INVESTIGATION ON MECHANICAL AND MICROSCOPIC CHARACTERISTIC OF SEAWEED-BASED HYDROGEL FOR THREE-DIMENSIONAL BIOPRINTING

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INVESTIGATION ON MECHANICAL AND MICROSCOPIC CHARACTERISTIC OF SEAWEED-BASED HYDROGEL FOR THREE-DIMENSIONAL BIOPRINTING

This report is submitted in accordance with requirement of the Universiti Teknikal Malaysia Melaka (UTeM) for Bachelor Degree of Manufacturing Engineering (Hons.)

by

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2024

DECLARATION

I hereby, declared this report entitled “INVESTIGATION ON MECHANICAL AND MICROSCOPIC CHARACTERISTIC OF SEAWEED-BASED HYDROGEL FOR THREE-DIMENSIONAL BIOPRINTING” is the result of my own research except as cited in references.

Signature : ……

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Date : 20 JUNE 2024

APPROVAL

This report is submitted to the Faculty of Manufacturing Engineering of Universiti Teknikal Malaysia Melaka as a partial fulfilment of the requirement for Degree of Manufacturing Engineering (Engineering Materials) (Hons). The member of the supervisory committee is as follow:

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ABSTRAK

Penggunaan hidrogel yang diperoleh daripada rumpai laut, yang dicirikan oleh sifat seperti gel, telah mendapat perhatian dalam bahan bio-dakwat kerana keupayaannya untuk meniru sifat mekanikal tisu hidup. Terdapat potensi besar hidrogel yang dihasilkan daripada rumpai laut untuk pencetakan bio, menawarkan laluan yang menjanjikan untuk pengganti mesra alam dan mampan untuk bahan dakwat bio sintetik tradisional. Namun, terdapat kekurangan pemahaman yang ketara tentang amalan terbaik untuk menyediakan dan bekerja dengan bahan ini apabila ia berkaitan dengan pencetakan bio. Objektif kajian ini adalah untuk membangunkan bio-dakwat berasaskan rumpai laut untuk pencetakan bio, mengoptimumkan parameter pencetakan bio untuk bahan berasaskan rumpai laut, dan mengkaji sifat mekanikal dan mikrostruktur cetakan berasaskan rumpai laut yang terhasil. Ciri-ciri mekanikal hidrogel, termasuk kekuatan dan ketahanannya terhadap ubah bentuk, dinilai dengan ujian tusukan. Tambahan pula, struktur mikro dan ciri hidrogel berasaskan rumpai laut telah diperiksa menggunakan (SEM), yang memberikan resolusi analisis imej yang lebih besar. didapati bahawa kelikatan bioink meningkat dengan bioink berasaskan rumpai laut. Formulasi hidrogel berasaskan rumpai laut terbaik untuk penyemperitan ialah 6.72dPas untuk 1% rumpai laut dan 1% alginat. Kekuatan bioink berasaskan rumpai laut meningkat, sehingga 1.60Mpa. Analisis mikrostruktur 1%rumpai laut dan 1% alginat mempamerkan saiz dan jumlah keliangan yang lebih besar yang diperlukan untuk kultur sel dalam perancah. Hasil daripada kajian itu mungkin menyumbang kepada kemajuan variasi biomaterial baharu yang meningkatkan kultur sel dalam rawatan perubatan, terutamanya dalam menggunakan bahan sumber tempatan.

ABSTRACT

The utilization of hydrogels derived from seaweed, characterized by gel-like properties, has gained prominence in bio-ink materials due to their ability to emulate the mechanical attributes of living tissues. There is tremendous potential of hydrogels generated from seaweed for bio-printing, offering a promising route for environmentally friendly and sustainable substitutes for traditional synthetic bio-ink materials. Nowadays, there is a significant lack of understanding about the best practices for preparing and working with these materials when it comes to bio-printing. The objective of this study is to develop a seaweed-based bio-ink for bio-printing, optimize the bio-printing parameters for the seaweed-based materials, and examine the mechanical and microstructural properties of the resulting seaweed-based prints. The mechanical characteristics of the hydrogel, including its strength and resistance to deformation, were evaluated by puncture tests. Furthermore, the microstructure and characteristic of the seaweed-based hydrogel were examined using (SEM), which provided a greater resolution of image analysis.it was found that the viscosity of the bioink increases with seaweed based bioink. The best seaweed-based hydrogel formulation for extrusion was 6.72dPas for 1% seaweed and 1% alginate. Strength of the seaweed based bioink increases, up to 1.60Mpa. Microstructural analysis of 1%nseaweed and 1% alginate exhibit larger size and amount of porosity which is necessary for cell culture in the scaffold. The results from the study may contribute to the advancement of new biomaterial variations that improve cell culture in medical treatments, especially in utilizing locally resourced materials.

DEDICATION

This thesis is dedicated to the unwavering support and encouragement of my family, whose love has been my anchor throughout this academic journey. To my parents, **Kirno bin Muhaini** and **Masjunita binti Wagiman**, your sacrifices and belief in my abilities have been my greatest motivation.

I also dedicate this work to my friends and Supervisor **Dr. Masni Azian Binti Akiah**

who have shared their wisdom, challenged my thinking, and stood by me during the highs and lows of this research endeavour. Your guidance has been invaluable.

Lastly, this thesis is dedicated to the pursuit of knowledge and the quest for a better understanding of the world. May the findings presented here contribute, even if in a small way, to the collective body of knowledge.

ACKNOWLEDGEMENT

Praise and gratitude are due to Allah, The Highest, for generously granting His blessings, good health, endurance, wisdom, and guidance to sustain me during the course of my research.

I would like to express my deepest gratitude to Dr. Masni Azian binti Akiah, my supervisor, for their invaluable guidance, unwavering support, and mentorship throughout this research journey. Their expertise, constructive feedback, and encouragement have been instrumental in shaping the trajectory of this work. I express my appreciation to my friends and colleagues who established a supportive network, offering both intellectual and emotional encouragement. Your companionship significantly enhanced the significance of this academic pursuit. I extend my thanks to the administration of UTeM, particularly the Faculty of Industrial and Manufacturing Technology and Engineering, for granting me the research opportunity that enabled the expansion of my knowledge and experience.

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LIST OF ABBREVIATION

SEM	-	Scanning Electron Microscopy
3D	-	3-Dimensional
LAB	-	Laser Asisted Bioprinting
STL	-	Stereolithography
PAAM	-	Polyacrylamide
GelMa-	-	Gelatin Methacryloyl
FTKIP	-	Fakulti Teknologi dan Kejuruteraan Industri dan Pembuatan
CaCl	-	Calcium Chloride

CHAPTER 1

1 INTRODUCTION

1.1 Background Research

In recent years, there has been a significant advancement in the field of bio-printing, which involves the use of 3D printing technology for the purpose of fabricating biological tissue and organs (Murphy et al., 2018). Three-dimensional (3D) bioprinting is an advanced technology utilised for the creation of biological constructs that possess a hierarchical architecture resembling their natural counterparts. The artificial development of living functional tissues has the potential to fulfil the existing demand for tissue replacement and organ transplantation. Consequently, bioprinting is increasingly being recognised as a promising approach by medical professionals and researchers worldwide, offering the possibility of enhancing the quality of life for individuals afflicted by various diseases (Matai et al., 2020).

Scaffolds play a vital role in establishing a favourable microenvironment for cell attachment, growth, and metabolism. These scaffolds are constructed using bioinks, which consist of cells and biopolymers. Bioinks can be crafted from synthetic or natural materials, with optimal diffusivity, nutrient permeability, and controlled biodegradability being essential considerations in their formulation (Marta Anna Szychlinska et al., 2022). In bioprinting, hydrogel have been used as the scaffold build-up material. Hydrogels are polymers derived from seaweed that exhibit gel-like characteristics which have been shown to be useful as bio-ink materials owing to their ability to simulate the mechanical properties of living tissues (Yamamoto et al., 2020). The development of bioink derived from seaweed entails the incorporation of alginate, a naturally occurring polymer found in brown seaweed shows in Figure 1.1. A seaweed based bioink has been created by scientists, primarily consisting of alginate. This bioink is biocompatible and possesses the ability to form a stable gel without the need for heat. The process involves the preparation of alginate solutions in water, with the addition of various components to regulate the ink's texture (Arnold et al., 2021). Scaffolds play a vital role in establishing a favourable microenvironment for cell attachment, growth, and metabolism. These scaffolds are constructed using bioinks, which consist of cells and biopolymers. Bioinks can be crafted from synthetic or natural materials, with optimal

diffusivity, nutrient permeability, and controlled biodegradability being essential considerations in their formulation (Marta Anna Szychlinska et al., 2022).

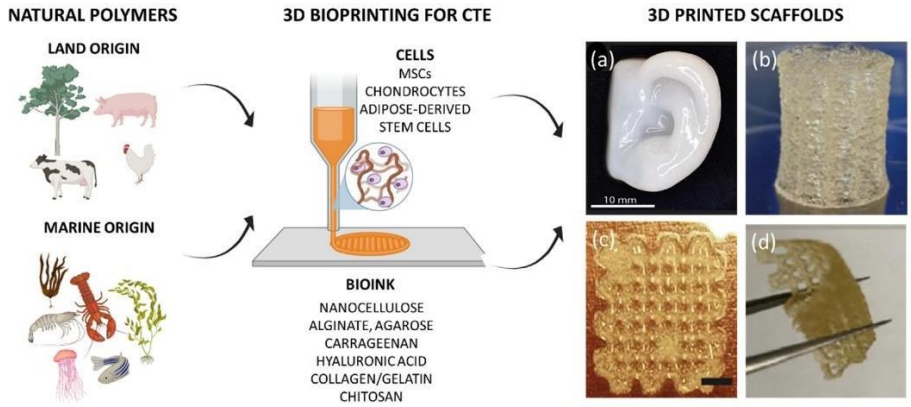

Figure 0.1 The sources that can be used in 3D bio-printing (Gungor-Ozkerim et al., 2018a)

Marine macroalgae, often referred to as seaweed, has gained recognition as a promising bio-ink material because to its distinctive mechanical properties, characterised by notable flexibility and biocompatibility (Lin et al., 2019). In addition, a variety of seaweed polysaccharides are used within the medical and pharmaceutical sectors to provide diverse biological properties. The use of seaweed polysaccharide-based nanoparticles, microspheres, and gels has been identified as having the capacity for prolonged and regulated drug administration, particularly for anticancer and anti-inflammatory medications (Chudasama et al., 2021).

The use of hydrogels derived from seaweed in the realm of bioprinting is an emerging interest among researchers which necessitates further scientific exploration into the mechanical characteristics and appropriateness of this substance for bioprinting applications. In addition, seaweed cultivation was introduced in Sabah since 1978 which has become an economically important natural resource for Malaysia (Ahemad & Raduan, 2006.). Hence, the study on hydrogels produced from seaweed is significant for the bioprinting advancement as well as the potential for local economic growth.

1.2 Problem Statement

Although seaweed-based hydrogels have the potential to serve as a more sustainable and environmentally friendly alternative to the conventional synthetic bio-ink materials, there is still a significant amount of information that needs to be learned on the most efficient ways for manufacturing and processing these materials for use in bio-printing applications. In addition, as seaweed-based hydrogel is a relatively new material, there is a dearth of information about the appropriate printing settings and parameters that yield structures that are mechanically robust and biocompatible. In order to accomplish this goal, research must be conducted on the effect of a variety of parameters on the mechanical characteristics and cellular adhesion of printed structures. These variables include the concentration of seaweed-based hydrogel, the concentration of gelatine, and the pace at which the printing is done (Webb et al. 2017). In addition, more research is needed to establish the necessary mechanical testing procedures that may be used to evaluate the mechanical characteristics of seaweed-based hydrogels and determine whether they are suitable for use in bioprinting especially suitability for cell culture.

In summary, the utilisation of hydrogel derived from seaweed in the context of bio-printing represents a very auspicious area of research, exhibiting substantial prospects for the development of sustainable and ecologically conscious substitutes to traditional synthetic bio-ink substances. Nevertheless, there remains a substantial amount of knowledge still to be acquired about the optimal methodologies for the preparation and manipulation of these substances in the context of bio-printing implementations. Additional investigation is necessary to enhance the mechanical characteristics and biocompatibility of printed structures by optimising printing parameters and hydrogel composition. Furthermore, it is imperative to identify suitable mechanical testing methodologies to assess the appropriateness of seaweed-based hydrogels for bio-printing purposes.

1.3 Objective

The main objective of this research is to ascertain the best printing settings for the use of seaweed-based hydrogels in bioprinting applications. The specific goals of this study are as follows:

I. To formulate the Seaweed-Based bioink for Bioprinting.
II. To optimize the formulation of Seaweed-based bioink parameters.
III. To investigate the mechanical and microstructural properties of seaweed-based print.

1.4 Project Scope

This research project focuses on further determination of materials and techniques for the use of seaweed-based hydrogels for bioprinting.

I. This research is restricted to solely red seaweed.
II. The supplement for the bio-ink will make use of several food-grade gelatine including sodium alginate, fish gelatine and bovine gelatine.
III. This study focuses on building the scaffold for potential cell culture, However, effectiveness for cell attachment growth is not included in this study.
IV. The process of bioprinting will be carried out using a customized hydrogel extruder that is attached to the Snapmaker 3D Printer.
V. Imaging is limited to microscopic imaging due to the inavailability dry freezer which is necessary for SEM imaging.

CHAPTER 2

2 LITERATURE REVIEW

2.1 Introduction of seaweed biomaterial

This literature review chapter will examine the several varieties of seaweed that are presently under investigation and their prospective applications, including the utilisation of seaweed-derived materials in diverse domains such as bio-printing and biomedical applications shown in Figure 2.1.

Seaweed-based materials are being studied for their potential application in bio-printing, a rapidly expanding industry that utilises 3D printing technology to fabricate living tissue and organs. The distinctive mechanical features of seaweed-based materials, including exceptional flexibility and biocompatibility, make them well-suited for utilisation as a bio-ink. Furthermore, seaweed-derived materials have demonstrated promise for application in other sectors, including the manufacturing of compostable plastics, pharmaceuticals, and even edible goods.

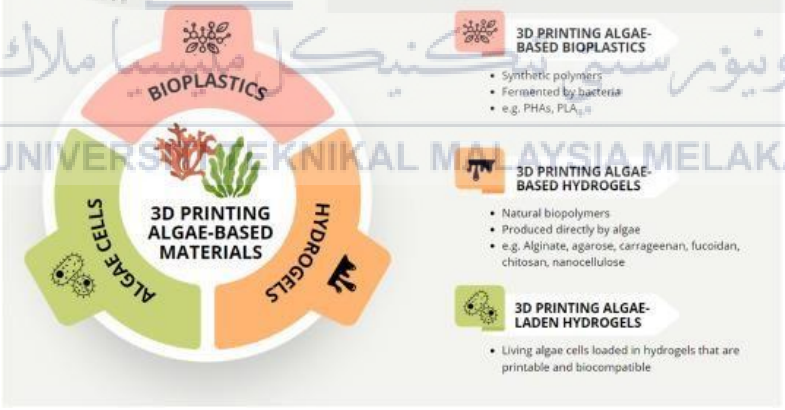

Figure 2.1: 3D printing Algae-based materials.

The possible applications of seaweed-based materials and their mechanical properties will also analyxed. This encompasses an examination of the current techniques and challenges associated with the production of seaweed-derived fillers, including the extraction and purification process of the necessary components from seaweed. In addition, this chapter will

examine the many mechanical testing techniques employed to assess the appropriateness of seaweed-derived materials for bio-printing and other uses.

The primary objective of this literature review chapter is to offer a thorough comprehension of the existing research on materials derived from seaweed and their prospective applications in the future. The study outlined in this chapter emphasises the promise of seaweed as a sustainable resource for various uses and underscores the necessity for additional research and development in this domain. The utilisation of seaweed-based products holds the capacity to fundamentally transform several sectors and actively contribute to the advancement of a more environmentally sustainable future.

