TÜRKİYE CUMHURİYETİ
ADANA ALPARSLAN TÜRKEŞ SCIENCE AND TECHNOLOGY UNIVERSITY

GRADUATE SCHOOL
BIOENGINEERING DEPARTMENT

Design of Multifunctional Cryogel-Based Wound Dressings Incorporating *Artocarpus lakoocha* Extract

Ahmet Burak ALTUNSÖZ

YÜKSEK LİSANS TEZİ

ADANA 2025

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YÜKSEK LİSANS TEZİ

THESIS ADVISOR
ASSOC. PROF. DR. Sibel ÖZDAŞ

SECOND ADVISOR
PROF. DR. GÖZDE BAYDEMİR PEŞİNT

ADANA, 2025

DECLARATION OF CONFORMITY

In this thesis study, which was prepared following the thesis writing rules of Adana Alparslan Türkeş Science and Technology University Institute of Graduate School, I declare that I provide all the information, documents, evaluations and results in accordance with scientific ethics and moral codes without resorting to any means or assistance that would be contrary to scientific ethics and traditions. I also declare that I refer to all of the articles I used in this study with appropriate references and accept all moral and legal consequences if a situation is found contrary to my statement regarding my work.

30/10/2025

[İmza]

Ahmet Burak ALTUNSÖZ

ÖZET

Design of Multifunctional Cryogel-Based Wound Dressings Incorporating *Artocarpus lakoocha* Extract

Ahmet Burak ALTUNSÖZ
Yüksek Lisans, Biyomühendislik Anabilim Dalı

Danışman: Doç. Dr. Sibel ÖZDAŞ
II. Danışman: Prof. Dr. Gözde BAYDEMİR PEŞİNT
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Özellikle kronik ya da geç iyileşen yaralarda etkili ve biyoaktif yara bakım sistemlerine duyulan ihtiyaç göz önünde bulundurulduğunda, terapötik potansiyeli yüksek bitki bazlı bileşiklerin kullanımı giderek daha fazla önem kazanmaktadır. Bu bağlamda, güçlü antioksidan ve antimikrobiyal özellikleriyle bilinen *Artocarpus lakoocha* (*AL)* ekstresinin kriyojel bazlı bir taşıyıcı sistemle entegre edilmesi, yara iyileşmesini desteklemek için yenilikçi bir yaklaşım sunmaktadır. Bu çalışmada *AL* ekstresinin poli(vinil alkol) (PVA) ve jelatin (Jel) bazlı kriyojel matrislerle birleştirilerek biyouyumlu ve antimikrobiyal özellikli yara örtüsü geliştirilmesini amaçlamaktadır.

Çalışma kapsamında PVA ve Jel esaslı kriyojeller düşük sıcaklıkta polimerizasyon teknikleriyle sentezlenmiştir. Daha sonra bu kriyojeller, *AL* ekstresi ile fonksiyonelleştirilerek gözenekli, esnek ve yüksek sıvı tutma kapasitesine sahip bir yara örtüsü elde edilmiştir. Elde edilen kriyojeller FTIR, SEM, BET ve şişme testi ile karakterize edilmiştir. Ayrıca, kriyojellerin biyomedikal uygulamalara uygunluğu in vitro hücre kültürü (HaCaT) çalışmaları ile detaylı olarak değerlendirilmiştir.

Elde edilen sonuçlar, yüksek sıvı emilimi, nefes alabilirlik ve biyouyumluluk gibi yara bakımına yönelik ideal özellikler sunmuştur. Bu yaklaşım, bitki entegreli yara örtüsü materyalleri alanındaki literatür boşluğunu doldurmakla kalmayıp, aynı zamanda yerli ve erişilebilir doğal kaynakların kullanımıyla düşük maliyetli, ölçeklenebilir biyomedikal ürünlerin geliştirilmesine katkı sağlamaktadır.

