ANKARA UNIVERSITY

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BASIC BIOTECHNOLOGY

MASTER OF SCIENCE THESIS

BIOSYNTHESIS OF MULTI-MATERIAL SCAFFOLD AND ITS APPLICATION IN CONTROLLED BIOACTIVE MOLECULES DELIVERY

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September

2023

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ABSTRACT

Master of Science Thesis

Biosynthesis of multi-material scaffold and its application in controlled bioactive molecules delivery.

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Implantable polymeric hydrogels have been widely investigated for drug delivery systems. Collagen and Alginate are one of the most abundant natural polymers, and their biocompatibility and biodegradability make them a favourable material for implantable hydrogel fabrication. The addition of degradable polymeric microspheres can improve the hydrogel drug release behaviour. Poly caprolactone (PCL) have been studied for the use in drug delivery systems, and they can act as drug delivery vehicles to improve the delivery of different types of therapeutic agents. In this work, Vascular Endothelial Growth Factor (VEGF) and Rapamycin loaded PCL microparticles were fabricated by double emulsion technique, a Col/Alg hydrogels fabricated using 3D printing approach, and. Several tests including scanning electron microscopy, in vitro releasing, were utilized to characterize the microspheres. In addition, the physical properties of hydrogels, including mechanical properties, degradation properties, were investigated. The resulting VEGF-loaded microspheres had average sizes of 900 2000 nm and the in vitro release test confirms the sustained stable releasing profile of VEGF over a time of 15 days. On the other hand, the tensile properties and degradability of hydrogels, were readily tuned by increasing the concentration of Alginate without changing the concentration of collagen. These results imply that these implantable hydrogels are suitable candidates for applications in the fields of drug delivery and organ transplantation.

2023, 74 pages

Keywords: Vascular Endothelial Growth Factor VEGF; Rapamycin; organ transplantation; tissue engineering; 3D bioprinting.

ÖZET

Yüksek Lisans Tezi

Çoklu materyal içerikli doku iskelelerin üretimi ve kontrollü materyal saliminin optimizasyonu

Israa Abdulrahman

Ankara Üniversitesi, Biyoteknoloji Enstitüsü

Dr. Öğr. Üyesi Duru Aras Tosun

İmplant edilebilir polimerik hidrojeller, ilaç dağıtım sistemleri için yaygın olarak araştırılmaktadır. Kolajen ve Aljinat en bol bulunan doğal polimerlerden biridir ve biyouyumlulukları onları implant edilebilir hidrojel üretimi için uygun bir malzeme haline getirmektedir. Poli kaprolakton (PCL) ilaç dağıtım sistemlerinde kullanım için incelenmiştir ve farklı terapötik ajan türlerinin dağıtımını iyileştirmek için ilaç dağıtım araçları olarak hareket edebilirler. Bu çalışmada, VEGF ve Rapamisin yüklü PCL mikropartikülleri, 3D baskı yaklaşımı kullanılarak üretilen bir Col/Alg hidrojel olan çift emülsiyon tekniği ile üretilmiştir. Mikroküreleri karakterize etmek için taramalı elektron mikroskopisi, in vitro salınım gibi çeşitli testler kullanılmıştır. Ayrıca, mekanik özellikler, bozunma özellikleri de dahil olmak üzere hidrojellerin fiziksel özellikleri araştırılmıştır. Elde edilen VEGF yüklü mikrokürelerin in vitro salım testi VEGF'nin 15 günlük bir süre boyunca sürekli ve kararlı salım profilini doğrulamaktadır. Öte yandan, hidrojellerin gerilme özellikleri ve bozunabilirliği, kolajen konsantrasyonunu değiştirmeden Aljinat konsantrasyonunu artırarak kolayca ayarlanabilmiştir. Bu sonuçlar, bu implant edilebilir hidrojellerin biyoteknoloji alanlarındaki uygulamalar için uygun adaylar olduğunu göstermektedir.

2023, 74 sayfa

Anahtar Kelimeler: Vasküler Endotel Büyüme Faktörü VEGF; Rapamisin, biyomalzeme; doku mühendisliği; 3D biyobaskı.

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Israa Abdulrahman

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LIST OF ABBREVIATIONS

3D	3 Dimension
bFGF	Basic fibroblast growth factor
$CaCl_2$	Calcium chloride
ELISA	Enzyme-Linked Immunosorbent Assay
FGF	Fibroblast growth factor
HPLC	High-Performance Liquid Chromatography
M	Molar
mg	Milligram
ml	Millilitre
mm	Millimetres
mTOR	Mechanistic Target of Rapamycin
PCL	Polycaprolactone
PVA	Polyvinyl alcohol
SEM	Scanning Electron Microscope
UV	Ultraviolet
VEGF	Vascular Endothelial Growth Factor
µL	Microlite

1. INTRODUCTION

A biomaterial is a structured, natural, or synthetic substance made up of certain components that interact with biological systems to improve or change a normal or abnormal function of living organs or tissue. Biomaterials possess a diverse range of uses, encompassing the fields of biomedicine, pharmaceuticals, and food manufacture. Biomaterials have been successfully utilized in the fabrication of clinical implants materials since the 19th century (1).

The incorporation of biomaterials, cells, and bioactive compounds has been employed within the medical field to fabricate 3D biomaterial scaffolds, which serve to guide tissue regeneration and facilitate inclusion within the living system. Biomaterials are widely recognized for their ability to facilitate the interaction between therapeutic substances and the body's innate healing mechanisms. The construction of 3D biomaterial scaffolding involves the use of three unique categories of biomaterials, namely ceramics, polymers, and metals (2).

To this end, many distinct types of 3D biomaterial scaffolds have been developed, each constructed from a unique biomaterial and employing a unique manufacturing technique (Figure 1.1). Biomaterial scaffolds serve as a conducive medium for the regeneration of tissues and organs, functioning as a structural framework for tissue development. These scaffolds are commonly packed with cells and occasionally supplemented with bioactive substances such as growth factors. The cell-seed scaffolds can be cultivated in vitro to facilitate tissue synthesis, after which they can be transplanted into the affected region. Alternatively, they can be surgically inserted into the wounded region, leveraging the organism's inherent mechanisms to trigger tissue or organ repair in vivo (2).

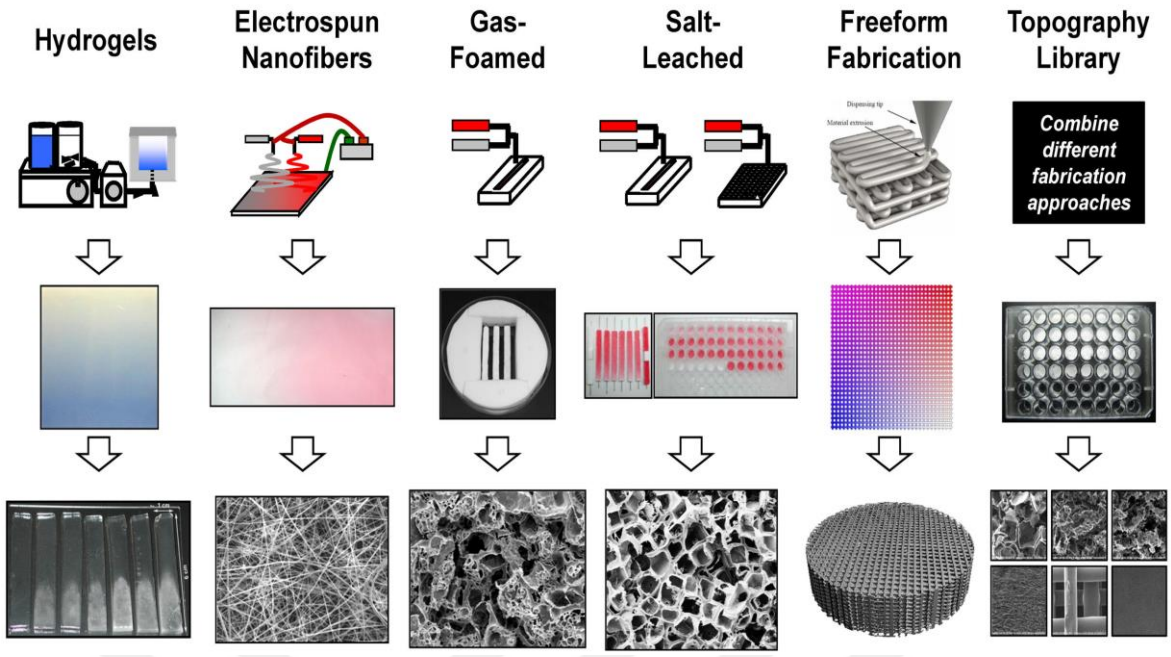

Figure 1.1. Biomaterial 3D scaffolds fabrication approaches

Until near future, monitoring of the physiological reactions caused by directly transplanted biomaterial scaffolds remains a challenge, as the reaction is random and lacks deliberate regulation. In order to precisely regulate biological responses, biomaterial design and manufacture should be founded on an understanding of the physiology of the targeted organ or tissue(3). Numerous methodologies are currently under investigation for precisely medication and/or cell delivery, employing diverse biomaterials as carriers to effectively elicit a decisive physiological response. To effectively assist normal organ/tissue repair, a mix of therapeutic agents and/or cells were delivered along with an appropriate carrier (scaffold) with specified microscale and nanoscale features, a well-adjusted biodegradation profile, and unique biological markers (4).

Biomaterials intended for medical applications should possess optimal biocompatibility with the biological system, hence minimizing any potential adverse effects. Additionally, these biomaterials should exhibit appropriate porosity and possess intricately interconnected structures to facilitate cellular growth and metabolic processes. Also, it is imperative that the biodegradability or bio absorbability of the materials align with the regulated breakdown rate required for practical medicinal purposes. Furthermore, it is imperative that the mechanical qualities exhibit adequate strength to endure various forms of stress, while simultaneously possessing a surface composition that facilitates effective cellular adhesion. These objects

should possess the capability to be readily transformed into a diverse range of three-dimensional forms (4).

Polymer-based biomaterials, such as hydrogels, have been employed in the development of drug delivery strategies for targeted administration of biomolecules. This strategy aims to mitigate the adverse effects, such as toxicity, hypertension, edema, and mortality, that are commonly associated with the systemic administration of therapeutic agents. Hydrogels are polymeric networks that are cross-linked and exhibit the property of non-dissolvability, while demonstrating the ability to undergo swelling when exposed to aquatic environments. These materials provide a milieu that closely mimics the extensively hydrated condition of native tissues, rendering them very promising contenders for applications in regenerative medicine and drug delivery (2).

Polymeric biomaterials (i.e., hydrogels) for tissue 3D scaffolds and drug delivery devices have been developed through the study of several crosslinking methods, including ionic, physical, and chemical cross-linking. Limiting systemic exposure to a particular medicine while simultaneously delivering the drug to the intended tissue is how a hydrogel improves the therapeutic efficacy of pharmaceutical therapies. Hydrogel scaffolds offer several benefits, including the capacity to modify their physical and mechanical characteristics as well as their degradability. These scaffolds also serve as a framework for facilitating diverse physicochemical interactions with encapsulated medicines, thereby regulating their dynamics (5).

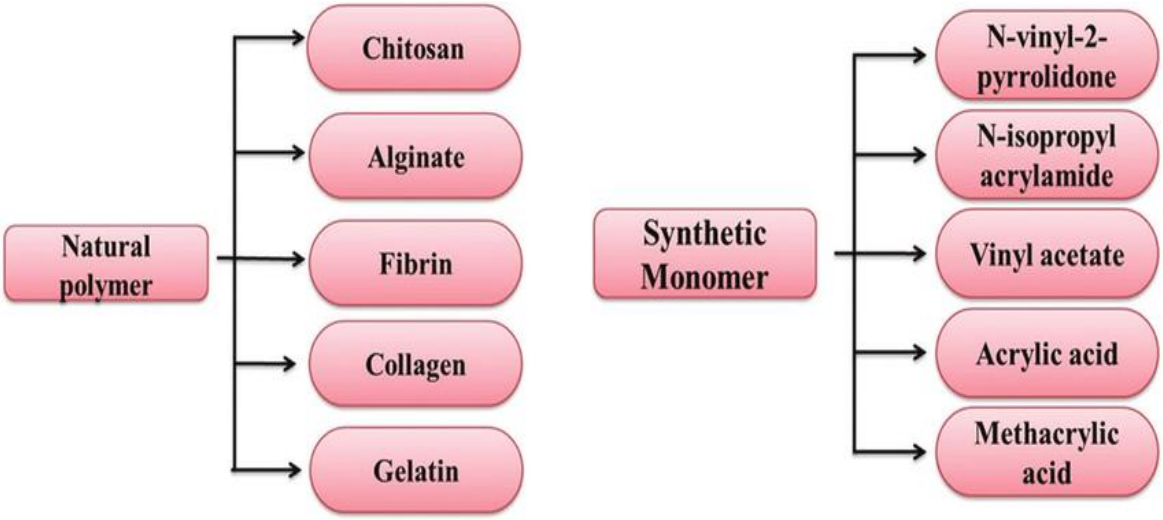

Figure 1.2. Examples of natural and synthetic biomaterials used as pharmaceutical carriers.

Hydrogels exhibit favourable characteristics for 3D bioprinting owing to their high velocity, which contributes to their biocompatibility and facilitates the maintenance of shape fidelity following gelation. Nevertheless, hydrogels possess certain limitations, particularly concerning their mechanical and physical characteristics, hence constraining their suitability as inks for bioprinting. The integration of microparticles into multi-material hydrogels has resulted in enhanced printing potential and performance of hydrogel scaffolds. The fabrication of hydrogels composed of multiple materials have the potential to enhance the physiomechanical characteristics of hydrogels (6).

The incorporation of microparticles has the potential to enhance the regulated and prolonged release kinetics of biomolecules, therefore enhancing their efficacy in the sector of drug delivery. Hydrogels could be used in multifactorial pathological cases (such as spinal cord damage and organ transplantation) by including microparticles carrying various pharmaceutical molecules, which can make the hydrogels respond to a varied physiological stimulation (7).

The design and fabrication of a hydrogel scaffold are contingent upon the exact anatomical region being targeted, as well as the particular physiological responses being sought. The current research aimed to design and fabricate a hydrogel specifically tailored for the purpose of ovarian tissue transplantation, with the objective of enhancing angiogenesis and preventing the occurrence of burning out syndrome in the ovarian follicle after transplantation. Vascular endothelial growth factor (VEGF) has been chosen as an enhancer of angiogenesis, while Rapamycin, an immunosuppressant, has been selected to inhibit the mechanistic target of the rapamycin (mTOR) signalling pathway which is crucial in the activation of ovarian follicles.

2. LITERATURE REVIEW

2.1.HYDROGELS AS DRUG DELIVERY SYSTEMS

Hydrogel-based scaffolds possess inherent appeal for the purpose of controlled drug administration due to their capacity for modifiable qualities during fabrication, as well as their compatibility with secure implantation, discharge, and decomposition processes. Hydrogels are defined as highly moist interconnected meshes consisting of natural, synthetic, or semi-synthetic polymers that are cross-linked through physical or chemical techniques. This specific type of materials is utilized for targeted drug delivery owing to their remarkable characteristics, such as enhanced biocompatibility, drug encapsulation capabilities, precise control over drug release in terms of both spatial and temporal aspects, as well as adaptability to diverse physicochemical conditions. Moreover, hydrogels possess the capability to encapsulate and provide medications with diverse characteristics, including small compounds, amino acids, and nucleic acids(8, 9).

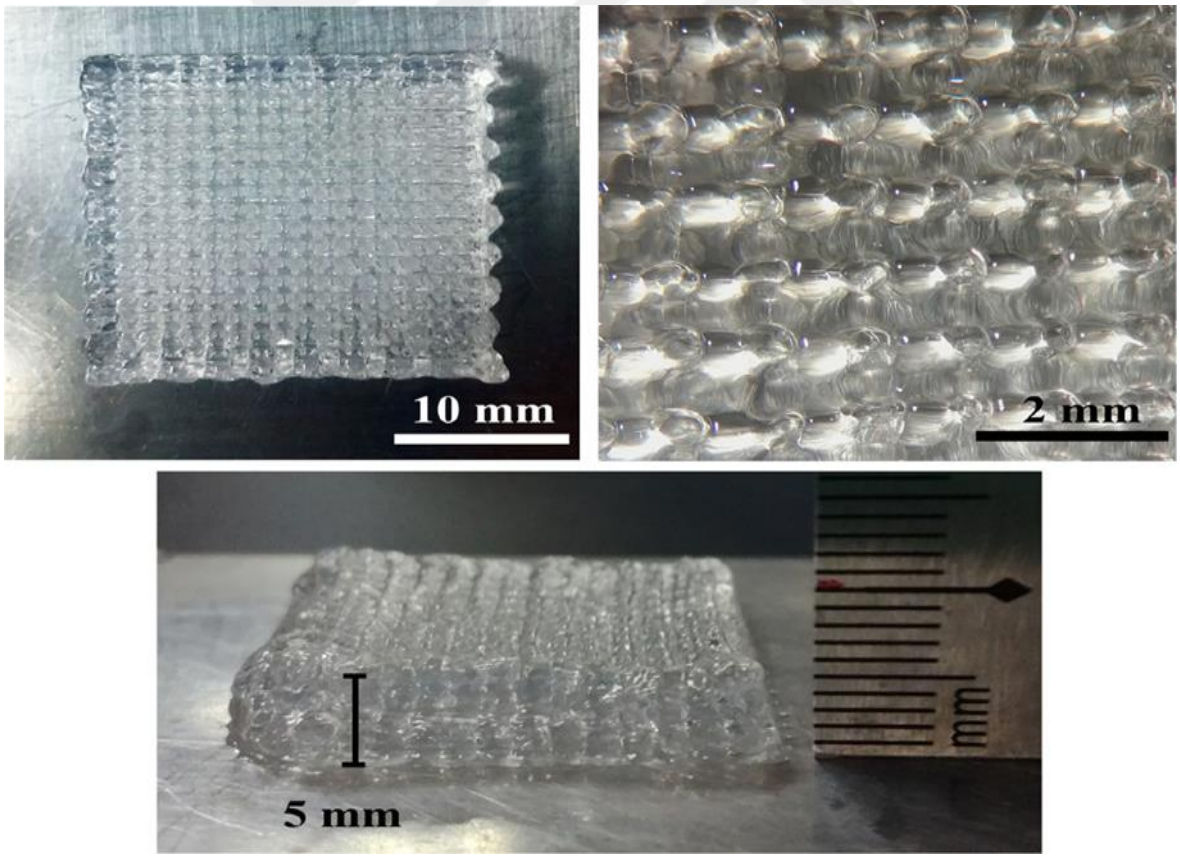

Figure 1.3. 3D network structure of polymer-based hydrogels

The unique physical attributes exhibited by hydrogels received considerable interest due to their potential utility in the sector of drug delivery. Hydrogel drug delivery systems are frequently categorized into two distinct groups: natural and synthetic. Hydrogels originating from natural sources encompass hydrogels formulated utilizing collagen, chitosan, alginate, fibrin, gelatine, or hyaluronic acid. On the other hand, synthetic hydrogels, such as poly (ethylene glycol) (PEG) or poly (vinyl alcohol), are commonly utilized in the production of hydrogel scaffolds (8).

2.2.MECHANISMS OF DRUG RELEASING FROM HYDROGELS

Pharmaceutical agents can be released by two mechanisms: disintegration from the entrapped particles followed by diffusion through the hydrogel, or the liberation of the particles from the hydrogel.

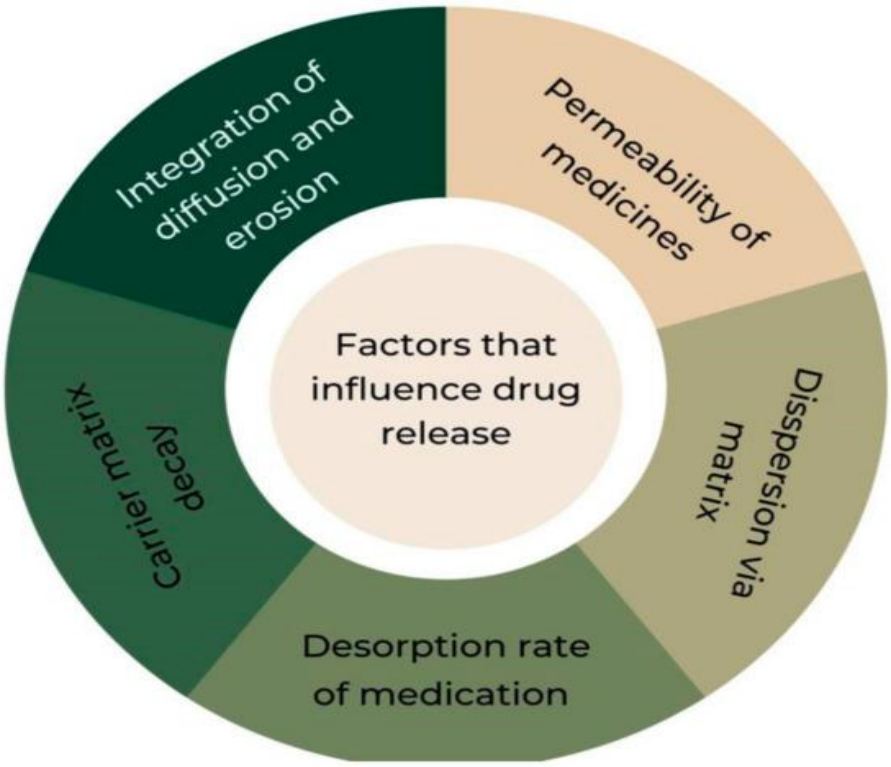

Figure 1.4. Factors influencing drug release from drug carrier.

2.2.1. Controlled release through mechanical deformation

The stress-induced deformation of the network framework induces the release of the encapsulated medication. The deformation of a network of fibers can be accomplished

through a variety of methods, such as mechanical deformation or the utilization of deformations caused by ultrasonic and electromagnetic fields. In each of these methodologies, a notable consideration about mechanical deformation pertains to the progressive deterioration of hydrogels, resulting in eventual mechanical breakdown. The potential remedy for this issue may lie in the utilization of self-healing hydrogels. An illustration of this concept is the use of alginate hydrogels that are crosslinked with divalent cations. These hydrogels possess the ability to regenerate under physiological conditions after ultrasound destruction. This unique characteristic enables the repetitive and precise release of tiny molecules, proteins, and condensed oligonucleotides (10).