2.1.1 Type of Seaweed used in bioink formulation.

The choice of seaweed for bioink formulation is a crucial factor in determining the success of 3D bioprinting applications. The intrinsic variability among different varieties of seaweed gives rise to unique characteristics that have a substantial influence on important aspects of bioink functionality, such as the ability to be printed, the strength of the structure, and how compatible it is with living organisms.

Agarose, alginate, carrageenan, and agar are often used seaweeds in bioink formulation. Agarose, obtained from red algae, is frequently used due to its exceptional gelation characteristics and ability to work well with a wide variety of cells. Alginate, derived from brown algae, is widely recognised for its adaptability and natural compatibility with living organisms, making it appropriate for a wide range of cell types (Lee & Mooney, 2012). Carrageenan, derived from red algae, has distinct rheological characteristics that are essential for improving the thickness and durability of the bioink (Zhou et al., 2014) shown in Figure 2.2. Furthermore, agar, derived from red algae, is highly regarded for its ability to form a gel and its compatibility with many types of cells.

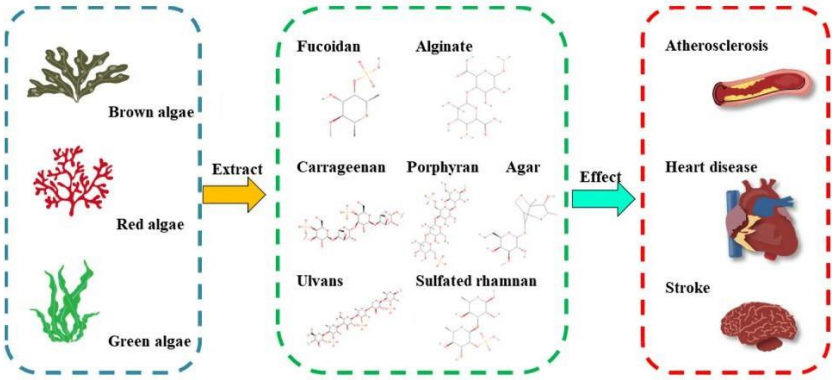

Figure 2.2 The type of seaweed used in Bioink formulation (Zhou et al., 2014).

When it comes to the formulation of bioink, the exact choice of seaweed required is determined by the properties that are being targeted by the bioink. This selection procedure is heavily influenced by several factors, including printability, mechanical strength, and the ability to provide supportive conditions for cell growth. For optimising the performance and biocompatibility of the 3D bio printed constructions, researchers take a thorough approach, taking into careful consideration the parameters while producing an appropriate bioink.

Researchers are dedicated to making progress in the field of three-dimensional bioprinting, as seen by the careful and complex selection procedure mentioned above. The precision and effectiveness of bioink formulations are ensured across a wide variety of applications, ranging from tissue engineering to regenerative medicine, thanks to this. There is a high probability that current research endeavours may reveal other seaweed candidates for bioink formulation as the area continues to undergo development. This will further increase the possibilities and capabilities of 3D bioprinting technology.

2.1.2 Properties of seaweed relevant to bioink development

Seaweed's properties are crucial in the advancement of bioink formulation for the purpose of 3D bioprinting applications. The distinct attributes of different seaweed varieties have a substantial influence on the composition and effectiveness of bioinks, affecting important factors including the capacity to be printed, the strength of the structure, and the compatibility with living organisms.

Agarose, obtained from red algae, is a crucial element in the advancement of bioink due to its remarkable ability to form a gel and its compatibility with a wide variety of cells (Lukowski & Milojevich, 2016). The gelation process is remarkable for its capacity to generate

enduring formations in bioprinting, hence enhancing the overall structural integrity of the 3D-printed constructs.

Alginate, derived from brown algae, is a widely used seaweed in the creation of bioink due to its versatility and natural ability to be compatible with living organisms (Lee & Mooney, 2012). Alginate-based bioinks have been widely used in diverse tissue engineering applications because of their ability to accommodate a wide range of cell types and promote cell growth within the printed structures.

Carrageenan, a product of red algae, imparts distinct rheological characteristics that are essential for maintaining the viscosity and stability of bioink (Zhou et al., 2014). The rheological properties of carrageenan-based bioinks play a crucial role in establishing ideal printability and ensuring accuracy in the 3D bioprinting process.

Furthermore, agar, derived from red algae, is highly esteemed for its ability to form a gel and its compatibility with many types of cells (Lee, J. H., Kim, H. W., & Chung, H. Y, 2016). The gelation process of agar-based bioinks plays a crucial role in enhancing the mechanical integrity of the printed constructs, which is essential for the fabrication of biomimetic and functional tissues.

The selection of seaweed for bioink production depends on the required properties of the bioink, including factors such as its ability to be printed, its mechanical durability, and its ability to support cell growth (Gungor-Ozkerim et al., 2018b). Researchers employ a methodical strategy to assess these characteristics to enhance the efficiency and compatibility with living organisms of 3D bio printed structures.

As the field of 3D bioprinting progresses, further research efforts are expected to reveal more characteristics of seaweed that can be utilised for the development of bioink. This endeavour holds the potential to broaden the scope and enhance the functionalities of 3D bioprinting technologies, stimulating advancements in tissue engineering, regenerative medicine, and various other biomedical applications.

2.1.3 Seaweed sourcing

Seaweed sourcing constitutes a pivotal facet within the seaweed industry, encompassing the cultivation and harvesting of seaweed for diverse applications, including food, pharmaceuticals, and other industrial uses. The global landscape of seaweed exploitation has witnessed substantial expansion in recent years, with countries adopting varied approaches

such as natural resource harvesting and seaweed aquaculture (Buschmann et al., 2017) (Rebours et al., 2014).

Japan emerges as a prominent participant in the seaweed farming sector, contributing 1.15% to the overall global seaweed production. Notably, Japan's focus lies in the cultivation of laver (nori, Porphyra tenera) and wakame (Undaria pinnatifida) (Buschmann et al., 2017). In contrast, the European, Canadian, and Latin American seaweed industries predominantly rely on the extraction of seaweeds from natural habitats. These regions face the imperative of formulating comprehensive, long-term management plans to ensure the sustainable exploitation of their seaweed resources (Rebours et al., 2014) shown in figure 2.3.

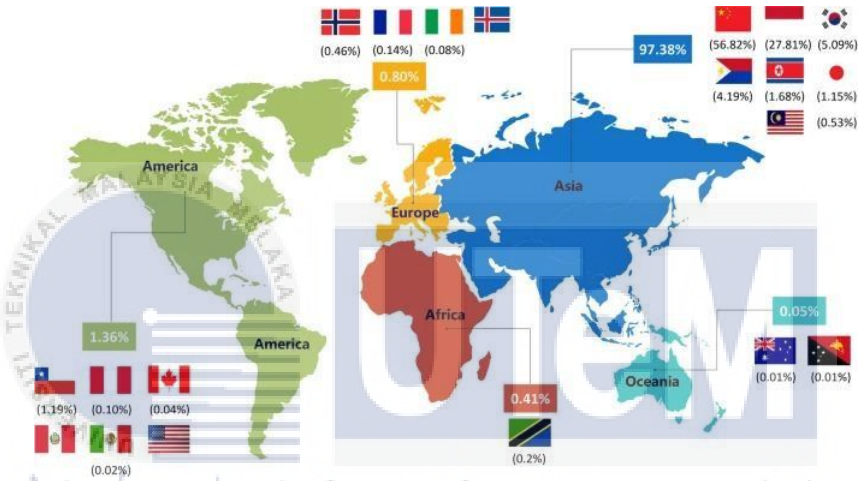

Figure 2.3 Seaweed production in the year 2019 (FAO 2021)

Advancements in seaweed farming technologies over the past two decades have been noteworthy, leading to the introduction of processed seaweed products into the market. The inherent richness of seaweed in various biologically active substances renders it an appealing raw material for applications in the food, medicine, and chemical industries (Buschmann et al., 2017). The market for algal products exhibits a projected growth trajectory, anticipated to ascend from approximately $4 billion to $5.2 billion by 2023 (Buschmann et al., 2017).

Prominent examples of edible seaweeds frequently employed in human consumption encompass various types:

Table 2.1 Types of algae with their representative species and culinary applications (Buschmann et al., 2017).

Type of Algae	Representative Species	Culinary Applications
Brown Algae	Laminaria digitata, Sargassum fusiformis	Diverse use in culinary contexts, including salads, soups, and as a flavour enhancer.
Red Algae	Porphyra spp.	Contribution to the production of agar used in desserts and as a thickening agent in sauces.
Green Algae	Chlorella, Spirulina	Prevalent in the production of nutraceuticals and cosmetics. Serve as a protein source in vegetarian and vegan products.

To ensure the sustainable exploitation of seaweed resources, it is imperative to institute national regulations delineating optimal practices for seaweed harvesting, management, and cultivation. Dissemination of this knowledge to coastal communities is essential (Rebours et al., 2014). The procurement of seaweed biomass through cultivation necessitates advancements in technology and management practices, institutional modifications, and the implementation of suitable environmental and social frameworks (Rebours et al., 2014).

2.2 Bioink development

The area of 3D bioprinting has experienced significant advancements, with the development of bioink emerging as a crucial element in creating intricate and functioning tissues. Bioinks, the substances utilised in 3D bioprinting, have a crucial impact on the outcome of this groundbreaking technique. This essay offers a thorough examination of the latest progress in the development of bioink, with a specific emphasis on crucial elements and their influence on the rapidly growing field of 3D bioprinting.

Bioinks commonly comprise a blend of biomaterials, cells, and other constituents specifically formulated to imitate the extracellular matrix (ECM) of natural tissues. Hydrogels

are frequently selected due to their ability to create a conducive setting for cell proliferation and allow for accurate cell placement throughout the printing process. Commonly used in

bioink formulations include natural polymers such as alginate, agarose, and collagen, as well as synthetic polymers like polyethylene glycol (PEG) (Murphy & Atala, 2014a).

The latest progress in bioink research underscores the need to guarantee optimal cell viability and functionality. To improve the integration of cells inside the bioink matrix, one can optimise the rheological properties of the ink, regulate the crosslinking kinetics, and provide cell-friendly additives. These enhancements facilitate the effective production of live and functional tissues (Olgun et al., 2019).

Researchers are increasingly exploring the functionalization of bioinks to impart specific properties to the printed constructs. Incorporating bioactive molecules, growth factors, and nanoparticles into the bioink enhances its biological and mechanical properties. These additions can facilitate cell signaling, promote tissue regeneration, and improve the overall performance of the 3D-printed structures (Jia et al., 2016).

The progress in bioink development has resulted in the production of bioinks that consist of many materials and multiple types of cells. This allows for the printing of intricate and diverse tissues. Researchers strive to replicate the complex microenvironments present in natural tissues by integrating several cell types and materials into a single bioink. The utilisation of this method is essential in the creation of organs that contain a variety of cell types and perform specific tasks (Ouyang et al., 2015).

The development of bioink is intricately linked with the advancement of bioprinting processes. The utilisation of extrusion-based, inkjet-based, and laser-assisted bioprinting techniques poses distinct problems and prospects for the development of bioink formulations. Customising bioinks to match the precise demands of individual bioprinting methods is crucial for attaining precise and consistent outcomes.

While significant strides have been made in bioink development, challenges persist. Achieving optimal mechanical properties, scalability, and long-term stability of printed constructs remains a focus of ongoing research. Additionally, efforts are underway to develop personalized bioinks, accounting for individual patient variations in tissue composition and function.

2.3 **Bioink formulation**

The formulation of bioink assumes a central role within the domain of 3D bioprinting, an avant-garde technology amalgamating biological substrates with engineering principles for the fabrication of intricate living structures. This innovative paradigm holds significant promise in diverse domains, including tissue engineering, regenerative medicine, and pharmaceutical development, fostering potential breakthroughs in the realm of personalized medicine. This discourse explores the fundamental constituents and considerations integral to bioink formulation, elucidating recent progressions and their prospective implications.

Bioinks conventionally comprise biocompatible elements that furnish a supportive matrix for living cells throughout the bioprinting procedure. Key constituents encompass hydrogels, mirroring the characteristics of the extracellular matrix, along with cells, often sourced from the intended tissue. A variety of biomaterials, such as alginate, gelatine, hyaluronic acid, and synthetic polymers, are harnessed, each conferring distinct advantages concerning biocompatibility, mechanical attributes, and degradation kinetics (Mandrycky et al., 2016).

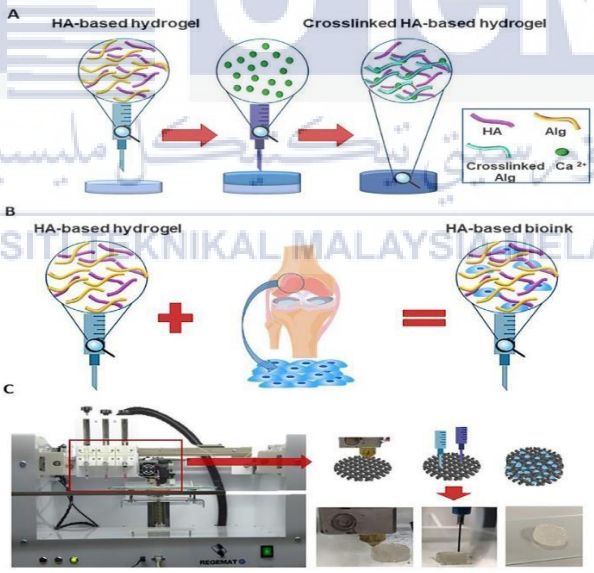

Figure 2.4 shows: Bioink Formulation (Mandrycky et al., 2016).

Maintaining cellular viability and functionality within the bioink is imperative for the success of tissue engineering endeavours. Scholars concentrate on optimizing the rheological characteristics of the bioink to facilitate the bioprinting process while safeguarding cell integrity shown in Figure 2.4. Furthermore, formulations of bioink are tailored to create a

microenvironment conducive to cell proliferation, differentiation, and the manifestation of tissue-specific functions (Skardal et al., 2016).

The stability of the printed structure is ensured through the implementation of crosslinking mechanisms, which lead to the solidification of the bioink after deposition. Common strategies involve physical crosslinking, such as temperature or pH-induced gelation, and chemical crosslinking utilizing agents like calcium chloride or UV light. Researchers strive to strike an equilibrium between attaining structural integrity and mitigating any deleterious effects on cell viability (Gungor-Ozkerim et al., 2018c).

Recent progress in bioink formulation is centred on augmenting the biomimetic qualities of printed constructs and enhancing their mechanical attributes. The integration of bioactive molecules, growth factors, and nanomaterials into bioinks is employed to amplify cellular responses and functional outcomes (Chimene et al., 2016). Additionally, there is ongoing research to devise bioinks that incorporate multiple materials and cell types, aiming to emulate the heterogeneous nature of native tissues and thereby enabling more precise tissue modelling.

Bioink formulation stands as a pivotal aspect of 3D bioprinting, playing a key role in advancing the realms of tissue engineering and regenerative medicine. The interdisciplinary character of this domain, amalgamating principles from biology and engineering, continues to spur innovative developments that exhibit promise in addressing intricate healthcare challenges.

2.4 **Biocompatibility of Seaweed-Based Bioink**

Within the dynamically evolving domain of 3D bioprinting, the pursuit of materials that exhibit biocompatibility has prompted an exploration into bioinks derived from seaweed. Seaweed, abundantly present in marine ecosystems, emerges as a sustainable substitute for conventional components employed in bioink formulations (Hinton et al., 2015). This discourse delves into the assessment of biocompatibility intrinsic to seaweed-based bioinks, scrutinizing their potential to revolutionize the landscape of tissue engineering while concurrently bolstering the sustainability quotient of bioprinting technologies (Buschmann et al., 2017).

