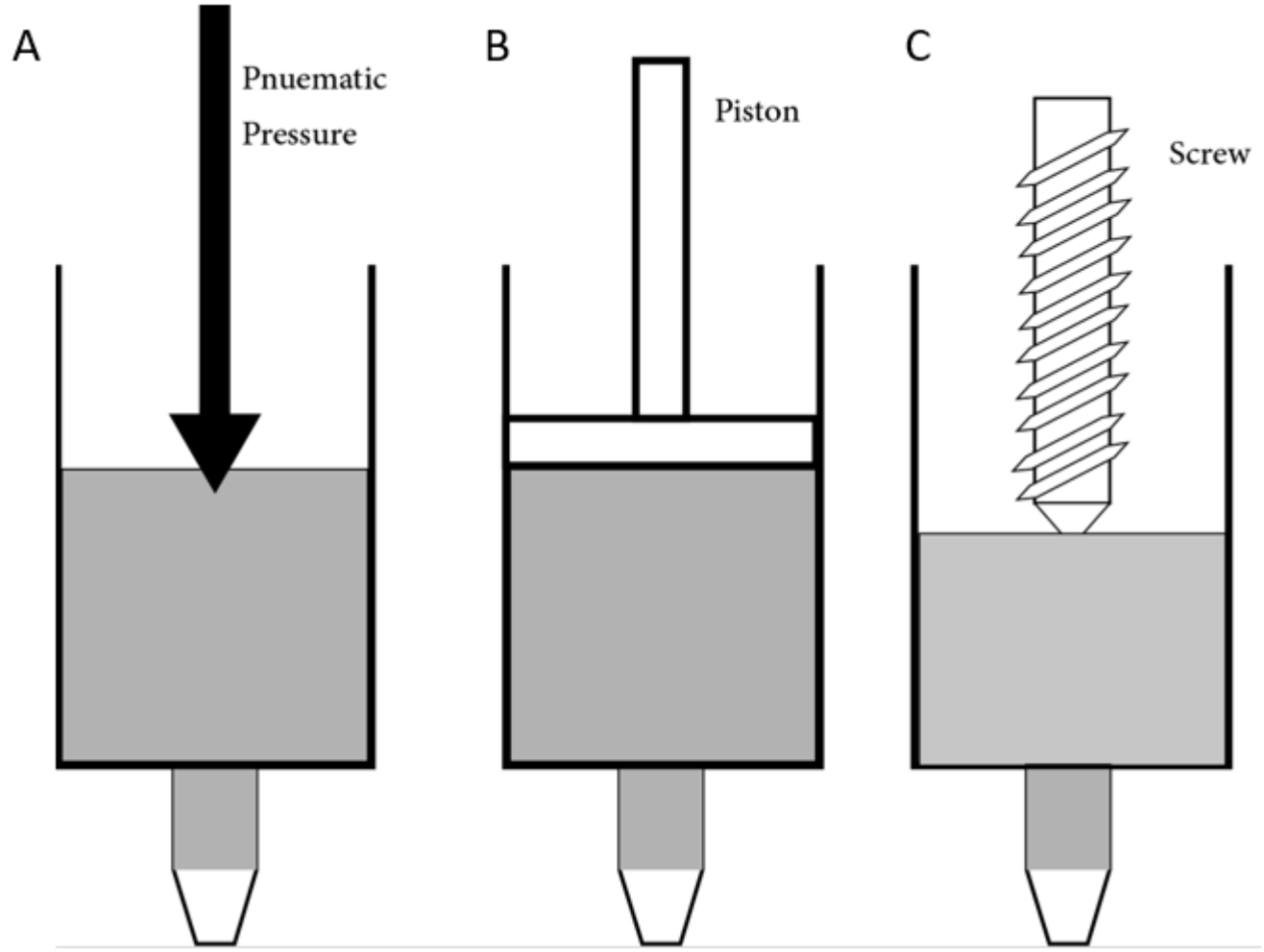

Figure 5: Schematic diagram of EBBP (A) Pneumatic Pressure EBBP (B) Piston EBBP (C) Screw EBBP

Overall, Bio-Printing equipment incorporates printing materials like scaffold, bio-ink, and other additive elements to form tissue by employing any specific Bio-Printing technique. These procedures differ in their level of accuracy, stability, and tissue viability.

Table 5: Brief comparison of various techniques available for 3D Bio-Printing

Inkjet based 3D Bioprinting	Successive droplets of bioink are expelled from a surface under the influence of heat, piezoelectricity, or electromagnetic fields.	Human chondrocytes Dermal fibroblasts Keratinocytes	Collagen Fibrinogen Dimethacrylate	Thermal	Enhanced tissue integration and improved wound healing	[55][94]
Laser Assisted Bioprinting	The bottom of the ribbon is filled with bioink and cell suspension, which is subsequently vaporized by a laser pulse and sent onto the receiving substrate.	Mesenchymal stem cells (MSCs) Fibroblasts Keratinocytes	Collagen Nano-hydroxyapatite	Photo-polymerization	For the regeneration of soft tissue, bone, and skin grafts, self-assembling cell sheets in a tubular form were developed.	[66][95]
Stereolithography based 3D Bioprinting	Using light, Photocurable Bioink is cured in layers.	Mesenchymal cells Osteoblasts C2C12 Skeletal muscle cells Fibroblasts BrCa and MSC's MCF-7 Breast cancer cells	GelMA GelMAan PEGDA dnHA	Photo-polymerization	High cell survival and accelerated proliferation of BrCa by macromolecules released by MSCs resulted in a successful model for post metastatic breast cancer research. advancement in bone investigation.	[75] [96]
Extrusion based 3D Bioprinting	Bioink is dispensed through a nozzle while being pushed by mechanical and pneumatic forces.	Fibroblasts Keratinocytes Chondrocytes MSCs HUVECs	Gelatin Alginate Chitosan GelMA Collagen Fibrinogen	Thermal Calcium chloride assisted reaction pH assisted Photo-polymerization Fibrinogen-Thrombin assisted reaction	For the reconstruction of facial wounds, cartilage, and endothelialized myocardium-on-a-chip, patient-specific tissue construct is used.	[86] [97]–[101]

CHAPTER 3: METHODOLOGY

Many research investigations have been conducted on the development of 3D bioprinting technology. Numerous techniques have so far been successfully created and used in the area of Bio-Printing. Yet additional pertinent strategies, in addition to the ones that have been mentioned, are commonly used in this market. A rising variety of cutting-edge Bio-Printing processes have also been created by fusing existing ones, which are presently available [102], [103].

