Faculty of Industrial and Manufacturing Technology and Engineering

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

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Master of Science in Manufacturing Engineering

2024

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

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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

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

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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.

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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.

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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.

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LIST OF ABBREVIATIONS

UTeM	-	Universiti Teknikal Malaysia Melaka
TE	-	Tissue Engineering
3D	-	Three-dimensional
AM	-	Additive Manufacturing
CAD	-	Computer-aided Design
EBB	-	Extrusion-based Bioprinting
FDM	-	Fused Deposition Modelling
ECM	-	Extracellular Matrix
$CaCl_2$	-	Calcium Chloride
HA	-	Hyaluronic Acid
GelMA	-	Metracrylate Gelatin
PEG	-	Polyethylene Glycol
PCL	-	Polycaprolactone
$BaCl_2$	-	Barium Chloride
UV	-	Ultraviolet
HAMA	-	Hyaluronic Acid Metracrylate
AlgMA	-	Alginate Metracrylate
PEGMA	-	Poly(ethylene glycol) Metracrylate
SEM	-	Scanning Electron Microscopy
$CaSO_3$	-	Calcium Sulphite
$CaSO_4$	-	Calcium Sulfate
$NaCl_2$	-	Sodium Chloride
FRESH	-	Freeform Reversible Embedding of Suspended Hydrogels

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

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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
°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

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pH	-	Degree of acidity or alkalinity of a solution
rpm	-	Revolutions per minute
$wt\%$	-	Weight percentage

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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.

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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 (GeIMA), polyethene 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 uses 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.

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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.