2.2.2. Controlled release through swelling

A hydrogel's mesh size expands as it swells. Temperature, glucose, ph, light, electric field, and ionic strength are just a few of the environmental factors that might affect how swelling behaves. The response time for macroscopic hydrogels is somewhat slow for swelling-controlled systems because water diffuses slowly. Changes in swelling and drug release are anticipated to take several minutes for hydrogels that are 1 mm in thickness. In order to expedite the response time, it is possible to diminish the diffusion length through the reduction of hydrogel size or the creation of interconnecting macropores inside the hydrogel structure (11-13).

2.2.3. Controlled release through network degradation

Disruption in polymers can happen either in the backbone or through cross-links and is commonly caused by hydrolysis or enzyme activity. Hydrogel degradation, sometimes referred to as erosion, entails the reduction in polymer mass. This process can occur either concurrently throughout the entire mass of the hydrogel or, more desirably, on the hydrogel's surface. Mass and surface erosion can be employed as distinct mechanisms for regulating medication release. Numerous hydrogels experience significant erosion in mass due to the permeability of their mesh structure, allowing for the passage of water or enzymes that facilitate the process of degrading (14, 15).

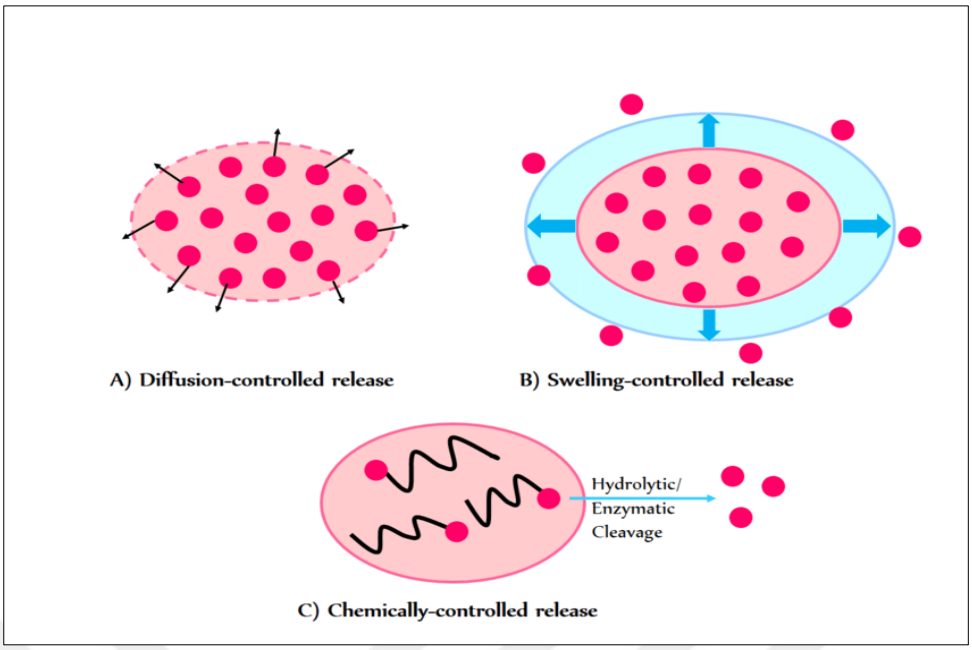

Figure 1.5. Bioactive molecule releasing mechanisms from hydrogel.

2.3.RELEASE KINETICS

The in vitro assessment of drug release kinetics involves submerging samples of the hydrogel-drug delivery system in a release medium that largely simulates physiological circumstances, specifically with regards to pH, temperature, and CO2 concentration. The drug quantity is measured by taking samples of the supernatants at various times. When the amount of the drug is known, a cumulative release graph is usually made to show how much of the drug is released as time passes. Release profiles provide significant insights pertaining to the kinetics of release, the quantity released at different periods, and potential indications on the underlying release processes (5).

From a theoretical perspective, a diverse array of polymers, medicines, release factors, and kinetics can be employed to accomplish many forms of controlled release patterns. These include explosive, biphasic, pulsatile, or sustained release, all of which adhere to zero-order kinetics. The choice of release strategy has a considerable impact on the characterization of drug-hydrogel combinations. The detailed rationalization and standardization of tests for each drug-hydrogel system has significant importance(5).

Ideally, it is recommended to customize these assays according to the specific requirements of the intended application and afterwards validate them within the context of the real

application. In order to facilitate a more thorough examination of the cumulative curves, additional tools are required. The aforementioned technologies comprise mathematical algorithms that can be applied for the analysis of version data. In addition, it is essential to utilize appropriate statistical analysis methods to determine any significant differences among the experimental groups and/or variables(5).

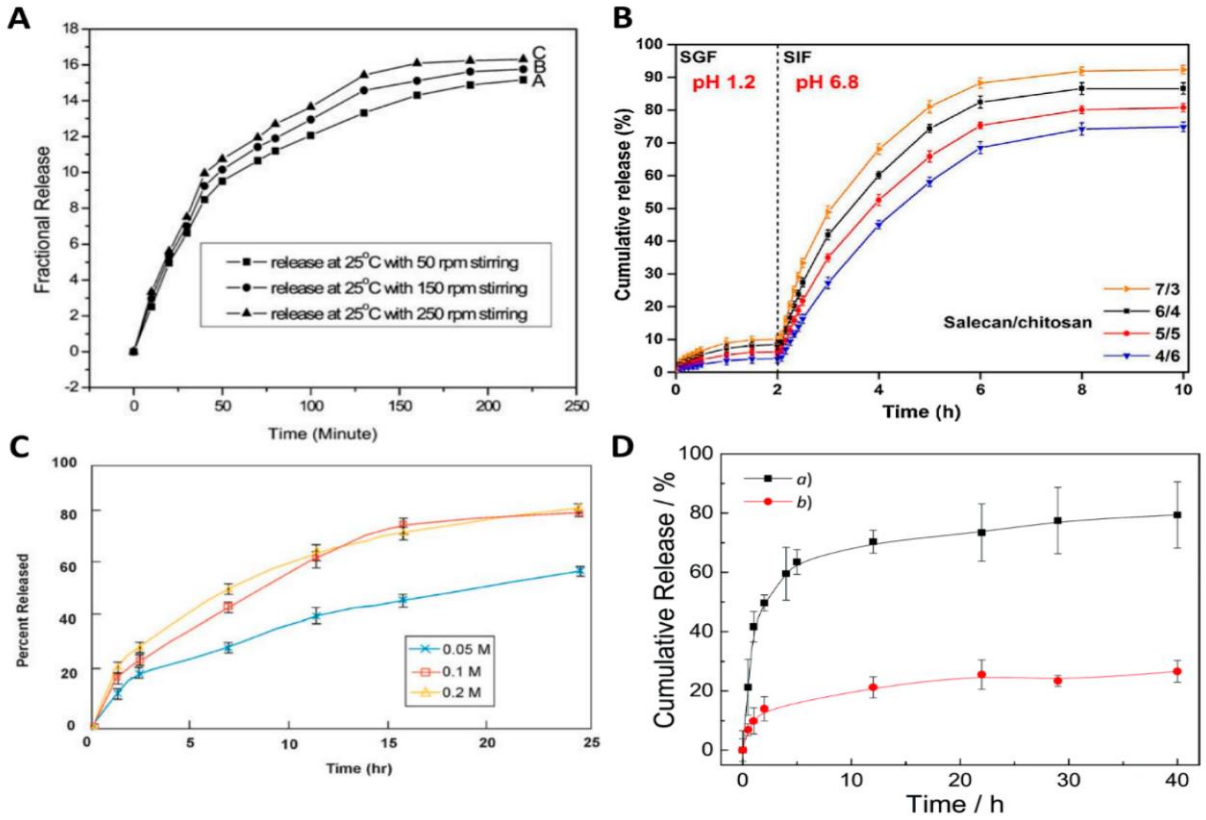

Figure 1.6. Factors affecting in vitro drug release. (A) different stirring rates (B) simulated gastric fluid for the first two hours and simulated intestinal fluid (SIF) from the second hour. (C) phosphate buffers at different molarities. (D) PBS.

2.4. ASSESMENT OF IN VITRO AND IN VIVO HYDROGEL RELEASE KINETICS:

2.4.1. In vitro:

The utilization of in vitro saline release assays holds significant importance in the comprehension of drug release kinetics and processes within hydrogel drug delivery systems. Nevertheless, there are several constraints associated with the evaluation of the efficacy of these systems in relation to certain target cells or tissues. In vitro testing is crucial for

assessing the appropriateness of the drug delivery strategy. Furthermore, in vitro experiments provide significant insights into the ideal pharmaceutical dosing range, which can later be translated to in vivo settings(5).

2.4.2. In Vivo:

Animal models play a vital role in understanding how living systems respond to drug delivery systems. They provide valuable insights before human clinical trials can begin. The main objective of conducting in vivo research, which mainly involves using mice models, is to establish a comprehensive biological framework for evaluating the effectiveness of administering medications and the potential for translating drug delivery systems. The primary objective of animal preclinical investigations is to evaluate the pharmacodynamics, performance of drug delivery devices in relation to dosage interval and toxicity, or to investigate the pharmacokinetics of drug release, encompassing the processes of drug uptake, distribution, metabolism, and elimination(5).

2.5.HYDROGEL APPLICATIONS IN DRUG DELIVERY AND DIFFERENT ADMINISTRATION ROUTES

Hydrogels possess the capacity to function as efficacious systems for the delivery of drugs through several routes of administration, encompassing oral, parenteral, ophthalmic, nasal, topical, brain delivery, and tissue engineering.

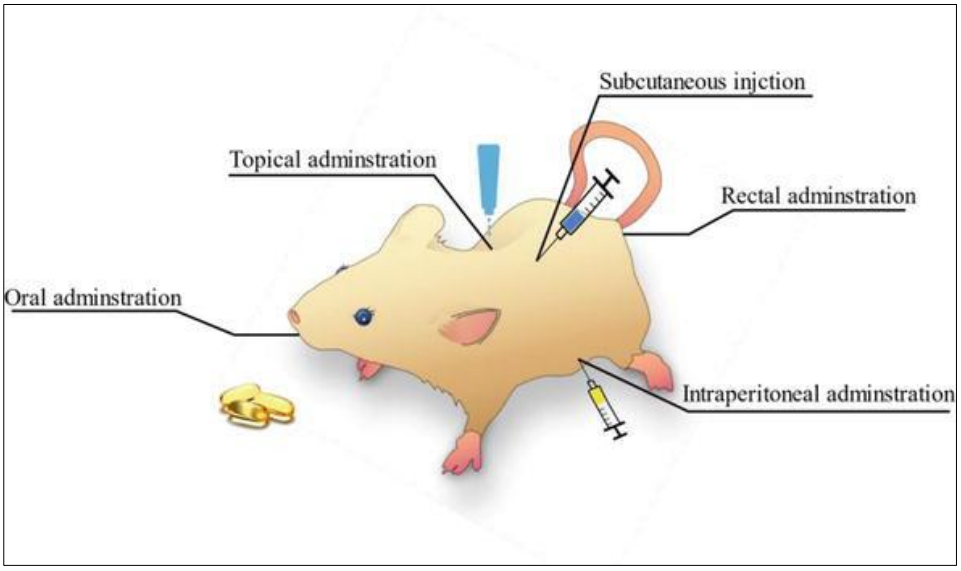

Figure 1.7. Hydrogel drug delivery administration routs.

2.6.COLLAGEN BASED HYDROGELS:

Connective tissue relies heavily on collagen as their principal protein component. Furthermore, collagen accounts for more than 30% of the protein in the human body (16). Because of its high biocompatibility and low immunogenicity, collagen-based hydrogel has been the subject of extensive research in the fields of bioengineering science. However, its inadequate mechanical characteristics restrict its applicability. Because collagen is so important to animals as a structure, it needs to have a lot of important features. These properties involve being stable at different temperatures, being strong, and being able to connect with other biomolecules in certain ways (17). Other biomaterials, such as alginate, chitosan, and hyaluronic acid, are commonly incorporated into collagen to increase its mechanical characteristics (18).

Collagen, either independently or in conjunction with other substances derived from the extracellular matrix, assumes a significant role in the physiology and behavioural patterns of cells originating from connective tissue. Keratinocytes and fibroblasts play crucial roles in the process of wound healing in the skin. Hence, the utilisation of collagen hydrogels as a substrate for cellular cultivation enhances cellular adhesion, migration, proliferation, and differentiation in both physiological and pathological contexts. The interactions between collagen and cells play a significant role in the processes of wound healing and adult tissue remodelling. Collagen is known to induce cell differentiation and preserve cell phenotypic, making these interactions crucial in these biological phenomena.

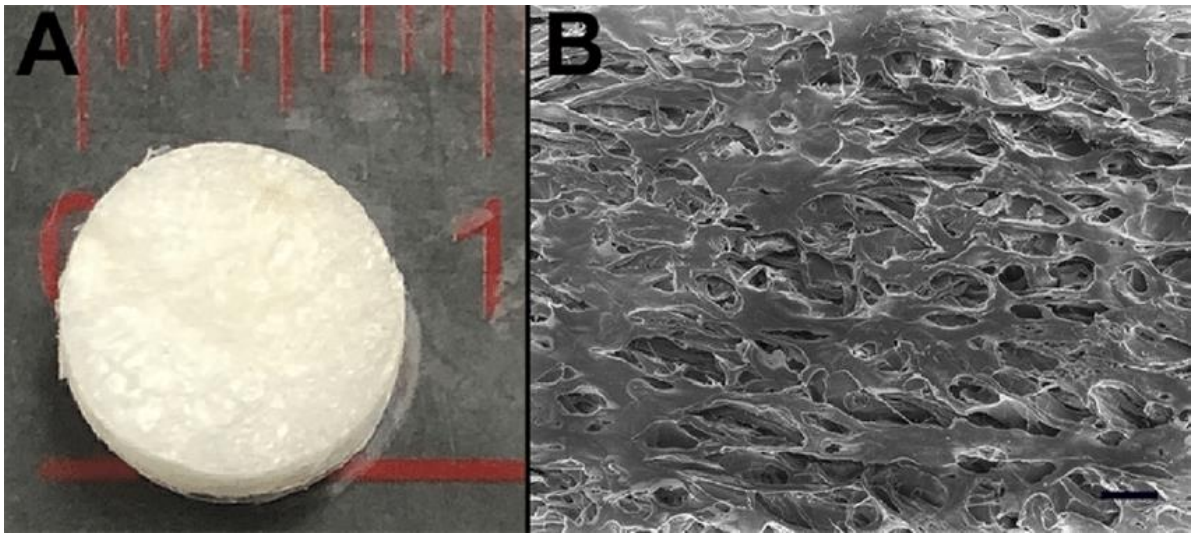

Figure 1.8. Picture displayed the structure of collagen scaffold.

2.7.COLLAGEN BASED HYDROGELS AS DRUG DELIVERY SYSTEMS:

Collagen is extensively utilized biopolymers for angiogenesis factor administration. Many studies tested the in vivo release of VEGF from collagen hydrogels and proved that the ionic interaction between VEGF and collagen helps preserve VEGF bioactivity and prolonged release. This is analogous to how growth factors in living tissue are maintained and enhanced by the ECM through sequestration (19, 20). The primary constraint associated with collagen-based hydrogels has historically pertained to their inferior mechanical characteristics.

Alvarez and colleagues created collagen-silica tiny materials that transport two antibacterial. The researchers encased gentamicin and rifamycin in silica nanostructures. These microscopic particles were then added to collagen hydrogels to test their medication delivery for severe injuries infection. Collagen networks slow silica nanoparticle dissolution. The nanocomposite releases gentamicin for over a week and has excellent antibacterial properties. However, high-concentration silica particles with rifamycin can change collagen hydrogel architecture. Rifamycin is released and absorbed on positive-charged collagen fibres. This interaction reduces composite antibacterial efficacy (21).

In a laboratory-based study, the experimental group, comprising a complicated hydrogel, demonstrated a significant wound healing rate of 98% in rats diagnosed with type II diabetes by the fourteenth day, resulting in nearly complete wound repair. Moreover, it is noteworthy to mention that synthetic collagen demonstrates significant potential as a catalyst for polymerization processes. Zhang et al. conducted an experimental study in which they synthesised a hydrogel utilising collagen as the fundamental constituent. The hydrogel utilised in this study consisted of polymer matrices incorporating pyrogallol and a transition metal, namely silver. The hydrogel exhibits a remarkable ability for self-catalysis, eliminating the need for external stimuli and resulting in the formation of a rigid interconnecting polymer network (IPN). Simultaneously, the hydrogel demonstrated notable antibacterial properties due to the incorporation of silver ion particles. Furthermore, the hydrogel composed of collagen demonstrated notable haemostatic properties (22).

In their study, Olivetti et al. developed collagen hydrogels that were subjected to modification using dodecenylsuccinic anhydride (DDSA). The objective of this modification was to enhance the transport efficiency of the hydrophobic anti-inflammatory medication

simvastatin. The study concluded that the modified collagen hydrogels successfully maintained their fibrous and porous architecture. Furthermore, the hydrogels exhibited enhanced hydrophobic characteristics and exhibited promise as a vehicle for hydrophobic pharmaceutical compounds (23).

The wound healing ability is significantly reduced when using hydrogels made solely of collagen, compared to composite hydrogels that incorporate additional components. The mechanical properties of collagen-based hydrogels do not meet the desired standards. Collagen has been employed in the field of skin tissue engineering to enhance the properties of various natural and synthetic polymers. These polymers include hyaluronic acid (HA), chitosan, alginate, polyvinyl alcohol (PVA), and polyethylene glycol (PEG). The aim of this endeavour was to improve the overall effectiveness of these substances for application in the field of tissue engineering.

Polyvinyl alcohol (PVA) demonstrates exceptional mechanical properties. In the work conducted by the group Wang, polyvinyl alcohol (PVA) and collagen were employed as materials to produce hydrogels. The incorporation of polyvinyl alcohol (PVA) into hydrogels has been seen to result in an augmentation of their tension properties. When comparing pure collagen hydrogels with combined hydrogels, it was seen that the stress experienced by the latter increased significantly. Specifically, the stress rose from 40 to 6 kPa under a strain of 33%. The available information suggests that the inclusion of polyvinyl alcohol (PVA) is vital in bestowing hydrogels with remarkable mechanical characteristics through its facilitation of the creation of improved network entanglements. Several experiments, including as permeability tests, scanning electron microscopy (SEM) analysis, and water retention assessments, have yielded empirical evidence indicating that the microstructure of mixed hydrogels is solid and typified by tightly interconnected networks. The aforementioned findings are consistent with the mechanical property outcomes derived from the corresponding hydrogels (24).

A novel hydrogel composed of collagen and alginate microspheres encapsulating proteins has been created for potential use in retinal therapies. Bovine serum albumin (BSA) was utilised as a representative model for drugs. The composite hydrogel exhibited comparable optical clarity and mechanical rigidity to the placebo hydrogels lacking microspheres. A

continuous release of Bovine Serum Albumin (BSA) was successfully achieved in a neutral phosphate buffer saline PBS for a duration of 11 days. The composite hydrogel promoted human corneal epithelial cell growth and had sufficient mechanical strength and excellent optical clarity to be used as a therapeutic lens for drug delivery and/or as a corneal substitute for transplantation to patients with corneal diseases (25).

2.8.ALGINATE BASED HYDROGELS:

Alginate is a naturally occurring organic polysaccharide with anionic properties and hydrophilic characteristics. It is obtained through the extraction process from specific bacterial strains and seaweed species. The utilization of alginate as a biomaterial has numerous benefits, such as its cost-effectiveness, simplicity in its manufacture, and absence of hazardous properties (26). Alginate-based biomaterials can be manufactured in various forms, such as hydrogels, foams, sponges, fibres, microspheres, and microcapsules, employing a wide range of production techniques. Alginate is extensively utilized in the field of 3D tissue engineering for a diverse range of uses. Such uses include drug delivery, wound recovery, as well as repair and regeneration of connective tissue (27).

Alginate demonstrates significant amounts of carboxyl groups and negative charges, leading to compromised mechanical properties and diminished flexibility. The negative impact of Alginate-based biomaterials on cell contacts limits their potential application in customised tissue defect therapies. One possible approach to address the constraints is by implementing modifications to alginate and its derivatives. This can involve the integration of appropriate materials and components. One possible approach involves the utilisation of crosslinkers, such as methacrylate and chitosan, for incorporation. The effectiveness of chitosan-based bionics is limited due to its limited solubility in aqueous solutions. The utilisation of acid solvents for dissolving chitosan carries the risk of detrimental impacts on cellular structures and growth hormone activity (27, 28).

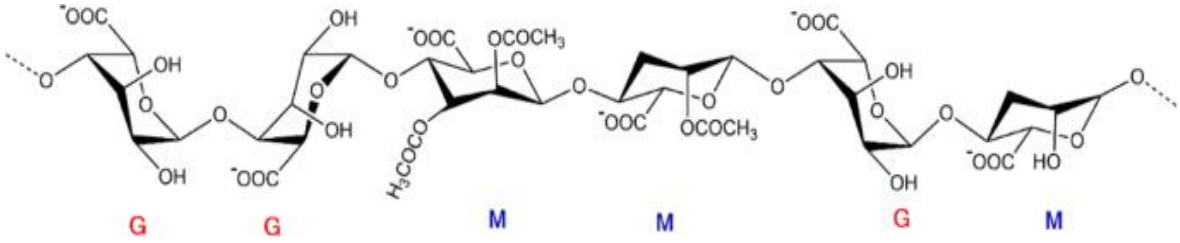

Figure 1.9. Chemical structure and conformation of guluronic acid (G) and mannuronic acid (M) residue in bacterial alginate

Alginate hydrogels have remarkable properties such as a substantial water content, absence of harmful effects, pliable texture, compatible with biological systems, and capability for degradation. These characteristics render them exceptionally well-suited for use as vehicles for tiny molecular weight medicines and macromolecules, including proteins and DNA, while also possessing the capacity to maintain or target their administration (29).

The load has the potential to become stationary or enclosed within the tiny spaces of the alginate hydrogel carriers. Alginate could create two distinct forms of gels, which is contingent upon the pH of the surrounding environment. The substance exhibits a reduction in volume under low pH conditions, namely within the stomach environment, and generates a dense acidic gel with high viscosity. This gel effectively retains the enclosed medications without releasing them. Upon entering the gastrointestinal system, characterised by an elevated pH, the alginic acid's dermal-like composition undergoes a transformation into a fluid-like viscous gel. This conversion leads to the rupture of the polymer system, facilitating the dissolution and subsequent release of the medicine. Hence, when administering drugs to the intended tissue, it is crucial to regulate the release of the drug over an extended duration to mitigate the risk of systemic adverse effects. The release of drugs from the pores of the hydrogel is facilitated through various mechanisms, such as diffusion-controlled, swell-controlled, chemically controlled, and environmentally responsible release (30).

