



**Faculty of Industrial and Manufacturing Technology and
Engineering**

**DEVELOPMENT AND ANALYSIS OF MODULAR-BASED
BIOPRINTER FOR THREE-DIMENSIONAL (3D) PRINT OF
HYDROGEL**

ABERNICE ANN ANAK ABERT ACHILLES NUNONG

اونيورسيتي تيكنيكل مليسيا ملاك
UNIVERSITI TEKNIKAL MALAYSIA MELAKA

Master of Science in Manufacturing Engineering

2024

**DEVELOPMENT AND ANALYSIS OF MODULAR-BASED BIOPRINTER FOR
THREE-DIMENSIONAL (3D) PRINT OF HYDROGEL**

ABERNICE ANN ANAK ABERT ACHILLES NUNONG

**A master project submitted
in partial fulfillment of the requirements for the degree of
Master of Science in Manufacturing Engineering**



Faculty of Industrial and Manufacturing Technology and Engineering

UNIVERSITI TEKNIKAL MALAYSIA MELAKA

UNIVERSITI TEKNIKAL MALAYSIA MELAKA

2024

DEDICATION

Only

my beloved father, Abert Achilles Nunong

my appreciated mother, Aida Renuan

my adored brothers, Aidan Newman and Aichner

for giving me moral support, money, cooperation, encouragement and also understandings

Thank you so much



ABSTRACT

Bioprinting is an emerging technology to produce biologically adaptive tissues and organs particularly useful in clinical treatment, requiring the replacement of body parts. The alternative to producing biologically adaptive material has been studied using additive manufacturing (AM) technology due to its ability to create complex geometry. However, building soft biological tissues using a three-dimensional (3D) print technology has been significant challenge in bioprinting. Furthermore, the accessibility and customisation of the existing bioprinter has its own limitation. Moreover, the existing bioprinter experiences vibration issue and volume of syringe which impacts on printing quality. Therefore, this study aims to develop the Modular-based Syringe Extruder (MSE) on the 3D printer technology to enable customised 3D printing of biomaterial. A sodium alginate with 2% (w/v) represents a medium viscosity, ideal for hydrogel print. The printing variable will be investigated for seven printing parameters, which are nozzle shape, nozzle size, layer height, print speed, infill percentage, flow rate and retraction effect. Furthermore, the optimisation of the printing parameters would have been performed using the $L_8(2^7)$ orthogonal array. The quality of the printed would have been measured based on the scoring system. Based on Taguchi method, the data collected from the experiment was analyzed using concept of signal-to-noise ratio (SNR). Response data reveals that the print speed and retraction effect is the most significant factor that effects the printing quality. On the other hand, response graph shows that conical nozzle shape, 18G nozzle size, 0.8 mm layer height, 4 mm/s print speed, 15% infill percentage, 100% flow rate and no retraction effect are the optimal printing parameter in order to print high quality of printed structure. Printing soft biomaterials such as sodium alginate has been a major challenge due to their susceptibility to gravitational collapse. To overcome the issue, a Freeform Reversible Embedding of Suspended Hydrogels (FRESH) method was developed, providing solution by holding the sodium alginate in gelatin slurry support bath during printing. This method polymerizes the biomaterial for crosslinking, resulting in a well-structured scaffold build-up. The experiment involved printing four different structures, including grid square, circle, zigzag and blood vein profile, all of which were successfully printed five layers of 3D printed alginate scaffold. Compression testing and microstructure analysis were conducted to evaluate strength and porosity of the hydrogel. It was found that the stress value was 0.015 ± 0.003 MPa when the hydrogel was compressed up to 70% before failure. Furthermore, microstructure analysis of the scaffold revealed a high porosity (25-255 μm), which creates an ideal environment for cell attachment and migration. Overall, this research contributes valuable knowledge to the field of bioprinting and its potential applications in clinical treatments, particularly in the context of replacing degenerated body parts.