Seaweed, colloquially referred to as macroalgae, inherently boasts biocompatible attributes attributed to its historical use as a safe consumable by humans and its application in

traditional medicine. The integration of materials derived from seaweed into bioink formulations aligns with the escalating demand for sustainable and environmentally conscious alternatives within the realm of biomedical research. Notably, alginate, a polysaccharide extracted from brown seaweeds, stands out as a prominent illustration, acknowledged for its exceptional biocompatibility and gel-forming characteristics, rendering it a preferred selection in the constitution of seaweed-based bioinks.

The biocompatibility inherent in seaweed-based bioinks transcends their natural origin. These bioinks frequently demonstrate rheological properties conducive to the 3D bioprinting process, furnishing essential structural support for the resultant printed constructs. As an illustration, alginate undergoes ionotropic gelation, facilitating swift crosslinking in the presence of divalent cations such as calcium, thereby imparting stability to the printed structures (Gao et al., 2015). The adaptability of seaweed-based bioinks enables the encapsulation of diverse cell types while concurrently preserving high cell viability throughout and following the bioprinting procedure (Mantha et al., 2019).

In addition to their role in structural support, seaweed bioinks frequently encompass bioactive compounds with potential therapeutic advantages. Seaweed is replete with polysaccharides, proteins, and other bioactive molecules that may contribute to heightened cell proliferation, differentiation, and overall tissue functionality. This dual functionality of seaweed-based bioinks, encompassing both structural support and bioactive cues, positions them as auspicious candidates for advancing the capabilities of 3D bio printed tissues.

The biocompatibility inherent in seaweed-based bioinks represents a noteworthy advancement in the pursuit of sustainable and biologically pertinent 3D bioprinting. Leveraging the inherent advantages of seaweed not only conforms to the tenets of eco-friendly bioprinting but also introduces novel prospects for fabricating functional and environmentally conscientious bioengineered tissues. As investigations in this domain advance, seaweed-based bioinks have the potential to assume pivotal roles in shaping the forthcoming paradigm of regenerative medicine and tissue engineering.

2.5 **Mechanical Properties of Seaweed-Based Bioink Scaffold**

The biomechanical characteristics of bioink scaffolds are crucial factors determining their appropriateness for applications in tissue engineering. Bioinks derived from seaweed, sourced from marine algae, have gained recognition for their distinctive combination of

biocompatibility and environmental sustainability. This article delves into the intricate mechanical features of seaweed-based bioink scaffolds, elucidating their profound significance and potential transformative impact on the domain of tissue engineering.

The efficacy of tissue engineering is contingent upon the precise replication of the inherent biomechanical milieu found in tissues and organs. Mechanical stimuli exert substantial influence over various cellular behaviours, encompassing critical aspects such as adhesion, proliferation, and differentiation. Striking an intricate balance between structural robustness and pliability is imperative for the seamless integration of bioink scaffolds within the intricate and dynamic microenvironment of the human body (Murphy & Atala, 2014b).

In essence, this exploration not only sheds light on the biomechanical intricacies of seaweed-based bioink scaffolds but also underscores their strategic importance in advancing the field of tissue engineering. The distinctive combination of biocompatibility and sustainability exhibited by these bioinks positions them as promising candidates for further exploration and application in the complex realm of regenerative medicine.

Seaweed-derived bioinks, often incorporating alginate or agarose extracted from marine algae, confer unique mechanical attributes to resulting scaffolds. Alginate, a prevalent selection, is renowned for its gel-forming capabilities via ionotropic gelation, bestowing stability upon the bioink(Gao et al., 2015). The ultimate mechanical properties of the bioink scaffold are influenced by variables such as the concentration of seaweed-derived components, crosslinking methodologies, and the integration of reinforcing elements like nanoparticles or fibres.

Ensuring compatibility with the 3D bioprinting process is imperative for the mechanical properties of seaweed-based bioink scaffolds. Achieving optimal printability mandates a delicate equilibrium between viscosity and shear-thinning behaviour, facilitating meticulous layer-by-layer deposition. Concurrently, the scaffold must uphold structural integrity throughout and post the printing process to ensure adherence to the intended design (Ozbolat & Hospodiuk, 2016).

Given the diverse mechanical characteristics exhibited by different tissues, a tailored approach to bioink scaffold design becomes imperative. Seaweed-based bioinks afford versatility in fine-tuning mechanical properties to align with specific tissue requisites. For instance, cardiac tissue might derive benefits from a softer scaffold to replicate heart

compliance, whereas bone tissue engineering may necessitate a more rigid structure to support load-bearing functionalities (Murphy & Atala, 2014b).

The mechanical characteristics of bioink scaffolds derived from seaweed are pivotal in determining their effectiveness for tissue engineering applications. Achieving an optimal balance between structural integrity, printability, and the incorporation of tissue-specific mechanical signals is crucial for maximizing the capabilities of seaweed-based bioinks. As ongoing research advances in this domain, these bioinks have the potential to assume a leading role in the development of biomimetic and sustainable scaffolds, thereby making significant contributions to the progress of regenerative medicine.

2.6 Seaweed-Based Materials for 3D-Printing

The incorporation of materials derived from seaweed into 3D printing processes has emerged as a compelling avenue for sustainable and biocompatible innovations. Seaweed, abundant in marine ecosystems, represents a renewable resource with unique properties, rendering it an appealing candidate for a diverse array of applications. This essay undertakes an exploration of the utilization of seaweed-based materials in 3D printing, delving into their potential contributions to sustainability, biocompatibility, and the broader landscape of additive manufacturing.

Seaweed's rapid growth, minimal resource requirements, and adaptability to diverse marine environments position it as an ecologically friendly and sustainable raw material for 3D printing. In contrast to traditional plastics derived from fossil fuels, seaweed-based materials present a renewable alternative capable of mitigating the environmental impact associated with conventional manufacturing processes (Bixler & Porse, 2011).

Inherent biocompatible characteristics distinguish seaweed-based materials, rendering them suitable for various biomedical applications in 3D printing. Notably, alginate, a polysaccharide derived from brown seaweed, exemplifies this trait. Its capacity for ionotropic gelation facilitates a biomimetic environment, closely resembling the extracellular matrix and supporting cell viability (Gao et al., 2015). Consequently, seaweed-based biomaterials hold substantial promise for advancing bioprinting technologies and tissue engineering.

Seaweed-based materials demonstrate versatility across various 3D printing processes, encompassing fused deposition modelling (FDM), stereolithography (SLA), and selective laser sintering (SLS). Alginate, for instance, exhibits shear-thinning behaviour, facilitating precise

extrusion in FDM systems, while its responsiveness to crosslinking methods aligns with SLA applications (Gao et al., 2015). This adaptability positions seaweed-based materials as valuable resources applicable to a wide range of 3D printing technologies shown in Figure 2.5.

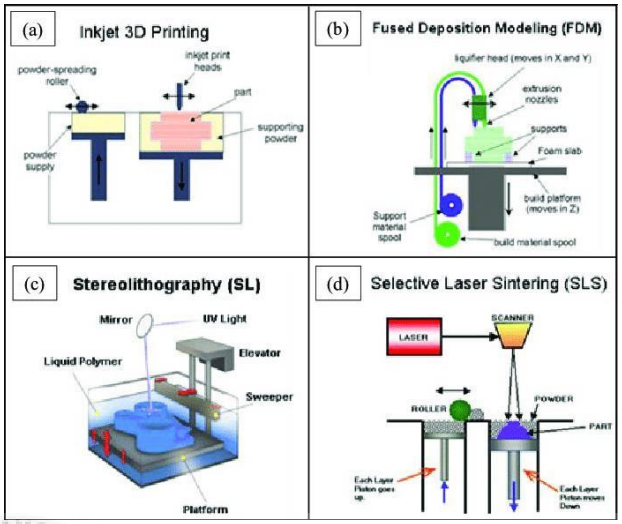

Figure 2.5 shows 3D printing process (Gao et al., 2015).

The notable biodegradability of seaweed-based materials assumes significance within the framework of a circular economy. As 3D printing gains traction, addressing considerations related to end-of-life becomes imperative. Seaweed-based filaments and resins, owing to their biodegradable nature, offer a sustainable solution to mitigate the environmental impact associated with plastic waste generated by traditional 3D printing materials (Gao et al., 2015).

Despite the promising attributes inherent in seaweed-based materials, persistent challenges necessitate continued attention. Ongoing research and development efforts are imperative to ensure consistent quality and mechanical properties, optimize printability across diverse 3D printing technologies, and address scalability issues. Furthermore, there is a need for exploration into synergies with other sustainable materials and the enhancement of seaweed-based composites to unlock novel possibilities for advanced applications.

Seaweed-based materials have emerged as transformative elements in the realm of 3D printing, presenting a sustainable, biocompatible, and versatile alternative to traditional materials. As technological advancements unfold, the integration of seaweed-based materials into 3D printing processes holds the potential to revolutionize industries, spanning from biomedicine to sustainable product design. The convergence of seaweed and 3D printing epitomizes innovative strides toward a more sustainable and environmentally conscious future.

2.6.1 Viscosity influences the printing process.

Viscosity is a crucial parameter that profoundly impacts the behaviour and performance of hydrogel bioinks in the context of 3D printing applications. As these bioinks are extruded through printing nozzles, their viscosity dictates how they flow, adhere, and form layers, directly influencing the precision and fidelity of the final printed structures. When bioinks possess low viscosity, they tend to spread rapidly upon deposition. This uncontrolled spreading can lead to inaccuracies in the printed shape, as well as a loss of resolution in fine details. Maintaining the intended structure becomes challenging, as the bioink may fail to hold its shape and details, compromising the overall quality of the printed object (Billiet et al., 2012).

Conversely, bioinks with inadequate viscosity—too high or too low—can struggle to maintain structural integrity between successive layers. Insufficient viscosity may result in poor adhesion between layers, which could eventually cause deformation or collapse of the printed structure over time. This issue is particularly critical in complex prints or those requiring precise layer alignment and stability (Ozbolat & Hospodiuk, 2016). Achieving an optimal viscosity range is therefore essential for ensuring precise and reliable layer-by-layer deposition in 3D bioprinting. An ideal viscosity allows the bioink to flow smoothly and uniformly through the nozzle, facilitating accurate placement and adherence of each layer. This not only enhances the structural integrity of the printed constructs but also supports their functional performance and biocompatibility, critical factors in biomedical applications (Hinton et al., 2015).

Achieving an optimal viscosity range is critically important in the realm of 3D bioprinting for several fundamental reasons. The viscosity of hydrogel bioinks directly governs their flow behaviour during the extrusion and deposition processes. When the viscosity is appropriately tuned, it ensures that the bioink flows uniformly through the printing nozzle, facilitating precise and consistent deposition of each layer. This uniform flow is essential for maintaining the intended shape fidelity and resolution of the printed structures, which is particularly crucial for creating intricate and complex geometries. Moreover, effective viscosity control enhances the overall quality of the printed constructs. Bioinks with well-controlled viscosity exhibit improved structural integrity between layers, minimizing the risk of deformation or collapse over time. This stability is vital for the functionality and longevity of tissue-engineered constructs in biomedical applications. (Ganewatta et al., 2018)

In the development of hydrogel bioinks, researchers strive to optimize viscosity to balance ease of extrusion with structural stability. Too low viscosity can lead to spreading upon deposition, compromising the printed structure's accuracy and detail resolution. Conversely, bioinks with excessively high viscosity may encounter challenges in extrusion, such as nozzle clogging or inconsistent material flow, resulting in reduced print resolution. (Zhang et al., 2021) Therefore, ongoing research focuses on refining viscosity modulation techniques to achieve precise control over bioink behavior during printing. This includes exploring additives, cross- linking agents, and formulation adjustments to tailor viscosity to specific printing requirements. Such advancements are crucial for expanding the capabilities of 3D bioprinting technology, enabling the fabrication of tissues and organs with intricate architectures and functional complexity. The printable bioink viscosity is in range 6 dPas-11 dPas (Aktas et al., 2014).

2.6.2 Parameters affecting printing process.

The field of additive manufacturing, commonly known as 3D printing, has experienced remarkable growth, presenting unparalleled capabilities in prototyping, product development, and even tissue engineering. However, achieving precision in the 3D printing process relies on a complex interplay of numerous parameters. This article delves into the pivotal parameters that exert substantial influence on the 3D printing process, encompassing factors such as material properties, printer settings, and environmental conditions.

The selection of printing material stands as a fundamental determinant of success in any 3D printing endeavour. Material characteristics, including viscosity, melt flow, and thermal properties, wield a profound impact on the intricacies of the printing process. Different materials necessitate specific processing temperatures and extrusion rates to ensure proper layer adhesion and structural integrity. Additionally, considerations such as material shrinkage during cooling must be meticulously addressed to prevent undesirable warping and distortion (Gibson et al., 2010).

Printer settings encapsulate a wide array of parameters, each playing a critical role in shaping the final output. Variables such as layer thickness, printing speed, and nozzle diameter contribute to determining the resolution and surface finish of printed objects. Furthermore, the infill density, representing the amount of material within the object, holds sway over both mechanical strength and printing time. Attaining precision in printer calibration, including bed

levelling and nozzle alignment proves essential for maintaining accuracy throughout the entirety of the printing process (Bikas et al., 2016).

Environmental variables, such as humidity and airflow, exert a considerable impact on the 3D printing process. Materials with hygroscopic tendencies, capable of absorbing moisture from the air, may undergo alterations in viscosity and extrusion behaviour, resulting in defects during printing. Furthermore, maintaining control over the printing environment proves instrumental in mitigating issues related to temperature fluctuations and external contaminants that could compromise the structural integrity of the printed object (Moetazedian et al., 2020).

The successful printing of intricate geometries often necessitates the incorporation of support structures. Parameters pertaining to support material, including its solubility or ease of removal, directly influence subsequent post-processing steps. The optimization of support structures becomes imperative for achieving precision in intricate designs, especially when dealing with overhangs or suspended features (Kuang et al., 2019).

Post-processing procedures, encompassing activities such as curing, annealing, or surface finishing, constitute an additional set of parameters that impact the final product. A nuanced understanding of the material-specific requirements for post-processing is indispensable for enhancing mechanical properties, aesthetic appeal, and overall functionality.

Achieving precision in 3D printing necessitates a meticulous equilibrium attained through the thoughtful examination and refinement of multiple parameters. The success of the printing process is contingent upon the comprehensive optimization of material properties, printer settings, temperature regulation, environmental factors, support structures, and post-processing considerations. As technological advancements in additive manufacturing persist, a profound comprehension of these parameters proves essential for fully harnessing the expansive capabilities of this technology across diverse industries.

2.6.3 Optimization strategies.

As additive manufacturing, commonly known as 3D printing, continues its transformative impact on various industries, the imperative to optimize the process has assumed a central role. Optimization strategies play a pivotal role in augmenting efficiency, curtailing costs, and elevating the overall performance of additive manufacturing technologies. This essay scrutinizes key optimization strategies applied in additive manufacturing,

encompassing elements such as design optimization, process parameters, materials, and considerations in post-processing.

At the core of additive manufacturing optimization lies the design paradigm. Design for Additive Manufacturing (DfAM) entails tailoring designs to suit the specific capabilities and constraints inherent in 3D printing technologies. This involves harnessing intricate geometries, minimizing the need for support structures, and optimizing part orientation to amplify build efficiency and curtail material usage. DfAM not only enhances the manufacturability of components but also unveils prospects for lightweight structures and innovative functionalities (Gao et al., 2015).

Precise adjustment of process parameters is imperative for achieving prints of high quality. Variables such as layer thickness, printing speed, and temperature settings exert influence over the resolution, mechanical properties, and surface finish of printed objects. Systematic experimentation and analysis enable manufacturers to discern optimal parameter combinations, ensuring reproducibility and consistency in the printing process. Continuous monitoring and fine-tuning of these parameters contribute significantly to the optimization of print quality (Bikas et al., 2016).