Sodium alginate exhibits a distinct chain architecture composed of several carboxyl –COO- groups. In aqueous solutions, these entities have a propensity to aggregate, leading to the adhesive nature of the hydrogel. As a result, sodium alginate has demonstrated its efficacy as a beneficial pharmaceutical carrier for the therapeutic targeting of mucosal tissues. The pH sensitivity exhibited by sodium alginate can be attributed to the presence of the carboxylate (-COO-) group. Under acidic conditions, the carboxylate group (-COO-) undergoes a transformation into a carboxylic acid group (-COOH). This alteration results in

an augmentation of the hydrogen bond contact between the carboxyl groups of sodium alginate molecules. As a result, the molecular chain undergoes a reduction in size or shrinking (31).

In the domain of guided drug delivery systems, the phenomenon of rapid and excessive release of drug molecules from alginate-based hydrogels, which are generated by cross-linking with calcium, is a frequently seen challenge in typical conditions. In recent years, there has been a notable focus on improving the efficacy of alginate salt-based hydrogels as vehicles for drug delivery. alginate has the ability to create chemical complexes with a range of polymers, such as polyvinyl alcohol (PVA), gelatine, methyl cellulose, and collagen (31).

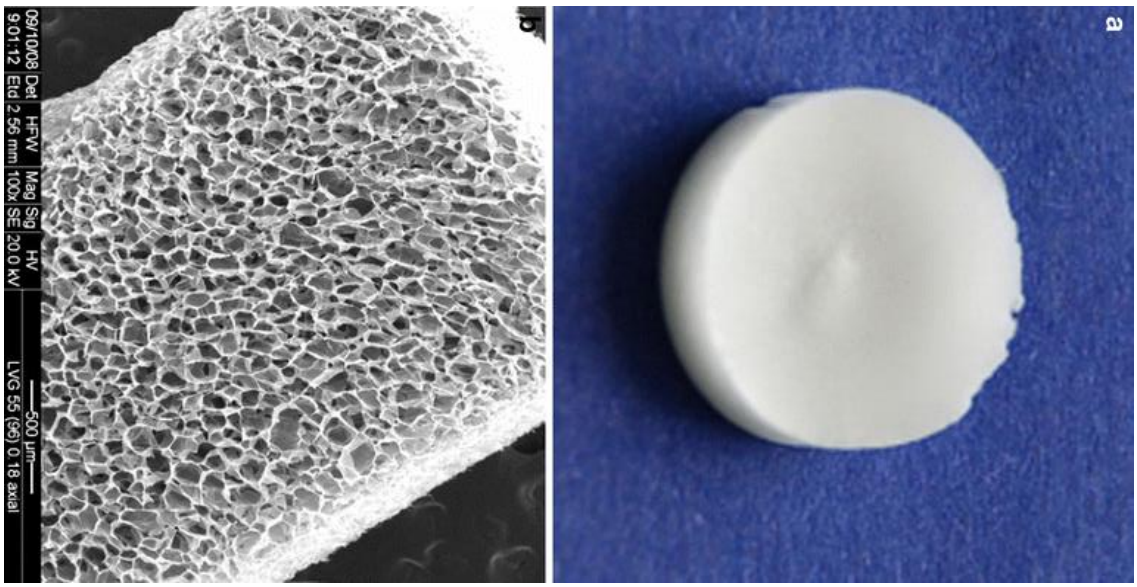

Figure 1.10. Picture displayed the structure of alginate scaffold.

2.9.THREE-DIMENSION 3D BIOPRINTING

The term "3D printing" encompasses many technologies that utilize automated methods for dropping materials and fabricate tangible three-dimensional objects. The layer-by-layer procedure, which is carefully regulated, renders 3D printing the most contemporary and adaptable technique for fabricating polymer hydrogel scaffolds of the highest standard. In addition, the utilization of design/computer-aided manufacturing (CAD/CAM) software allows the creation of diverse configurations, a range of networks, varying pore sizes, multilayers, and various production procedures.

The inherent adaptability of this system holds promise for regulating the rates at which drugs are released. Furthermore, the novel capability of transforming computed tomography images into computer models has the potential to produce three-dimensional bio scaffolds, which are essentially scaffolds that possess the intricate structure and diverse composition characteristic of living tissues and organs. In these instances, a diverse range of active chemicals, such as growth factors, can be included to augment the desired physiological reactions.

One of the primary challenges in the field of 3D printing involves the identification of biocompatible materials that possess the necessary characteristics for the printing process and fulfil the mechanical strength criteria for scaffolds utilised in tissue engineering. Hydrogel materials possess the ability to meet specific requirements and demonstrate considerable promise as biocompatible materials in the field of 3D bioprinting. The classification of natural bio-inks encompasses protein-based bio-inks, polysaccharide bio-inks, and bio-inks derived from the extracellular matrix (ECM) of decellularized organs (32, 33).

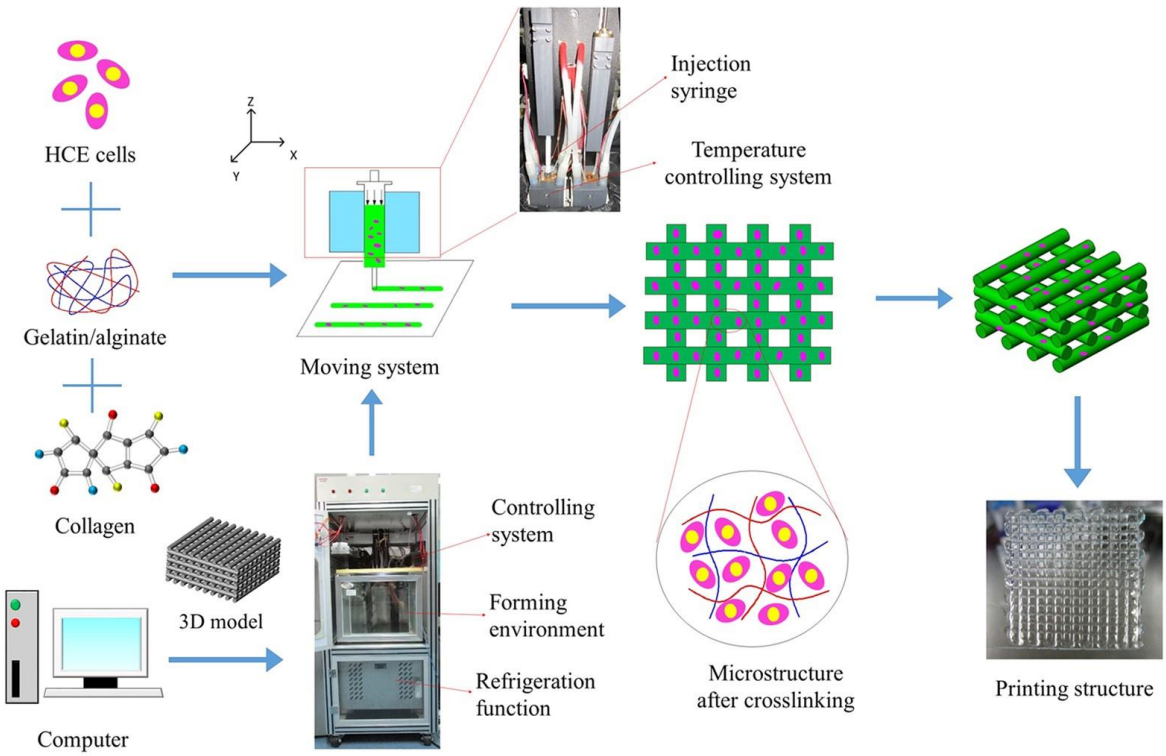

Figure 1.11. Illustration shows the 3D bioprinting steps.

Collagen, being one of the most extensively utilized bioinks, has garnered significant attention within the field of tissue engineering and pharmaceutics fields. Nevertheless, the excessive stiffness of the collagen-based scaffolds that are formed may impede the migration of cells, while the adhesive nature of proliferating collagen can lead to blockages in the printer's nozzle. Multiple approaches have been explored to enhance collagen bioinks, such as incomplete crosslinking, modification by chemicals, and the incorporation of a supplementary promoting polymer materials (34, 35).

The use of collagen accompanied by other materials has gained significant popularity in the field of 3D printing. In order to optimize the quality of printed lines, it is possible to combine collagen and alginate salts to produce hydrogels that exhibit superior structural and biological characteristics. The research conducted by Niu et al. employed a bio-ink composed of sodium alginate, gelatin, and collagen (SA/Gel/C) hydrogel to fabricate a bionic scaffold for full-thickness skin (36, 37).

The biocompatibility of alginate salts, as a copolymer, is evidenced by their ability to effectively enhance cell proliferation. Therefore, it is often regarded as an ideal precursor for applications in the field of 3D bioprinting. The use of alginate derivatives as bioink for the fabrication of hydrogel scaffolds presents a challenge in maintaining the structural integrity and form fidelity of the produced hydrogels, mostly due to their insufficient viscosity. The low viscosity of the formed filaments renders them susceptible to collapse and fusing. When alginate hydrogels are cross-linked using calcium-based crosslinkers, their mechanical qualities are weakened, rendering them vulnerable to gravitational collapse. The collapse has implications not only on the structural integrity of the hydrogels but also on the precision and faithfulness of their three-dimensional printing. One of the main obstacles encountered in the use of alginate hydrogels as bioink in the context of 3D bioprinting pertains to their comparatively limited printing speed (38).

2.10. HYDROGEL CROSSLINKING:

2.10.1. Physical crosslinking:

2.1.1.1 Ionic crosslinking

The synthesis of ionized cross-linked hydrogel scaffolds is founded on a fundamental principle, involving the incorporation of polymers that can undergo ionization with compounds possess opposite polarity. The mechanical characteristics of polymer scaffolds, as well as the molecular mass of the polymers and the degree of cross-linking, can be effectively controlled by manipulating parameters such as the quantity of polymers or ion levels.

Alginate derivatives are a well-established class of native macromolecules that possess numerous advantageous properties, including biocompatibility and non-thrombogenicity. As a result, it is widely employed across various industries such as cosmetics, healthcare, and food processing. Alginate is comprised of a significant amount of carboxyl groups, which have the ability to undergo cross-linking through the introduction of positively charged donors like calcium chloride (CaCl2). Enhancing the mechanical properties of the alginate hydrogels can be achieved by augmenting the concentration of Ca2+ ions incorporated within the network (39-41).

2.10.2. Hydrogen bonding

The use of hydrogen bonding as a crosslinking method for the fabrication of implantable hydrogels is a promising approach due to its elastic properties and vulnerability to rupture under high temperatures. Moreover, this methodology confers inherent therapeutic attributes, thermoplastic behavior, and recyclability to the hydrogels. A limitation commonly seen in hydrogen bonding crosslinked hydrogels is their restricted repellent to water, as the presence of water can result in the disruption of the hydrogen bonds between polymer crosslinks (42, 43).

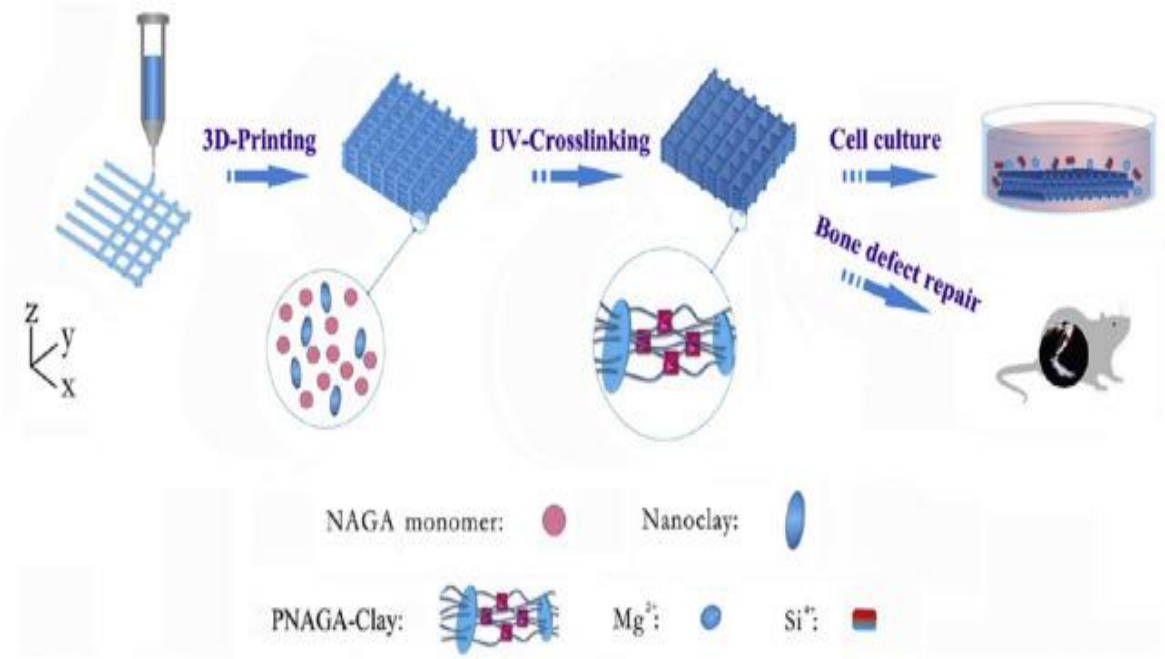

Figure 1.12. Hydrogel composed of N-acryloyl glycinamide (NAGA) monomer and nanoclay was produced by 3D-printing technique towards the construction of load-bearing tissue engineering scaffolds for bone defect repair (6).

2.10.3. Chemical crosslinking

2.1.1.2 Enzymatic reaction

Enzymatic crosslinking serves as a viable alternative approach for the fabrication of hydrogel scaffolds, namely in the realm of protein-based gel materials. Enzymes obtained from various plant and animal sources, such as horseradish peroxidase (HRP), glucose oxidase (GOx), and laccase, have been utilized to promote the establishment of covalent linkages inside hydrogels (44).

2.1.1.3 Photo-initiated crosslinking

The application of photo-initiated crosslinking is widely acknowledged as the primary method for manufacturing injectable and implantable hydrogels. It is imperative to maintain fine control over the viscosity and crosslinking dynamics of the hydrogel matrix. The rationale for this design is to ensure that the system is localized to the specific implantation location, rather than exhibiting diffusion to the adjacent regions. The activation mechanism in most hydrogel systems involves the utilization of ultraviolet (UV) light to induce the generation of reactive oxygen species or ions (45).

2.11. PARTICULATE DRUG DELIVERY SYSTEMS

Microspheres are particulate materials characterized by their ability to exhibit free-flowing properties. These materials consist of biodegradable polymers, which can be either natural or synthetic. Hence, it is imperative for microspheres to possess a particle size that is less than 1000μm. The utilization of this approach is of utmost importance in ensuring the accurate and controlled administration of medicinal substances to their designated target site, hence ensuring a sustained and predetermined release throughout a specified duration. These devices facilitate the accurate delivery of tiny quantities of active drugs, while simultaneously reducing the diffusion of the drug in the vicinity of the targeted organ or tissue (46).

Particulate drug delivery systems offer pre- and post-application protection for unstable medications, safeguarding them until they reach their intended site of action. They offer the capability to modify the drug's in vivo impact, pharmacological profile, delivery within tissues, and interaction with cells. They offer a controlled release mechanism for the medication. Some examples of pharmaceutical substances that are commonly used in medical treatments include growth factors and immunosuppressants

Microparticles drug delivery systems used usually are natural or synthetic polymers. Different preparation techniques are used to fabricate particulate polymers such as Single emulsion technique, Double emulsion technique, or Polymerization (46).

2.11.1. Double emulsion technique

This methodology entails the creation of a dual or multiple emulsion with layers of water-oil-water (w/o/w) or oil-water-oil (o/w/o). These formulations are highly suitable for pharmaceuticals that demonstrate both water solubility and insolubility, as well as for peptides, proteins, and vaccines. The technique is applicable to both naturally occurring and artificially produced polymers.

In brief, with respect to water-in-oil-in-water (w/o/w) emulsion, the aqueous protein solution is dispersed inside the lipid-soluble organic phase. The protein solution has the potential to include active constituents. The continuous phase typically comprises a polymer solution that envelops the protein present in the scattered aqueous phase. Prior to being added to the

aqueous poly vinyl alcohol (PVA) solution, the emulsion undergoes either homogenization or sonication. As a result, the phenomenon of double emulsion production takes place. Subsequently, the emulsion undergoes solvent removal by either solvent evaporation or solvent extraction techniques. The solvent evaporation method involves either maintaining the emulsion at lower pressure or utilizing agitation techniques to facilitate the evaporation of the organic phase. Following this, the emulsion is placed into a substantial volume of water, so promoting the dispersion of the organic phase. The solid microspheres are acquired by a filtration procedure, subsequently undergoing a washing step utilizing n-hexane, acetone, or another suitable organic solvent. The aforementioned phase bears considerable significance due to its pivotal role in efficiently eliminating any residual oil that might be present on the surface of the microsphere (46).

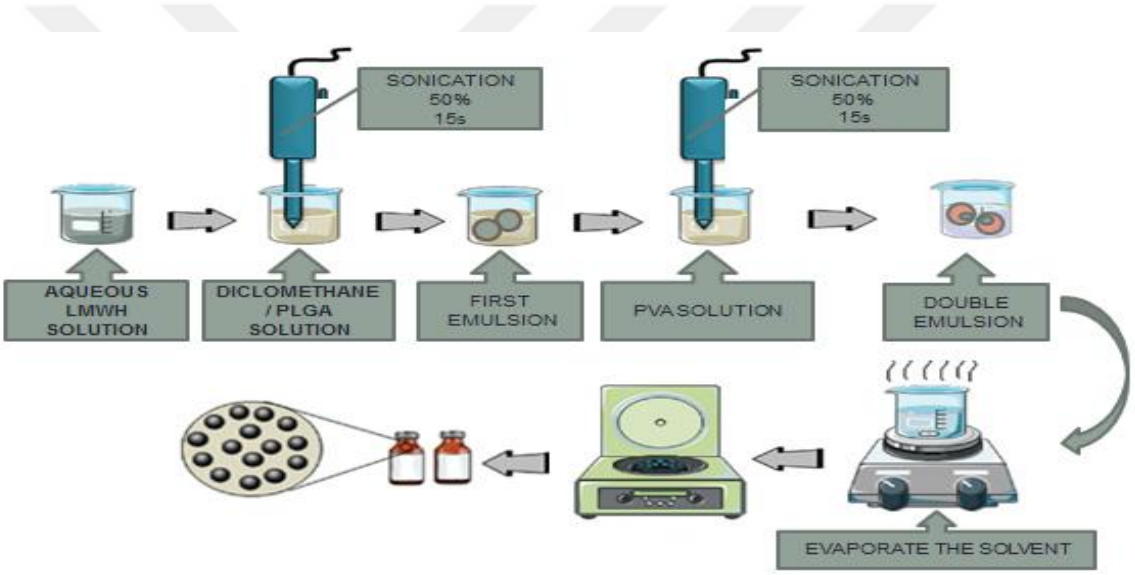

Figure 1.13. Production and characterization microspheres by double emulsion method.

2.12. MULTIPLE MATERIAL HYDROGEL DELIVERY SYSTEM APPLICATION IN ORGAN TRANSPLANTATION

Organ transplantation has been considered a medical miracle since the last century, as thousands of people with various end-stage organ failures have been treated (47, 48) However, Organ transplantation has been associated with all rejection patterns of organ/tissue transplantation, which have been clarified as a consequence of cell-mediated and/or antibody-mediated tissue injuries. As a result, transplantation surgeons' have

dominated various immunological considerations in order to understand the dilemma of transplant rejection as the primary cause of graft failure (49, 50).

One of the most common causes of organ engraftment rejection is inadequate or absent angiogenesis. Repair and regeneration require the ability to restore blood flow circulation, and consequently oxygen and nutrient supply to ischemic or injured organs (51).

Thomas Starzl, stated in his historical record of the first three cases of liver transplantation that "the provision of a viable and little damaged homograft is certainly the most critical single component in the determination of success." As a result, the goal of organ transplantation should be to avoid graft IRI (52).

Complexity pathophysiology of IRI, it is challenging to effectively integrate basic scientific and creative principles into clinical therapy(53). Recently, various machine perfusion systems, including hypothermic machine perfusion (HMP), hypothermic oxygenated perfusion (HOPE), and normothermic machine perfusion (NMP), have been implemented in medical strategies for enhancing transplant viability. Research has demonstrated their reliability and applicability. Even though these studies strengthen the field of transplant restoration, all methods of device blood flow still involve a period of ischemia when the graft is being retrieved and retransplanted (54).

Numerous therapy methods have been proposed and evaluated to overcome this challenge, Guo, Zhiyong, and his colleagues created a method of ischemia-free organ transplantation (IFOT) and evaluated its adequacy in participants who got liver transplants. Unfortunately, there has been moderate success (55).

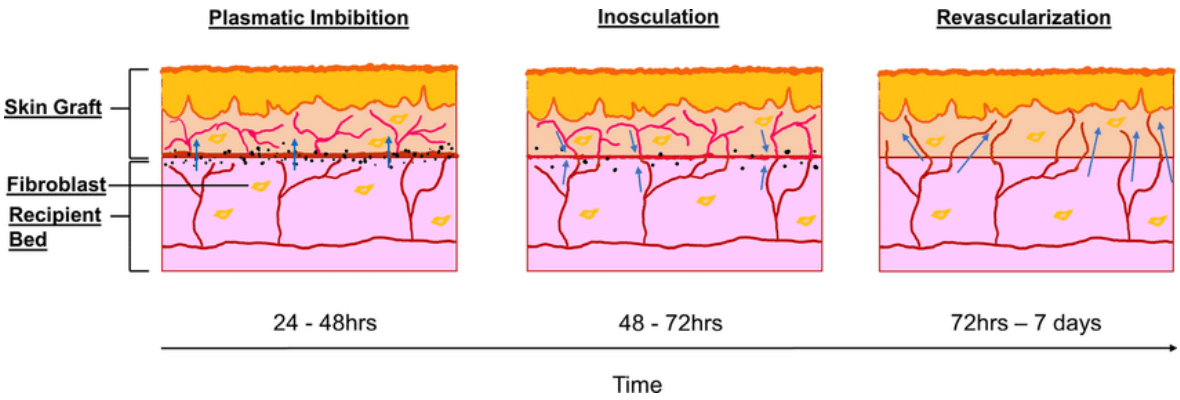

Figure 1.14. Skin graft implantation illustration showing the ability of transplant to initiate vasculogenesis and angiogenesis between the graft and the recipient bed within 7 days.