Anahtar Kelimeler: Yara örtüsü, *Artocarpus lakoocha,* jelatin, kriyojel

ABSTRACT

Design of Multifunctional Cryogel-Based Wound Dressings Incorporating *Artocarpus lakoocha* Extract

Ahmet Burak ALTUNSÖZ

M.Sc., Department of Bioengineering

Supervisor: Assoc. Prof. Dr. Sibel ÖZDAŞ

Co-Supervisor: Asst. Prof. Dr. Gözde BAYDEMİR PEŞİNT

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Considering the growing need for effective and bioactive wound care systems, particularly in chronic or slow-healing wounds, the utilization of plant-based compounds with high therapeutic potential has gained increasing importance. In this context, the integration of *Artocarpus lakoocha* (AL) extract—known for its strong antioxidant and antimicrobial properties—into a cryogel-based carrier system offers an innovative approach to support wound healing. This study aims to develop a biocompatible and antimicrobial wound dressing by incorporating AL extract into poly(vinyl alcohol) (PVA) and gelatin (Gel)-based cryogel matrices.

Within the scope of the study, PVA and Gel based cryogels were synthesized using low-temperature polymerization techniques. Subsequently, these cryogels were functionalized with AL extract, resulting in a porous, flexible wound dressing with high liquid retention capacity. The obtained cryogels were characterized using FTIR, SEM, BET, and swelling tests. In addition, the suitability of the cryogels for biomedical applications was thoroughly evaluated through in vitro cell culture (HaCaT) studies.

The results demonstrated ideal wound care properties such as high fluid absorption, breathability, and biocompatibility. This approach not only fills a gap in the literature on plant-integrated wound dressing materials but also contributes to the development of cost-effective and scalable biomedical products through the use of locally available and accessible natural resources.

Keywords: Wound dressing materials, *Artocarpus lakoocha,* gelatin, cryogel

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LIST OF ABBREVIATIONS

PVA : Polyvinyl Alcohol

Gel : Gelatin

AL	: *Artocarpus lakoocha*
BET	: Brunauer-Emmett-Teller
LDL	: Low-density Lipoprotein
HDL	: High-density Lipoprotein
VEGF-2	: Vascular Endothelial Growth Factor-2
PDGF-BB	: Platelet-derived Growth Factor
TGF-β	: Transforming Growth Factor-β
RPMI	: Roswell Park Memorial Institute
FBS	: Fetal Bovine Serum
PBS	: Phosphate Buffered Saline
SEM	: Scanning Electron Microscopy
MTT	: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
DAPI	: 4',6-diamidino-2-phenylindole
PVA/Gel	: Polyvinylalcohol/gelatin

LIST OF SYMBOL

µL : Microliter

mL : Mililiter

mm : Millimeter

m_d : Weight of The Dry Cryogel

m_{sw} : Weight Of The Swollen Cryogel

1. INTRODUCTION

The skin is the largest organ that protects the body against external factors; It is a basic defense system with thermoregulation, immune defense, and mechanical barrier functions (Farley et al., 2012). Wounds that occur as a result of the disruption of the integrity of this barrier can cause both physiological dysfunctions and serious complications such as infection (Ramasastry and Sai, 2005). Lack of proper wound care increases the burden of infection-related disease worldwide and in some cases leads to mortality (Dai et al., 2011). Given the prevalence of chronic non-healing wounds and their economic burden on health systems, effective wound management has become a global priority (Gaspar et al., 2019).

Wound healing; It is a four-stage dynamic process: hemostasis, inflammation, proliferation and restructuring. In order for this process to be completed successfully, the materials used must have biocompatible and non-toxic properties (Alven et al., 2020). Although traditional wound dressings—such as gauze and silver-containing materials—offer basic protective functions; they are inadequate in maintaining moisture balance, exudate management, and immunomodulation (Long et al., 2022; Roy et al., 2010). This situation adversely affects the success of treatment, especially in chronic or complex wound types.

Modern biomaterials have been developed to overcome these limitations. Biopolymer-based functional wound dressings not only provide physical protection but also offer properties that promote tissue regeneration (White et al., 2012). Especially with the integration of plant extracts into these structures; it acquires antioxidant, antibacterial, and healing properties (Ahmad et al., 2022; Pelin et al., 2023). In this context, *Artocarpus lakoocha (A. lakoocha, AL),* which is one of the remarkable natural resources, has the potential to support wound healing thanks to its rich phytochemical content. However, the use of this plant in wound dressings has not yet been adequately researched (Liu and Jia, 2018).