In the realm of additive manufacturing, the criticality of material selection cannot be overstated, and the scope of optimization extends beyond the mere choice of materials. The formulation of materials with customized properties, such as enhanced strength, flexibility, or thermal conductivity, facilitates the optimization of end-use applications. Furthermore, delving into advanced materials, including composites and multi-material printing, presents opportunities for refining material characteristics and broadening the spectrum of producible components (Ligon et al., 2017).

Topology optimization stands out as a potent technique involving the mathematical determination of the optimal distribution of material within a designated design space to meet specified performance criteria. Through the strategic removal of excess material from non-critical areas, topology optimization achieves a reduction in weight and material consumption while preserving structural integrity. This approach not only enhances the efficiency of additive manufacturing processes but also contributes to sustainable and resource-efficient manufacturing practices (Kladovasilakis et al., 2021).

Post-processing steps are integral to the holistic optimization of the final product. Techniques such as annealing, surface finishing, and heat treatment hold the potential to

significantly enhance the mechanical and aesthetic properties of printed components. Optimizing post-processing methodologies, including the advancement of automated and efficient post-processing workflows, ensures that printed components meet or surpass specified performance standards Zhu et al. (2021).

Optimization strategies within the realm of additive manufacturing are intricate and cover a spectrum of considerations, including design, process parameters, material selection, and post-processing techniques. The incorporation of these strategies empowers manufacturers to elevate efficiency, curtail costs, and produce products with enhanced performance attributes. As advancements in additive manufacturing technologies persist, the pursuit of optimization remains a dynamic and integral facet, playing a pivotal role in unlocking the complete potential of 3D printing across various industries.

2.7 Application of Seaweed-Based Bioink in Tissue Engineering

Tissue engineering stands at the forefront of regenerative medicine, presenting a revolutionary approach to restoring, maintaining, or enhancing tissue function. A pivotal component in tissue engineering is bioink, a substance serving as the "ink" in 3D bioprinting processes. This discourse delves into the utilization of seaweed-based bioink in tissue engineering, emphasizing its potential advantages concerning sustainability, biocompatibility, and versatility.

Seaweed-based bioinks primarily leverage polysaccharides extracted from marine algae, prominently alginate and agarose. Alginate, a polysaccharide derived from brown seaweed, is particularly favoured due to its biocompatibility and gel-forming properties. These bioinks often incorporate additional elements such as cells, growth factors, and other bioactive substances to establish an environment conducive to tissue development (Gao et al., 2015).

The application of seaweed-based bioinks resonates with the escalating emphasis on sustainability within biomedical applications. Seaweed, a renewable resource flourishing in marine environments, demands minimal cultivation resources. In comparison to conventional bioinks derived from animal or synthetic sources, seaweed-based bioinks offer a more environmentally friendly alternative, thereby contributing to the advancement of sustainable practices in tissue engineering (Mantha et al., 2019).

A fundamental requisite for a successful bioink is biocompatibility, ensuring its ability to support cell viability and functionality. Seaweed-based bioinks, particularly those

incorporating alginate, demonstrate exceptional biocompatibility. Alginate's capability for ionotropic gelation results in a gel-like matrix that closely mimics the natural extracellular matrix (ECM), creating an environment conducive to cell adhesion, proliferation, and differentiation (Gao et al., 2015).

The versatility of seaweed-based bioinks in 3D bioprinting processes is noteworthy. Their rheological properties, characterized by shear-thinning behaviour, enable precise extrusion and layer deposition, facilitating the generation of intricate and complex tissue structures. Furthermore, seaweed-based bioinks often exhibit rapid crosslinking, ensuring stability in the printed constructs both during and after the bioprinting process (Skardal et al., 2010).

Seaweed-based bioinks harbour significant potential across a broad spectrum of tissue engineering applications (figure 2.6). Their utilization has been explored in the bioprinting of diverse tissues, including skin, cartilage, blood vessels, and more intricate organs. The adaptability of seaweed-based bioinks to various 3D bioprinting technologies empowers researchers to customize the printing process according to specific tissue requirements, propelling the field closer to the realization of functional and implantable tissues (Chimene et al., 2016).

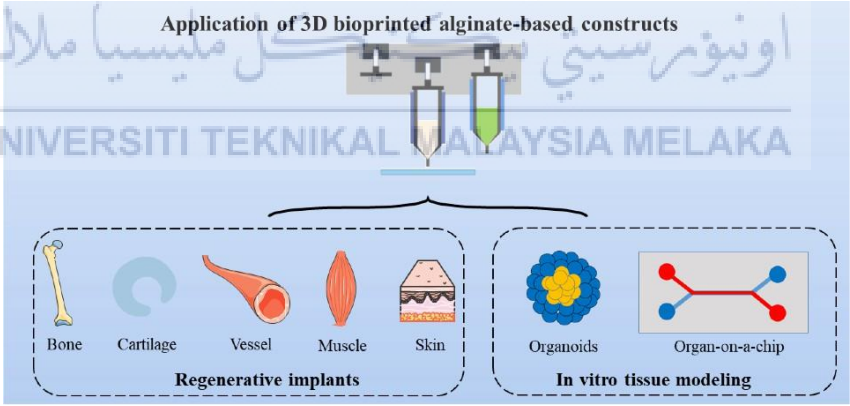

Figure 2.6 The application of 3D bio printed alginate based (Chimene et al., 2016).

Bioinks derived from seaweed present a sustainable and biocompatible approach in the realm of tissue engineering, in harmony with the tenets of environmentally conscious bioprinting shown in Figure 2.6. Originating from renewable marine sources and possessing the capacity to replicate the extracellular matrix (ECM) while fostering cellular activities, these bioinks emerge as valuable materials within the domain of regenerative medicine. Ongoing

research, delving into the extensive capabilities of seaweed-based bioinks, positions them strategically to play a central and transformative role in shaping the future landscape of tissue engineering and regenerative medicine.

2.8 Regulatory and Safety Considerations

The integration of seaweed-based bioinks as promising materials in tissue engineering necessitates a meticulous transition from laboratory advancements to clinical applications, underscored by careful consideration of regulatory and safety aspects. This discourse delves into the regulatory landscape and safety parameters associated with the utilization of seaweed-based bioinks, underscoring the imperative for compliance, ethical practices, and thorough risk assessments.

The regulatory trajectory for bioinks, inclusive of those derived from seaweed, entails strict adherence to established guidelines and standards. Regulatory entities, such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), set exacting requirements for the approval and commercialization of medical products. Seaweed-based bioinks undergo comprehensive evaluation, encompassing criteria such as biocompatibility, stability, and long-term safety assessments, to secure regulatory clearance for clinical applications (FDA, 2017).

Ensuring the biocompatibility of seaweed-based bioinks stands as a pivotal safety consideration. While seaweed-derived materials are generally acknowledged for their biocompatible nature, variations in composition and processing methods may influence their interaction within the human body. Rigorous testing, including both in vitro and in vivo studies, is imperative to evaluate cell viability, tissue response, and the potential for immunogenic reactions. A thorough and exhaustive biocompatibility assessment is essential to ensure the safety of patients undergoing bioink-based implants (ISO 10993-1, 2018).

Comprehensive understanding of the enduring stability and degradation characteristics of seaweed-based bioinks is imperative. Regulatory authorities mandate meticulous evaluations of degradation profiles to anticipate the durability of implanted constructs and the potential release of degradation by-products. Research endeavours must address concerns related to potential toxicity or adverse effects linked to degradation products, ensuring the enduring safety of the implanted biomaterials (ASTM F1635-11, 2011).

Beyond the technical and regulatory domains, ethical considerations stand as integral elements in the secure development and application of seaweed-based bioinks. This encompasses transparent reporting of research findings, the acquisition of informed consent from research participants, and the responsible and ethical utilization of bioprinting technologies. Collaborative engagements involving researchers, regulatory bodies, and ethical review boards are indispensable to navigate the intricate ethical landscape surrounding tissue engineering and bioink applications (Groll et al., 2016).

Conducting comprehensive risk assessments throughout the development and application phases of seaweed-based bioinks is essential. Identifying potential risks, such as contamination, unintended immune responses, or unanticipated material interactions, enables the implementation of effective mitigation strategies. Regular monitoring, implementation of quality control measures, and continuous post-market surveillance contribute to the ongoing assessment of risks and the refinement of safety protocols (ISO 14971, 2019).

The potential of seaweed-based bioinks in advancing tissue engineering is considerable, but their secure and efficient integration into clinical applications necessitates a robust framework encompassing regulatory compliance and safety considerations. Adherence to well-established regulatory standards, comprehensive biocompatibility assessments, ethical scrutiny, and the implementation of vigilant risk management practices are indispensable. In the ongoing exploration of the therapeutic capabilities of seaweed-based bioinks, a resolute commitment to regulatory and safety principles is fundamental, paving the path for responsible and impactful progress in the field of regenerative medicine.

2.9 Conclusion and Future Directions

Seaweed-based bioinks have emerged as a promising and sustainable alternative in the realm of tissue engineering, offering distinctive advantages such as biocompatibility, versatility, and ecological sustainability. The transition from laboratory exploration to clinical application has underscored the transformative potential of these bioinks in reshaping the landscape of regenerative medicine. Nevertheless, akin to any groundbreaking technology, addressing challenges and considerations is imperative to guarantee effective implementation.

The exceptional biocompatibility of seaweed-based bioinks, particularly those incorporating alginate, stands out, facilitating the establishment of biomimetic environments conducive to cellular growth and tissue development. Their sustainable derivation from marine

ecosystems aligns with the global trend towards environmentally conscious practices in the realm of medical research and application. The versatility of seaweed-based bioinks across various 3D bioprinting technologies has created opportunities for the precise fabrication of intricate tissue structures.

Prioritizing regulatory compliance and safety considerations is of utmost importance in the translation of seaweed-based bioinks from laboratory settings to clinical application. Stringent adherence to established standards, thorough biocompatibility assessments, and ethical practices are essential prerequisites to ensure patient safety and the seamless integration of these bioinks into clinical practice.

To propel the utilization of seaweed-based bioinks in tissue engineering, several key avenues of research and development need to be explored. First and foremost, there is a critical need for further research to optimize the mechanical properties of these bioinks. This includes addressing challenges associated with printability, ensuring structural integrity, and effectively mimicking tissue-specific mechanical cues. Additionally, investigating the incorporation of seaweed-based materials into composite bioinks, along with other biomaterials or nanoparticles, holds promise for enhancing the overall properties of the bioink to cater to diverse tissue engineering requirements.

Another crucial area of exploration involves conducting extensive studies on the long-term stability and in vivo performance of seaweed-based bioinks. Understanding how these bioinks behave over extended periods within the complex physiological environment is essential for their successful clinical translation. Furthermore, efforts should be directed towards scaling up the production of seaweed-based bioinks to enable broader clinical applications. Developing cost-effective and scalable manufacturing processes will be pivotal for the commercialization of these bioinks.

The ongoing innovations in 3D bioprinting technologies are anticipated to significantly influence the application of seaweed-based bioinks. Advances in printing methods, such as multi-material printing and the integration of vascular networks, will further enhance the complexity and functionality of the tissues that can be printed. Beyond the laboratory setting, the initiation of clinical trials and translational research represents a crucial step to validate the safety and efficacy of seaweed-based bioinks in human subjects. This comprehensive approach to research and development will contribute to unlocking the full potential of seaweed-based bioinks in advancing tissue engineering practices.

In summary, the convergence of sustainability, biocompatibility, and precision in tissue engineering is evident in the utilization of seaweed-based bioinks. Despite current challenges, continuous research endeavours and prospective directions offer the potential to fully harness their capabilities, ushering in a transformative era in regenerative medicine. This foresees seaweed-based bioinks assuming a central role in addressing intricate healthcare demands.

CHAPTER 3

3 METHODOLOGY

3.1 Introduction

The methodology section will delineate a systematic and sequential protocol for executing the procedures involved in this study. A comprehensive elucidation of the methods and tools employed in this project will be provided. Each stage will be expounded upon in Chapter 3, encompassing the meticulous implementation of the optimal strategy aimed at realizing the objectives of the project.

3.2 Project Flowchart

The flowchart of this project is illustrated in Figure 3.1. The suitability of the process for fabricating the seaweed-based hydrogel component is duly established in accordance with the facilities available at the University.

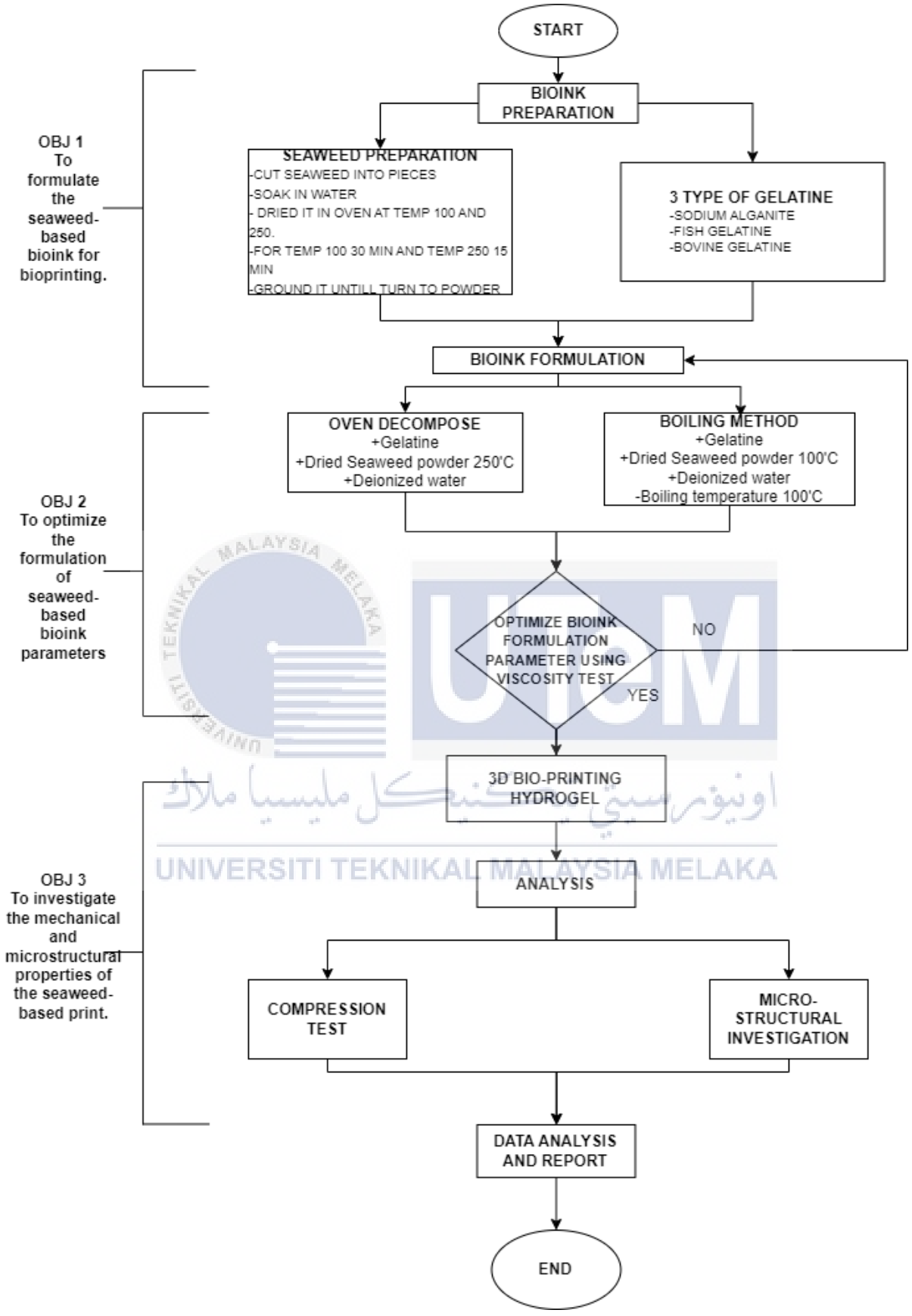

Figure 3.1 The flowchart of this project.