One of the most important contributors to organs transplantation failure is the postponed or lacking vascularization process. It is important for the process of repair and regeneration to have the capability to restore blood circulation (neoangiogenesis), and with it, oxygenation, and nutrients able to be delivered to organs that have been injured (56, 57).

Another pattern of transplant rejection is known as pathological injury, and it occurs when antibodies and/or T cells are responsible for causing the injury in the transplant. To reduce the risk of rejection reactions following transplantation, it is possible to achieve ideal matching of major histocompatibility complex (MHC) antigens, to take medications that inhibit the immune system in general, or to induce a level of acceptance in the recipient (58).

2.12.1. ANGIOGENESIS MEDIATED FACTORS

Angiogenesis is a complex process mediated by a multi-step cascade driven by various biological factors, nonetheless, VEGF can promote neoangiogenesis on its own. Moreover, VEGF therapeutic angiogenesis administration into ischemic tissue can enhance blood flow. As a result, delivering or incorporating VEGF into tissue grafts may minimize/prevent the damage caused by chronic hypoxia by promoting angiogenesis (59).

Angiogenesis is important in numerous physiological processes, especially healing process, tissue regeneration, and the progress of certain diseases. To boost neoangiogenesis, biomaterials may be employed as scaffolding to enable vascular infiltration for tissue regeneration, or they can be employed to transport proangiogenic agents and cells in a regulated manner (60).

2.12.2. MECHANISTIC TARGET OF RAPAMYCIN

Immunosuppressants are drugs used to hinder the immune system's capabilities to respond to immunogens. To prevent transplant rejection, these medications are administered to organ and cell therapy recipients. Such drugs like, antibodies, corticosteroids, and mammalian target of rapamycin (mTOR) inhibitors Rapamycin was approved as an immunosuppressive drug for transplant recipients by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) (61, 62).

2.12.3. ANGIOGENIC DRUG DELIVERY

Bioactive molecules have been extensively employed to address the challenges encountered by drug-delivery strategies aimed at augmenting angiogenesis. The systemic injection method is the most used technique for administering angiogenic medication in medical applications (63). Nevertheless, this approach presents several factors to be considered. Several medicines have a short half-life and elimination occurs relatively fast. Therefore, systemic administration growth factors in single or multiple dosages are promptly removed from the body, for instance, elimination half-lives of injected VEGF and bFGF are less than 1 hour (64). As a result, it may be necessary to provide the medicine in a series of large dosage over a short period of time to keep the drug's concentration and effectiveness within the target dosing range. This raises questions about the safety of administering these angiogenic agents systematically (63-65).

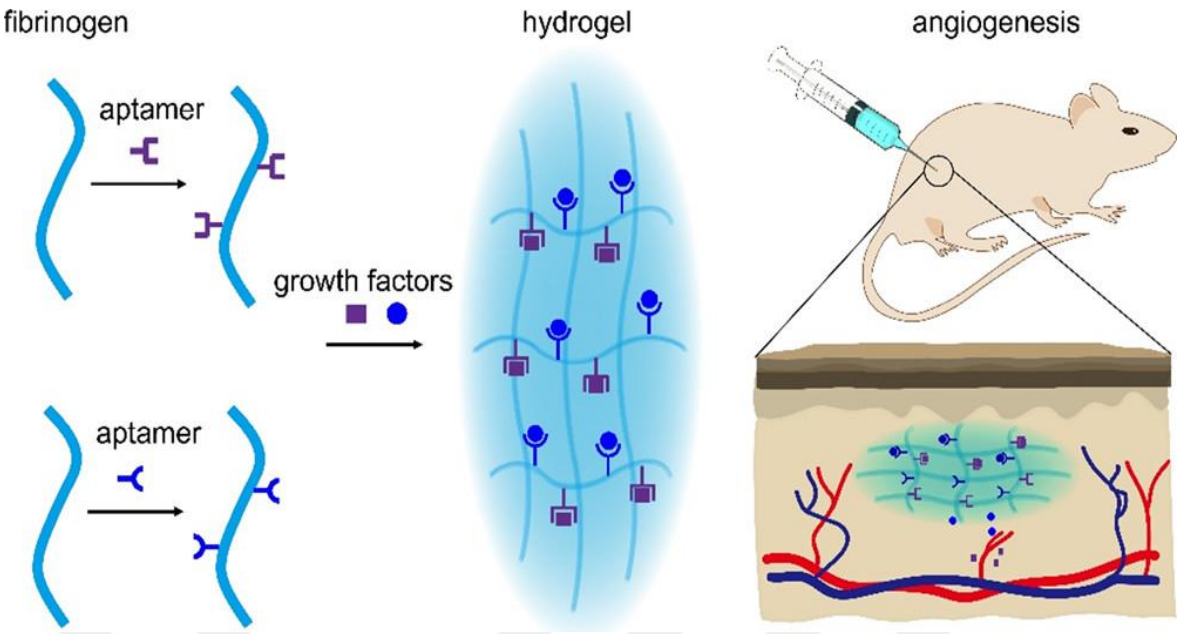

Figure 1.15. Demonstrating the administration of hydrogel-based growth factor via in situ injection as an effective method for drug delivery and regenerative medicine.

Inordinate doses of angiogenic factors can have adverse consequences on the circulatory system. For instance, VEGF, a powerful hyperpermeability molecule, can trigger increases in the vascular permeability of blood vessels (66). In one research, overexpression of VEGF via virus vector caused sever death among the experimental animals. The animals established severe edema in their organs as a result of increasing vascular permeability. In another research, both VEGF and FGF proteins produced hypotension and subsequent death of the experimental animals for the same reason. To address many of these concerns, biomaterials may be used for localized, controlled and sustained drug delivery (67).

There is a wide range of approaches for incorporating and releasing growth factors from polymers composites. Hydrogels, nanostructured scaffolds, and microparticles are all examples of tissue engineering that can be manufactured using biomaterials. Mechanical performance is improved by porous nanostructured and hydrogels, yet microparticles are effectively and easily injectable (68, 69).

2.12.4. RAPAMYCIN DRUG DELIVERY

Rapamycin has been shown to minimize of post transplantation kidney injuries. It has been revealed that Rapamycin-Loaded microspheres present a successful approach for releasing rapamycin in a time-dependent manner into the circulation (70, 71).

3. AIMS AND OBJECTIVES

In numerous cases of organ or tissue transplantations, repeated systemic administration of angiogenic or immunosuppressant medications is necessary. This is mostly owing to the high elimination ratio and short half-life of these agents. Nevertheless, the administration of medications through systemic means has the possibility of encountering significant consequences, such as the development of edema, toxicity, hypertension, and even mortality. However, it is imperative to decrease the quantity and frequency of administrations to enhance the socioeconomic consequences linked to repeated injections and mitigate the side effects that may arise.

In response to this requirement, we are developing an implantable drug delivery system (IDDS) consisting of poly (caprolactone) (PCL) microspheres suspended within a collagen/ alginate implantable hydrogel. Prolonged release of drugs with tailored characteristics can be accomplished by encapsulating drugs (VEGF and Rapamycin) within PCL microspheres. PCL is a biodegradable and biocompatible copolymer used in many FDA-approved therapeutic devices.

Herein, the aim of this study is to establish an extrusion 3D printing based composite collagen-alginate gel scaffolds with multiscale pore structures and containing hydrophobic VEGF/rapamycin loaded polycaprolactone microspheres. Therefore, administering VEGF to tissue grafts may reduce the damage caused by immunological reactions by inhibiting mechanistic target of rapamycin (mTOR) pathway, and prolonged hypoxia by increasing blood vessel formation rate.

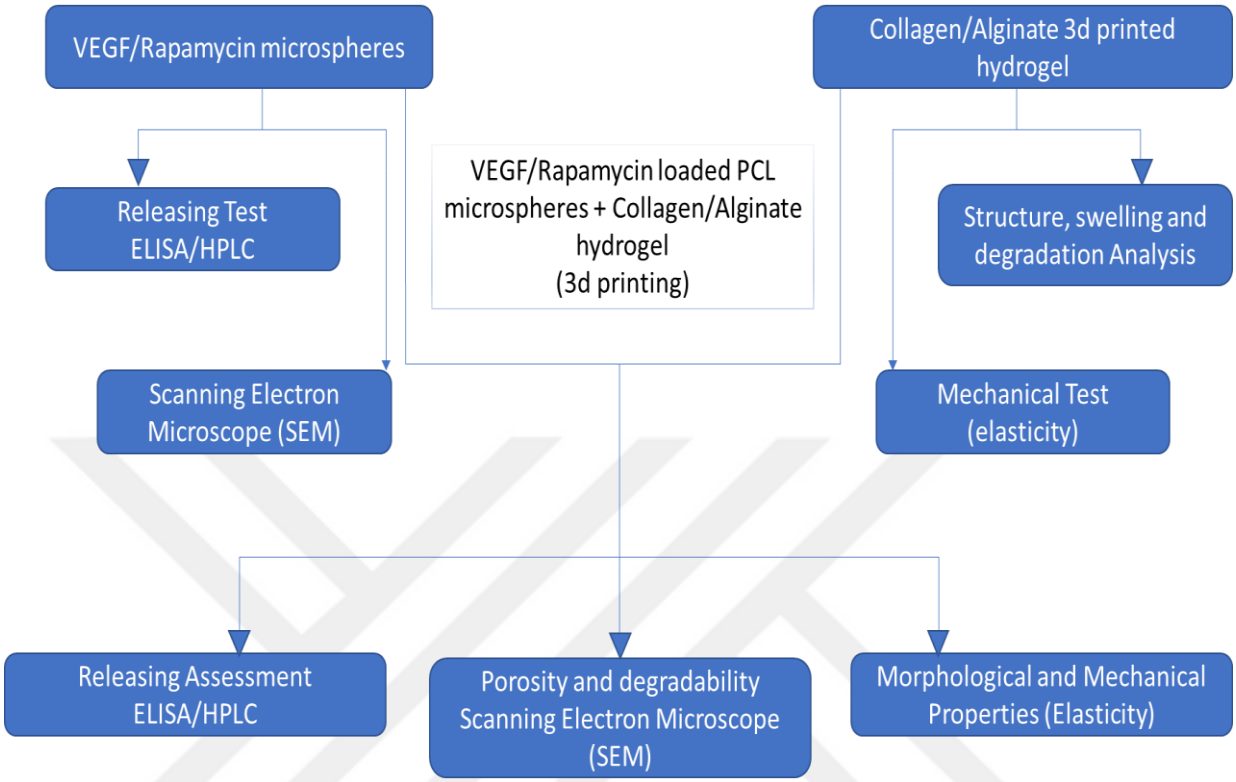

Figure 1.16. Schematic representation of the research strategy used for this study.

4. MATERIALS AND METHODS

4.1. MATERIALS

4.1.1. Chemicals

Polycaprolactone (PCL) (Capa^{R}, 6400 UK), polyvinyl alcohol (PVA) (ALDRICH, MW = 125.000), Dichloromethane (Sigma Aldrich), High purity collagen (AR-GE Merkezi, Turkiye), Medium Viscosity Sodium Alginate (Sigma-Aldrich), Methyl Cellulose (Vegrano, Turkiye), CaCl2, Vascular Endothelial Growth Factor VEGF (sigma), Rapamycin (Bioshop, Canada), Phosphate Buffer Saline (PBS), Penicillin/Streptomycin (Gibco), ELISA (Elabscience Human VEGF-A Elisa kit), polyvinyl alcohol (PVA) MW 1.5.104 (Fluka, USA). Lipase (Sigma Aldrich), Distilled water from the Lab, Dulbecco's Modified Eagle Medium (DMEM) (Gibco).

4.1.2. Equipment

Ultrasonic homogenizer system (BANDELIN SONOPLUS), magnetic stirrer (IKA RH digital), centrifuge Thermos scientific SL 8R), various volume automatic pipettes, 0.21 mm 3d bioprinter needle, universal testing machine (SHIMADZU), scanning electron microscope SEM (LEO 438 VP), spectrophotometer, digital calliper, Analytical balance.

4.2. METHODS

4.2.1. Microsphere fabrication

4.2.1.1 Preparation of Polyvinyl alcohol (PVA) solutions

Two concentrations (4% in 8 ml ddH_2O, and 0.3% in 200 ml ddH_2O) of Polyvinyl alcohol (PVA) solution prepared. The solutions were heated 120^oC and stirred vigorously 450 rpm for 5 hours until all the PVA was dissolved in the water.

4.2.1.2. VEGF/Rapamycin-Loaded Polycaprolactone (PCL) Microcapsule

PCL microcapsules were produced using the double emulsion method. Briefly, the first emulsion (W/O) was formed of 10% w/v PCL + Dichloromethane solution (77.3 mg PCL +761 µl DCM). (0.5mg Rapamycin in 0.1mL 0.5 µg VEGF) were added, separately, into the PCL/DCM solution, sonicated at 50 Hz frequency for 1 min. The first emulsion was added to the solution of polyvinyl alcohol (4% w/v PVA) and again sonicated at 50 Hz frequency. Afterwards, the (w/o/w) solution added to 50 ml of an aqueous solution of 0.3% PVA (w/v), and stirred at room temperature, 250 rpm, overnight so as to evaporate the organic solvent DCM. Next day, Microcapsules collected by centrifugation, and lyophilized after washing with Tris-HCL. The produced microcapsules were stored at -20°C until the release analysis of bioactive agents from microcapsules.

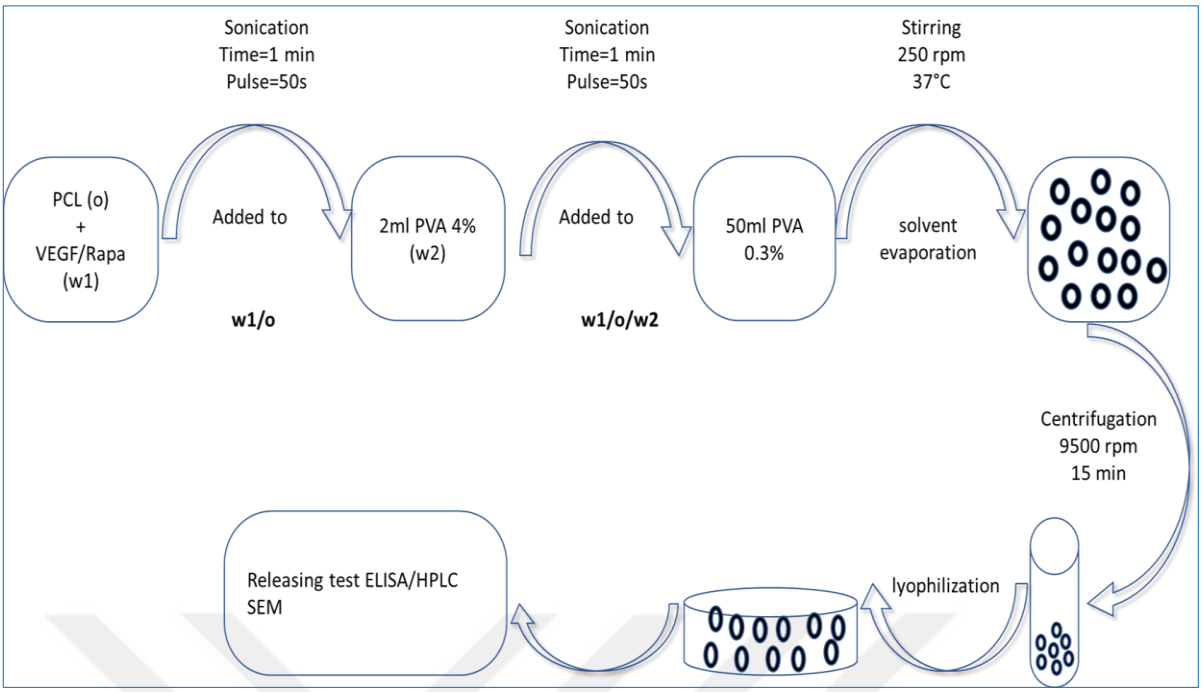

Figure 4.1. Schematic representation of the double emulsion microsphere fabrication used for this study.

4.2.2. 3D printing of hydrogel scaffold

4.2.2.1. Printing ink preparation

A combination of three distinct polymer biomaterials were used as bioprinting bioinks in this study: collagen, alginate, methyl cellulose. Four bioink solutions were prepared:

1- Solution (1): Collagen (0.82 mg/mL), Alginate (2% w/v).

2- Solution (2): Collagen (0.82 mg/mL), Alginate (1% w/v), Methyl Cellulose (4%w/v).

3- Solution (3): Collagen (0.82 mg/mL), Alginate (4% w/v), Methyl Cellulose (2%w/v).

4- Solution (4): Collagen (0.82 mg/mL), Alginate (4% w/v), Methyl Cellulose (2%w/v), Rapamycin (1, 0.5, 0.1 mg)

4.2.2.2. 3D printing

The hydrogel structures were printed using a bioprinter that was controlled by temperature and pressure. Printing parameters for all solutions were set as shown in table 4.1.

4.2.2.2.1. Collagen-Alginate bioprinting (S1)

The hydrogel solution was loaded into extrusion cartridges, which were subsequently attached to the printer's carriage, and extruded through a 0.4 mm needle. The scaffolds were designed as a rectangle (4*2) cm in size with 3D-Builder software, and then 5 layers of hydrogel were made. Parameters of the printing process were as follow: (Speed = (6mm/s), Layer Height = (0.4mm), Pressure = (10psi), Temperature = (14^{o}C)). The samples were then crosslinked by adding 500 µL of CaCl2 (0.5M) to the printed scaffold (immediately after printing) 5 minutes.

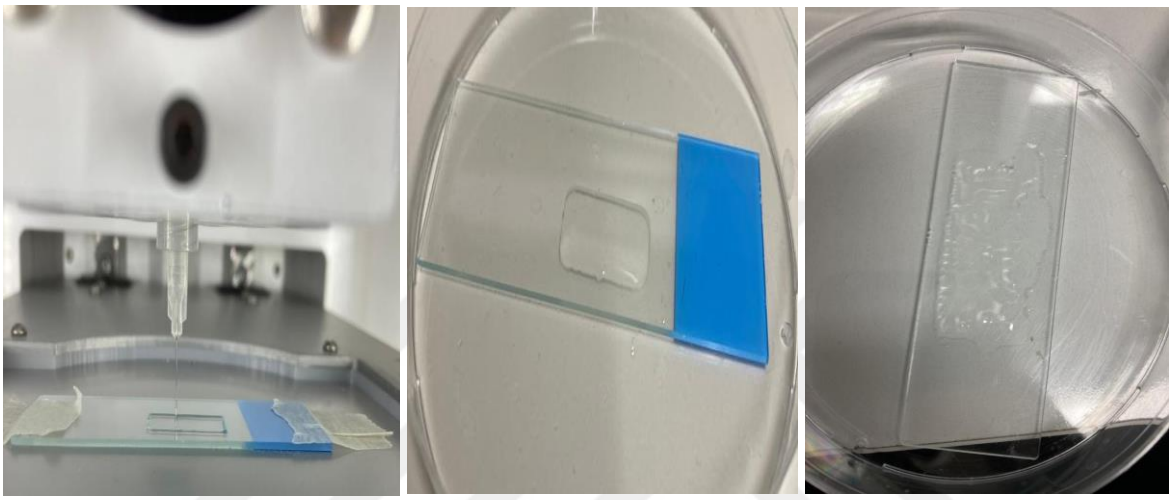

Figure 4. 2. Photographs during printing and crosslinking of C/ALG scaffolds

4.2.2.2.2. Collagen-Alginate-Methyl cellulose bioprinting (S2, S3 & S4)

The hydrogel solution was loaded into extrusion cartridges, which were subsequently attached to the printer's carriage, and extruded through a 0.21 mm needle. The scaffolds were designed as a rectangle (4*2) and (1*1) cm in size with 3D-Builder software, and then 5 layers of hydrogel were made. Parameters of the bioprinting process (Speed = (6mm/s), Layer Height = (0.21mm), Pressure = (10psi), Temperature = (7^{o}C)). The samples were then crosslinked by adding 500 µL of CaCl2 (0.5M) to the printed scaffold (immediately after printing) 5 minutes.

Solution (4) was prepared using the parameters outlined in Table 4.1, with the exception that it included three distinct concentrations of Rapamycin (1, 0.5, 0.1 mg).

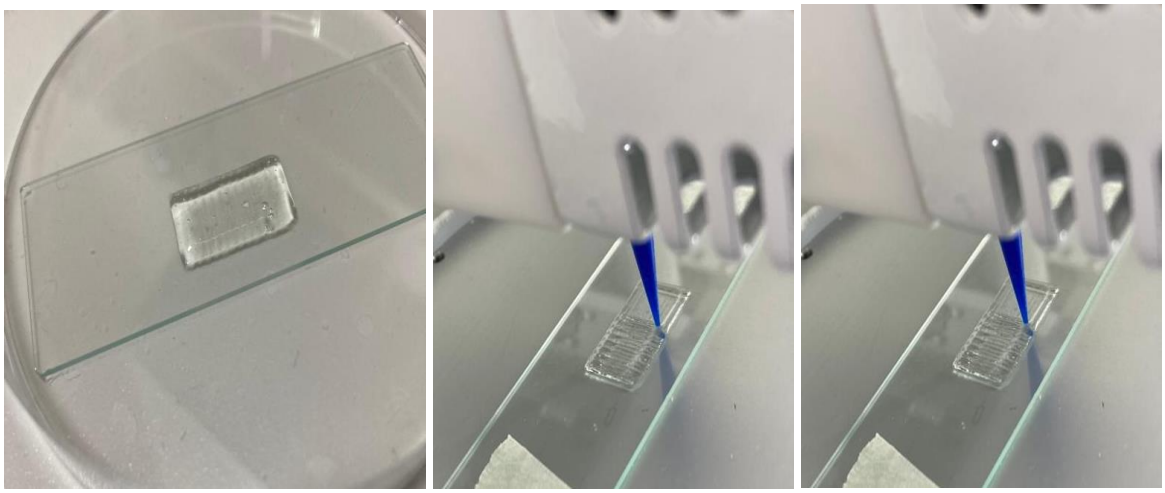

Figure 4.3. Photographs during printing and crosslinking of C/ALG/MC scaffolds

Table 4.1. Summary of hydrogel 3D printing parameters

	Solution (1)	**Solution (2)**	**Solution (3&4)**
Speed (mm/s)	6	6	6
Layer Height (mm)	0.21	0.4	0.25
Pressure (psi)	10	10	10
Temperature (°C)	7	14	14
Infill density (%)	30	30	30
Size (mm)	4*2	4*2	1*1

4.2.3. Characterization of samples

4.2.3.1. PCL Microcapsules morphological properties

Morphological properties were evaluated with a scanning electron microscope, in Ankara University Faculty of Medicine, Department of Histology-Embryology. PCL samples collected after lyophilization were placed on double-sided carbon tapes adhered to SEM

sample holders. The samples, which were attached to the stall, were then kept under vacuum in the Sputter Coater device for 10 minutes, and immediately after the 20 nm gold plating application, they were visualized with the LEO 438 VP brand SEM device at 20 kV.