The objective of this project was to create a 3D Bio-Printer that uses a syringe to more accurately dispense bioinks in order to print high resolution constructions. Extrusion is dependent on the coordinated movement of a construct platform or the dispensing head when dispensing bioink in X-Y [104]. Relying on this strategy, a syringe pump was developed specifically that was mounted on the XZ-axis of the gantry, for the accurate dispensing of bioink. In principle, piston-based extrusion method was employed in which an actuator was attached to the piston. The actuator was responsible for the movement of the piston to achieve proper dispensing of the bioink. Basically, the motion of the actuator depends on the electric signal which makes the attached piston move toward forward position. When actuator rotates due to the electric signal, the movement of the piston occurs which causes the material to flow outside. In the case of Bio-Printing when the bioink was fed to the syringe, the movement of actuator due to the electric pulse causes the piston to propel frontward pushing the bioink downwards. In this way the bioink dispenses out from the nozzle.

3.1 Gantry (XYZ axis)

The first step in the whole procedure was to make the gantry of the Bio-Printer so that the movement in all three XYZ axis could be assured. The stepper motors were used to serve the purpose of actuation. The X,Z axis were responsible for the motion in right-left and up-down direction. The platform was used as Y-axis, to and fro motion of the platform served as the third axis. In this way movement of the syringe pump in all three directions was achieved.

3.2 Assembly

The assembly of the Bio-Printer involved mechanical endstops which were installed to avoid structural failure of the whole assembly. A heat bed was installed to maintain the specific temperature if needed. A thermistor was also programmed to monitor the temperature changes.

3.3 Development of the syringe pump

A syringe pump was developed for the dispensing of the desired material from the nozzle of the syringe. The syringe pump was mounted on the XZ-axis of the assembly to ensure the precise movement in left-right and up-down fashion so that the pump can dispense the material on the exact location where it is needed to be placed. It was operated with the help of stepper motor when electric signals were applied. The stepper motor was attached to the lead screw with the help of coupling which was rotated so that the plunger of the syringe could move frontward. In this way the plunger pushed the bioink present in the syringe downward so that it could be dispensed out through the nozzle.

The clamp of the syringe pump was made universal which allowed the syringes of varying sizes to fit in easily [105]. The flow through the nozzle was monitored carefully to avoid any discrepancy during the Bio-Printing procedure. Also the nozzle selection is crucial in better printability and high resolution of the construct [82].

3.4 Syringe-based Bio-Printer

After the development of the syringe pump and assembly required, the necessary equipment required for the Bio-Printer was completed. Next step was combining assembly with syringe pump, so the syringe pump was mounted on the XZ axis of the assembly with the help of the 3D printed parts which hold the syringe pump in place. The assembly and syringe pump were combined in such a way that movements of the whole apparatus could get synchronized. The main purpose for synchronizing the movements was that the syringe pump and assembly could move in a coordinated manner. The viscosity and density of the bio-ink, its liquid phase, the dispense rate, and other special substance-specific qualities like the ability to cross-link with the printed layer must all be carefully considered when using a syringe-based Bio-Printer to produce high-quality prints. The two most important variables in a syringe-based Bio-Printer (SBBP) are flow rates and nozzle size, hence they need careful consideration. High-viscosity materials may be used to build components with stronger structural support, while low-viscosity materials can be used to create conditions that are better for maintaining cell viability and function. The main issue arises when printing with high viscosity material is nozzle clogging, the nozzle gets clogs, and the material finds it difficult to come out from the nozzle which affects the printability adversely. The flow rate is very crucial when Bio-Printing scaffolds as placing the cells at specific sites results in higher

resolution of the print. The flow rate and nozzle size are the most crucial factors in syringe-based Bio-Printer so need special attention. Syringe-based Bio-Printing is a rapid method for achieving high cell densities. For the SBBP to increase resolution, it must be viscid. The significant shear stress was the reason for the poor cell viability.