The flowchart presented in Figure 3.1 outlines the procedural steps involved in the synthesis of seaweed-based materials through bio-printing. The initial step involves the preparation of bioink, which is divided into two pathways: seaweed preparation and the use of three types of gelatine. In the next stage, the bioink formulation is determined by combining seaweed powder, gelatine, and deionized water. Seaweed based hydrogel was prepared using 2 method which is oven decompose and boiling method. For oven decomposed method, the usage of the seaweed is seaweed that have been dried at temperature 250°C for 15 minutes. For boiling method, the usage of seaweed powder that have been dried at temperatures 100°C for 30 minutes. And it will be boiled at 100°C until all the mixture dissolves.

Following this, the subsequent stage focuses on optimizing bioink formulation parameters, achieved through a viscosity test. Hydrogel within viscosity range of 6-11 dPas is proceeded for bioprinting. Then the characteristic of the seaweed-based hydrogel was evaluated with compression tests and microstructural investigations using Scanning Electron Microscopy (SEM).

The subsequent phases involve comprehensive data analysis and reporting. However, if the materials do not meet the desired specifications, an iterative process is initiated. This process focuses on refining the material type, parameters, and experimental methodology to address any shortcomings and enhance the overall synthesis process.

3.3 Preparation of Bio-ink

The process of preparing seaweed-based bioink involves several steps. First, red seaweed is collected from the FTKIP lab. Next, the dried algae are treated to remove external impurities such as sand, stones, and dried marine organisms shown in figure 3.2.

Figure 3.2 Raw red seaweed.

Following this, the algae will undergo a washing procedure utilizing flowing deionized water for a duration of five minutes to diminish the salt content, which has the potential to adversely affect the ultimate gelling characteristics of carrageenan. The pre-treated algae will

then be subjected to drying in an oven set at 250 °C for 15 minutes shown in Figure 3.3, this is for decomposed seaweed powder. Subsequently, the seaweed powder was also prepared using a boiling method. The solution is heated to a temperature of 100°C shown in figure 3.4. Prior to carrageenan extraction, the dried and pre-treated algae will undergo a process of dehydration and sanitization. The desiccated seaweed can subsequently be finely ground for a duration of five minutes using a food processor or blender until achieving a finely powdered state.

Figure 3.3 Oven Decompose seaweed

Figure 3.4 Seaweed powder for boiling method

Subsequently, a mixture comprising 2% of gelatine powder and 1%, 2% and 3% of ground seaweed powder will be introduced into 100ml of deionized water, where it will be stirred until complete dissolution using a magnetic stirrer. After that, the following step was repeated using 100 °C seaweed powder and boiling it at 100 °C until the mixture dissolved. Following this, the resulting solution will be transferred into an amber bottle with a securely fastened lid to avert any risk of contamination. At this point, the prepared mixture is poised for insertion into the syringe for the printing process.

3.4 Optimization of Bioink formulation Parameters

Optimizing bioink formulation parameters is crucial for ensuring the success of bioprinting processes, where cells are printed in a supportive matrix (bioink). Here are some key parameters and strategies for optimization.

3.4.1 Bioink formulation parameters

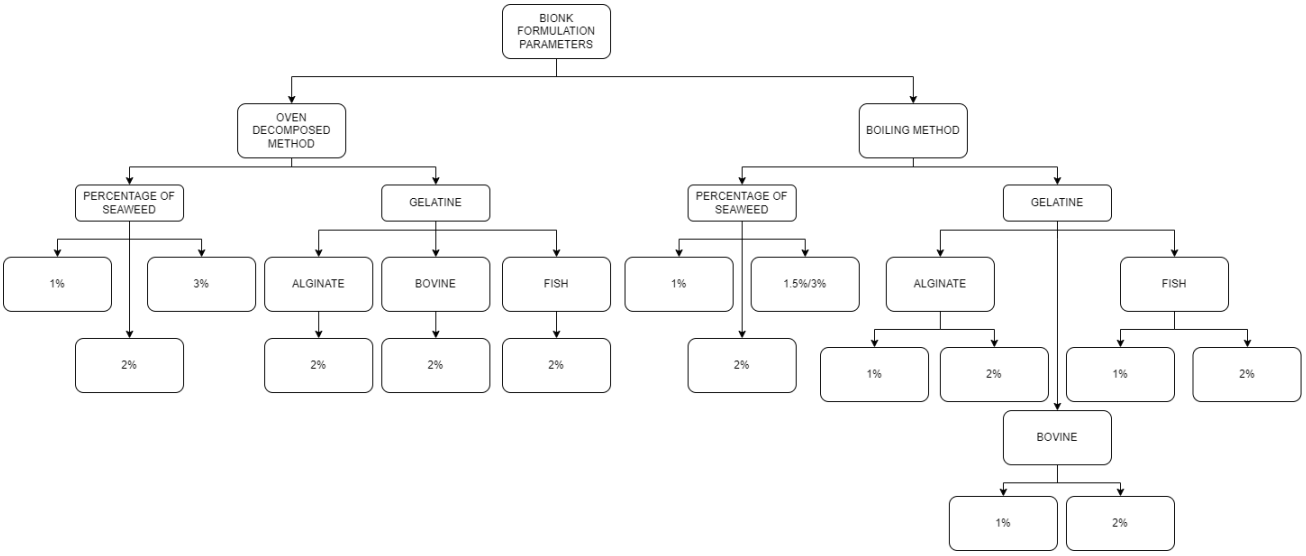

Figure 3.5 shows: Tree diagram for factor effecting Bioink formulation.

3.4.2 Optimization data evaluation

After identifying the most influential parameters, an experimental validation will be conducted to obtain the optimum bioink quality by running an experiment for 1 specimens of each factor Bioink hydrogel that have been illustrated at Table 3.2, 3.3 and 3.4.

Table 3.1: List of parameters in optimization the formulation by using oven decompose method.

Oven decomposes method							
No	**Percentage of seaweed powder (%)**	**Percentage of gelatine powder (%)**	**Temperature (°C)**	**DATA ANALYSIS**	**Sodium alginate**	**Fish gelatine**	**Bovine gelatine**
1	**1**	**2**	**25**		**Viscosity Data**	**Viscosity Data**	**Viscosity Data**
2	**2**	**2**	**25**				
3	**3**	**2**	**25**				

Table 3.2 List of parameters in optimization the formulation by using boiling method with 1% of gelatine.

Boiling method						
Percentage of seaweed powder (%)	**Percentage of gelatine powder (%)**	**Temperature (°C)**	**DATA ANALYSIS**	**Sodium alginate**	**Fish gelatine**	**Bovine gelatine**
1	**1**	**100**		**Viscosity Data**	**Viscosity Data**	**Viscosity Data**
2	**1**	**100**				
3	**1**	**100**				

Table 3.3 List of parameters in optimization the formulation by using boiling method with 2% of gelatine

Boiling method						
Percentage of seaweed powder (%)	**Percentage of gelatine powder (%)**	**Temperature (°C)**	**DATA ANALYSIS**	**Sodium alginate**	**Fish gelatine**	**Bovine gelatine**
1	**2**	**100**		**Viscosity Data**	**Viscosity Data**	**Viscosity Data**
1.5	**2**	**100**				
2	**2**	**100**				

3.5 Testing and Analysis

3.5.1 Viscosity Test

Viscosity testing is a method employed to assess the flow characteristics and consistency of fluids, particularly hydrogels, utilizing a viscometer—a specialized device designed for this purpose. The primary objective of conducting viscosity tests on hydrogels is to gain insights into their flow behaviour, assess their suitability for specific applications, and refine their formulation to achieve the desired viscoelastic properties. In the context of bioprinting applications, the determination of hydrogel viscosity is a critical parameter influencing the printability and overall performance of bioinks. A study conducted at a shear rate of 1.4/s elucidated the viscosity characteristics of a hydrogel formulation both in the absence and presence of gelatine. Specifically, in MilliQ water, the viscosity was measured at **23048 mPa**, while the incorporation of gelatine resulted in a discernible increase to **34932 mPa** (Gungor-Ozkerim et al., 2018a; Tian et al., 2021). The recommended printable bioink viscosity is in range **6 dPas-11 dPas** (Aktas et al., 2014).

This research provides valuable insights into the nuanced effects of gelatine inclusion on hydrogel viscosity, thereby contributing to the informed selection of an optimal hydrogel composition for bioink formulations in bioprinting endeavours. The observed viscosity variations underscore the significance of fine-tuning hydrogel formulations, a crucial aspect in achieving precision and efficacy in bioprinting applications for tissue engineering and regenerative medicine. By performing viscosity testing, researchers can optimize hydrogel formulations to attain the desired viscoelastic properties, ensuring proper flow during processing or application. This testing procedure also plays a pivotal role in quality control, maintaining batch-to-batch consistency, and assessing the impact of various additives or processing parameters on the rheological properties of hydrogels.

Figure 3.6 Viscometer that used in testing viscosity of Bioink.

3.5.2 Compression Test

Compression testing serves as a crucial method for assessing the mechanical attributes of hydrogels, aiding in the determination of their compressive strength, deformation characteristics, and resilience. By subjecting hydrogel samples to escalating compressive stresses, researchers can identify the point at which deformation or failure occurs, providing insights into the material's load-bearing capacity. The analysis of deformation enables the calculation of the compressive modulus, reflecting the material's stiffness under compression. Additionally, assessing the resilience of hydrogels helps gauge their capacity to absorb and release energy during deformation.

This testing approach facilitates the optimization of hydrogel formulations, assesses their suitability for specific applications, and validates theoretical models. A comprehensive understanding of hydrogel mechanical behaviour through compression testing is imperative to ensure structural integrity, performance, and diverse applications across various sectors.

The widely utilized ASTM D575 standard is commonly employed for compression testing of materials, including hydrogels. Although this standard, not specifically tailored for characterizing hydrogel compressive properties, often serves as a starting point, it is essential to adapt testing parameters or explore alternative standards better suited to the unique characteristics of hydrogels. The sample size that we use is (d=28.6 mm and the h=12.5±0.5 mm) (figure 3.8). The testing, utilizing the equipment employed for puncture tests, will be conducted in the FTKIP laboratory, adhering to laboratory guidelines.

Figure 3.7 Shimadzu Tensile Testing Machine

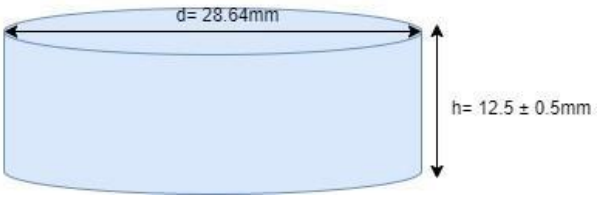

Figure 3.8 Specimen size follow ASTM D575

3.5.3 Micro-structural Investigation under SEM

As the project approaches its conclusion, it is imperative to perform specific analyses immediately upon the completion of the printed part. This step is crucial to observe the interaction of seaweed within the hydrogel. The internal structure of the seaweed-based hydrogel and the overall printing quality can be assessed by employing a scanning electron microscope (SEM), specifically the Hitachi SU5000 model. SEM is an electron microscope that utilizes a focused electron beam to scan the surface of a specimen, generating high-resolution images.

This technique allows for a detailed examination of the internal structure of the seaweed-based hydrogel, providing insights into how the seaweed components interact. SEM offers exceptional magnification and resolution, enabling a thorough analysis of various materials, including metals, ceramics, polymers, and biological samples. Its widespread application spans diverse fields, encompassing materials science, biology, and semiconductor manufacturing, where it is utilized to study material microstructures, identify defects, and analyse surface properties. Both analyses will be carried out in the FTKIP Laboratory.

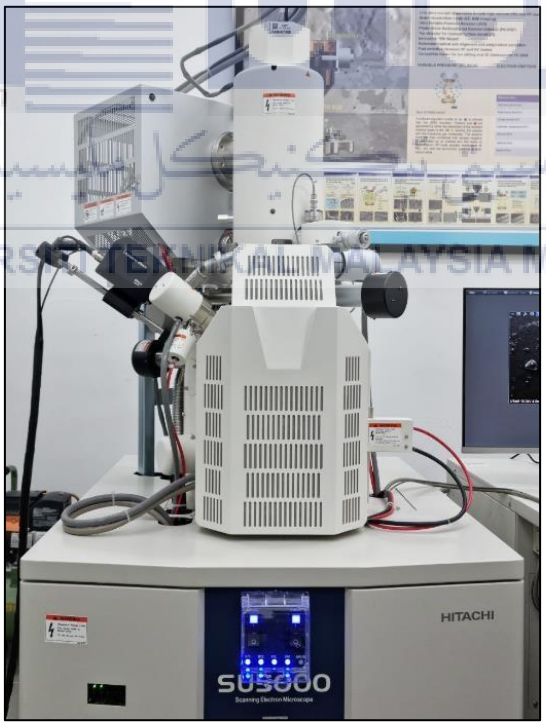

Figure 3.9 Hitachi SU5000 SEM Machine

3.6 Expected result.

The objective of incorporating the crosslinker module in conjunction within crosslinking methods and utilizing extrusion-based bioprinting on the Snapmaker 3D printer is to fabricate a 5-layer scaffold. When focusing on hydrogel printing, the anticipated outcomes encompass guaranteeing the stability of the structure, attaining a polished surface texture (figure 3.11), and fostering desirable porosity (figure 3.10). The critical optimization of bioink formulation parameters is vital to pinpoint the factors influencing the printed scaffold.

Above all, ensuring the structural integrity of the printed hydrogel is of utmost importance, indicating its capacity to maintain the intended shape without significant deformations. This is crucial for precision and functionality, especially in applications where the hydrogel serves as a scaffold for tissues or implants subject to compression testing. Additionally, achieving a seamless surface finish is essential to minimize flaws on the external structure of the hydrogel. This holds particular significance in fields such as tissue engineering and medical implants, where the visual quality of the final product is a key consideration. Lastly, the presence of favourable porosity within the cross-sectional structure is a desired outcome. This porosity is particularly advantageous for tissues or scaffolds, playing a pivotal role in supporting essential processes like cell survival and growth, which will be scrutinized through microstructural analysis under SEM of the cross-sectionally printed scaffold.

Figure 3.11 Expected hydrogel Scaffold

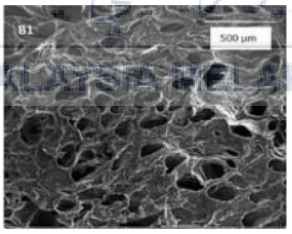

Figure 3.10 Expected cross sectional of hydrogel structural under SEM.

CHAPTER 4

4 RESULT AND DISCUSSION

4.1 Viscosity test.

Figure 4.1 Perform the viscosity test at Lab

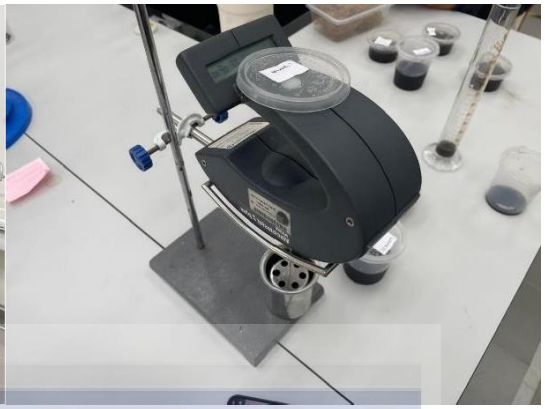

Figure 4.2 Viscometer device

A viscosity experiment was conducted using a viscometer to determine the optimal concentration of seaweed in a bio-ink mixture (figure 4.1). The objective was to measure the viscosity of the bio-ink at different seaweed concentrations (0%, 1%, 2%, and 3%). These viscosity measurements provided valuable insights into how the bio-ink flows, aiding in the selection of the most suitable formulation. A rotational viscometer equipped with multiple spindles was employed for the viscosity tests (figure 4.2). Bio-ink samples were prepared by adding varying amounts of seaweed to a base ink composed of 2% Sodium Alginate. Care was taken to ensure accurate measurement and thorough mixing of all materials to maintain consistency in the samples tested.