PEMBANGUNAN DAN ANALISIS BIOPENCETAK BERASASKAN MODUL BAGI PENCETAKAN HIDROGEL TIGA-DIMENSI (3D)

ABSTRAK

Biopencetakan adalah teknologi yang sedang berkembang untuk menghasilkan tisu dan organ yang dapat menyesuaikan dengan tubuh secara biologis, khususnya dalam perawatan klinikal yang memerlukan penggantian bahagian tubuh. Alternatif untuk menghasilkan bahan yang dapat disesuaikan dengan manusia telah dikaji menggunakan teknologi pembuatan tambahan kerana keupayaannya untuk mencipta geometri kompleks. Namun, membina tisu biologi lembut menggunakan teknologi pencetakan tiga-dimensi (3D) telah menjadi cabaran besar dalam biopencetakan. Selain itu, kebolehcapaian dan penyesuaian pencetak sedia ada mempunyai hadnya sendiri. Tambahan pula, pencetak sedia ada mengalami isu getaran dan isu isipadu suntikan yang memberi impak kepada kualiti pencetakan. Oleh itu, kajian ini bertujuan untuk membangunkan extruder gel-cairan berdasarkan modul pada teknologi pencetak 3D untuk membolehkan pencetakan 3D biomaterial yang disesuaikan. Natrium alginat akan digunakan sebagai biomaterial yang akan dicetak. Natrium alginat dengan 2% (w/v) mewakili kelikatan sederhana, sesuai untuk pencetakan hidrogel. Pemboleh ubah pencetakan akan disiasat untuk tujuh parameter pencetakan, iaitu bentuk muncung, saiz muncung, tinggi lapisan, kelajuan cetak, peratusan infill, kadar limpahan, dan kesan retraksi. Selanjutnya, pengoptimuman parameter pencetakan akan dilakukan menggunakan pengaturan ortogonal $L_8(2^7)$. Kualiti bahan yang dicetak akan diukur berdasarkan sistem penilaian. Berdasarkan kaedah Taguchi, data yang dikumpulkan dari eksperimen dianalisis menggunakan konsep signal-to-noise ratio (SNR). Data respons menunjukkan bahawa kelajuan cetak dan kesan retraksi adalah faktor yang paling signifikan yang memberi kesan kepada kualiti pencetakan. Selain itu, graf respons menunjukkan bahawa bentuk muncung kon, 18G saiz muncung, 0.8 mm tinggi lapisan, 4 mm/s kelajuan cetak, 15% peratusan infill, 100% kadar limpahan dan tanpa kesan retraksi adalah parameter pencetakan yang optimum untuk mencetak struktur yang berkualiti tinggi. Pencetakan biomaterial lembut seperti natrium alginat telah menjadi cabaran besar kerana mudah runtuh akibat graviti. Untuk mengatasi isu ini, kaedah Freeform Reversible Embedding of Suspended Hydrogel (FRESH) telah dibangunkan, memberikan penyelesaian dengan mengekalkan natrium alginat dalam larutan gelatin semasa pencetakan. Kaedah ini mempolimerisasi biomaterial untuk crosslinking, menghasilkan struktur bingkai yang terbentuk dengan baik. Eksperimen melibatkan pencetakan empat struktur berbeza, termasuk kotak grid, bulatan, zigzag, dan profil saluran darah, yang semuanya berjaya dihasilkan dalam lima lapisan bingkai alginat 3D yang dicetak. Ujian mampatan dan analisis mikrostruktur telah dijalankan untuk menilai kekuatan dan keliangan hidrogel. Didapati nilai tekanan ialah 0.015 ± 0.003 MPa apabila hidrogel dimampat sehingga 70% sebelum kegagalan. Analisis mikrostruktur pada perancah menunjukkan keliangan yang tinggi (25-255 μm), yang mewujudkan persekitaran yang ideal untuk pelekatan dan penghijrahan sel. Secara keseluruhan, penyelidikan ini menyumbang pengetahuan berharga kepada bidang biopencetakan dan potensinya untuk digunakan dalam rawatan klinikal, terutamanya dalam konteks menggantikan bahagian tubuh yang telah rosak.

ACKNOWLEDGEMENT

First and foremost, I would like to express my deepest gratitude to my supervisor and co-supervisor, Dr. Masni Azian Binti Akiah and Prof. Madya. Ts. Dr. Shajahan Bin Maidin for their unwavering support, guidance, and invaluable expertise throughout the course of this research. Their encouragement and constructive feedback have been instrumental in shaping the direction of this thesis, and I am truly grateful for the mentorship they have provided.

I extend my sincere thanks to the laboratory staff for their generous assistance during the experimental phase of this study. Their technical expertise and willingness to help have been instrumental in the successful execution of the experiments and I am grateful for their contributions.