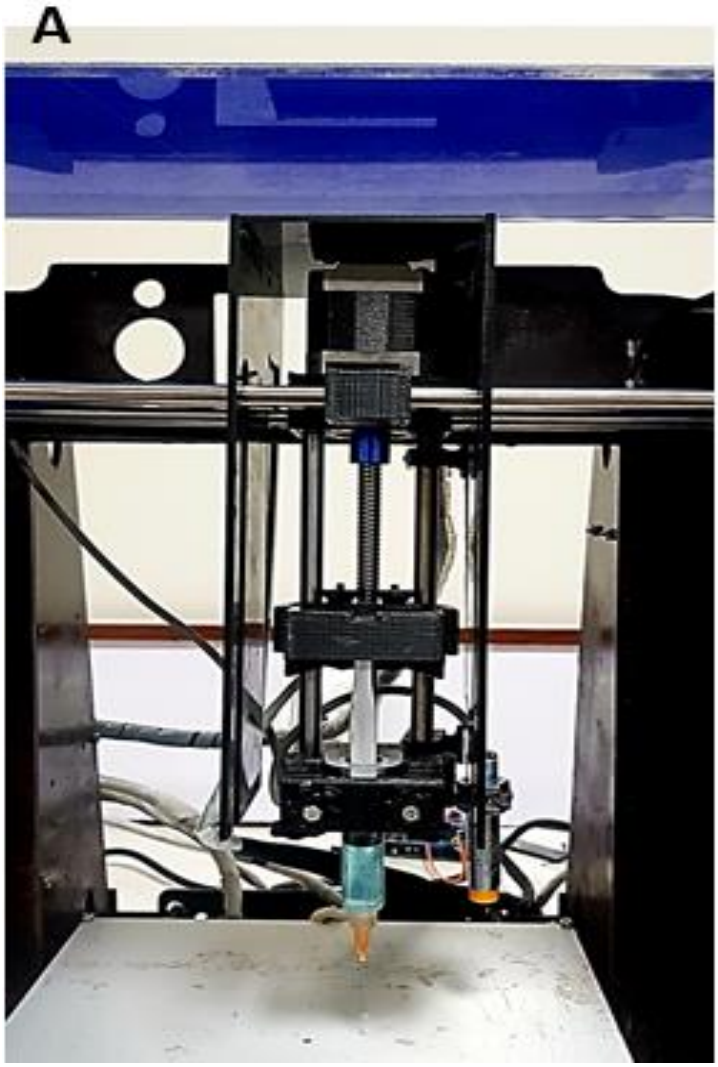

Figure 6: Syringe pump mounted on the assembly

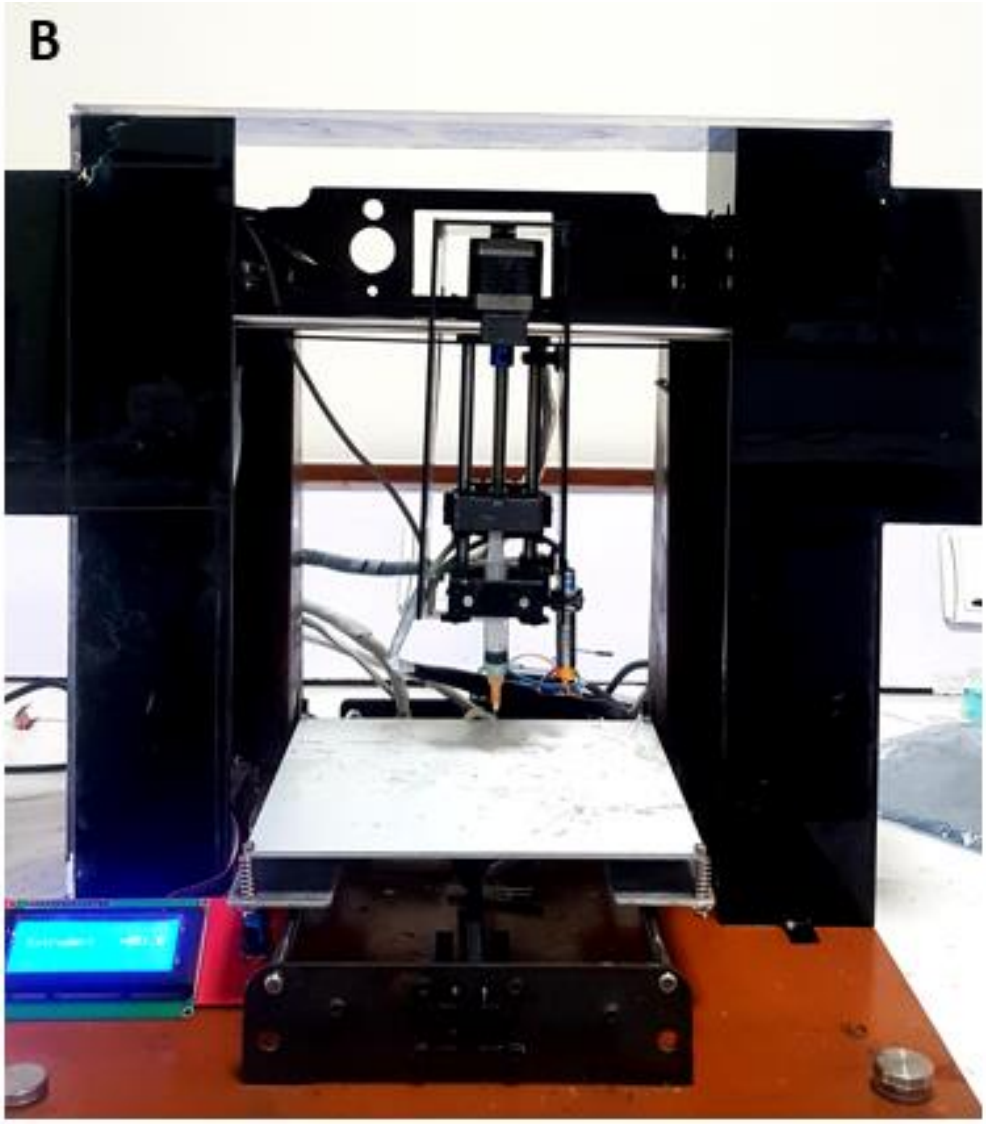

Figure 7: Whole assembly of Bio-Printer ready for 3D Bio-Printing.

3.5 Testing

After the successful development of 3D Bio-Printer we tested it. By checking its movement in all axes to verify accuracy in its movement. The initial testing of movement was done on the software named Pronterface. After that calibration of the syringe pump was made so that it dispenses the material accurately in layer-by-layer manner.

3.5.1 Layer width

After that, the test was conducted to check the layer width so that the thickness of each strand can be determined. This layer width was then used in slicing software so that the dispenser extruded the accurate amount of material for each layer to print the construct. For this purpose, syringe pump with the needles of three different gauges i.e 23G, 25G and 27G was tested.

To check the layer width, a single layer was printed with all these three needles. A composition of 7% Alg-8% Gel was used as it exhibited good mechanical strength, high printability and gelation properties [106]. A model was designed following the protocol on the fusion 360 and model was then imported to Repetier Host software. In order to precisely place components and specify printer limitations, Repetier Host is utilised with 3D printer for its visual representation. Another software named slic3r was then employed to analyze the printing parameters of the model. Then, based on a variety of inputs and after considering the printing geometries and constraints, Slic3r generates a print path known as the G-Code. After printing the single layer, the print's image was taken through the camera and evaluated using ImageJ software, and the width of the layer was determined by taking many measurements and averaging them out. The layer width will be greater than the internal diameter of the needle tip due to the viscous gel pooling; as a result, this layer width represents the real nozzle diameter parameter required by Slic3r to precisely slice solid objects.