4.2.3.2. Releasing test of VEGF and Rapamycin

For this, microcapsules and hydrogels loaded with bioactive molecules (VEGF &Rapa) were taken into Eppendorf tubes (5 mg) and 6 culture well plate respectively and incubated in 1 ml of PBS (Phosphate Buffered Saline; pH 7.4) at 37^{o}C. At various times (7, 14, 21, 28 days) the supernatant was collected, and incubation was continued by adding fresh PBS. Encapsulation efficiencies and release kinetics of the PCL microcapsules were studied with ELISA kit for VEGF, and HPLC for rapamycin.

4.2.3.3. ELISA VEGF releasing

Determination of VEGF in the supernatant was performed using the growth factor ELISA kit. Briefly, VEGF standards and sample's supernatants were placed on the microplate coated with VEGF monoclonal antibody included in the kit for VEGF determination. The microplate was incubated for 90 minutes for VEGF to bind with the coated antibody on the plate. Biotinylated Detection solution was added to the wells to bind with the second antibody, then the microplate was incubated for 60 minutes. Then HRP added to the wells and incubated for 30 min. The plate was washed 5 times with washing buffer, then substrate solution was added to the wells and incubated for 15 min. After incubation, colour change was detected with a microplate reader at a wavelength of 475 nm. Calibration curve and sample concentrations calculated using excel.

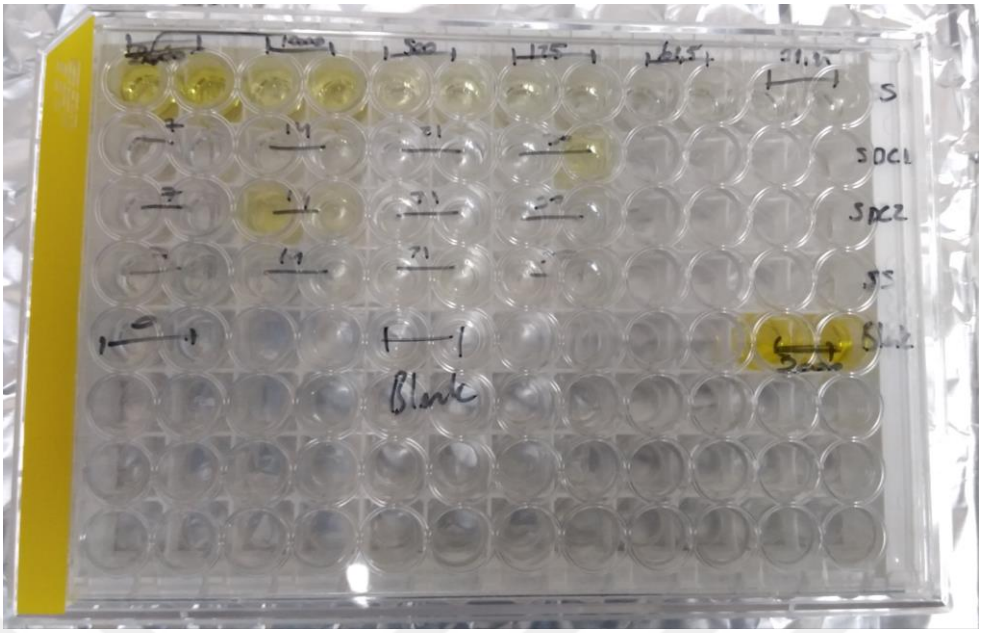

Figure 4.4. 96-well microplate image of VEGF-A release assay with ELISA kit

4.2.3.4. Rapamycin HPLC releasing test

The analysis was conducted using an analytical Knauer ODS column of dimension 4.6 x 150 mm size of 5 mm. The temperature within the column was established at 55°C. The solvent system employed a mobile phase consisting of 70% methanol. A volume of 10 µl of rapamycin was dissolved in 1 ml dichloromethane (DCM), and subsequently administered into the apparatus. The isocratic flow rate was set at 1 ml/min. rapamycin detected by UV absorption 278 nm. The detection of rapamycin was achieved through the utilization of UV absorption at a wavelength of 278 nm.

4.2.4. Mechanical Testing of C/ALG and C/ALG/MC Scaffolds

The mechanical properties of C/ALG and C/ALG/MC scaffolds were tested with SHIMADZU material testing device (AGS – X). For uniaxial tensile testing, each scaffold is mounted on the machine's clamps with a 50 N load cell. The original length (L0) and thickness were measured using digital calliper. Then, the tensile test was started, and the scaffolds were stretched at a stretching speed of 5 mm/min at room temperature until the specimen yield point. Finally, the modulus was calculated from the initial linear regions of the stress-strain curves.

Figure 4.5. Photographs taken during tensile testing with 50N Heads for printed C-ALG scaffolds.

4.2.5. Collagen-Alginate-Methyl cellulose hydrogel degradation test

For degradation test, the hydrogels were weighed (W0) and then were incubated in 1 mL sterile PBS buffer and lipase enzyme seperatly at 37 °C. At predetermined time scheduled (1, 3, 7, 14, 21 days) the hydrogels were taken out of solutions and put in 20°C. after taking all the samples out of solutions, freez drying was conducted and the samples were weighed (Wt). Three parallel hydrogels were performed to obtain averaged values. The degradation behavior of the hydrogels was expressed according to the following equation:

$$\text{Weight loss } (\%) = (W0 - Wt)/W0 \times 100\%$$

W0 = initial weight, Wt = weight after freeze-dry

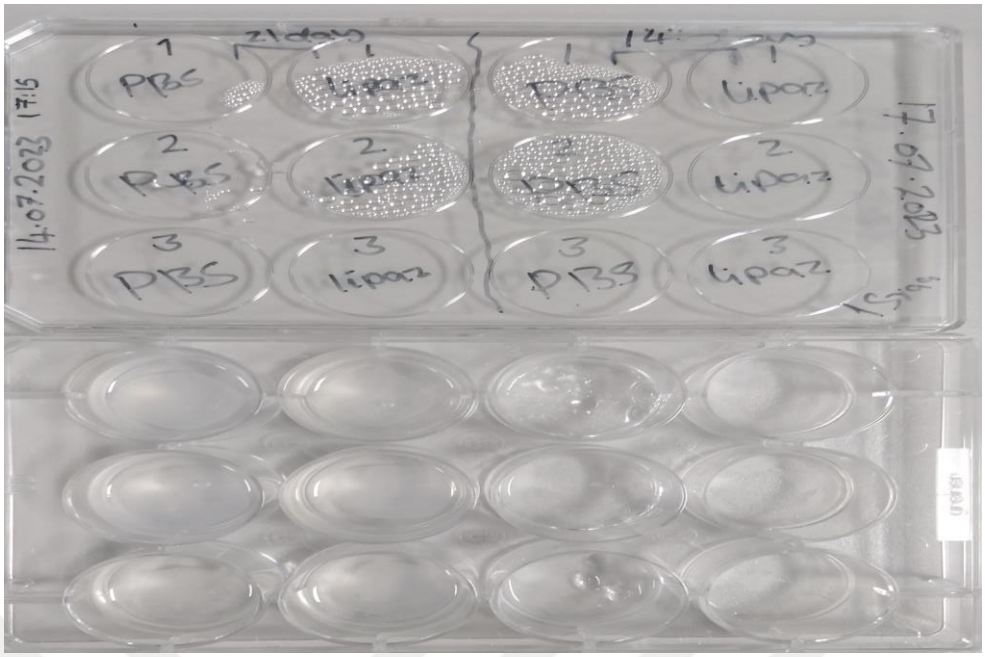

Figure 4.6. Collagen/Alginate/Methyl cellulose degradation test

4.2.6. Hydrogel scaffold's structure stability test

After 3D printing, all samples were incubated in 0.5 M CaCl2 solution for 10-15 mins at 37^{o}C for polymers cross-linking and then made ready for use. The samples prepared by 3D printing were incubated in cell culture medium prepared with DMEM Low Glucose + 1% Penicillin/Streptomycin for 21 days under sterile conditions in order to evaluate the long-term durability in cell culture medium. Structural integrity assessments of the constructs were performed observationally on days 7, 14 and 21.

5. RESULTS

5.1. CHARACTERIZATION OF PCL-MICROCAPSULES MORPHOLOGY AND PARTICLE SIZE

The morphology of PCL-microcapsules has been characterised through scanning electron microscopy (SEM) analysis. It was determined that the PCL-microparticles loaded VEGF/Rapamycin, separately, were spherical in shape and had a homogeneous size distribution. Similar findings were obtained about the morphology of PCL-microcapsules loaded VEGF/Rapamycin.

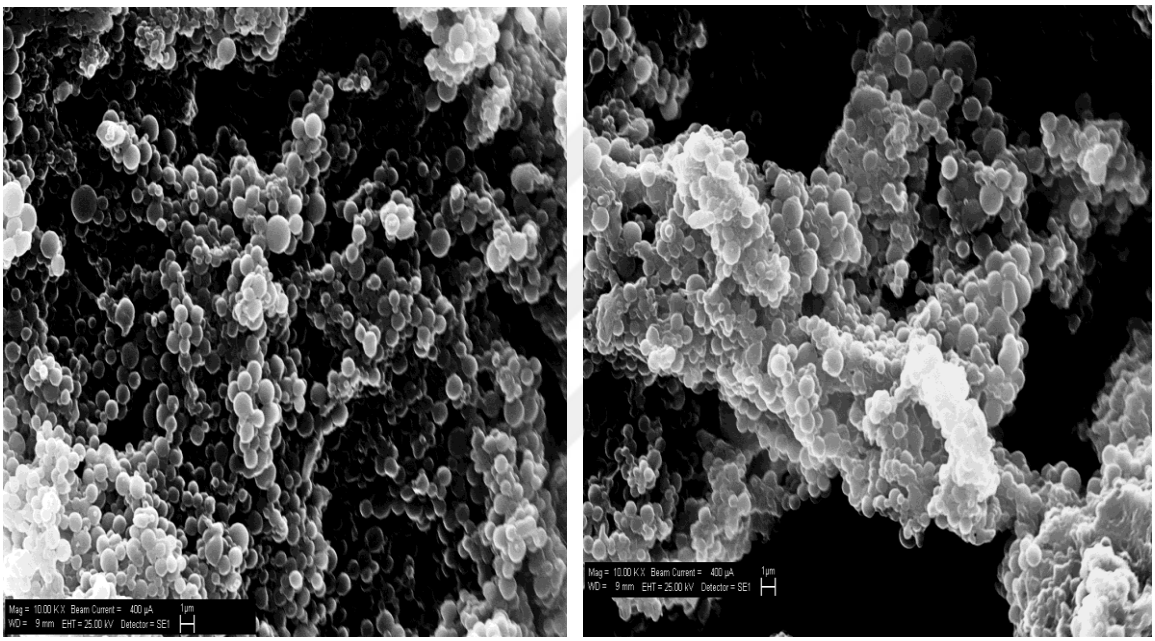

Figure 5.1. Representative SEM pictures of PCL microspheres (1000x magnification)

5.2. IN VITRO VEGF/RAPAMYCIN RELEASING KINETICS

To investigate whether PCL microcapsules and hydrogels could prolong release of VEGF/Rapamycin, several release experiments were performed.

5.2.1. VEGF ELISA Releasing test

Free VEGF concentrations at multiple time points were determined with ELISA. The accumulative release profiles of gradually and fast groups are shown. VEGF release was prolonged and more gradual in the slow-release group in comparison with the fast release

group. VEGF was constantly released for at least 2 weeks at concentrations relevant for vasculogenesis (The ED50 of VEGF is typically 1–6 ng/ml) (72).

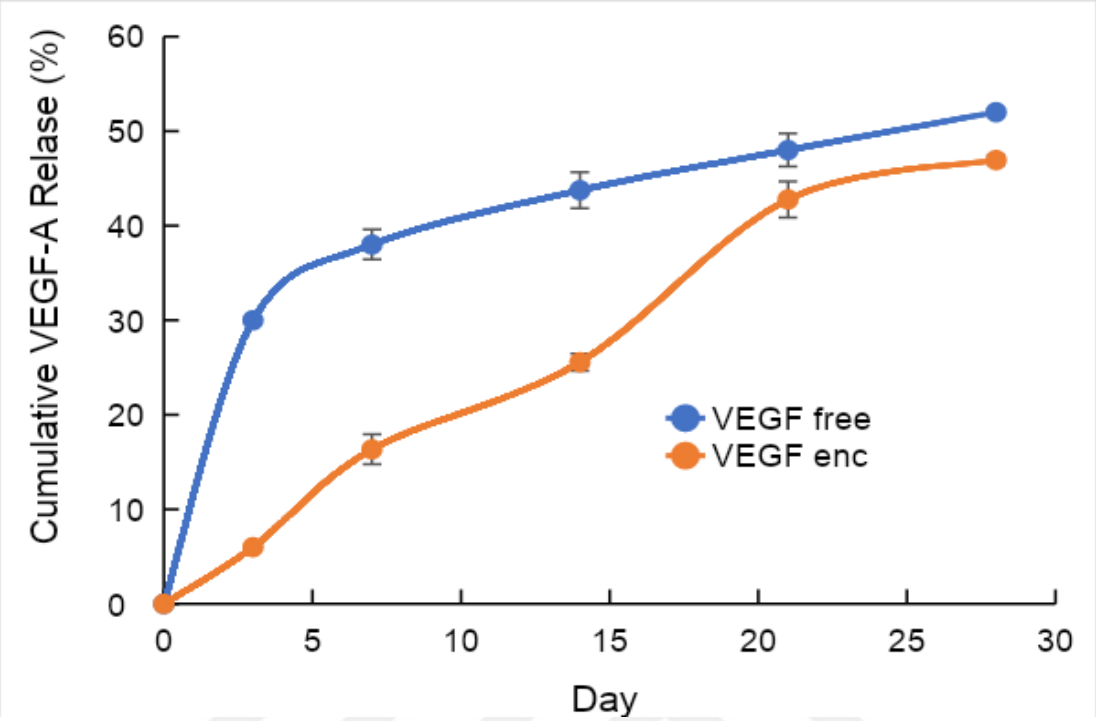

Figure 5.2. Determination of VEGF release from VEGF-loaded PCL microparticles in free and encapsulated with 3D-printed C/ALG/MC scaffold.

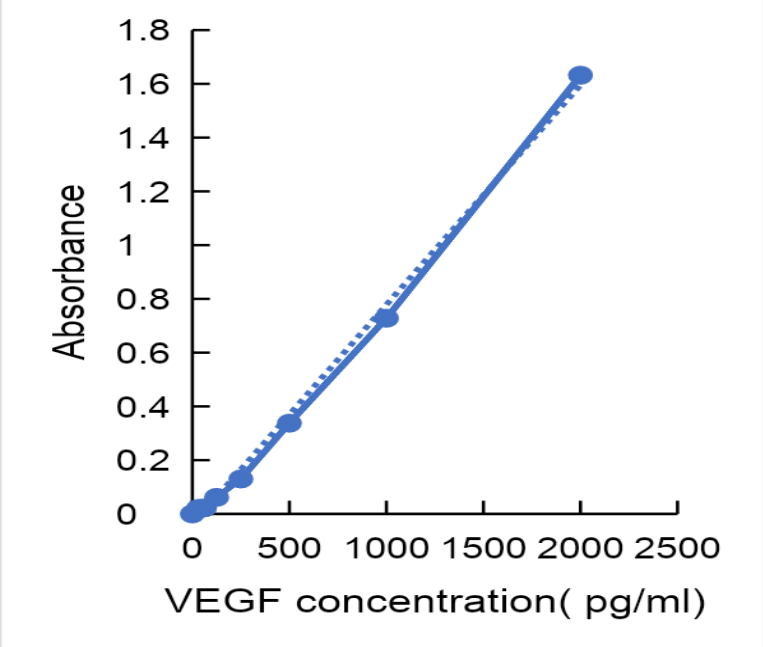

Figure 5.3. Calibration curve created for quantitative determination with the ELISA assay.

5.2.2. Rapamycin HPLC releasing test.

The rapamycin release test for encapsulated microparticles and hydrogels were conducted separately. No peak detected when analysing with HPLC Fig 5.4.

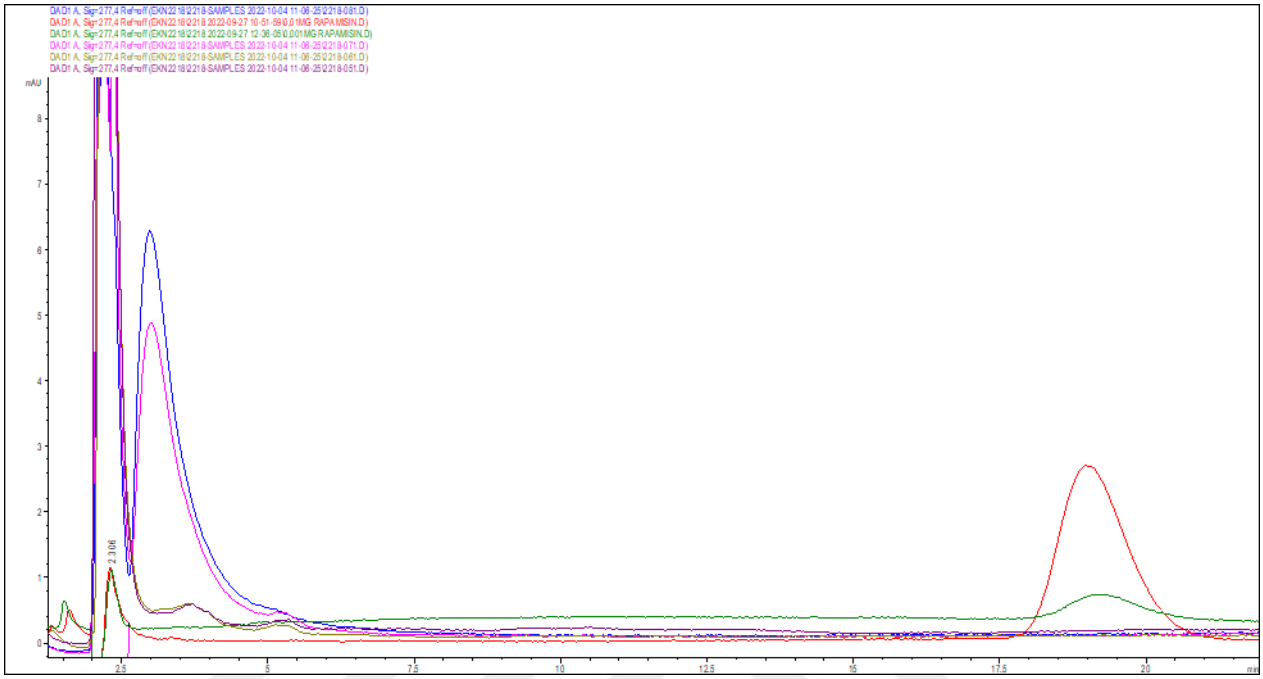

Figure 5. 4. Rapamycin loaded samples HPLC analysis.

5.3. 3D PRINTED SCAFFOLD MECHANICAL PROPERTIES

The mechanical properties of C/ALG and C/ALG/MC scaffolds were characterized by tensile test. The mean curve of the C/ALG scaffold (Figure 5.5.) showed a peak stress of 0.0136 Mpa and an average Young's modulus of 0.2417 Mpa. The addition of Mythl Cellulose to the scaffold reduced the Young's modulus to an average of 0.1407 Mpa while reducing the ultimate tensile strength to 0.0130 Mpa (Figure 5.6.). The mechanical properties of C/ALG scaffolds are significantly increased when C/AlG is pressed at low temperature 7ºC compared to C/ALG/MC which is 14ºC.