4.1.1 Viscosity Measurements

After each bio-ink sample was loaded into the viscometer chamber, the spindle was submerged to begin the viscosity measurement. Once the sample reached a stable state, the rotational speed was set, and the viscosity was recorded. The viscosity measurements were expressed in units of pascal-seconds (Pas) or decipascal-seconds (dPas), which are units used to quantify dynamic viscosity.

4.1.2 Results

For the various seaweed concentrations, the viscosity test produced the following results:

Table 4.1 Viscosity result for oven decompose method.

OVEN DECOMPOSE METHOD (250°C)							
No	**Percentage of seaweed powder (%)**	**Percentage of gelatine powder (%)**	**Temperature (°C)**	**DATA ANALYSIS**	**Sodium alginate**	**Fish gelatine**	**Bovine gelatine**
1	**1**	**2**	**25**		3.78 dPas	<0.3 dPas	<0.3 dPas
2	**2**	**2**	**25**		5.53 dPas	<0.3 dPas	<0.3 dPas
3	**3**	**2**	**25**		6.75 dPas	<0.3 dPas	<0.3 dPas

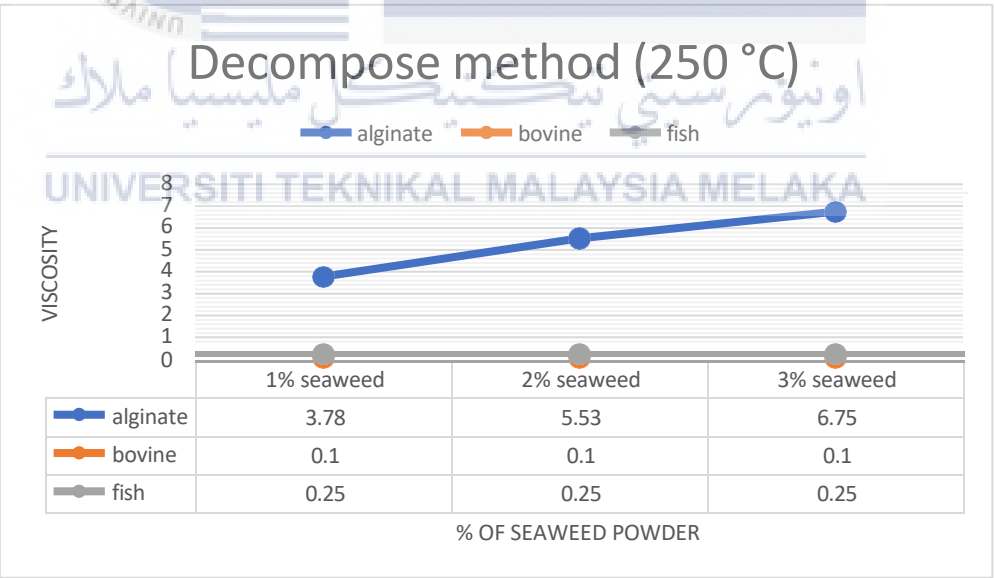

Figure 4.3 Graph for viscosity of oven decomposed method

After the experiment, hydrogels containing bovine gelatine solidified in cold temperatures and liquefied at room temperature, whereas those with fish gelatine remained liquid consistently with minimal viscosity change. Hydrogels formulated with sodium alginate showed increased viscosity with higher seaweed concentrations.

Table 4.2 Viscosity result for boiling method 2% of gelatine

BOILING METHOD							
No	**Percentage of seaweed powder (%)**	**Percentage of gelatine powder (%)**	**Boiling Temperature (°C)**	**DATA ANALYSIS**	**Sodium alginate**	**Fish gelatine**	**Bovine gelatine**
1	**1**	**2**	**100**		7.21 dPas	0.44 dPas	0.54 dPas
2	**1.5**	**2**	**100**		14.05 dPas	1.52 dPas	1.62 dPas
3	**2**	**2**	**100**		17.0 dPas	2.74 dPas	2.94 dPas

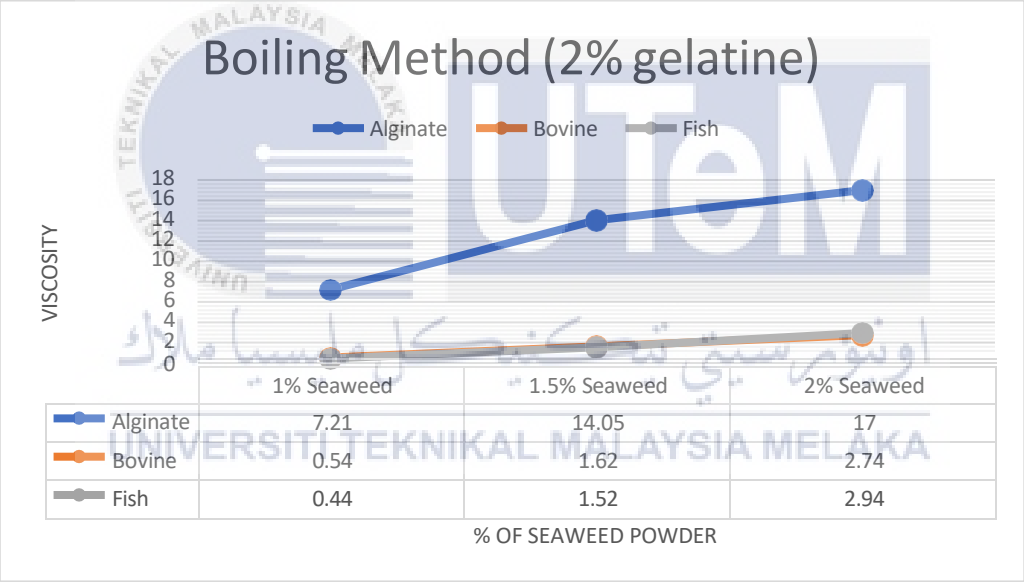

Figure 4.4 Graph for viscosity of Boiling method using 2% of gelatine

After the experiment, hydrogels made with bovine and fish gelatin formed a jelly-like consistency at room temperature. In contrast, hydrogels containing sodium alginate maintained their viscosity for several hours at room temperature. The 3% seaweed was elimated because its significantly making it difficult to stir.

Table 4.3 Viscosity result for boiling method 1% of gelatine

BOILING METHOD							
No	**Percentage of seaweed powder (%)**	**Percentage of gelatine powder (%)**	**Boiling Temperature (°C)**	**DATA ANALYSIS**	**Sodium alginate**	**Fish gelatine**	**Bovine gelatine**
1	**1**	**1**	**100**		6.72 dPas	0.84 dPas	0.74 dPas
2	**2**	**1**	**100**		10.04 dPas	1.65 dPas	1.74 dPas
3	**3**	**1**	**100**		23.00 dPas	2.54 dPas	2.64 dPas

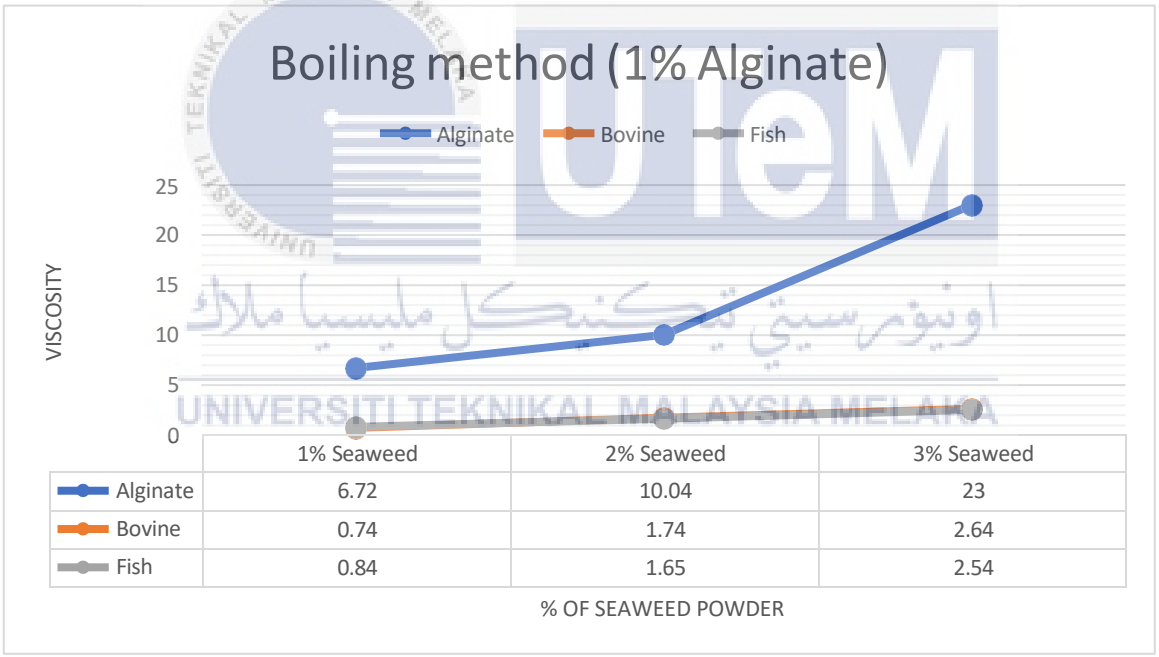

Figure 4.5 Graph for viscosity of Boiling method using 1% of gelatine

By the experiment, increasing the percentage of seaweed resulted in higher viscosity of the bioink. However, the viscosity remained relatively stable for bioinks containing bovine and fish gelatin, rendering these materials unsuitable for this experiment.

4.1.3 Reaction to crosslinker (CaCl)

At the conclusion of the experiment, formulations incorporating sodium alginate as a gelling agent exhibited a favorable reaction with $CaCl_2$. In contrast, formulations utilizing bovine and fish gelatin did not demonstrate any reaction with $CaCl_2$. This outcome highlights that bovine and fish gelatin are not a suitable choice for these studies due to their lack of interaction with calcium chloride, which is crucial for cross-linking and structural integrity in scaffold build up. Therefore, sodium alginate proves to be a more effective gelling agent in this context.

4.1.4 Selection of bioink formulation.

Based on the viscosity test results, three formulations were selected as they fell within the optimal viscosity range of 6-11 dPas, as suggested by (Aktas et al., 2014). These formulations include (1) 1% seaweed and 1% alginate with a viscosity of 6.72 dPas, (2) 2% seaweed and 1% alginate with a viscosity of 10.04 dPas, and (3) 1% seaweed and 2% alginate with a viscosity of 7.21 dPas. Additionally, a fourth formulation consisting of 2% alginate without seaweed was included for comparison.

These selected formulations will proceed to further testing, including compression tests and microstructural imaging, to evaluate their mechanical properties and structural characteristics. The viscosity range criterion ensures that the bioinks are suitable for 3D printing applications, maintaining adequate flow properties during deposition.

4.2 Compression test

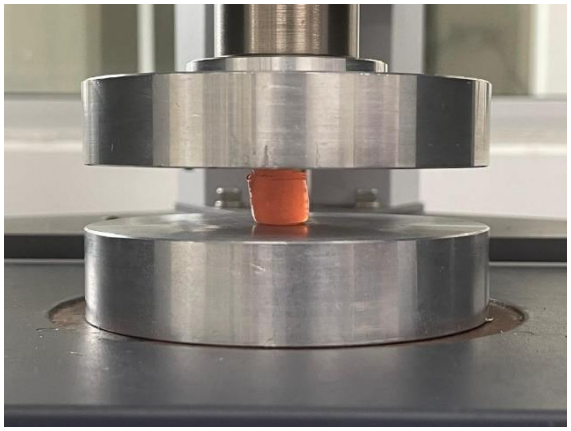

Figure 4.6 Before compression

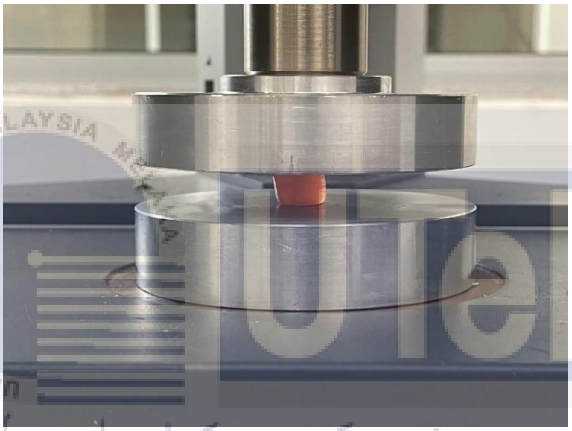

Figure 4.7 After compression

The mechanical properties and compressive strength of four different bio-ink samples were evaluated using a compression test (figure 4.6 and figure 4.7). These samples had the following formulations: - (1) 2% sodium alginate, (2) 1% seaweed and 1% sodium alginate, (3) 1% seaweed and 2% sodium alginate, and (4) 2% seaweed and 1% sodium alginate. The compression tests were conducted according to ASTM D575 standards, using cylindrical samples with dimensions of diameter (d) 28.64mm and height (h) 12.5 ± 0.5mm. Each bio-ink sample was placed into the compression test apparatus and compressed at a constant rate of 60 mm/min until the specified compression level based on predetermined percentages was achieved.

The maximum stress experienced by each bio-ink sample during compression was determined by calculating the applied force divided by the cross-sectional area of the sample. The stress values at each compression level were then measured and expressed in megapascals (MPa).

1. **2% sodium alginate**

The experiment results displayed a graph depicting the maximum stress at a strain percentage of 450%. Notably, break point was observed during the experiment. The objective was to evaluate the compressive properties of the hydrogel formulation, particularly its ability to withstand shear stress until failure. The findings indicate that the hydrogel formulation exhibited substantial shear stress at a strain of 450%, suggesting strong mechanical stability under compression. Specifically, the bio-ink sample compressed of its original height showed a maximum stress value of 1.52 MPa and stress break point at 0.45 MPa.

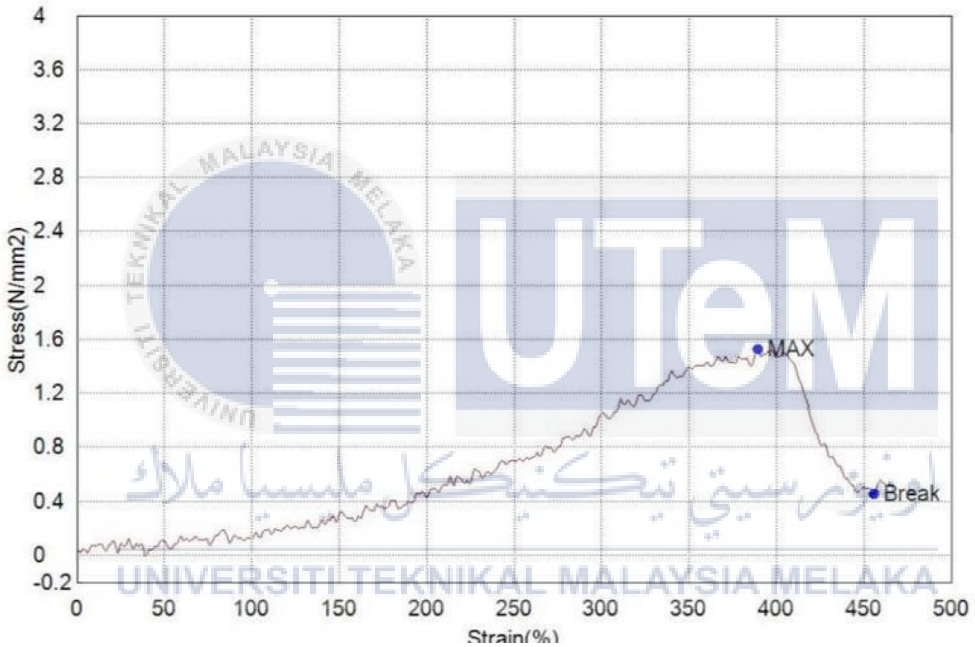

Figure 4.8 Graph stress/ strain for sample 1

2. **1% seaweed and 1% sodium alginate**

The experiment results were represented in a graph showing the maximum stress at a strain of 480%. It's important to note that failure occurred during the experiment. The goal was to assess the compressive properties of the hydrogel formulation, focusing on its ability to withstand shear stress until failure. The results indicate that the hydrogel formulation demonstrated significant shear stress at a strain of 480%, suggesting robust mechanical stability under compression. Specifically, the bio-ink sample compressed from its original height showed a maximum stress value of 1.60 MPa, with a stress break point occurring at 1.23 MPa.