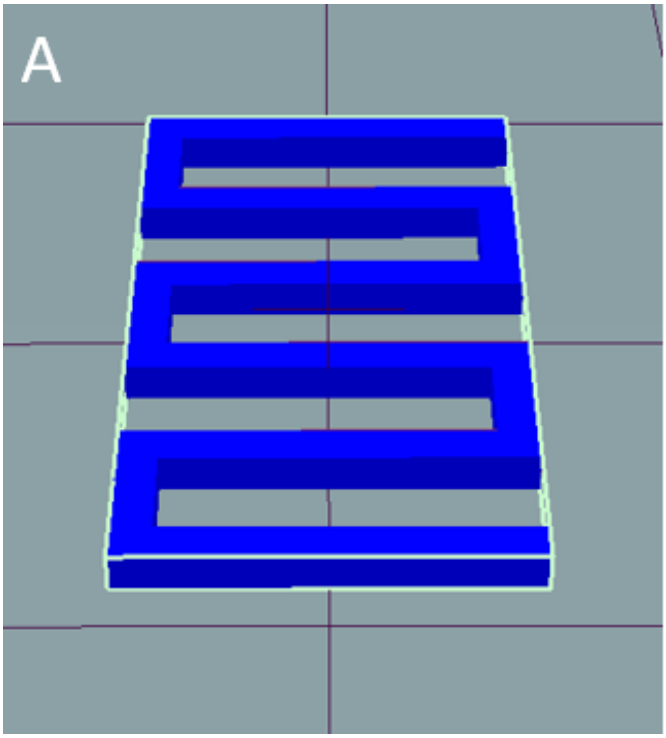

Figure 8: Model for layer width test

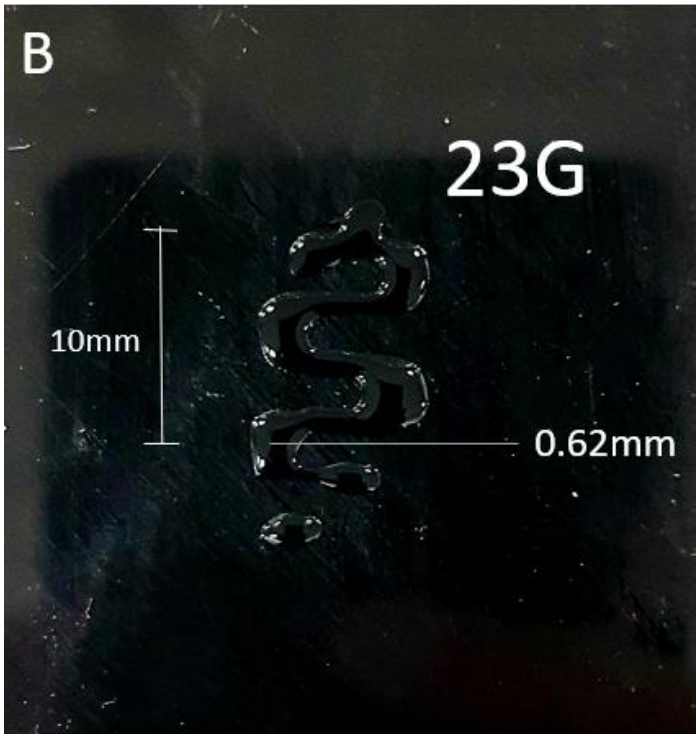

Figure 9: Printed single layer using 23G needle and analyzed through ImageJ software

Figure 10: Printed single layer using 25G needle and analyzed through ImageJ software

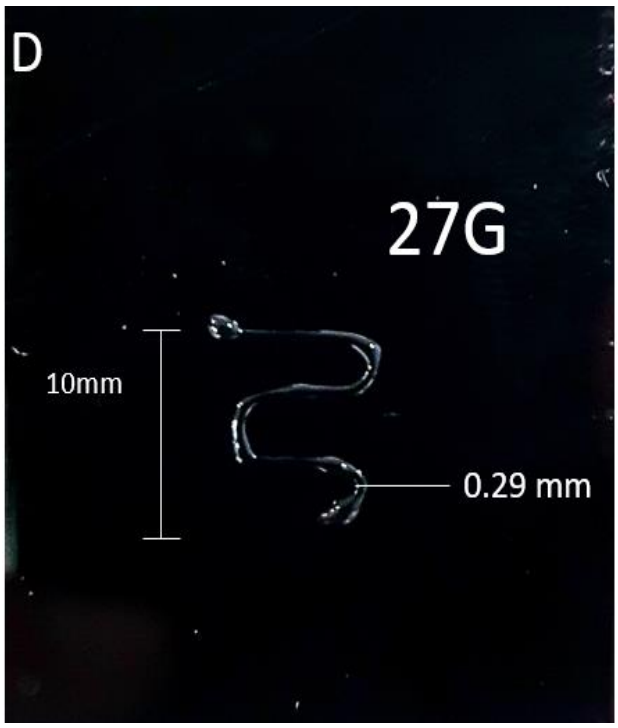

Figure 11: Printed single layer using 27G needle and analyzed through ImageJ software

As it is shown in the above figure, the material spread out a little on the bed due to the comparatively higher inner diameter of 23G needle (0.40 mm) delaying the gelation of material (Figure 2B). A proper layer was printed with 25G needle (0.30 mm) without flowing of material (Figure 2C). In case of 27G needle, the inner diameter (0.23 mm) is comparatively lower due to which material got clog inside the needle and not printed the layer properly.

3.5.2 Accuracy

A design of multilayer 1 x 3 grid was made in Fusion 360, sliced with the help of Slic3r using the optimized print parameters and layer width found earlier and printed (Figure 3). The multilayer 1 x 3 grid was printed using 23G and 25G needles. The size and printed area of each grid were then determined using the same method for obtaining images and ImageJ analysis. The printing accuracy for each needle gauge was calculated by comparing the printed area, Ai, to the designed grid area, A, using the equation below.

$$\text{Printing accuracy (\%)} = \left[1 - \frac{|Ai - A|}{A}\right] \times 100$$