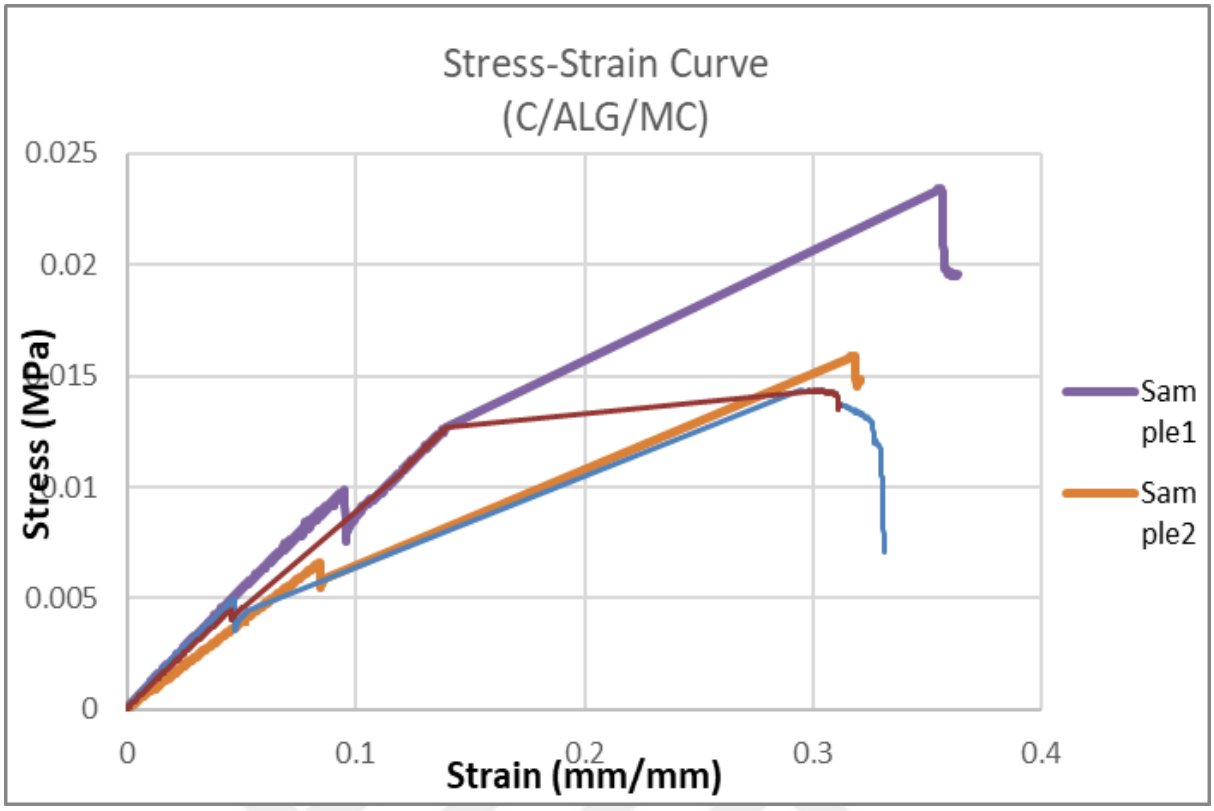

Figure 5.5. Stress-strain curve of collagen-alginate-methylcellulose scaffold

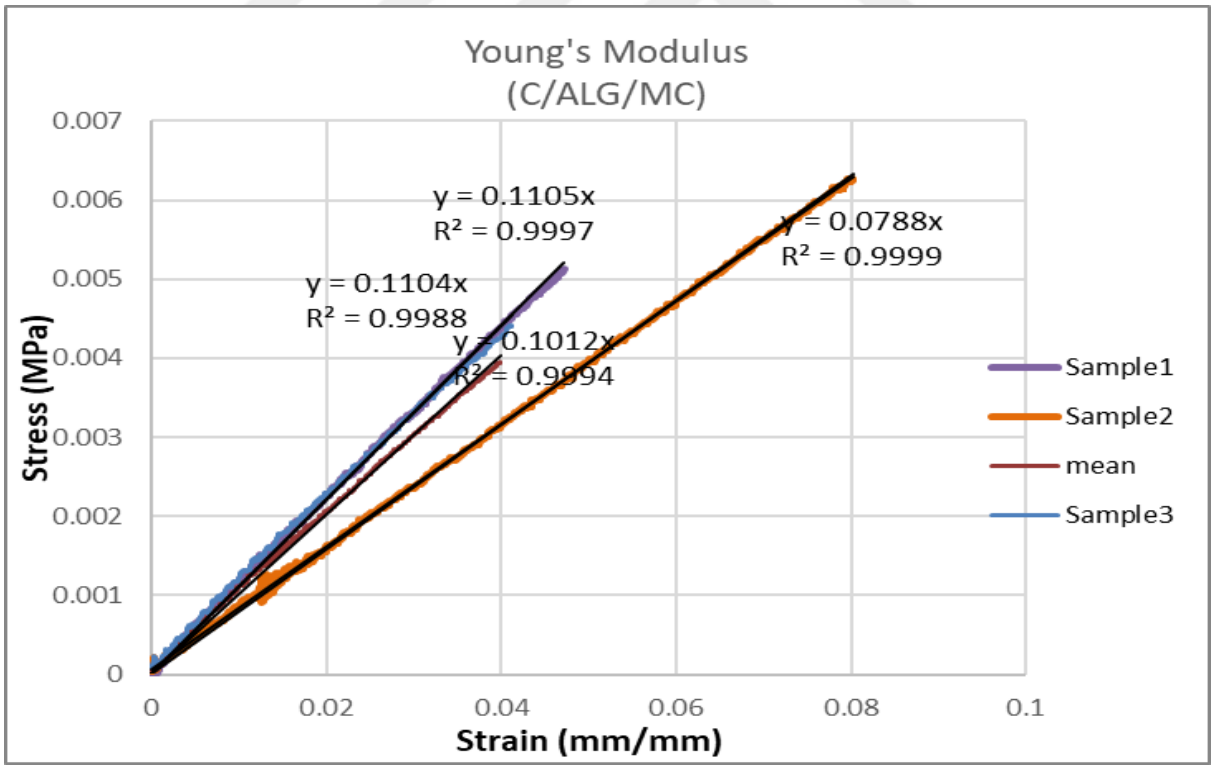

Figure 5.6. Elastic Modulus Measurement of collagen-alginate-methylcellulose scaffold

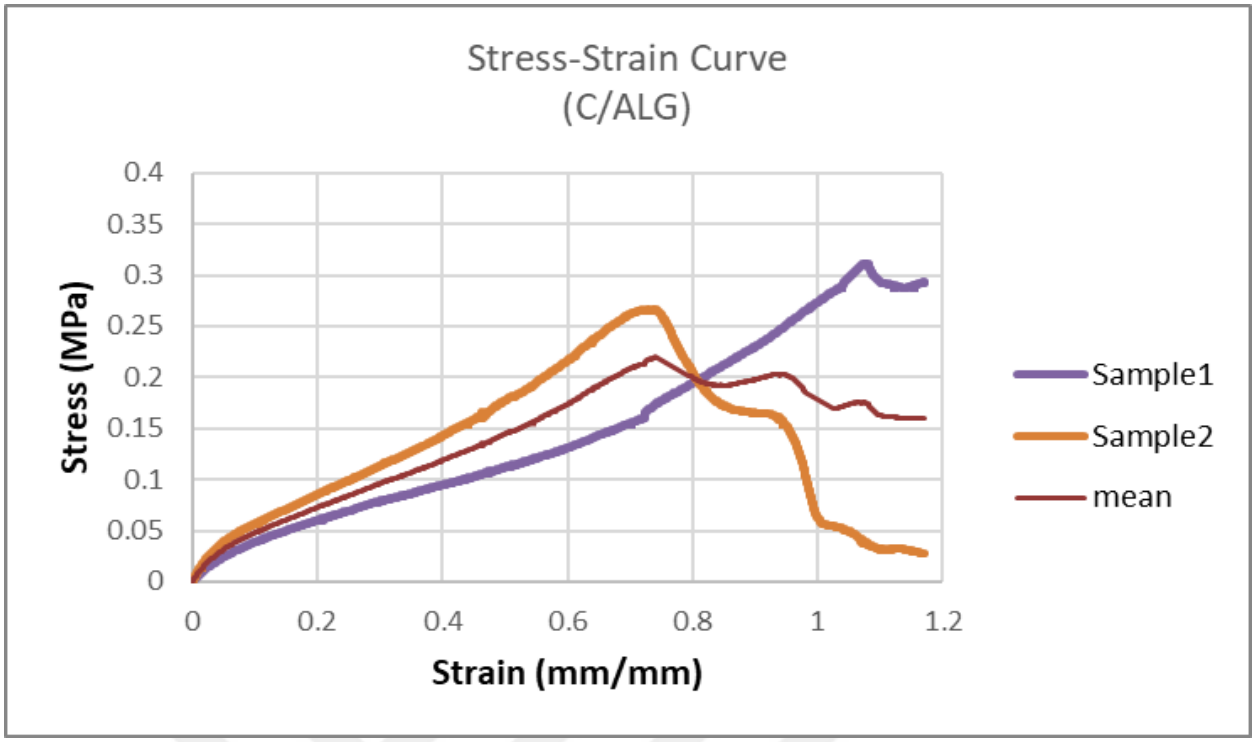

Figure 5.7. Stress-strain curve of collagen-alginate scaffold

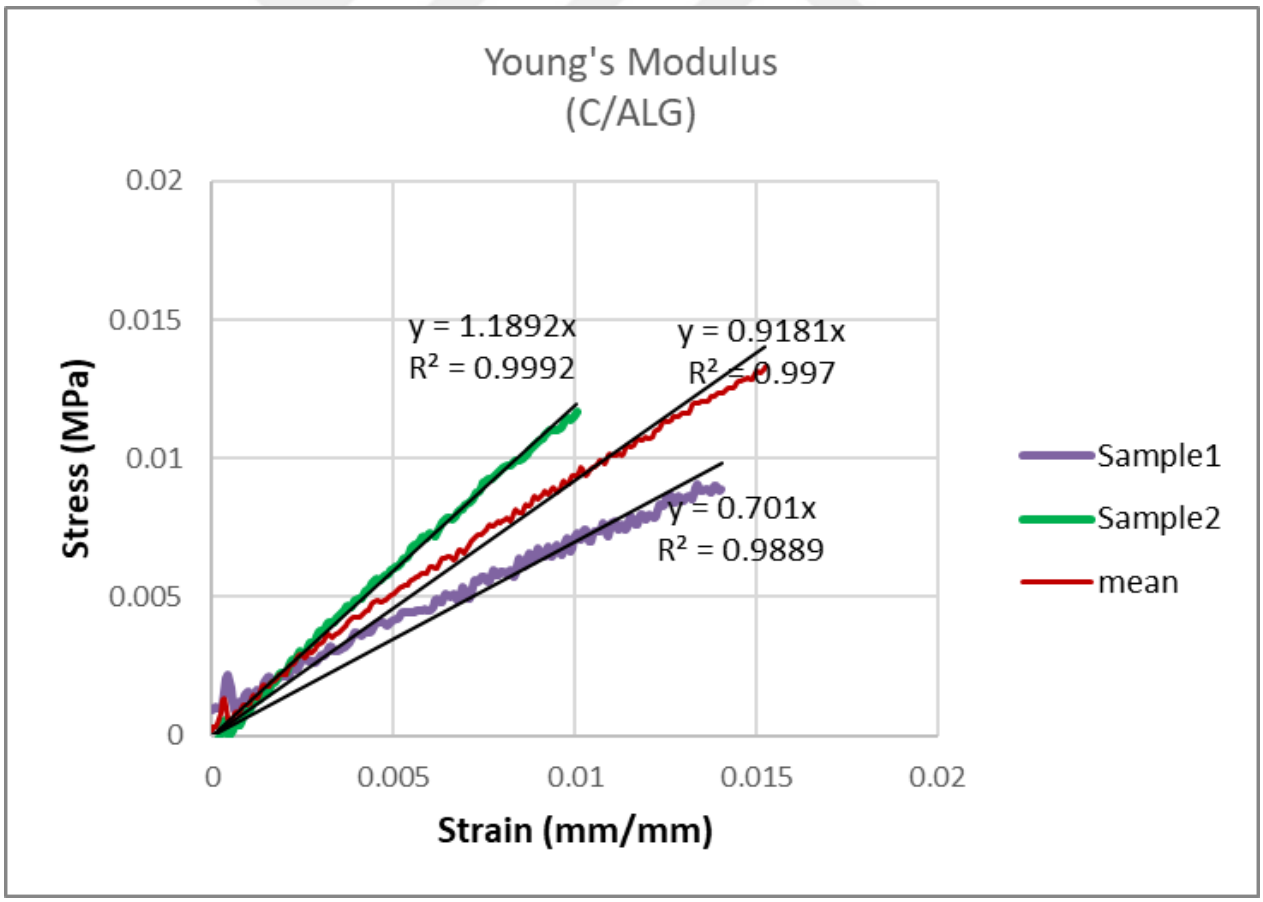

Figure 5.8. Elastic Modulus Measurement of collagen-alginate scaffold

5.3. HYDROGELS DEGRADABILITY PROPERTIES

The degradation rate of hydrogels with different alginate concentrations (2% and 4% w/v) was examined by maintaining hydrogels in sterile PBS and Lipase separately for 21 days Fig 5.9. and Fig 5.10. No obvious hydrogel shrinkage and degradation was observed with 4%Alg, while Shrinkage and degradation of 2% Alg hydrogels was observed.

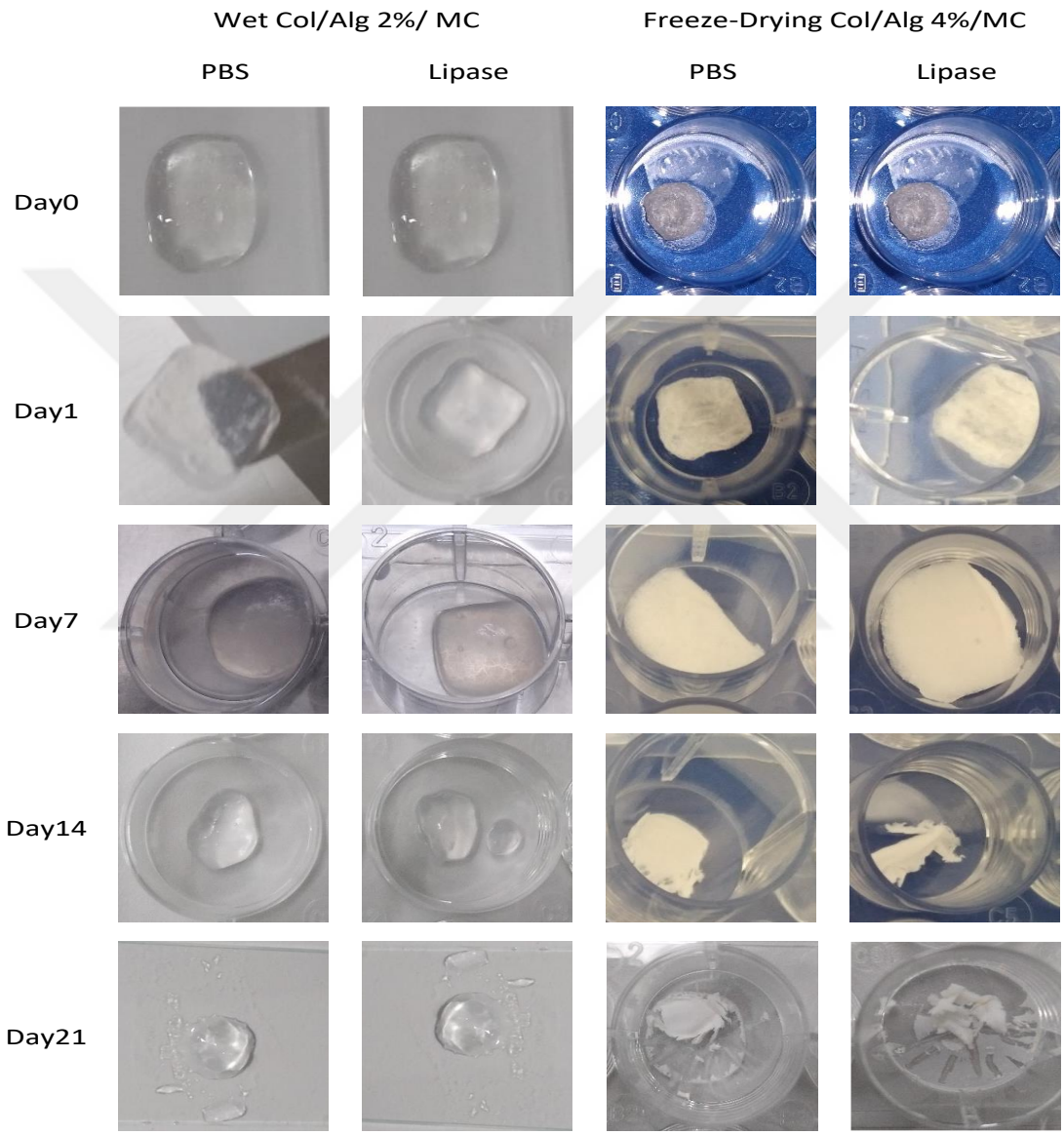

Figure 5.9. Collagen/2%Alginate/Methyl Cellulose hydrogel before and after soaking in PBS and Lipase for the period of 21 days.

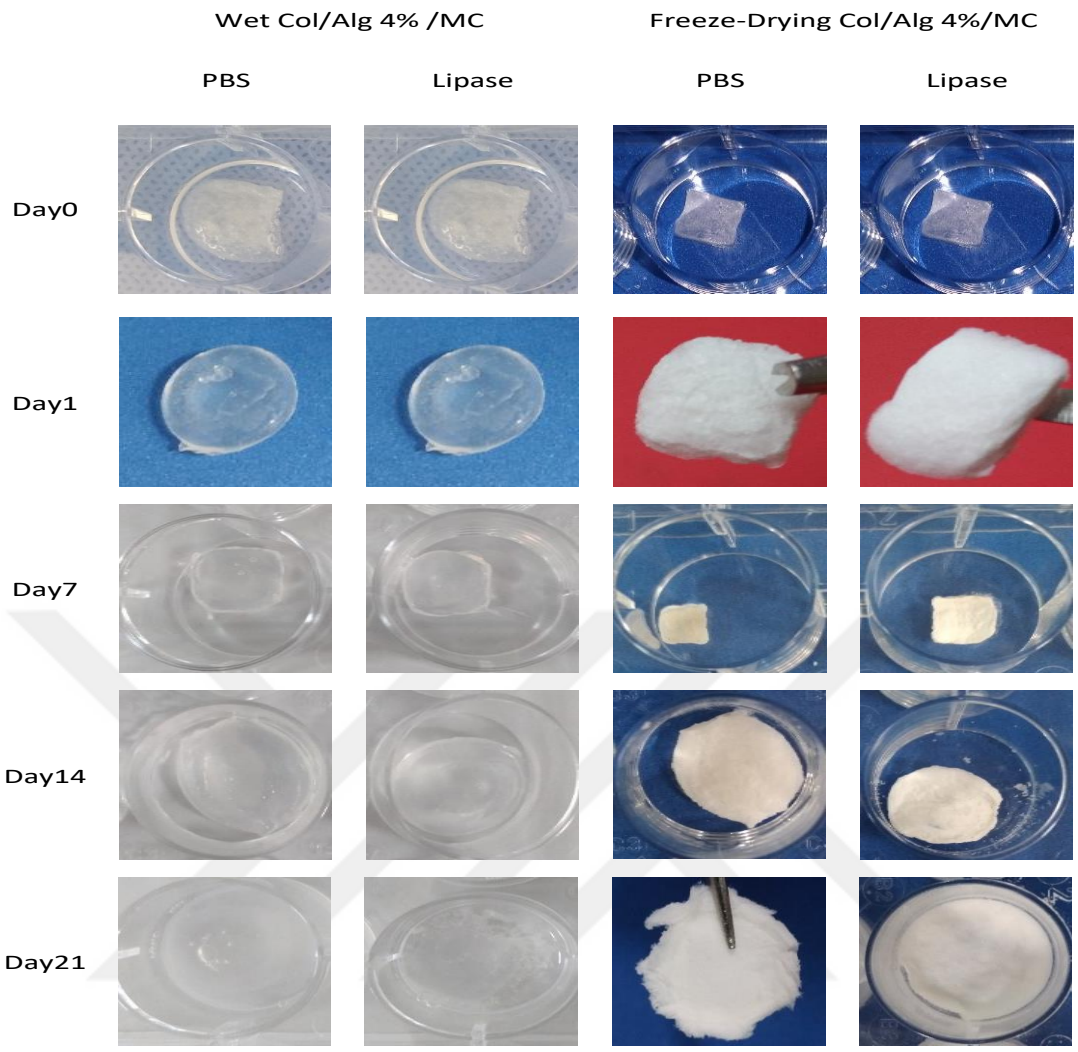

Figure 5. 10. Collagen/4%Alginate/Methyl Cellulose hydrogel before and after soaking in PBS and Lipase for the period of 21 days.

Since the delivery of the bioactive agent from the microspheres depends on the degradation of the hydrogels, their degradation was investigated in vitro up to 21 days. The PBS and enzymatic in vitro degradation of Col/2%Alg/MC hydrogel samples were assessed by measuring the percentage of weight loss from the hydrogels Fig .5.11. Col/2%Alg/MC hydrogels show gradual degradation in proportion to immersion time. Hydrogels soaked in PBS showed greater degradation ratio in compare with hydrogels soaked in Lipase. The in vitro degradation of the Col/2%Alg/MC hydrogels was also followed by scanning electron microscope (SEM) to check the loss of polymer integrity. It was observed that at the end of the incubation period, 2% alginate hydrogel structure had completely degraded.

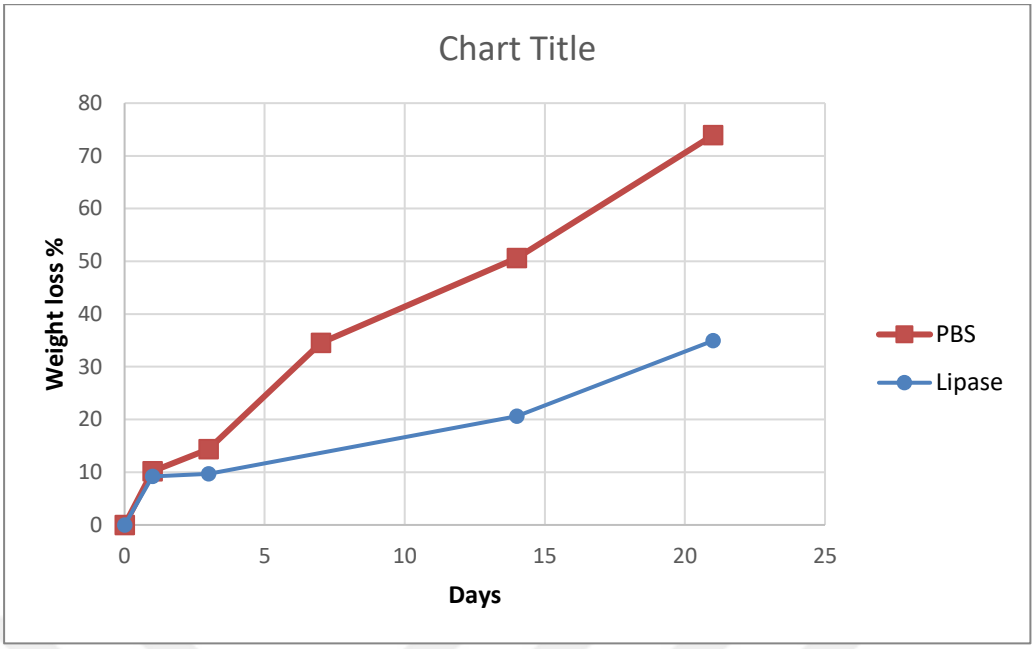

Figure 5. 11. Degradation ratios of Collagen/2%Alginate/Methyl Cellulose hydrogels after soaking in PBS and Lipase for the period of 21 days

5.4. HYDROGEL STRUCTURE STABILITY PROPERTIES

The stability of the collagen alginate hydrogels was evaluated in PBS and cell culture medium to detect their stability for use in cell or tissue culture experiments later. However, it was found that the printing solution (Col/1%ALG) used in the preliminary study was able to maintain its 3D structure for only 3-4 days after 3D printing and then started to lose its 3D structure in cell culture medium as shown in figure 5.11. To overcome this problem, two ways were followed: Firstly, the concentration of ALG (1% w/v) used in the preliminary study was increased to (2&4%w/v). Secondly, Methyl cellulose (MC), a material with high viscosity required for 3D printability and gelling at 37°C, was added to the printing solution.