Special thanks to my dear friends, Rprakash Ramanathan, Kennedy Mekantus and Khairun Najmi bin Kamaludin for being a constant source of encouragement and motivation. Their insightful discussions, feedback, and willingness to lend a helping hand have been invaluable during this process. Their friendship has made this academic journey even more enjoyable.

Last but not least, I would like to express my profound gratitude to my family. Their love, encouragement, and unwavering belief in me have been the driving force behind my pursuit of knowledge and academic excellence. Their sacrifice and understanding have been crucial in enabling me to focus on my studies and achieve this milestone.

TABLE OF CONTENTS

PAGES

DECLARATION	
APPROVAL	
DEDICATION	
ABSTRACT	i
ABSTRAK	ii
ACKNOWLEDGEMENTS	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	vii
LIST OF FIGURES	ix
LIST OF ABBREVIATIONS	xii
LIST OF SYMBOLS	xiv
LIST OF APPENDICES	xvi
LIST OF PUBLICATIONS	xvii

CHAPTER

1. INTRODUCTION	
1.1 Background of Study	1
1.2 Problem Statements	3
1.3 Research Objectives	4
1.4 Research Scopes	5
1.5 Research Questions	5
1.6 Significance of Study	6
1.7 Thesis Outline	6
2. LITERATURE REVIEW	
2.1 Background	8
2.2 Bioprinter Mechanism	10
2.3 Extrusion-based Bioprinting (EBB)	12
2.3.1 Advantages of EBB	13
2.3.2 Disadvantages of EBB	15
2.4 Hydrogel	16
2.4.1 Types of Hydrogel	16
2.4.1.1 Alginate	18
2.4.2 Rheological Properties	20
2.4.2.1 Viscosity	21
2.4.3 Crosslinking Method	22
2.4.3.1 Thermal Crosslinking	22
2.4.3.2 Physical Crosslinking	23
2.4.3.3 Chemical Crosslinking	24
2.5 Printing Parameter	26
2.5.1 Syringe Volume	27
2.5.2 Nozzle Shape	29
2.5.3 Nozzle Size	30
2.5.4 Layer Height	32

2.5.5	Print Speed	33
2.5.6	Infill Percentage	35
2.5.7	Flow Rate	36
2.5.8	Retraction	38
2.5.9	Previous Study on Printing Parameter	38
2.6	Optimisation of Printing Parameter	41
2.6.1	Parameter Optimisation Index (POI)	41
2.6.2	Response Surface Methodology (RSM)	42
2.6.3	Machine Learning (ML)	43
2.6.4	Taguchi Method	44
2.7	Evaluation of Hydrogel	47
2.7.1	Printing Accuracy	47
2.7.2	Compression Testing	48
2.7.3	Microstructure Analysis	49
2.8	Summary	50

3 METHODOLOGY

3.1	Introduction	53
3.2	Design Cconcept Development	56
3.2.1	Quality Funtion Deployment (QFD)	56
3.2.1.1	Voice of Custcomer (VOC)	56
3.2.1.2	User Requirement	59
3.2.1.3	Technical Requirement	61
3.2.1.4	House of Quality (HOQ)	63
3.2.2	Concept Generation	66
3.2.2.1	Design 1	67
3.2.2.2	Design 2	68
3.2.2.3	Design 3	69
3.2.2.4	Design 4	70
3.2.3	Concept Selection	71
3.2.4	Detailed Design	74
3.2.5	Exploded View and Bill of Material (BOM)	74
3.2.6	Fabrication of Extrusion System	76
3.2.7	Snapmaker © 3D Printer	80
3.2.8	Modification and Execution	81
3.3	Material Preparation	82
3.3.1	Sodium Alginate	82
3.3.2	Gelatin Slurry Support Bath	83
3.3.3	Hydrogel Viscosity Characterisation	84
3.4	Design of Experiment (DOE)	85
3.4.1	Taguchi Method	87
3.4.2	Analysis of Printing Quality	88
3.4.3	Optimisation of Printing Parameter	90
3.4.4	Validation of Printing Parameter	91
3.5	Printing in Gelatin Slurry Support Bath	91
3.6	Printing Accuracy	92
3.7	Compression Testing	93
3.8	Microstructure Analysis	95