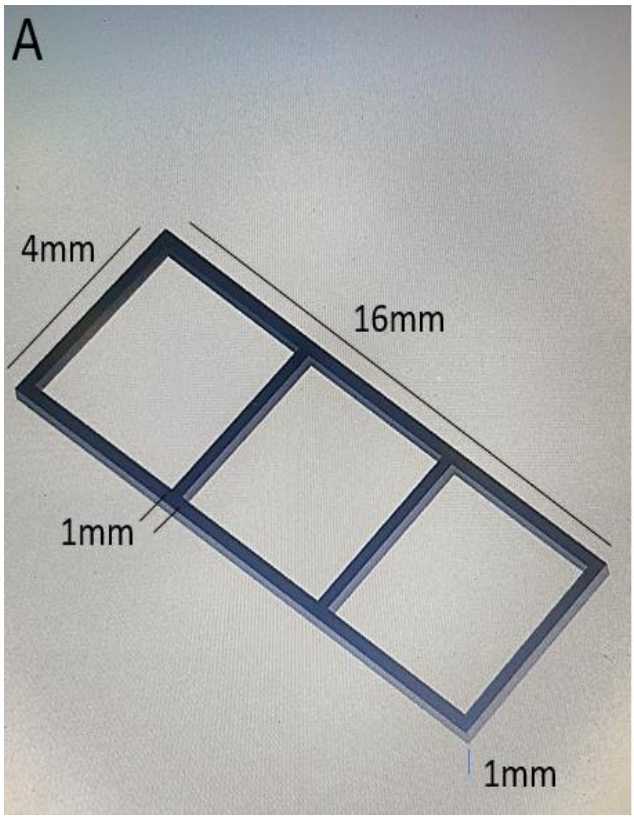

Figure 12: The model of 1 x 3 grid designed in Fusion 360

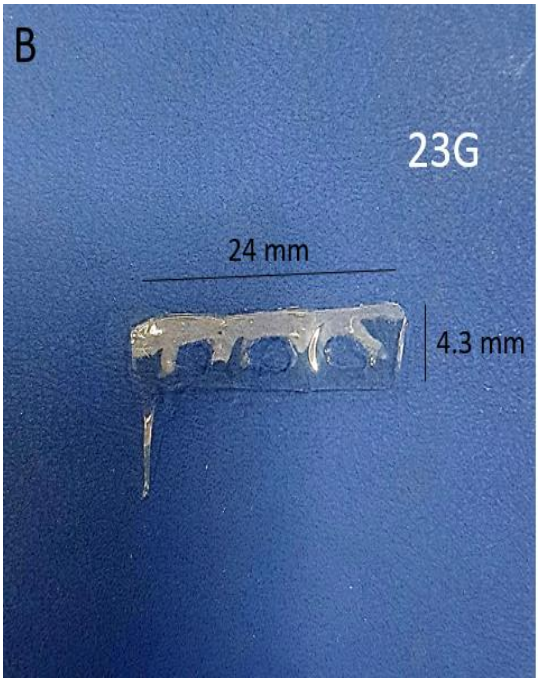

Figure 13: Print of 23G needle

Figure 14: Print of 25G needle.

3.5.3 Compression testing

For the compression test cubes of 5 mm thickness of the 7% Alg-8% Gel was prepared. Four samples were taken to perform the test after an interval of time. Thus, compression on the samples were made in different duration to check the strength of the samples after the specific time. Uniaxial compression test was performed at the loading rate of 1 mm/s.