MC was incorporated into the C/ALG structure at a concentration of 4% w/v. In this way, it was aimed both to increase the print quality during 3D printing and to maintain the structural integrity of the 3D structure in long-term culture. The scaffolds obtained after increasing the ALG concentration to (2&4%) and the addition of MC were found to be stable FBS, and cell culture medium for more than 21 days as shown in figure 5.11.

Figure 5. 12. 3D printed hydrogels produced for integrity analysis of Col/ALG (C: 0.82 mg/mL; ALG 1% w/v).

Figure 5. 13. 3D printed hydrogels produced for integrity analysis of Col/ALG (C: 0.82 mg/mL; ALG 2% w/v; MC 4% w/v).

6. DISCUSSION AND CONCLUSIONS

It is aimed that the tissue scaffold to be produced will be porous in a way to induce angiogenesis of the organ/tissue graft and have the feature to realize the controlled release of VEGF/rapamycin. It is planned that the hydrogel 3D scaffold contains poly (caprolactone) (PCL) microparticles containing active ingredients to produce collagen-based porous tissue scaffold with 3D printing technique and to provide simultaneous VEGF/rapamycin-controlled release.

6.1. VEGF/RAPAMYCIN LOADED PCL MICROSPHERES

In this study we have developed VEGF loaded PCL microparticles, this polymer was selected based on previous work of Karina et al. who achieved a sustained release of VEGF for 4 weeks (73). The release pattern of the VEGF Microspheres was evaluated through a comprehensive screening procedure spanning a period of three weeks. The time-based aspect is significant in the vascularization process after biomaterial implantation, as it plays an essential role in deciding the pattern of growth factor release in vivo (74). The Enzyme-Linked Immunosorbent Assay (ELISA) technology was utilised to measure the amount of Vascular Endothelial Growth Factor (VEGF) released. The acquired data was subsequently represented graphically as normalised cumulative values of Vascular Endothelial Growth Factor (VEGF). The comprehensive release of the encapsulated VEGF is represented by the overall cumulative release observed over a period of 21 days. The microspheres exhibited a consistent release profile characterised by no significant initial burst release. The chosen formulations were considered suitable as they effectively demonstrated sustained release for several weeks, these formulations were suitable for our purposes.

Various procedures have been employed thus far for the fabrication of polymer microspheres with a diameter less than 1000 mm. The double emulsion technique is considered a good approach for the encapsulation of pharmaceutical ingredients(75). The methodology utilised in this study involved the formulation of PCL microspheres loaded with VEGF while considering the hydrophilic properties of VEGF. The results demonstrated that the encapsulation of (VEGF) occurs within the inner aqueous phase (w1) of the double emulsion layers, this step serves to prevent the diffusion of VEGF into the external aqueous phase (w2). To enhance the biological performance of vascular endothelial growth factor (VEGF),

a supplementary concentration of 0.3% polyvinyl alcohol (PVA) was utilised. This was done to facilitate the formation of a protective coating around the VEGF particles.

The addition of a stabiliser to the external aqueous phase is crucial for achieving the desired formation of well-defined spherical with smooth surface microspheres during the emulsification process (76), Polyvinyl alcohol (PVA) was selected as a stabilizer for the purpose of creating the aqueous phase. due to its low toxic effects, favourable solubility in water, and being accessible in various molecular weights. Additionally, PVA is known for its biocompatibility and capacity to undergo biodegradation. The major function of the stabiliser is to inhibit the coagulation of microspheres during the process of emulsification, hence improving the stability of the primary emulsion and facilitating the encapsulation of molecules (77).

Scanning electron microscopy (SEM) analysis (figure) was applied to study morphology, surface state, and size of the microspheres. Spherical and polydisperse particles having a smooth surface in diameters of length between 900 and 2000 nm were obtained. The micrographs do not show any pores on microspheres (Figure 5.1).

The rapamycin loaded microspheres, which were fabricated using the aforementioned technique of double emulsion, exhibited an unfortunate outcome during the analysis of release kinetics using HPLC. It was observed that no peak was detected. Therefore, we hypothesised that the insufficient dosage of rapamycin may have been the underlying cause. We have made the decision to increase the concentration of the dose. However, prior to implementing this change, it is necessary to determine the optimal dose that will effectively achieve our intended objective. A study has determined that the administration of 1mg/kg of rapamycin intravenously could effectively inhibit the mTOR pathway(78). Unfortunately, the suppression of mTOR pathway is linked to severe implications, including dyslipidaemia and hyperglycaemia. These effects arise from the nonspecific biodistribution and high doses that occurs following the systemic administration of Rapamycin(79). In the current study, three different doses (0.1, 0.5, and 1 mg) of Rapamycin incorporated into the Col/Alg printing solution and (1*1) mm rectangle scaffolds were printed. The releasing kinetics of Rapa from those scaffolds studied by HPLC and the no peak was detected.

6.2. COLLAGEN/ALGINATE/METHYL CELLULOSE HYDROGEL SCAFFOLDS

Since collagen is the major extracellular matrix (ECM) protein of connective tissue, it was chosen as the material from which the scaffold to be used as a carrier will be produced, however collagen bioprinting has mostly been limited to the extrusion 3D printing due to low viscosity and slow polymerization, the thing led to form weak collagen hydrogels. However, in one study, a 3D hydrogel scaffold was fabricated from a bioink compromised of collagen/methyl cellulose and showed greater capacity of high stability and performance using extrusion-based 3D bioprinters. In this direction, a 3D printed tissue scaffolds with stable structure were obtained by using collagen/alginate/methyl cellulose bioink and CaCl2 as crosslinker in the preliminary study carried out based on the examples in the literature in the production of collagen-based tissue scaffolds with 3D printing. Collagen is stiffer and more resistant to deformation, while sodium alginate hydrogels degrade quickly because of their high water-absorbing capacity. Hydrogels generated in this way can successfully achieve locally sustained drug release by retaining therapeutic medicines at target areas for extended periods of time (80, 81).

A rapid breakdown of the hydrogel was seen within the 14-day when the content of Alginate Alg was 2% Fig 5.9. This phenomenon was notably demonstrated through the enhanced efficiency and swiftness of the hydrolysis degradation of alginate by PBS. The rate of alginate degradation is influenced by various factors, including the methods employed for gelation, and the specific crosslinking strategy utilised. Calcium has been frequently employed in the process of alginate gelation. To improve the printability and extend the degradation time of the hydrogel, the concentration of alginate was raised to 4%. Additionally, increasing the concentration of Alg led to increasing the viscosity of the bioink which make the ink can be extruded smoothly through a nozzle without clogging.

Alginate derivative polymers in reaction with water solutions form hydrogels by divalent bonding (82). Thereby, mechanical features of the Col/Alg hydrogels can be modifying by increasing the crosslinker concentration, exposing time (83), and increasing the concentration of alginate (84). As shown in Fig 5.8. increasing the alginate concentration from 2% to 4% enhanced the gel structural stability for up to 21 days, which make it potential for promoting long term angiogenesis after organ transplantation.

Mechanical tensile testing was conducted directly after printing to prevent hydrogels from drying, which can significantly affect the mechanical behaviour of hydrogels. The tensile strength values of various scaffolds were assessed as shown in Figs. 5.5., 5.6., 5.7. and Fig. 5.8. The mean curve of the printed Col/ALG scaffold demonstrated a peak stress of 0,22 Mpa, and a mean Young's modulus of 0,92 Mpa at a printing temperature of 7°C. Following the addition of Methyl Cellulose to the Col/ALG scaffold, the Young's modulus decreased significantly to a mean of 0,10 Mpa while the ultimate tensile strength decreased to reach 0,014 Mpa at a printing temperature of 14°C.

In order to make sure whether this decrease is due to the addition of Methyl Cellulose or due to the increasing of the printing temperature, molding for both scaffolds (Col/ALG & Col/ALG/MC) was performed under room temperature and under same parameters. After molding, the tensile strength values of the scaffolds were assessed. The mean curve of the Col/ALG scaffold demonstrated a peak stress of 0,0136 Mpa, and a mean Young's modulus of 0,24 Mpa. The results showed that the addition of Methyl Cellulose to the scaffold decreased the Young's modulus to a mean of 0,14 Mpa while reducing the ultimate tensile strength to 0,0130 Mpa. Compared with the printing results, where the mechanical properties of Col/ALG scaffolds were significantly increased when Col/Alg was printed with low temperature (7 Celsius) compared to that of Col/ALG/MC, which was 14 Celsius, molding both hydrogels with the same parameters resulted with approximately similar mechanical properties for both hydrogels.

Therefore, the mechanical properties of the Col/Alg scaffold are unaffected by the addition of Methyl Cellulose, but the printability of the hydrogels was affected. We discovered that Col/Alg/MC hydrogels have superior mechanical properties compared to hydrogels existing in the literature, but whether or not they can sustain the organ biomechanical stress is still unknown due to a lack of data about the mechanical properties of the organs of experimental animals.

According to the findings of Liu and his colleagues, as the alginate concentration in the hydrogel increases, the hydrogel will have more crosslinking sites, resulting in a diminished degradation ratio (85). According to the provided information, in the event that the implanted hydrogel drug delivery system is unable to undergo degradation, it will result in persistent

tissue damage. An optimal hydrogel scaffold for drug delivery should possess a degradation rate that is uniform, in conjunction with the concurrent development of new tissue or the attainment of the desired physiological outcome. The degradation ratios of the hydrogel at lower levels (2%) are insufficient for effective bioactive molecule delivery.

The in vitro degradation of Col/Alg/MC hydrogels was studied by incubating them in a body fluid solution (PBS) and enzymatic solution (Lipase) and then monitoring their weight-losses and analyzing their biodegraded products following different incubation times. The in vitro PBS degradation rate of hydrogels is greater (70%) than the in vitro Lipase degradation rate (30%). The explanation for this can be elucidated by observing that the hydrogels immersed in phosphate-buffered saline (PBS) exhibited substantial swelling over time, whereas no discernible swelling was detected in the hydrogels immersed in Lipase Fig .5.11. As the swelling ratio of hydrogels increases, there is a corresponding rise in pore size, allowing for greater penetration of solution into the hydrogel. As a result of aqueous solution penetration, the hydrolysis of the hydrogel will be facilitated, which can break more hydrogen bonds in the hydrogel and lead to greater degradation rate of the hydrogel.

In order to achieve successful utilization in in-vitro cell or tissue culture, hydrogels must demonstrate structural integrity by offering a mechanically and biochemically tolerant milieu. In the present investigation, it was observed that the structural stability and degradation rate of Col/Alg/MC hydrogels exhibited a gradual decline over a span of multiple weeks when the concentration of Alg was elevated. This extended degradation period would facilitate a long period for conducting in-vitro testing of the hydrogels Figs .5.9, 5.10, 5.11, and 5.12.

6.3. LIMITATIONS OF THE STUDY

The most significant limitation of this study that should be considered is the difficulty to print hydrogels with constant initial weighs and frame. The Rapamycin releasing kinetics test was impacted by the non-homogeneous distribution of the desired concentration within the hydrogel which make it below the detection limit. In order to achieve a hydrogel with consistent weight and frame, it is recommended to employ a 3D printing extrusion technique that consistently dispenses equal amounts of solution during the process.

The optimal dosage of Rapamycin to be incorporated into the microspheres or hydrogels, as well as the subsequent evaluation of their efficacy in experimental animals, is yet to be determined. Therefore, it is necessary to conduct dosage form experiments in order to determine the suitable dose of Rapamycin to be used.

6.4. CONCLUSION

Growth factors (GF) utilisation in regenerative medicine and tissue engineering is often challenging due to their instability and short half-life. The delivery of growth factors with microcarriers can eliminate these problems. In the present study, we introduced VEGF loaded PCL microparticles into 3D printed multimaterial hydrogel scaffold. In the hydrogel scaffold, VEGF-loaded PCL microspheres were incorporated onto 3D-printed collagen/alginate scaffolds to achieve sustained drug release over several weeks. We used double emulsion method to fabricate the microspheres to obtain morphological stable particles. Additional PVA phase used as external stabilizer to prolong the duration of drug release and increase the encapsulation rate and drug-loading particles. The VEGF - loaded PCL microspheres exhibited good biodegradability over a relatively moderate period of time, leading to the release of the drug at effective concentrations.

The hydrogel system fabricated in this study designed as a semisolid containing water-swollen, chemically crosslinked, hydrophilic, degradable polymer. Like other elastomeric materials, the mechanical behaviour of the hydrogel in this study is mainly determined by the architecture of the hybrid polymer network of collagen and alginate. The proliferation and differentiation fate of cells interacting with scaffolds is significantly by the mechanical properties of the implanted scaffold. Ideally, scaffolds designed for utilisation as drug delivery carriers in tissue or organ transplantation should have mechanical properties that closely emulate those of the host organ. The attributes mentioned above showed that increasing the concentration of Alg and the crosslinking time can improve the stability and mechanical features of the printed hydrogels, which make collagen/alginate hydrogels attractive material systems for the delivery of a large range of therapeutics.

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APPENDIXES

APPENDIX 1: EXTENDED TURKISH SUMMARY OF THE THESIS

Giriş

Biyomalzeme, canlı organların veya dokuların normal veya anormal bir işlevini iyileştirmek veya değiştirmek için biyolojik sistemlerle etkileşime giren belirli bileşenlerden oluşan yapılandırılmış, doğal veya sentetik bir maddedir. Biyomalzemelerin, hücrelerin ve biyoaktif bileşiklerin bir araya getirilmesi, doku rejenerasyonunu yönlendirmeye ve canlı sisteme dahil olmayı kolaylaştırmaya hizmet eden 3D biyomalzeme iskeleleri üretmek için tıp alanında kullanılmıştır. Biyomalzemeler, terapötik maddeler ile vücudun doğuştan gelen iyileştirme mekanizmaları arasındaki etkileşimi kolaylaştırma yetenekleriyle yaygın olarak tanınmaktadır. 3D biyomalzeme iskele yapımı, seramik, polimer ve metal olmak üzere üç benzersiz biyomalzeme kategorisinin kullanımını içerir.

Hidrojeller gibi polimer bazlı biyomalzemeler, biyomoleküllerin hedeflenen uygulaması için ilaç dağıtım stratejilerinin geliştirilmesinde kullanılmıştır. Bu strateji, terapötik ajanların sistemik uygulamasıyla yaygın olarak ilişkilendirilen toksisite, hipertansiyon, ödem ve mortalite gibi olumsuz etkileri azaltmayı amaçlamaktadır. Hidrojeller çapraz bağlı polimerik ağlardır ve su ortamına maruz kaldıklarında şişme kabiliyeti gösterirken çözünmeme özelliği sergilerler.

Kolajen ve aljinat gibi hidrojeller, biyouyumluluklarına katkıda bulunan ve jelleşmenin ardından şekil doğruluğunun korunmasını kolaylaştıran yüksek hızları nedeniyle 3D biyobaskı için uygun özellikler sergiler. Bununla birlikte, hidrojeller, özellikle mekanik ve fiziksel özellikleriyle ilgili bazı sınırlamalara sahiptir, bu nedenle biyobaskı için mürekkep olarak uygunluklarını kısıtlamaktadır. Mikropartiküllerin çok malzemeli hidrojellere entegrasyonu, hidrojel iskelelerin baskı potansiyelinin ve performansının artmasıyla sonuçlanmıştır. Çoklu malzemelerden oluşan hidrojellerin üretimi, hidrojellerin fizyomekanik özelliklerini geliştirme potansiyeline sahiptir.

Bir hidrojel iskelenin tasarımı ve üretimi, hedeflenen anatomik bölgenin yanı sıra aranan belirli fizyolojik tepkilere de bağlıdır. Bu çalışma, anjiyogenezi artırmak ve transplantasyondan sonra yumurtalık folikülünde yanma sendromunun ortaya çıkmasını

önlemek amacıyla yumurtalık dokusu transplantasyonu için özel olarak tasarlanmış bir hidrojel geliştirmeyi amaçlamıştır. Vasküler endotelyal büyüme faktörü (VEGF) anjiyogenezi arttırıcı olarak seçilirken, bir immünosupresan olan Rapamisin, yumurtalık foliküllerinin aktivasyonunda çok önemli olan rapamisin (mTOR) sinyal yolunun mekanik hedefini inhibe etmek için seçilmiştir.

Hidrojel bazlı iskeleler, üretim sırasında değiştirilebilir nitelikleri ve güvenli implantasyon, boşaltma ve ayrıştırma süreçleriyle uyumlulukları nedeniyle kontrollü ilaç uygulaması amacıyla doğal bir çekiciliğe sahiptir. Hidrojeller, fiziksel veya kimyasal tekniklerle çapraz bağlanmış doğal, sentetik veya yarı sentetik polimerlerden oluşan yüksek nemli birbirine bağlı ağlar olarak tanımlanır.

Polivinil alkolün (PVA) hidrojellere dahil edilmesinin, gerilim özelliklerinin artmasına neden olduğu görülmüştür. Saf kolajen hidrojelleri kombine hidrojellerle karşılaştırıldığında, ikincisinin maruz kaldığı gerilimin önemli ölçüde arttığı görülmüştür. Mevcut bilgiler, polivinil alkolün (PVA) dahil edilmesinin, gelişmiş ağ dolaşıklıklarının oluşturulmasını kolaylaştırarak hidrojellere dikkate değer mekanik özellikler kazandırmada hayati öneme sahip olduğunu göstermektedir.

Aljinat, anyonik özelliklere ve hidrofilik özelliklere sahip, doğal olarak oluşan organik bir polisakkarittir. Belirli bakteri suşlarından ve deniz yosunu türlerinden ekstraksiyon işlemi ile elde edilir. Aljinatın biyomalzeme olarak kullanılmasının, maliyet etkinliği, üretimindeki basitlik ve tehlikeli özelliklerinin olmaması gibi çok sayıda faydası vardır. Aljinat bazlı biyomalzemeler, çok çeşitli üretim teknikleri kullanılarak hidrojeller, köpükler, süngerler, lifler, mikroküreler ve mikrokapsüller gibi çeşitli formlarda üretilebilir. Aljinat, 3D doku mühendisliği alanında çok çeşitli kullanımlar için yaygın olarak kullanılmaktadır. Bu kullanımlar arasında ilaç dağıtımı, yara iyileşmesi ve bağ dokusunun onarımı ve rejenerasyonu yer almaktadır.

"3D baskı" terimi, malzemeleri düşürmek ve somut üç boyutlu nesneler üretmek için otomatik yöntemler kullanan birçok teknolojiyi kapsar. Dikkatli bir şekilde düzenlenen katman katman prosedür, 3D baskıyı en yüksek standartta polimer hidrojel iskeleleri üretmek için en çağdaş ve uyarlanabilir teknik haline getirmektedir. Buna ek olarak, tasarım/bilgisayar destekli üretim (CAD/CAM) yazılımının kullanılması, çeşitli

konfigürasyonların, bir dizi ağın, değişen gözenek boyutlarının, çoklu katmanların ve çeşitli üretim prosedürlerinin oluşturulmasına olanak tanır.

Organ naklinin başarısız olmasındaki en önemli etkenlerden biri ertelenmiş veya eksik vaskülarizasyon sürecidir. Onarım ve rejenerasyon süreci için kan dolaşımının (neoanjiyogenezin) ve bununla birlikte oksijenasyonun ve besin maddelerinin yaralanmış organlara ulaştırılabilmesinin yeniden sağlanabilmesi önemlidir. Anjiyogenez, özellikle iyileşme süreci, doku rejenerasyonu ve bazı hastalıkların ilerlemesi gibi çok sayıda fizyolojik süreçte önemlidir. Neoanjiyogenezi artırmak için biyomalzemeler, doku rejenerasyonu için vasküler infiltrasyonu sağlamak üzere iskele olarak kullanılabilir veya proanjiyojenik ajanları ve hücreleri düzenlenmiş bir şekilde taşımak için kullanılabilirler.

Biyoaktif moleküller, anjiyogenezi artırmayı amaçlayan ilaç dağıtım stratejilerinin karşılaştığı zorlukların üstesinden gelmek için kapsamlı bir şekilde kullanılmaktadır. Sistemik enjeksiyon yöntemi, tıbbi uygulamalarda anjiyojenik ilaçların uygulanması için en çok kullanılan tekniktir. Bununla birlikte, bu yaklaşım dikkate alınması gereken çeşitli faktörler sunmaktadır. Birçok ilacın yarı ömrü kısadır ve eliminasyon nispeten hızlı gerçekleşir. Bu nedenle, tek veya çoklu dozajlarda sistemik uygulama büyüme faktörleri vücuttan derhal uzaklaştırılır, örneğin, enjekte edilen VEGF ve bFGF'nin eliminasyon yarı ömürleri 1 saatten azdır.

Amaç ve hedefler:

Birçok organ veya doku nakli, uygulanan ilaçların nispeten yüksek klirens oranı ve kısa yarı ömrü nedeniyle anjiyojenik veya immünosupresanların sistemik uygulamalar yoluyla tekrar tekrar uygulanmasını gerektirir. Bununla birlikte, sistemik olarak uygulanan ilaçlarla ilişkili ödem, toksisite, hipertansiyon ve ölüm gibi potansiyel olarak ciddi komplikasyonlar vardır. Bununla birlikte, tekrarlayan enjeksiyonlarla ilişkili sosyoekonomik etkiyi iyileştirmek ve ilişkili potansiyel komplikasyon riskini azaltmak için uygulama sayısını ve sıklığını azaltmaya ihtiyaç vardır.

Bu ihtiyacı karşılamak için, kolajen/aljinat implante edilebilir hidrojel içinde süspanse edilmiş poli (kaprolakton) (PCL) mikroküreлerden oluşan bir implante edilebilir ilaç dağıtım sistemi (IDDS) geliştiriyoruz. İlaçların (VEGF ve Rapamisin) PCL mikroküreleri içinde

kapsüllenmesi ile özel karakteristiklere sahip ilaçların sürekli salınımı sağlanabilir. PCL, FDA onaylı birçok terapötik cihazda kullanılan biyolojik olarak parçalanabilir ve biyouyumlu bir kopolimerdir.