3.9	Summary	95
4	RESULT AND DISCUSSION	
4.1	Introduction	98
4.2	Development of Extrusion System	98
4.3	Hydrogel Viscosity Characterisation	100
4.4	3D Bioprinting Sample	102
4.5	Analysis of Printing Quality	103
4.6	Optimisation of Printing Parameter	105
4.6.1	Response Data	105
4.6.2	Response Graph	106
4.6.3	Validation of Printing Parameter	107
4.7	Printing in Gelatin Slurry Support Bath	108
4.8	Printing Accuracy	110
4.9	Compression Testing	112
4.10	Microstructure Analysis	114
4.11	Summary	119
5	CONCLUSION AND RECOMMENDATION	
5.1	Conclusion	122
5.2	Recommendation for Future Studies	125
	REFERENCES	127
	APPENDICES	143



LIST OF TABLES

TABLE	TITLE	PAGE
Table 2.1	Hydrogels for bioprinting	17
Table 2.2	Printing parameter comparison between other researchers	40
Table 3.1	Customer needs to customise a 3D bioprinter	59
Table 3.2	Rate for customer needs	60
Table 3.3	Technical requirement to customise a 3D bioprinter	61
Table 3.4	Relationship between VOC, customer requirement and technical requirement	63
Table 3.5	Morphological chart	67
Table 3.6	Rate for concept evaluation	71
Table 3.7	Weightage for concept scoring	72
Table 3.8	Concept screening	72
Table 3.9	Concept scoring	73
Table 3.10	Bill of material for MSE	75
Table 3.11	Rotor measurement range	85
Table 3.12	The range of printing parameters	87
Table 3.13	Orthogonal array $L_8(2^7)$	88
Table 3.14	Scoring system for printing quality	90
Table 3.15	The summary of the problem statement, objectives, and methodology	96
Table 4.1	Viscosity of sodium alginate with various concentration	100
Table 4.2	Printing sample with concentration of 1%, 2% and 3% w/v sodium alginate	101
Table 4.3	Experimental parameter	102
Table 4.4	Scoring system for the printing quality	104
Table 4.5	Experimental parameter and recorded printing quality values	104

Table 4.6	Response data for printing parameters	106
Table 4.7	Printing with gelatin slurry support bath	109
Table 4.8	The relationship between objectives, methodology and result of the research	120
Table 5.1	Thesis conclusion	124



LIST OF FIGURES

FIGURE	TITLE	PAGE
Figure 2.1	Schematic representation of 3D printer (Shanmugam et al., 2021)	10
Figure 2.2	Schematic of extrusion-based bioprinting method (a) pneumatic-, (b) screw-, (c) piston-driven	13
Figure 2.3	3D architectures through swift continuous multi-material extrusion-based bioprinting. (a) Dual-layered cuboid block, (b) triple-layered cuboid block, (c) seven material containing pyramid (Liu et al., 2017)	15
Figure 2.4	Images of 3D printed structures with different concentrations of 2-10 wt% alginate hydrogels (Li et al., 2016)	20
Figure 2.5	Extrusion of agarose rod using thermal crosslinking method (Blaeser et al., 2013)	23
Figure 2.6	Bioprinted structure using physical crosslinking method (Ooi et al., 2018)	24
Figure 2.7	Image of filament (a-c) on glass and (d-f) within the support bath (Rahimnejad et al., 2022)	26
Figure 2.8	Shear stress on (a) conical nozzle and (b) cylindrical nozzle (Billiet et al., 2014)	30
Figure 2.9	The effect of (a) cell density and (b) size of the nozzle on printing quality and cell viability (Cidonio et al., 2019)	31
Figure 2.10	(a) Scheme of the potential calibration errors in the first layer: nozzle too distant and nozzle too near to printing platform, (b) The distance between nozzle and substrate influences the line width (He et al., 2016)	33
Figure 2.11	Result of bioink deposition at printing speed of 1-7 mm/s (Yenilmez et al., 2019)	34
Figure 3.1	Framework of research	55
Figure 3.2	List of issues in current bioprinter	57
Figure 3.3	House of quality (HOQ)	65
Figure 3.4	Design 1	68
Figure 3.5	Design 2	68