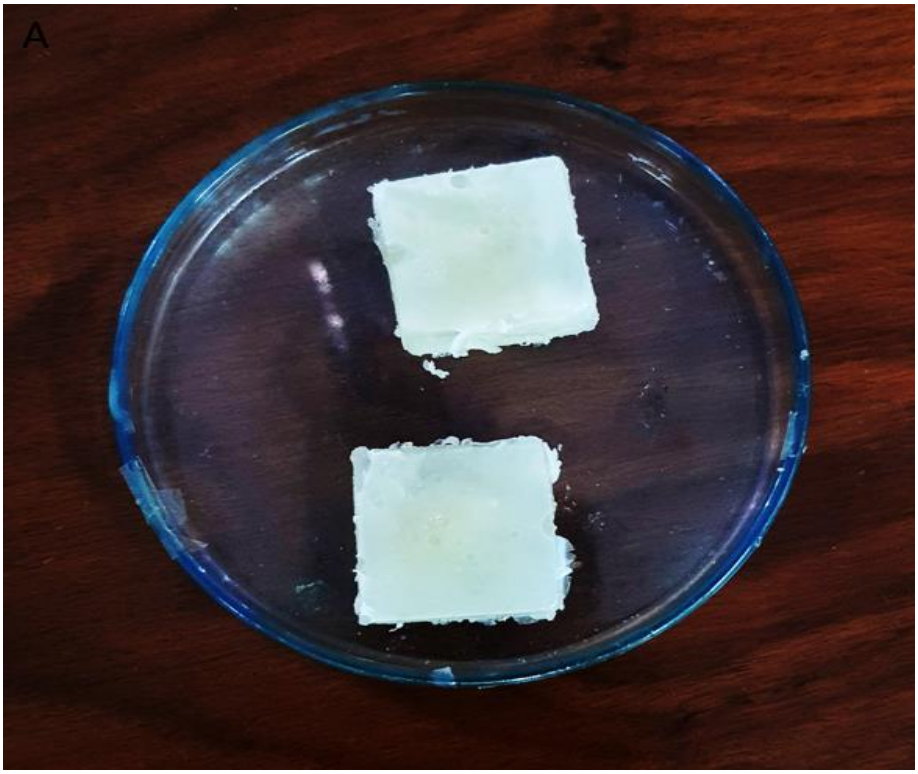

Figure 15: Cube shape sample for Compression testing

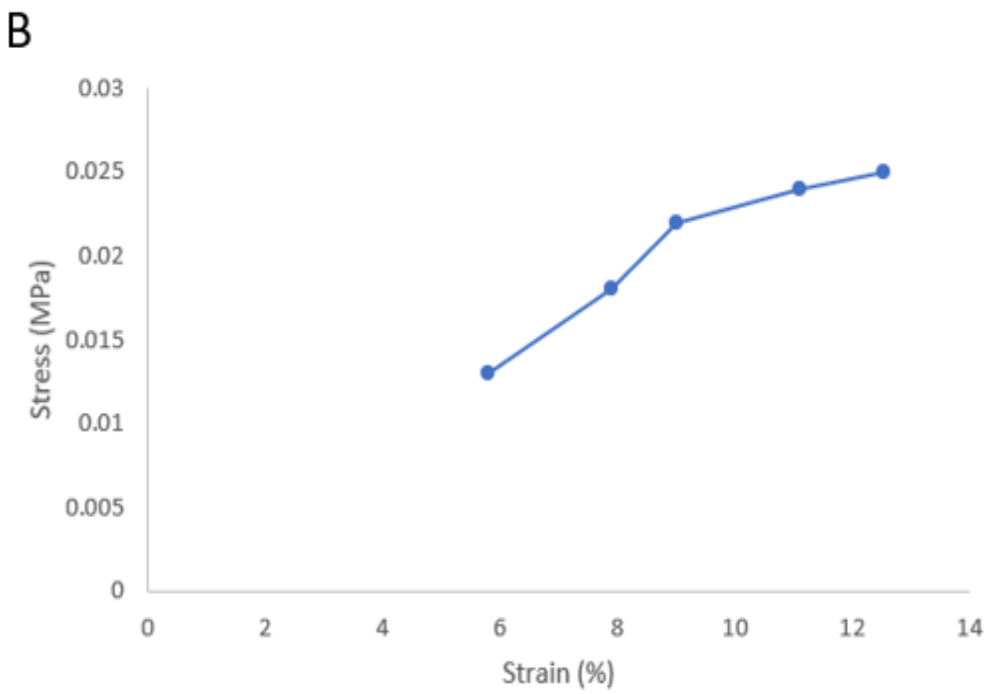

Figure 16: Stress-Strain Curve of compressional testing

3.5.4 Tensile Testing

With the help of tensile testing, mechanical characteristics of cell-free 3D printed alginate/gelatin hydrogels were then determined using a dumbbell-shaped model, which was created and built with dimensions of 3 mm in thickness, 7 mm in width, and 21 mm between grips.

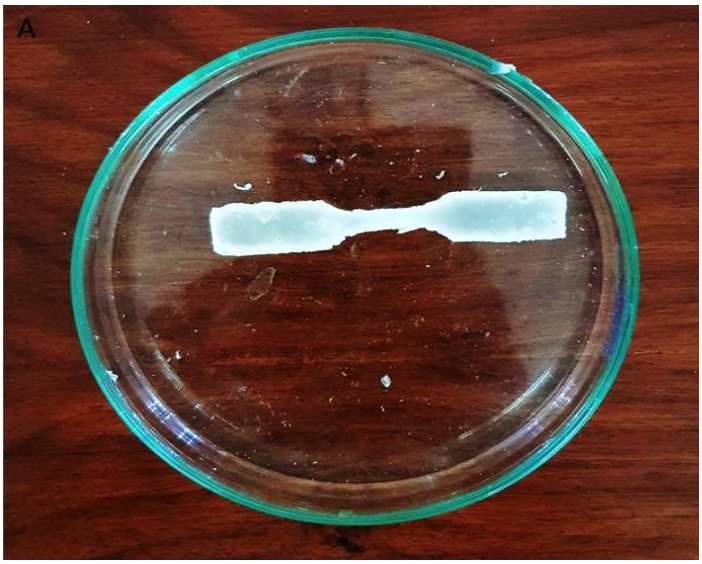

Figure 17: Dumbbell shape sample prepared for tensile testing

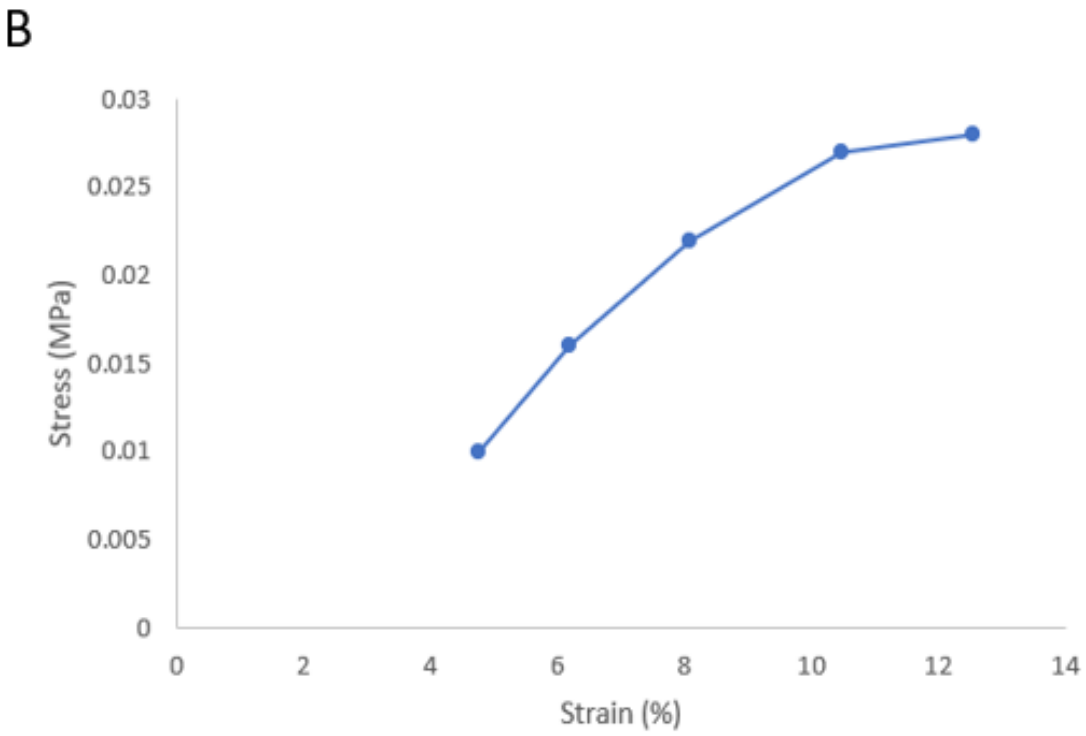

Figure 18: Stress-Strain Curve of tensile testing

3.5.5 Mechanical Properties

After the compression testing was performed, we noticed the decrease trend in the compressive modulus of blend after 2 hours, 4 hours, 6 hours and 10 hours respectively.

CHAPTER 4: RESULTS

4.1 Layer Width

It was observed that the layer width decreases with the increase in the gauge of needle. Like in our case, the lowest layer width observed was 0.29 mm with 27G. But there is a problem in using the higher gauge needle which is needle gets clog and material was unable to extrude due to small inner diameter of needle.