Bu çalışmanın amacı, çok ölçekli gözenek yapılarına sahip ve hidrofobik VEGF/rapamisin yüklü polikaprolakton mikroküreler içeren ekstrüzyon 3D baskı tabanlı kompozit kolajen-aljinat jel iskeleler oluşturmaktır. Bu nedenle, doku greftlerine VEGF uygulanması, rapamisinin mekanik hedef (mTOR) yolağını inhibe ederek immünolojik reaksiyonların neden olduğu hasarı ve kan damarı oluşum oranını artırarak uzun süreli hipoksiyi azaltabilir.

Metotları

PCL mikropartiküller çift emülsiyon yöntemi kullanılarak üretilmiştir. Kısaca, metilen klorür içinde çözülen PCL çözeltisinin içine biyoaktif ajanlar (0.1mL içinde 0.5 mg Rapamisin ve 0.5 µg VEGF-A) ilave edilerek emülsiyon 1 dk boyunca 50 Hz frekansta sonikasyona (Bandelin electronic GmbH) tabi tutulmuştur. İlk emülsiyon polivinil alkol (%4 w/v PVA) çözeltisinin içerisine eklenmiştir ve aynı özelliklerde sonikasyona tabi tutulmuştur. Çift emülsiyonda organik çözücülerin uzaklaşması ve PCL kapsüllerin homojenleştirilmesi için %0.3 w/v PVA çözeltisi içerine ilave edildikten sonra 24 saat karıştırıcıda bırakılmıştır. 24 saat sonrası santrifüj ile toplanan mikrokapsuller, Tris-HCL ile yıkandıktan sonra liyofilize edilmiştir. Biyoaktif ajanların mikrokapsüllerden salım analizine kadar, üretilen yapılar -20°C'de saklanmıştır.

Mikrokapsulların morfolojik özellikleri Ankara Üniversitesi Tıp Fakültesi Histoloji-Embriyoloji Anabilim Dalı bünyesinde bulunan taramalı elektron mikroskobuyla (Scanning Electrone Microscope; SEM) değerlendirilmiştir. Liyofilize edildikten sonra toplanan PCL örnekler SEM örnek tutucuları (stab) üzerine yapıştırılan çift taraflı karbon bantlar üzerine konulmuştur. Staba tutturulan örnekler daha sonra Sputter Coater cihazında 10 dakika vakum altında tutulmuş, 20 nm altın kaplama uygulamasının hemen ardından ise 20 kV'da LEO 438 VP marka SEM cihazı ile görüntülenmiştir.

PCL mikrokapsüllerden salım kinetiği tayini VEGF ve rapamisin yüklü mikropartiküller ile ayrı ayrı ve olası sinerjistik etkileri belirlemek için bir arada gerçekleştirilmistir. VEGF ve rapamisin ile yüklenen mikrokapsüller ependorf tüplerine alınmış (5 mg) ve 1 mL PBS

(Phosphate Buffered Saline; pH 7.4) içerisinde 37°C'de inkübe edilmiştir. Çeşitli zamanlarda (7, 14, 21, 28 gün) örnekler santrifüjlenerek süpernatan toplanmış ve yerine taze PBS eklenmesi ile inkübasyon devam ettirilmiştir. Süpernatanda VEGF tayini, büyüme faktörünün ELISA kiti kullanılarak gerçekleştirilmiştir (Elabscience Human VEGF-A Elisa kit). Kısaca, VEGF tayini için, kitte bulunan VEGF monoklonal antikoru ile kaplanmış mikroplakaya VEGF standartları ve süpernatan örnekleri yerleştirilmiştir. Süpernatanda bulunan VEGF'in plakadaki kaplı antikor ile bağlanması için mikroplaka 90 dk inkübe edilmiştir. İkinci antikorun bağlanması için kuyucuklara Biotinylated Detection çözeltisi eklenerek mikroplaka 60 dk inkübe edilmiştir. Yıkamayla bağlanmayan enzim-bağlı antikorun ayrılmasının ardından HRP konjugatının bağlanması için 30 dk inkübasyon gerçekleştirilmiştir. Yıkamayla bağlanmayan HRP konjugat ayrılmasının ardından substrat çözeltisi kuyucuklara eklenmiş ve 15 dk süreyle inkübe edilmiştir. İnkübasyon sonrası mikroplaka okuyucu ile 475 nm dalga boyunda renk değişimi kaydedilmiştir.

Hidrojel iskeleleri üç boyutlu (3B) baskı ile elde edilmiştir. Kollajen tip I/aljinat ve yapıya uzun süreli stabilitenin sağlanması amacıyla eklenen metil selülozdan elde edilen biyomürekkebin kullanımı ile 3B baskı gerçekleştirilmiştir. 3B baskıların elde edilmesinde Ankara Üniversitesi Biyomedikal Mühendisliği Bölümü'nde mevcut 3B AXO biyoyazıcı cihazı kullanılmıştır. Üretim sırasındaki başlangıç baskı parametreleri şu şekildedir: Kollajen (0.82 mg/mL), Aljinat (1% w/v), CaCl2 (3% v/v), Basınç: 0.6 bar, Hız: 2.5 mm/s, Katman kalınlığı: 170 µm, Nozzle: 0.34 G, Baskı sıcaklığı: 25°C.

Kollajen, Aljinat ve Mythl Selüloz olmak üzere uc farklı solüsyon hazırlandı. Tozlardan oluşan uc karışım ayrı ayrı 10 mL distile suda karıştırılarak 250 rpm hızında bir gece bekletildi.

Çözüm 1: Kollajen (0,82 mg/mL), Aljinat (%2 w/v)

Çözüm 2: Kolajen (0,82 mg/mL), Aljinat (%2 w/v), Mythl Selüloz (%4 w/v)

Çözüm 3: Kolajen (0,82 mg/mL), Aljinat (%4 w/v), Mythl Selüloz (%4 w/v)

Çözüm 4: Kolajen (0,82 mg/mL), Aljinat (%4 w/v), Mythl Selüloz (%4 w/v), Rapamycin (0.1, 0.5, 1 mg)

Hidrojel kompozit yapılarını yazdırmak için bir 3D Bioprinter sistemi (Axo C2, Axolotl Biosystems) kullanıldı. Hidrojel karışımı, bir iğneden ekstrüzyon için 3D yazıcının baskı taşıyıcısına yerleştirilmiş bir ekstrüzyon kartuşuna yerleştirildi. İskeleler 3D-Builder yazılımı ile dikdörtgen şeklinde tasarlanmış ve ardından 5 kat baskı yapılmıştır. Bundan sonra numuneler, 5 dakika boyunca basılı yapı iskelesi üzerine 500 uL CaCl2 (0.5M) eklenerek CaCl2 ile çapraz bağlandı.

C/ALG ve C/ALG/MC iskelelerinin mekanik özellikleri SHIMADZU malzeme test cihazı (AGS – X) ile test edilmiştir. Hidrojel numunelerin orijinal uzunluk (L0) ve kalınlık ölçüldü. Daha sonra çekme testine geçildi ve iskeleler oda sıcaklığında numune kopma noktasına gelene kadar 5 mm/dk germe hızında gerildi. Son olarak, modül, gerilim-gerinim eğrilerinin başlangıçtaki doğrusal bölgelerinden hesaplandı.

Bulgular:

Kollajen dokusunun major ekstraselüler matriks (ECM) proteini olması nedeniyle bir taşıyıcı olarak kullanılacak doku iskelesinin üretileceği malzeme olarak seçilmiştir. Üretilecek doku iskelesinin doku anjiogenezini indükleyecek şekilde gözenekli yapıda ve ayrıca VEGF/rapamisin'in kontrollü salımını gerçekleştirecek özellikte olması hedeflenmiştir. Kollajen temelli gözenekli doku iskelesinin 3B baskı tekniği ile üretimi ve eş zamanlı VEGF/rapamisin kontrollü salımının sağlanması için etken maddeleri içeren poli(kaprolakton) (PCL) mikropartikülleri içermesi planlanmıştır.

Çift emülsiyon yöntemiyle elde edilen mikropartiküllerin küresel yapıda olduğu ve homojen boyut dağılımına sahip olduğu belirlenmiştir. PCL-mikrokapsüllerin morfolojisi taramalı elektron mikroskobu (SEM) analizi ile karakterize edilmiştir. VEGF/Rapamisin yüklü PCL-mikropartiküllerinin ayrı ayrı küresel şekilli olduğu ve homojen bir boyut dağılımına sahip olduğu belirlenmiştir. VEGF/Rapamisin yüklü PCL-mikrokapsüllerin morfolojisi hakkında da benzer bulgular elde edilmiştir.

PCL mikrokapsüllerinin ve hidrojellerinin VEGF/Rapamisin salınımını uzatıp uzatamayacağını araştırmak için çeşitli salınım deneyleri gerçekleştirilmiştir. Çoklu zaman noktalarındaki serbest VEGF konsantrasyonları ELISA ile belirlenmiştir. Kademeli ve hızlı grupların birikimli salım profilleri gösterilmiştir. VEGF salınımı yavaş salınan grupta hızlı

salınan gruba kıyasla daha uzun süreli ve daha kademeli olmuştur. VEGF en az 2 hafta boyunca sürekli olarak ilgili konsantrasyonlarda salınmıştır (vaskülogenez (VEGF'nin ED50'si tipik olarak 1-6 ng/ml'dir).

Kolajen aljinat hidrojellerin stabilitesi ilk olarak PBS ve hücre kültürü ortamında değerlendirilerek ileride her iki hücre kültürü deneyinde de kullanılmak üzere stabiliteleri tespit edilmiştir. Ancak ön çalışmada kullanılan baskı solüsyonunun (C/1%ALG) 3D baskıdan sonra sadece 3-4 gün boyunca 3D yapısını koruyabildiği ve sonrasında hücre kültürü ortamında 3D yapısını kaybetmeye başladığı görülmüştür. Bu sorunun üstesinden gelmek için iki yol izlenmiştir: İlk olarak, ön çalışmada kullanılan ALG (%1 w/v) konsantrasyonu (%2w/v)'ye yükseltildi. İkinci olarak, 3D basılabilirlik ve 37°C'de jelleşme için gerekli yüksek viskoziteye sahip bir malzeme olan Metil selüloz (MC) baskı çözeltisine eklenmiştir.

MC, C/ALG yapısına %4 w/v konsantrasyonunda dahil edildi. Bu sayede hem 3D baskı sırasında baskı kalitesinin artırılması hem de 37°C'de MC'nin hidrojel yapısından dolayı uzun süreli kültürde 3D yapının yapısal bütünlüğünün korunması amaçlanmıştır. ALG konsantrasyonunun %2'ye çıkarılması ve MC ilavesinden sonra elde edilen iskelelerin hem FBS hem de hücre kültürü ortamında 21 günden fazla stabil kaldığı görülmüştür.

Çeşitli iskelelerin gerilme mukavemeti değerlendirildi. C/ALG iskelesinin ortalama eğrisi, 7°C'lik bir baskı sıcaklığında 0,22 Mpa'lık bir tepe gerilimi ve 0,92Mpa'lık bir ortalama Young modülü gösterdi. C/ALG yapı iskelesine Mythl Selüloz eklenmesinin ardından, Young modülü önemli ölçüde azalarak ortalama 0,1012 Mpa'ya düşerken, nihai gerilme mukavemeti 14°C'lik bir baskı sıcaklığında 0,014 Mpa'ya düştü.

Biyoaktif ajanın mikroküre lerden verilmesi hidrojellerin bozunmasına bağlı olduğundan, bozunmaları in vitro olarak araştırılmıştır. Farklı aljinat konsantrasyonlarına (%2 ve %4 w/v) sahip hidrojellerin bozunma hızı, hidrojellerin 21 gün boyunca steril PBS ve Lipaz içinde ayrı ayrı tutulmasıyla incelenmiştir. 4'lük aljinat hidrojellerinin kuru ağırlığı, PBS ve Lipaz'da 21 gün boyunca önemli bir kütle kaybı göstermezken, %2'lik aljinat içeren hidrojeller 21 ve 14 gün sonra tamamen bozunmuştur.

Tartışma

3D baskı tekniği ile kolajen bazlı gözenekli doku iskelesi üretmek ve eş zamanlı VEGF/rapamisin kontrollü salınım sağlamak için aktif bileşenler içeren poli (kaprolakton) (PCL) mikropartikülleri içermesi planlanmaktadır. Bu çalışmada VEGF yüklü PCL mikropartikülleri geliştirdik, bu polimer VEGF'nin 4 hafta boyunca sürekli salınımını sağlayan Karina ve arkadaşlarının önceki çalışmasına dayanarak seçilmiştir.

VEGF Mikrokürelerinin salınım modeli, üç haftalık bir süreyi kapsayan kapsamlı bir tarama prosedürü ile değerlendirilmiştir. Salınan Vasküler Endotelyal Büyüme Faktörü (VEGF) miktarını ölçmek için Enzim Bağlantılı İmmünosorbent Testi (ELISA) teknolojisi kullanılmıştır. Elde edilen veriler daha sonra Vasküler Endotelyal Büyüme Faktörünün (VEGF) normalize edilmiş kümülatif değerleri olarak grafiksel olarak gösterilmiştir. Kapsüllenmiş VEGF'nin kapsamlı salımı, 21 günlük bir süre boyunca gözlemlenen genel kümülatif salım ile temsil edilmektedir. Mikroküreler, minimal bir ilk patlama salımı ile karakterize edilen tutarlı bir salım profili sergilemiştir. Seçilen formülasyonlar, birkaç hafta boyunca etkili bir şekilde sürekli salınım gösterdikleri için uygun kabul edilmiştir, bu formülasyonlar amaçlarımız için uygundur.

Bu çalışmada kullanılan metodoloji, VEGF'nin hidrofilik özelliklerini göz önünde bulundurarak, VEGF yüklü ve VEGF içermeyen PCL mikrokürelerinin formülasyonunu içermektedir. Vasküler endotelyal büyüme faktörünün (VEGF) enkapsülasyonu, çift emülsiyon tabakalarının iç sulu fazı (w1) içinde gerçekleşir, bu adım VEGF'nin dış sulu faza (w2) difüzyonunu önlemeye yarar. Vasküler endotelyal büyüme faktörünün (VEGF) biyolojik performansını arttırmak için %0,3 polivinil alkol (PVA) ilave konsantrasyonu kullanılmıştır. Bu, VEGF partiküllerinin etrafında koruyucu bir kaplama oluşumunu kolaylaştırmak için yapılmıştır.

Dış sulu faza bir stabilizatörün eklenmesi, emülsifikasyon işlemi sırasında iyi tanımlanmış küresel mikrokürelerin istenen oluşumunu sağlamak için çok önemlidir, Polivinil alkol (PVA), düşük toksik etkileri, suda elverişli çözünürlüğü ve çeşitli moleküler ağırlıklarda erişilebilir olması nedeniyle sulu fazın oluşturulması amacıyla bir stabilizatör olarak seçilmiştir. Ayrıca, PVA biyouyumluluğu ve biyolojik bozunmaya uğrama kapasitesi ile bilinmektedir. Stabilizatörün ana işlevi, emülsifikasyon işlemi sırasında mikrokürelerin

koagülasyonunu engellemek, dolayısıyla birincil emülsiyonun stabilitesini artırmak ve moleküllerin kapsüllenmesini kolaylaştırmaktır.

Kolajen, bağ dokusunun başlıca hücre dışı matris (ECM) proteini olduğundan, taşıyıcı olarak kullanılacak iskelenin üretileceği malzeme olarak seçilmiştir, ancak kolajen biyo-baskı, düşük viskozite ve yavaş polimerizasyon nedeniyle çoğunlukla ekstrüzyon baskı ile sınırlı kalmıştır, bu da zayıf kolajen hidrojellerin oluşmasına neden olmuştur. Bununla birlikte, bir çalışmada, kolajen/metil selülozdan oluşan bir biyomürekkepten bir 3D hidrojel iskelesi üretilmiş ve ekstrüzyon tabanlı 3D biyoyazıcılar kullanılarak daha yüksek stabilite ve performans kapasitesi göstermiştir. Bu doğrultuda 3D baskı ile kolajen bazlı doku iskelesi üretiminde literatürdeki örneklerden yola çıkılarak gerçekleştirilen ön çalışmada kolajen/aljinat/metil selüloz biyoink ve çapraz bağlayıcı olarak CaCl2 kullanılarak stabil yapıya sahip 3D baskılı doku iskeleleri elde edildi. Kolajen daha sert ve deformasyona karşı daha dirençli iken, sodyum aljinat hidrojeller yüksek su emme kapasiteleri nedeniyle çabuk bozunmaktadır. Bu şekilde üretilen hidrojeller, terapötik ilaçları hedef bölgelerde uzun süreler boyunca tutarak yerel olarak sürekli ilaç salınımını başarıyla sağlayabilir.

Aljinat (Alg) içeriği %2 olduğunda 14 gün içinde hidrojelin hızlı ve tamamen parçalandığı görülmüştür. Bu olgu, lipaz enzimi tarafından aljinatın enzimatik bozunmasının artan etkinliği ve çabukluğu ile belirgin bir şekilde gösterilmiştir. Aljinatın bozunma hızı, jelleşme için kullanılan yöntemler, aljinat konsantrasyonu ve kullanılan spesifik çapraz bağlama stratejisi de dahil olmak üzere çeşitli faktörlerden etkilenmektedir. Aljinat jelleşme sürecinde kalsiyum sıklıkla kullanılmaktadır. Yazdırılabilirliği iyileştirmek ve hidrojelin bozunma süresini uzatmak için aljinat konsantrasyonu %4'e yükseltilmiştir. Sonuç olarak, hidrojellerin 21 günün üzerinde uzun bir kullanım ömrü sergilediği gözlemlenmiştir.

Aljinat türevi polimerler su çözeltileriyle reaksiyona girerek iki değerlikli bağlanma yoluyla hidrojeller oluşturur. Böylece, Col/Alg hidrojellerinin mekanik özellikleri çapraz bağlayıcı konsantrasyonu, maruz bırakma süresi ve aljinat konsantrasyonunun artırılmasıyla değiştirilebilir. Aljinat konsantrasyonunun %2'den %4'e çıkarılması jelin yapısal stabilitesini 21 güne kadar artırmıştır, bu da organ naklinden sonra uzun vadeli anjiyogenezi teşvik etme potansiyelini artırmaktadır.

Mekanik çekme testi, hidrojellerin mekanik davranışını önemli ölçüde etkileyebilecek olan hidrojellerin kurumasını önlemek için baskıdan hemen sonra gerçekleştirilmiştir. Çeşitli iskelelerin gerilme mukavemeti değerleri Şekil (5.4.), (5.5.), (5.6.) ve Şekil (5.7.)'de gösterildiği gibi değerlendirilmiştir. Basılan C/ALG iskelesinin ortalama eğrisi, 7°C baskı sıcaklığında 0,22 Mpa'lık bir tepe gerilimi ve 0,92 Mpa'lık bir ortalama Young modülü göstermiştir. C/ALG iskelesine Mythl Selüloz eklenmesinin ardından, Young modülü önemli ölçüde azalarak ortalama 0,10 Mpa'ya düşerken, nihai gerilme mukavemeti 14°C baskı sıcaklığında 0,014 Mpa'ya ulaşmıştır.

Sonuç

Rejeneratif tıp ve doku mühendisliğinde büyüme faktörlerinin (GF) kullanımı, istikrarsızlıkları ve kısa yarı ömürleri nedeniyle genellikle zordur. Büyüme faktörlerinin mikro taşıyıcılarla verilmesi bu sorunları ortadan kaldırabilir. Bu çalışmada, VEGF yüklü PCL mikropartiküllerini 3D baskılı çok malzemeli hidrojel iskeleye ekledik. Hidrojel iskelede, VEGF yüklü PCL mikroküreleri, birkaç hafta boyunca sürekli ilaç salınımı elde etmek için 3D baskılı kolajen/aljinat iskelelere dahil edildi. Morfolojik olarak stabil partiküller elde etmek amacıyla mikroküreleri üretmek için çift emülsiyon yöntemi kullandık. İlave PVA fazı, ilaç salınım süresini uzatmak ve kapsülleme oranını ve ilaç yüklü partikülleri artırmak için harici stabilizatör olarak kullanılmıştır. VEGF yüklü PCL mikroküreleri nispeten ılımlı bir süre boyunca iyi bir biyolojik bozunabilirlik sergilemiş ve ilacın etkili konsantrasyonlarda salınmasına yol açmıştır.

Bu çalışmada üretilen hidrojel sistemi, su ile şişmiş, kimyasal olarak çapraz bağlanmış, hidrofilik polimer içeren yarı katı olarak tasarlanmıştır. Diğer kauçuk benzeri malzemeler gibi, bu çalışmadaki hidrojelin mekanik davranışı da esas olarak kolajen ve aljinattan oluşan hibrit polimer ağının mimarisi tarafından belirlenmektedir. İskelelerle etkileşime giren hücrelerin çoğalması ve farklılaşma kaderi büyük ölçüde iskelenin mekanik özelliklerine bağlıdır. İdeal olarak, doku veya organ nakli ile ilaç dağıtım aracı olarak kullanılacak iskeleler.

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1) Immunoinformatics Prediction of Epitope Based Peptide Vaccine against Clostridium perfringens Fructose Bisphosphate Aldolase Protein. immunome · March 2021

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1) 2017 A presentation during the 17th NAPRECA Symposium on the theme "**HARNESSING NATURAL PRODUCTS FOR THE WELL-BEING OF THE AFRICAN PEOPLE**" at Ras Amba Hotel, Addis Ababa, Ethiopia.

2) 2023 A presentation during 8th INTERNATIONAL ACADEMIC STUDENT STUDIES CONGRESS, Presentation title "**THE ESTABLISHMENT OF IN VIVO RABBIT PREMATURE OVARIAN FAILURE MODEL INDUCED BY CYCLOPHOSPHAMIDE**". Istanbul, Turkey.

3) **DYNAMIC FOLLOW-UP OF NEOVASCULARIZATION IN TWO DIFFERENT ORTHOTOPIC OVARIAN TISSUE TRANSPLANTATION SITES USING IN VIVO MAGNETIC RESONANCE IMAGING MRI PERFUSION**. American Society for Reproductive Medicine ASRM. America. October 2023.

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2023 A presentation during 8th INTERNATIONAL ACADEMIC STUDENT STUDIES CONGRESS, Presentation title "**THE ESTABLISHMENT OF IN VIVO RABBIT PREMATURE OVARIAN FAILURE MODEL INDUCED BY CYCLOPHOSPHAMIDE**". Istanbul, Turkey.