Figure 3.6	Design 3	69
Figure 3.7	Design 4	70
Figure 3.8	Detailed design of Modular-based Syringe Extruder (MSE)	74
Figure 3.9	Exploded view of the MSE with balloon numbering	75
Figure 3.10	Assembly of stepper motor mount	76
Figure 3.11	Assembly of stepper motor and leadscrew with shaft coupler	77
Figure 3.12	Assembly of slide mount gantry	77
Figure 3.13	Assembly of syringe mount and end syringe mount	78
Figure 3.14	Syringe assembly on extruder mechanism	78
Figure 3.15	Assembly of three-way valve	79
Figure 3.16	Assembly of nozzle holder with tubing	80
Figure 3.17	Actual fabricated extrusion system with Snapmaker © 3D printer	80
Figure 3.18	Snapmaker © 3D printer	81
Figure 3.19	Modification of G-code and M-code	82
Figure 3.20	Preparation of sodium alginate	83
Figure 3.21	Preparation of gelatin slurry support bath	84
Figure 3.22	Schematic of (a) HAAKE Viscotester 2 Plus, (b) experimental diagram of viscosity testing	85
Figure 3.23	Ishikawa diagram to improve printing quality	86
Figure 3.24	Single-layer square print design	89
Figure 3.25	3D model of (a) grid square, (b) circle, (c) zigzag and (d) blood vein profile	92
Figure 3.26	3D model of grid square structure	93
Figure 3.27	Cylindrical alginate hydrogel sample. All units are in millimeters	94
Figure 3.28	Compression testing on alginate hydrogel sample	94
Figure 3.29	Cross-section of dried printed scaffold (a) specimen 1, (b) specimen 2	95

Figure 4.1	Detailed design of (a) extrusion system, (b) luer-lock mechanism, (c) nozzle extrusion	99
Figure 4.2	Schematic detailed design with Snapmaker © 3D printer	100
Figure 4.3	Printing sample	103
Figure 4.4	Response graph	106
Figure 4.5	Printing sample based on optimal printing parameter	107
Figure 4.6	(a) CAD 3D model of a grid square, (b) printing without support bath	108
Figure 4.7	(a) CAD file of construct, (b) five layers printout using gelatin slurry support bath, (c) five layers of post gelatin removal at 37 °C	111
Figure 4.8	Printing accuracy of printed sodium alginate scaffold	112
Figure 4.9	Image of cylindrical hydrogel before, during and after compression test	112
Figure 4.10	Compressive stress-strain curve	113
Figure 4.11	500x magnification on printed structure specimen 1	115
Figure 4.12	2000x magnification on printed structure specimen 1	115
Figure 4.13	500x magnification on printed structure specimen 2	116
Figure 4.14	2000x magnification on printed structure specimen 2	116
Figure 4.15	500x magnification on printed structure specimen 1	117
Figure 4.16	3000x magnification on printed structure specimen 1	118
Figure 4.17	500x magnification on printed structure specimen 2	118
Figure 4.18	2000x magnification on printed structure specimen 2	119

LIST OF ABBREVIATIONS

<i>UTeM</i>	- Universiti Teknikal Malaysia Melaka
<i>TE</i>	- Tissue Engineering
<i>3D</i>	- Three-dimensional
<i>AM</i>	- Additive Manufacturing
<i>CAD</i>	- Computer-aided Design
<i>EBB</i>	- Extrusion-based Bioprinting
<i>FDM</i>	- Fused Deposition Modelling
<i>ECM</i>	- Extracellular Matrix
<i>CaCl₂</i>	- Calcium Chloride
<i>HA</i>	- Hyaluronic Acid
<i>GelMA</i>	- Metracrylate Gelatin
<i>PEG</i>	- Polyethylene Glycol
<i>PCL</i>	- Polycaprolactone
<i>BaCl₂</i>	- Barium Chloride
<i>UV</i>	- Ultraviolet
<i>HAMA</i>	- Hyaluronic Acid Metracrylate
<i>AlgMA</i>	- Alginate Metracrylate
<i>PEGMA</i>	- Poly(ethylene glycol) Metracrylate
<i>SEM</i>	- Scanning Electron Microscopy
<i>CaSO₃</i>	- Calcium Sulphite
<i>CaSO₄</i>	- Calcium Sulfate
<i>NaCl₂</i>	- Sodium Chloride
<i>FRESH</i>	- Freeform Reversible Embedding of Suspended Hydrogels