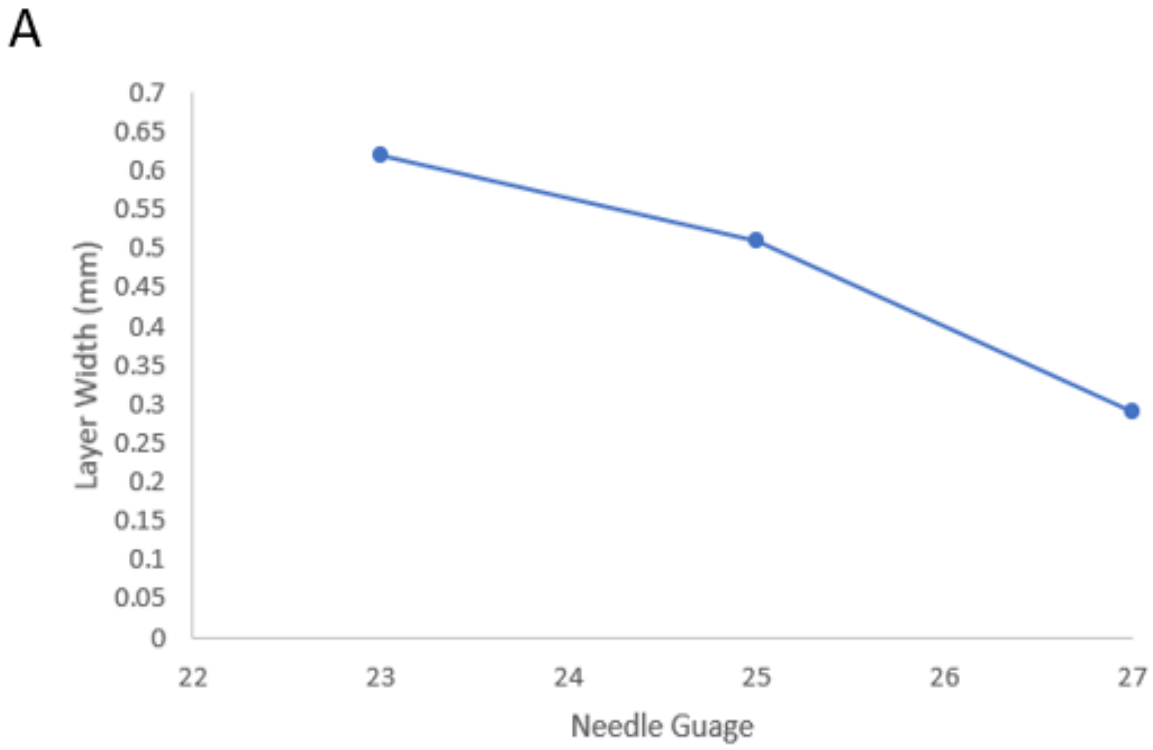

Figure 19: Effect of needle gauge on layer width when printing

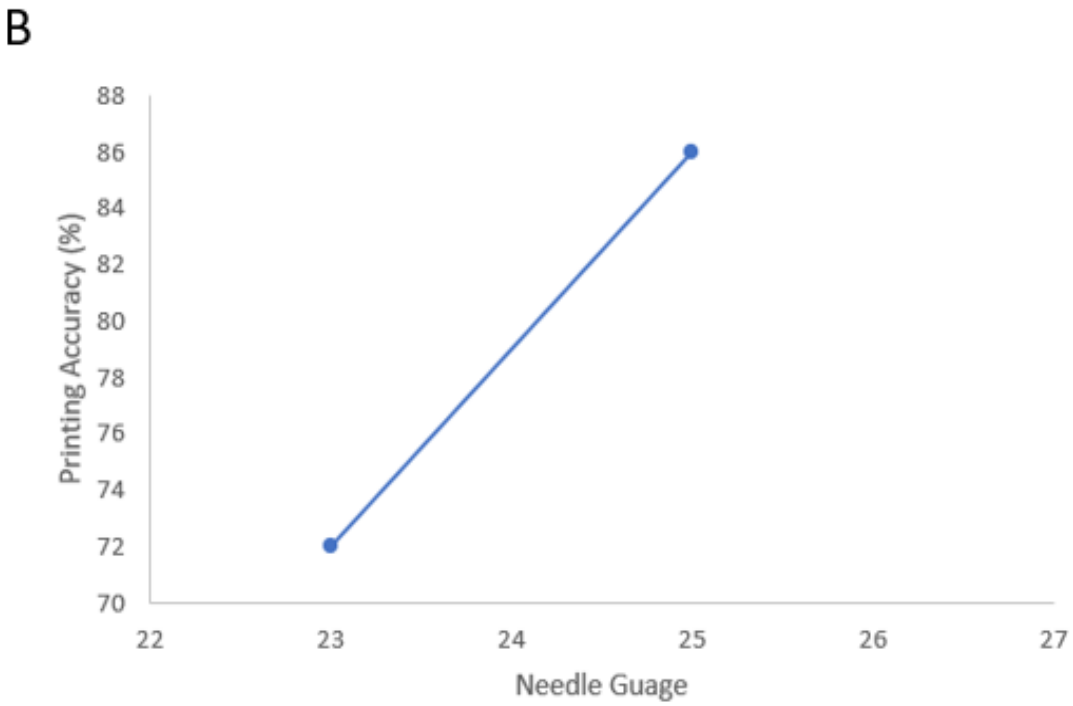

Figure 20: Effect of needle gauge on the printing accuracy.