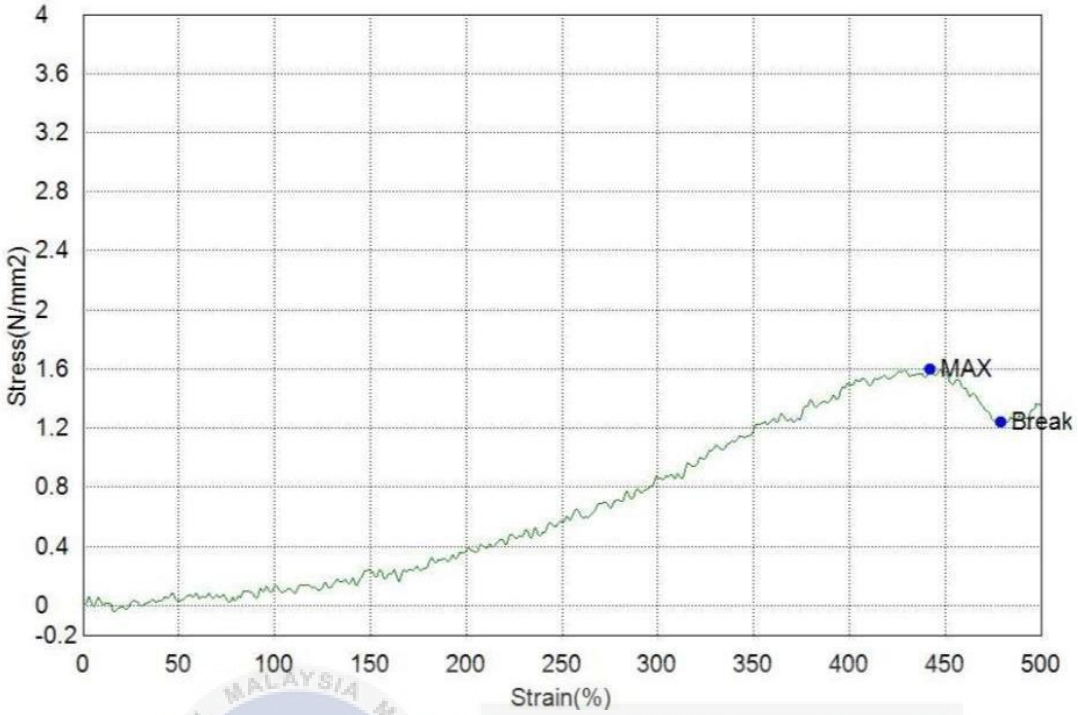

Figure 4.9 Graph stress/ strain for sample 2

3. 1% seaweed and 2% sodium alginate

The experimental results were illustrated on a graph showing the maximum stress at a strain of 320%. It's noteworthy that a failure point was observed during the experiment. The goal was to assess the compressive properties of the hydrogel formulation, specifically its capacity to endure shear stress until failure. The results indicate that the hydrogel formulation demonstrated considerable shear stress at a strain of 320%, indicating robust mechanical stability under compression. In particular, the bio-ink sample compressed from its original height exhibited a maximum stress value of 2.26 MPa, with a stress break point occurring at 1.84 MPa.

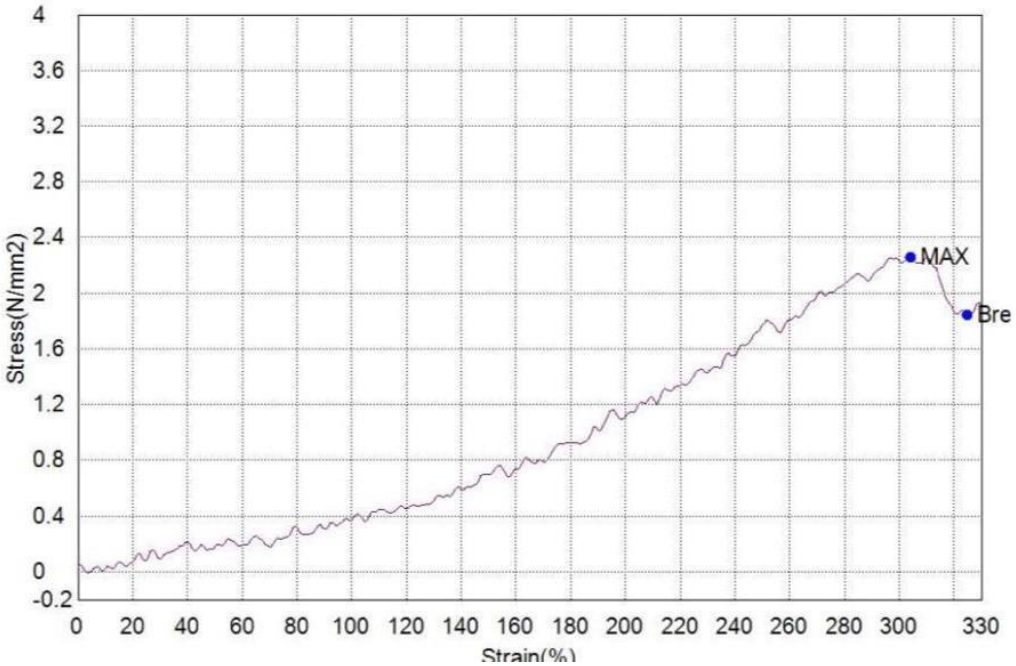

Figure 4.10 Graph stress/ strain for sample 3

4. 2% seaweed and 1% sodium alginate

The experimental findings were presented graphically, showing the maximum stress at a strain of 450%. Importantly, a point of failure was observed during the experiment. The aim was to assess the compressive properties of the hydrogel formulation, specifically its capability to endure shear stress until failure. The results suggest that the hydrogel formulation displayed significant shear stress at a strain of 450%, indicating robust mechanical stability under compression. More precisely, the bio-ink sample compressed from its original height exhibited a maximum stress value of 2.42 MPa, with a stress break point occurring at 1.65 MPa.

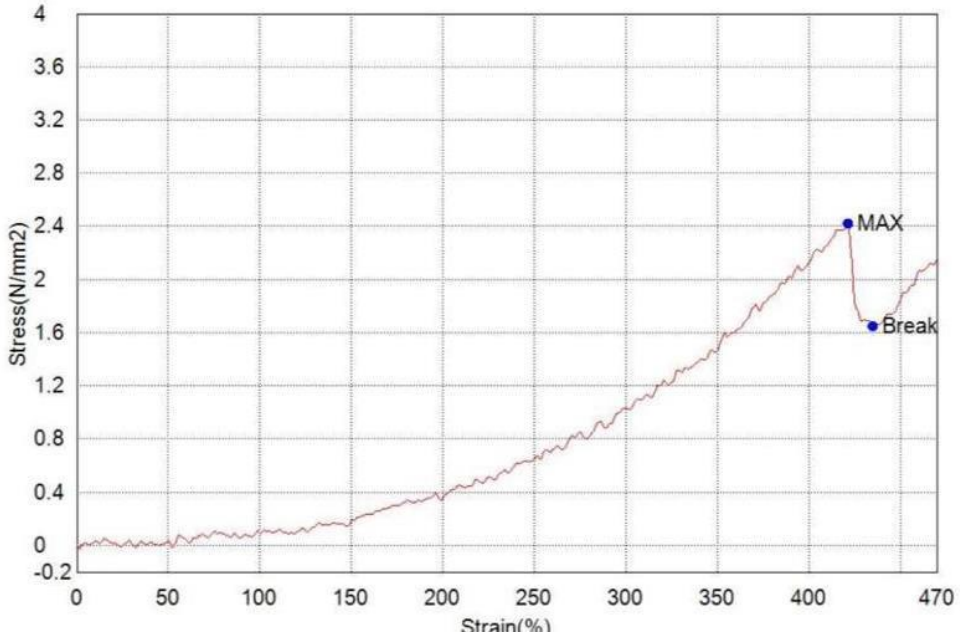

Figure 4.11 Graph stress/ strain for sample 4

Maximum and break stress point

Table 4.4 Maximum and break stress point

	Maximum stress (MPa)	Break point (MPa)
2% alginate	1.52	0.45
1% seaweed and 1% alginate	1.60	1.23
1% seaweed and 2% alginate	2.26	1.84
2% seaweed and 1% alginate	2.42	1.65

4.2.1 Mechanical Assessment

Advantages of high compression stress include enhanced structural integrity, where the hydrogel can endure greater mechanical loads without distortion, and improved scaffold stability, making it more resilient to mechanical wear and tear. Additionally, stronger hydrogels offer better durability, providing a stable scaffold for cell attachment. However, there are also drawbacks. Stiffer and denser hydrogels can hinder the diffusion of nutrients, oxygen, and waste products, impairing nutrient and waste exchange. High compression stress can also lead to printing challenges due to increased viscosity and stiffness, potentially resulting in poor print fidelity and resolution, complicating the fabrication process(Tozzi et al., 2016). Furthermore,

while greater mechanical strength is achieved, there may be a reduction in porosity, which can diminish the size and quantity of pores within the hydrogel matrix. (Cui et al., 2022)

The compression test findings show that the bio-ink samples with formulation of seaweed and sodium alginate have a significant compressive strength. The samples showed increasing stress values as seaweed percentage increased, indicating increased resistance to applied compressive forces. The bio-ink sample with formulation 1% alginate and 1% seaweed is the best formulation because it's had balance composition that make it strong and have benefit on porosity. It's because provides greater mechanical strength but can reduce the size and number of pores within the hydrogel matrix (Cui et al., 2022).

4.3 Printable result

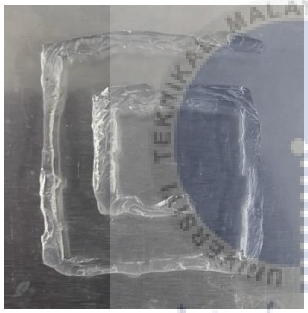

Figure 4.12 Print result for 2% alginate

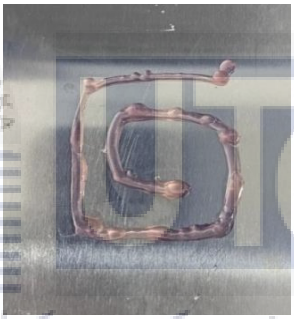

Figure 4.13 Print result for 1% seaweed and 2% alginate

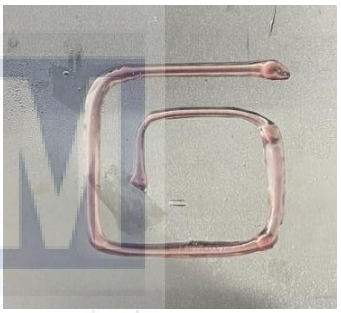

Figure 4.14 Print result for 1% seaweed and 1% alginate

Print result for 2% alginate

The inconsistency and lack of smoothness in the 3D printed results using a basic 2% alginate hydrogel formulation can be attributed to its low viscosity, which impairs the material's structural integrity during the printing process (figure 4.12). Alginate hydrogels rely on ionic crosslinking with calcium chloride (CaCl) to form stable structures. However, at low concentrations, the alginate solution may not achieve sufficient viscosity, leading to uneven extrusion and poor layer adhesion. This results in prints that are irregular and lack precision. Additionally, the rapid gelation upon contact with (CaCl) can cause clogging or inconsistent flow through the printer nozzle, further exacerbating the print quality issues. Increasing the concentration of alginate or optimizing the crosslinking conditions could enhance the viscosity and improve the overall print consistency and smoothness.

Print result for 1% seaweed and 2% alginate

The more consistent yet brittle and non-smooth print results observed with a higher viscosity hydrogel formulation are likely due to the increased viscosity of the alginate solution (figure 4.13). While higher viscosity can improve print consistency by providing better structural integrity and reducing deformation during printing, it can also lead to difficulties in extrusion. The increased resistance to flow can cause the hydrogel to clog the syringe or nozzle, leading to uneven extrusion and surface roughness. Furthermore, the higher viscosity may hinder the smooth layering of the material, resulting in a rougher texture and increased brittleness in the final print. To address these issues, it is crucial to balance the alginate concentration and crosslinking conditions to achieve optimal viscosity that ensures both smooth flow through the syringe and adequate structural stability without compromising the smoothness and flexibility of the printed object.

Print result for 1% seaweed and 1% alginate

Achieving the best print results with a seaweed-based hydrogel can be attributed to its optimal viscosity, which ensures consistent and smooth extrusion during the 3D printing process (figure 4.14). The well-balanced viscosity of this hydrogel formulation allows it to flow evenly through the printer's nozzle, minimizing clogs and disruptions that typically cause inconsistencies and surface roughness. The smooth extrusion facilitated by this ideal viscosity ensures that each layer is deposited uniformly, resulting in a high-quality print with fine detail and a smooth finish. The seaweed-based hydrogel's effective rheological properties strike a balance between fluidity and structural integrity, enabling it to maintain shape during printing while also adhering properly between layers. This combination of attributes—consistent flow, smooth deposition, and adequate mechanical strength—demonstrates the importance of fine-tuning hydrogel formulations to achieve optimal printing performance.

4.4 Micro-structural investigation under SEM

A Scanning Electron Microscope (SEM) was used to thoroughly analyze the microstructures of bio-ink samples, focusing on cross-sections and sheet portions of printed hydrogel scaffolds. The analysis was performed at various magnifications—500x, 1000x, and 3000x—to obtain detailed information about the sample's morphology and composition. At 500x magnification, SEM images displayed the overall structural integrity and distribution of the hydrogel matrix, highlighting macro-level uniformity and any significant defects. At 1000x magnification, the SEM allowed for a closer examination of finer details such as pore size, wall thickness, and surface smoothness. At the highest magnification of 3000x, the SEM provided an in-depth view of micro-scale features, including polymer chain alignment, crosslink density, and any microscopic imperfections or heterogeneities within the hydrogel. This multi-scale approach offered a comprehensive understanding of the structural characteristics and compositional details of the bio-ink, which is essential for optimizing its performance in bioprinting applications.

4.4.1 Sample preparation

The SEM examination involved preparing hydrogel samples with three formulations: (1% seaweed and 1% alginate), (1% seaweed and 2% alginate), and (2% seaweed and 1% alginate). The sample has been dried for 1 day (figure 4.15). These samples were analyzed to study how varying seaweed and alginate concentrations affect their structure and surface. SEM images at different magnifications revealed detailed surface topographies and porosity, providing insights into the hydrogels' mechanical stability and overall structure for bioprinting and biomedical applications

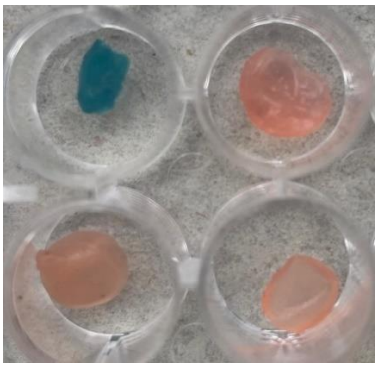

Figure 4.15 Hydrogel sample for SEM examination

4.4.2 Structural analysis

Under SEM analysis, surface characteristics of hydrogel samples revealed distinct pore structures observed at 1000x (figure 4.17) and 3000x magnifications (figure 4.18). At 1000x, interconnected pores were evident throughout the scaffold, suggesting uniform distribution. At 3000x, finer details of pore morphology, including size and shape variations, were observed along with the structure's integrity. These findings are critical for understanding the hydrogel's porosity, mechanical properties, and its potential applications in bioprinting and biomedical fields.