<i>CH</i>	- Chitosan
<i>LVE</i>	- Large Volume Extruder
<i>POI</i>	- Parameter Optimisation Index
<i>RSM</i>	- Response Surface Methodology
<i>ANOVA</i>	- Analysis of Variance
<i>ML</i>	- Machine Learning
<i>PLA</i>	- Polylactic Acid
<i>PCP</i>	- Progressive Cavity Pump
<i>PBS</i>	- Phosphate Buffered Saline
<i>Chi-Alg</i>	- Chitosan-Alginate
<i>QFD</i>	- Quality Function Deployment
<i>BOM</i>	- Bill of Material
<i>VOC</i>	- Voice of Customer
<i>HOQ</i>	- House of Quality
<i>NOSE</i>	- Nydus One Syringe Extruder
<i>MSE</i>	- Modular-based Syringe Extruder
<i>DOE</i>	- Design of Experiment
<i>OA</i>	- Orthogonal Array
<i>SNR</i>	- Signal-to-noise ratio

LIST OF SYMBOLS

μm	-	Micrometre
mm	-	Millimeter
$mPa.s$	-	Millipascal per second
%	-	Percentage
w/v	-	Weight per volume
cps	-	Centipoise
mW	-	Megawatt
E	-	Extrusion Value
F	-	Feed Rate
W	-	Watt
min	-	Minute
s	-	Seconds
$^{\circ}C$	-	Temperature
m	-	Meter
ml	-	Milliliter
mm/min	-	Millimeter per minute
mm/s	-	Millimeter per seconds
kPa	-	Kilopascal
G	-	Gauge
mm^2	-	Millimeter Square
Pa	-	Pascal
MPa	-	Megapascal
N/mm^2	-	Newton per millimeter square
USD	-	United State Dollar

<i>pH</i>	-	Degree of acidity or alkalinity of a solution
<i>rpm</i>	-	Revolutions per minute
<i>wt%</i>	-	Weight percentage



LIST OF APPENDICES

APPENDIX	TITLE	PAGE
Appendix A	Coding for Single Layer Square Print	143
Appendix B	Coding for Grid Square Print	144
Appendix C	Coding for Circle Print	145
Appendix D	Coding for Zigzag	146
Appendix E	Coding for Blood Vein Profile Print	147



LIST OF PUBLICATIONS

The followings are the list of publications related to the work on this dissertation/master project/project paper:

Abert, A.A. and Akiah, M.A., 2022. Development of modular-based bioprinter with three-way valve for hydrogel control. *Proceedings of Mechanical Engineering Research Day*, pp.26-27.

Abert, A.A. and Akiah, M.A., 2023. Preparation of Bioink for Hydrogel Printing in Additive Manufacturing. *Malaysian Journal on Composites Science and Manufacturing*, 12(1), pp.43-50.



CHAPTER 1

INTRODUCTION

1.1 Background of Study

Additive manufacturing (AM) is an advanced manufacturing process that can produce three-dimensional (3D) structures in a layer-by-layer approach through computer-aided design (CAD). This technology can fabricate a complex structure, replacing the traditional manufacturing method that uses moulds to fabricate a 3D structure. Recently, 3D printing technology has been considered in the medical field as it offers a promising alternative for fabricating soft tissues with patient-specific geometry. Such advancement would be revolutionary to clinical treatment requiring organ transplants (Mobaraki et al., 2020).

In bioprinting, the materials are deposited layer-on-layer to build a scaffold as the cell-cultured medium. Scaffolds are 3D porous structures with crucial features that support and direct the regeneration of specific tissues (Hutmacker, 2001). To provide the necessary function, the scaffolds need a porous structure that provides room for cell development and facilitates the movement of nutrients and waste products to and from the cells. The scaffold must be biocompatible, support cell functioning, and align with the development of cells and tissues. The scaffold must possess appropriate chemical and biological characteristics to facilitate cell attachment, growth, movement, specialization, and other cellular activities while demonstrating adequate mechanical strength to maintain structural stability. Scaffolds may have additional functional needs based on the unique application.

Hydrogel is an alternative material that can be used to build the scaffold. Its properties are suitable for cell culture and bioactive substances. Hydrogels comprise a water-rich network of polymers, mimicking the natural extracellular matrix (ECM) found in living tissues (Kyle et al., 2017). The unique properties of hydrogels, such as high-water content, biocompatibility, and tunable mechanical characteristics, make them well-suited for supporting cell growth and promoting tissue development (Li et al., 2018). Hydrogels can be categorised as natural or synthetic, each with distinct benefits. Hydrogels with inherent ligands encourage cell attachment and differentiation, often derived from ECM elements. Polymers obtained from non-mammalian sources, including alginate from brown seaweed, are frequently used as alternatives for cell culture (Bajaj et al., 2014; Li et al., 2014).