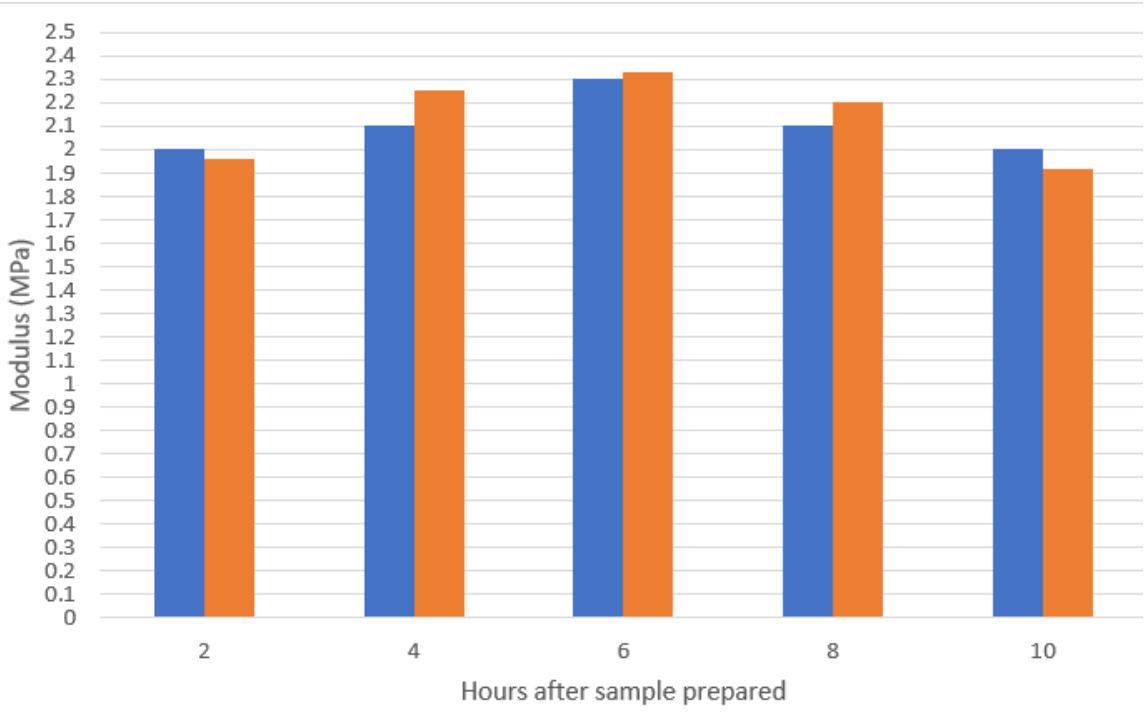

Figure 21: Compression Modulus with the passage of time.

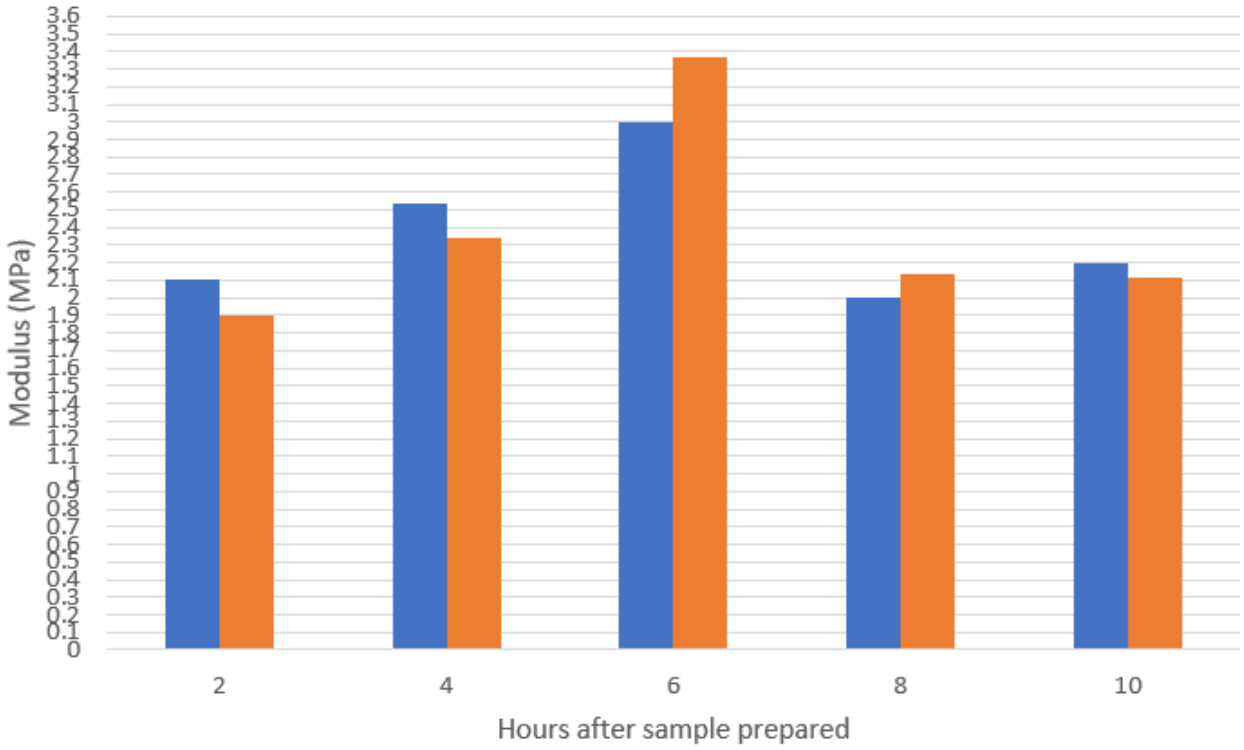

Figure 22: Tensile modulus with the passage of time

4.2 Accuracy

The results show that increasing the needle gauge resulted in more accurate printing, keeping in mind the needle clogging problem. The print made from 25G needle has higher accuracy than the print made from 23G needle.

Table 6: Comparison of data to check Parameter Optimization Index (POI).

Needle Gauge (G)	Inner Diameter (mm)	Layer Width (mm)	Printing Pressure (kPa)	Printing Accuracy (%)	POI
23	0.40	0.62	66	72	38
25	0.30	0.51	71	86	51
27	023	0.29	-	-	-

The Parameter Optimisation Index was derived [107] to aid in the investigation. It is advantageous to have a lesser layer width, high compression modulus, and low printing pressure. Thus, POI is given as

$$\text{POI} = \frac{Accuracy\,.\,Modulus}{Layer\ Width\,.\,Pressure}$$

4.3 Limitations

Many factors were found during the testing of SBBP which can affect the process of Bio-Printing. The needle gauge is a limiting factor, as higher resolution of the prints can be achieved only by using needle with more gauge, but this higher gauge needle did not allow material to extrude and get clog. It is a difficult task to find an optimum blend for printing which exhibits good mechanical strength, printability, and cell viability. By determining the shear stress in composition by measuring the viscosity via rheological research may assist better understand printability and cell survival.

CHAPTER 5: CONCLUSION AND FUTURE WORKS

5.1 Conclusion

The accuracy and resolution of the SBBP mainly depends on the needle gauge as the layer width plays the key role to precisely extrude the material in each layer. Also, the blend of bioink is the decisive factor for achieving higher printability and mechanical properties.

5.2 FUTURE WORKS

Further Optimization of the 3D Bioprinter will be carried out to tackle needle gauge problem and improving further layer width. The optimum blend of Bioink with cellular components in a physiologically simulated printing environment which exhibits good mechanical strength, printability and cell viability can further be explored.

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