1. 1% seaweed and 1% alginate.

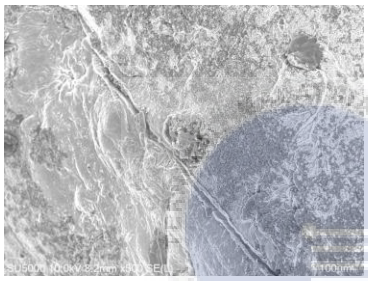

Figure 4.16 500x magnification

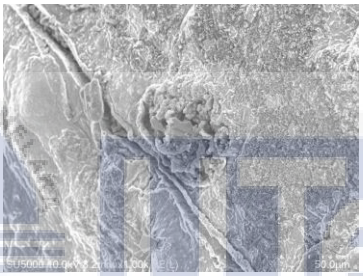

Figure 4.17 1000x magnification

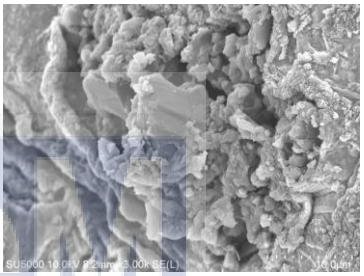

Figure 4.18 3000x magnification

The microstructural analysis of the hydrogel surface area revealed that the combination of 1% seaweed and 1% alginate produced a significantly more porous structure compared to other formulations. SEM images at different magnifications clearly showed a denser network of interconnected pores within the hydrogel matrix. This higher porosity indicates improved permeability, suggesting potential benefits for nutrient exchange and supporting cell growth in bioprinting. The detailed examination of these microstructures offers valuable insights into how varying concentrations of seaweed and alginate impact the physical and functional properties of hydrogels, essential for optimizing their performance in biomedical and tissue engineering applications.

2. 1% seaweed and 2% alginate.

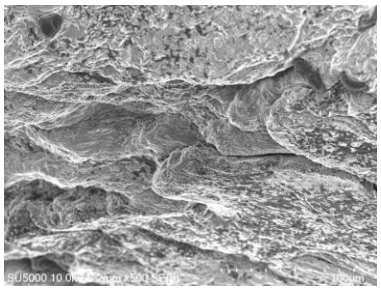

Figure 4.19 500x magnification

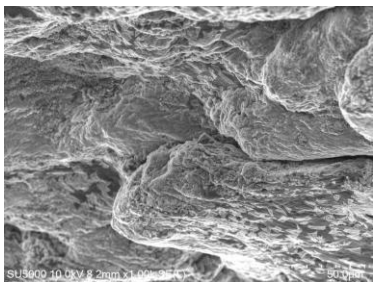

Figure 4.20 1000x magnification

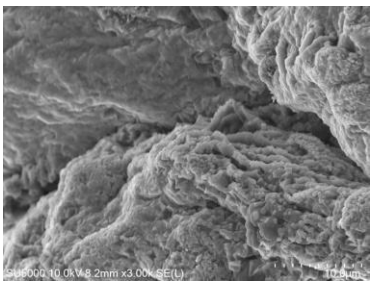

Figure 4.21 3000x magnification

The microstructure analysis of this formulation's surface area reveals a higher presence of cohesive particles and a less porous structure. This is primarily due to its elevated mechanical properties, which lead to smaller and more tightly packed pores within the hydrogel matrix. SEM images at different magnifications clearly illustrate a dense distribution of particles, indicating reduced pore connectivity and fewer empty spaces compared to formulations with lower mechanical strength. These characteristics suggest improved structural stability and durability, which are advantageous for applications requiring strong mechanical support in bioprinting and biomedical engineering. Understanding these microstructural features provides valuable insights into how varying mechanical properties affect the performance and utility of hydrogels in biomedical contexts.

3. 2% seaweed and 1% alginate.

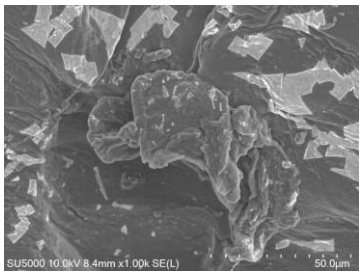

Figure 4.22 500x magnification

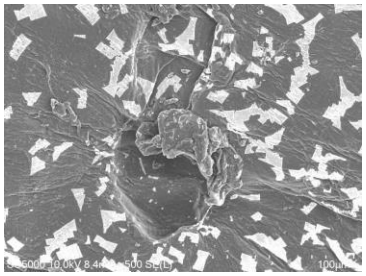

Figure 4.23 500x magnification

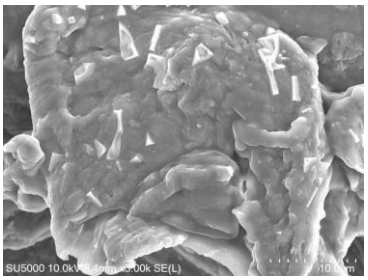

Figure 4.24 500x magnification

The microstructure of this formulation exhibits a dense and uniform composition, primarily due to its exceptional mechanical strength, which effectively minimizes structural porosity. SEM imaging at different magnifications clearly reveals a compact and homogeneous

arrangement within the hydrogel matrix, characterized by reduced void spaces and interconnected pores. This compactness enhances overall structural integrity and stability, making it suitable for applications requiring strong mechanical properties, such as load-bearing scaffolds in tissue engineering. The formulation's decreased porosity also suggests improved resistance to deformation and mechanical stress, highlighting its potential in biomedical applications where durability and reliability are critical factors. Understanding these microstructural characteristics is essential for optimizing hydrogel formulations tailored to specific biomedical and biotechnological needs.

4.4.3 Pore observations

Notably, when the mechanical strength of a material increases, it generally causes a decrease in the quantity and size of pores present within its structure. This change occurs because stronger mechanical properties require a more densely packed matrix. SEM analysis demonstrates that heightened strength results in a more compact and uniform organization of the hydrogel's components, leading to fewer empty spaces and smaller pores. This denser structure enhances the material's overall mechanical capabilities, including stiffness and durability, making it well-suited for applications where maintaining structural integrity is crucial, such as in biomedical implants and tissue engineering scaffolds.

4.4.4 Seaweed dominance

In the SEM analysis, the higher concentration or unique characteristics of seaweed particles may have overshadowed the visibility of gelatin strands in the micrographs. Seaweed particles, being more prominent or distinct in appearance, likely stood out prominently in the images, while gelatin strands, which are finer and less contrasting, may have been less visible. Additionally, there is a possibility that seaweed particles adhered or accumulated onto the gelatin strands, further reducing their visibility throughout the observed area. This highlights the importance of meticulous sample preparation and precise imaging techniques in SEM studies to accurately discern and examine different components within composite materials such as hydrogels, ensuring a comprehensive understanding of their structural composition and interactions.

CHAPTER 5

5 CONCLUSION

In conclusion, the validation of viscosity testing, compression testing, and SEM analysis have provided valuable insights into the properties and performance of the seaweed-based hydrogel used in 3D printing as bio-ink.

The Hydrogel preparation can be categorized into two methods: oven decomposition and boiling. These methods differ primarily in how they handle seaweed powder. In the oven decomposition method, seaweed powder is dried at 250°C. Conversely, the boiling method involves drying seaweed powder at 100°C and then boiling the solution at the same temperature. These distinct approaches are crucial for tailoring hydrogel properties based on the specific requirements of seaweed utilization and processing temperatures.

The viscosity tests were conducted on hydrogel formulations comprising varying concentrations of seaweed (0%, 1%, 2%, and 3%) combined with 2% of three types of gelatine: sodium alginate, fish gelatine, and bovine gelatine. The results showed that for bovine and fish gelatine, increasing seaweed concentration did not significantly affect viscosity, which ranged from 0.25 to 2.94 dPas. This finding provides critical insights into the flow characteristics of the hydrogel. In contrast, sodium alginate exhibited notable viscosity changes with seaweed concentration. Using the oven decomposition method, viscosity values increased from 2.78 dPas for 0% seaweed to 6.75 dPas for 3% seaweed, indicating the impact of seaweed concentration on viscosity and its potential applications. Under the boiling method, viscosity was divided into two categories based on alginate concentration. For 1% alginate, viscosity ranged from 6.72 dPas for 1% seaweed to 23 dPas for 3% seaweed. For 2% alginate, viscosities ranged from 7.21 dPas to 17 dPas across different seaweed concentrations. These findings supported the selection of optimal formulations (1% seaweed and 1% alginate), (2% seaweed and 1% alginate), and (1% seaweed and 2% alginate) for subsequent compression and SEM tests. These formulations were chosen because their printable hydrogel viscosity falls within the 6-11 dPas range, ensuring structural integrity during printing without requiring a support bath (Aktas et al., 2014). After conducting the viscosity test, I found that the boiling method yields the best results. This is because seaweed powder that has been oven-dried does not dissolve well in solution, reducing its effectiveness for hydrogel formation.

This formulation endows the hydrogel with outstanding self-supporting characteristics, ensuring it retains its shape throughout the printing process without collapsing or distorting. The presence of seaweed enhances the hydrogel's structural integrity by establishing a dense internal network. This advancement enhances efficiency and cost-effectiveness in 3D manufacturing workflows. By eliminating the need for support solutions, it paves the way for advancements in tissue engineering, regenerative medicine, and biomedical research, while also enabling the fabrication of intricate structures with new possibilities for innovation.

Compression tests were conducted according to ASTM D575 standards on cylindrical samples with a diameter (d) of 28.64 mm and height (h) of 12.5 ± 0.5 mm. The tests were performed at a compression rate of 60 mm/min to evaluate the mechanical properties of the hydrogels. Results showed maximum stress values of 1.52 MPa for the basic formulation with 2% alginate, 1.60 MPa for 1% seaweed and 1% alginate, 2.36 MPa for 1% seaweed and 2% alginate, and 2.42 MPa for 2% seaweed and 1% alginate. These findings indicate that increasing seaweed concentration correlates with higher maximum compression stress. Additionally, the break points were determined for each sample: 0.45 MPa for 2% alginate, 1.23 MPa for 1% seaweed and 1% alginate, 1.84 MPa for 1% seaweed and 2% alginate, and 1.65 MPa for 2% seaweed and 1% alginate. These results provide insights into the mechanical behaviour of the hydrogels under compression, highlighting the impact of seaweed content on their strength and resilience.

The incorporation of seaweed into the bio-ink formulation has significantly enhanced the strength and mechanical properties of the hydrogel compared to formulations without seaweed. Seaweed's inclusion as a pivotal component plays a crucial role in bolstering the overall robustness and structural integrity of the hydrogel. This improvement has been substantiated through comprehensive testing and analysis, which have consistently shown that hydrogels containing seaweed exhibit superior strength and resilience under various conditions. These findings underscore the importance of seaweed in enhancing the performance and durability of bio-ink formulations, thereby expanding their potential applications in biomedical research, tissue engineering, and beyond.

SEM analysis of the hydrogel's microstructure visually confirmed its quality and characteristics. Examination of the surface area clearly showed the presence of pores and the distribution of materials, confirming the hydrogel's porous structure and the incorporation of seaweed within its matrix. Moreover, an important finding from the analysis was that

increasing the hydrogel's mechanical strength led to a decrease in both the number and size of pores. This observation suggests that higher mechanical strength results in a more compact arrangement of the hydrogel's components, reducing overall porosity. These insights are crucial for understanding how changes in mechanical properties affect the structural integrity and performance of hydrogels in biomedical applications like bioprinting and tissue engineering. So, from all results obtained, I concluded that the best formulation for hydrogel is 1% seaweed and 1% alginate. It's because it can provide optimal viscosity, stable mechanical strength and good porosity of the structural for cell growth.

The data obtained from these validation and testing methods have greatly expanded our understanding of the properties and performance of seaweed-based hydrogels. These results form a solid foundation for further optimization of hydrogel formulations, ensuring quality control and customization for specific applications. The insights gained from these studies will aid in enhancing hydrogels for use in 3D printing, tissue engineering, and biomedical research. This knowledge not only helps in improving structural integrity and mechanical properties but also supports the development of innovative solutions tailored to various biomedical applications. Ultimately, these advancements are expected to drive progress in bioprinting technologies, regenerative medicine, and other fields that depend on advanced biomaterials.

5.1 Limitations and recommendation

One of the major challenges faced during this project was the limited power capacity of the NEMA stepper motor used in the 3D printer's extrusion system. This limitation posed significant difficulties when printing materials with much higher viscosity, such as hydrogel formulations. The NEMA stepper motor's restricted torque and power output made the precise and consistent extrusion of highly viscous materials a formidable task.

To address this issue and unlock the potential for superior hydrogel formulations, a critical upgrade is proposed. This upgrade aims to enhance the mechanical properties and internal structure of the hydrogels. By boosting the power output and torque capabilities of the stepper motor, the upgraded system would enable more efficient extrusion of highly viscous materials, resulting in hydrogel constructs with improved mechanical strength and stability.

Additionally, the enhanced system would support the creation of hydrogel structures with a more refined and interconnected porous network, which is vital for cell growth and tissue engineering applications. This network would promote cellular infiltration, nutrient exchange, and waste removal, thereby enhancing cell viability and proliferation within the hydrogel constructs.

Overall, the proposed upgrade has the potential to revolutionize 3D printing of hydrogel formulations. It allows the production of hydrogels with superior mechanical properties and optimized internal structures by overcoming the limitations of the NEMA stepper motor. These advancements would not only impact biomedical engineering but also pave the way for progress in tissue engineering, regenerative medicine, and other fields where hydrogels are crucial.

5.2 Commercialization Potential

The successful implementation and commercialization of this initiative could have significant impacts across various industries and applications. By overcoming the limitations of the NEMA stepper motor and enabling the printing of highly viscous hydrogel formulations with enhanced properties, numerous commercial opportunities can be unlocked.

Firstly, the improved 3D printing system can revolutionize the production of tissue engineering scaffolds in biomedical engineering. These enhanced hydrogel formulations can create a biomimetic environment conducive to cell growth and tissue regeneration. The improved mechanical properties and refined porous network would enable the fabrication of scaffolds with superior structural integrity and cell infiltration capabilities, facilitating the commercial production of patient-specific implants, such as bone scaffolds and cartilage constructs.

Furthermore, this enhanced system could have substantial implications for the pharmaceutical industry, where hydrogels are increasingly used as drug delivery carriers. The ability to print hydrogels with superior mechanical properties and optimized porous structures could lead to the development of advanced drug delivery systems. These systems would provide precise and controlled therapeutic release, enhancing treatment efficacy, reducing

adverse effects and supporting personalized medicine approaches.

Additionally, the upgraded 3D printing system could be utilized to produce bioactive devices, biosensors, and wearable technologies. Hydrogel-based sensors and devices can be integrated with electronic or biological components to create novel, multifunctional products. The improved printing capabilities would allow for the fabrication of complex structures with precise material property control, resulting in high-performance, customizable bioactive devices.

Overall, this initiative has significant commercialization potential in industries such as biomedical engineering, pharmaceuticals, and bioactive devices. By overcoming the NEMA stepper motor limitations and enabling the production of superior hydrogel formulations, the upgraded 3D printing system could lead to innovative products and solutions addressing critical challenges in healthcare, drug delivery, and bioengineering.

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