On the other hand, synthetic hydrogels have been developed to act as 'blank slate' materials and can be customised through chemical modifications to suit specific applications (Murphy and Atala, 2014; Bajaj et al., 2014). Currently, the synthetic hydrogels used in bioprinting include methacrylate gelatin (GelMA), polyethylene glycol diacrylate (PEGDA), and polylactic acid (PLA). Synthetic gels do not support cell adhesion. However, these hydrogels can be functionalized with ECM proteins to facilitate cell support.

An important consideration in using hydrogel for bioprinting is that it should be able to form and maintain a reproducible 3D structure with sufficient structural integrity (Chung et al., 2013). Similarly, an extrusion-based technique can be adopted to deposit the hydrogel in liquid form. Hydrogels can undergo physical, thermal and chemical cross-linking to enable the polymerization of the material for constructing a 3D structure. Hydrogel cross-linking is time-consuming, allowing the hydrogel to flow, spread, and perhaps deviate significantly from the intended design. The poor printability of hydrogels can lead to the collapse and failure to form a 3D structure of printed scaffolds. Printability is crucial as

variations between a printed scaffold and the optimal design might affect mechanical and biological characteristics like strength and cell functionality.

The motivation of this study is to advance medical research by developing customizable equipment for fabricating patient-specific soft tissues through additive manufacturing (AM). By improving the printability and structural integrity of 3D printed hydrogels, the study aims to support basic research in biomaterial development and processing mechanisms. This will ensure that the resulting scaffolds not only meet the complex biological and mechanical requirements necessary for tissue regeneration but also maintain the precision needed for clinical applications, such as organ transplants.

1.2 Problem Statements

The scaffold, serving as a vital medium for cell growth, plays a pivotal role in replicating the biological functions of healthy tissues to substitute damaged organs. However, the accessibility and customisation of the bioprinting process pose significant challenges, particularly concerning commercial bioprinters (Kahl et al., 2019; Tashman et al., 2022). The high cost of these bioprinters restricts their widespread use, limiting the exploration of crucial customisation in printing parameters. This is essential for evaluating the viability of hydrogel formulations in producing high-quality scaffolds.

Furthermore, the commonly used syringe pump extruders are mostly high resolution or low volume, as there is a trade-off between moving mass and high positioning precision (Bociaga et al., 2020). Commercial bioprinters use small syringe volumes to reduce carriage mass. However, this solution limits the printout size and affects printing productivity. Researchers may consider increasing the volume of the syringe as an alternative. However, additional weight may negatively affect printing quality. This is because attaching the liquid

extruder system directly to the 3D printer body affects the syringe plunger retraction and vibration control, leading to poor printing quality. Based on the previous study, the customised bioprinter has an issue of vibration, which was found to impact the quality of the fabricated scaffold (Pusch et al., 2018; Hinton et al., 2015).

Hydrogel, a key material in this investigation, presents inherent challenges in 3D printing due to its partial-liquid state (Hinton et al., 2015). Hydrogels typically have a high water content, which makes them flow easily. However, this can cause issues with maintaining shape and stability during printing. Therefore, there is a need to control the flow of the hydrogel through the printer nozzle with precise tuning to ensure consistent deposition and avoid clogging or dripping.

Printing parameters also plays a crucial role in achieving high-quality prints, especially for scaffolding constructs. Nowadays, scaffolding constructs have gained significant attention for their potential contribution to tissue structures. When designing scaffolding, each biomaterial is strongly influenced by several printing parameters. This is because the scaffolding structures include bioinks and microchannels that allow the diffusion of nutrients and oxygen. Thus, optimising the printing parameters is vital to achieve high printing quality.

In light of these challenges, there is a need for advancements in bioprinting technology, which allow extrusion system customisation, effective extrusion of liquid-based biomaterial, improved bioink capacity, and optimised printing parameters

1.3 Research Objectives

The objectives of the research are:

- i. To develop an extrusion system for liquid-based hydrogel printing.