Polymer-based systems with high water-holding capacity, especially hydrogels and cryogels are widely used in wound care (Ho et al., 2022; Razavi et al., 2019). Cryogels, with their porous and elastic structures, are advantageous in terms of exudate removal, gas exchange, and providing a moist microenvironment (Bereli et al., 2020). In addition, these structures contribute

to tissue engineering applications by showing similar properties to the extracellular matrix (Akin and Özmen, 2022).

Biopolymers such as PVA (poly(vinyl alcohol)) and gelatin are frequently preferred in cryogel systems due to their biodegradability and their structure that is open to functionalization (Savina et al., 2021; Vishnoi et al., 2021). The combination of these polymers with natural bioactive components such as *AL* extract enables the development of a new generation of wound dressings with high biological activity and suitable for clinical application.

This study aims to fill an important gap in the literature in terms of focusing on the development of wound dressing in the form of cryogel by combining *AL* extract, which is a phytochemically rich natural resource, with biocompatible polymers such as poly(vinyl alcohol) (PVA) and gelatin (Gel). Current studies have limitations on the wound healing effects of *AL*, and there is no comprehensive research on the integration of this extract with polymeric cryogel systems. In this context, the thesis not only presents a new biomaterial formulation, but also proposes a unique interdisciplinary approach by demonstrating that herbal bioactive components can be used effectively in tissue engineering-based wound care materials. The findings to be obtained will contribute to Türkiye's domestic and innovative medical equipment production and will also bring a new perspective to biotechnological applications in the field of wound management.

2. LITERATURE REVIEW AND THEORETICAL FOUNDATIONS

2.1. Skin Structure, Functions and Clinical Importance

2.1.1. Physiological Functions of the Skin

The skin is the human body's first line of defense against external factors and acts as a protective barrier (Farley et al., 2012). This structure, which protects the organism against a wide variety of environmental factors from mechanical traumas to chemical and biological threats, also plays vital roles in terms of thermoregulation, sensory perception and immune functions. The healthy integrity of the skin is considered an essential element in maintaining the body's homeostasis.

2.1.2. Deterioration of Skin Integrity: The Concept of Wound and Its Consequences

Wounds that cause the deterioration of this barrier are defined as damage to tissues caused by external or internal reasons that impair physiological structure and function (Ramasastry and Sai, 2005). Inadequacy of wound care causes millions of people around the world to become ill or die due to infections (Dai et al., 2011). For example, it is known that approximately 30-40 out of every 1000 surgical interventions develop infections, and some of these infections are life-threatening. Chronic non-healing wounds, on the other hand, have become a major public health problem, affecting approximately 2% of the US population (Gaspar et al., 2019).

2.2. Development of Wound Dressings and Applications of Biomaterials

2.2.1. Limitations of Traditional Methods

Traditional wound dressings – such as iodophore gauze, hydrocolloids and silver-containing materials – do not fully meet the basic requirements such as exudate absorption, moisture balance, infection prevention and air permeability. In addition, the lack of immunomodulatory properties of these dressings limits their contribution to the healing process and causes them to be ineffective, especially in complex or chronic wounds (Long et al., 2022). Its limiting properties, such as strong adhesion, insufficient moisture-holding capacity, and low gas permeability, mean that these traditional approaches are unable to respond to modern wound care needs (Roy et al., 2010; Waring et al., 2001). Today, bandages, gauze bandages and various

fixative materials are often used, especially in the treatment of superficial wounds. However, these materials; it does not adapt well to rough or hairy skin surfaces, increase the risk of infection by causing a gap between the wound and the dressing, and cannot provide adequate fluid absorption or deep wound coverage (Liu and Jia, 2018).

2.2.2. Modern Biomaterial-Based Approaches

In order to overcome such clinical limitations, a new generation of biopolymer-based, functional polymeric membranes is being developed using advanced tissue engineering techniques (White et al., 2012). Modern wound dressings promote not only protection from infection, but also the regeneration of dermal and epidermal tissues. These versatile biomaterials, designed with different functional properties, play an important role in accelerating the healing process and restoring the structural integrity of the skin (GasparPintiliescu et al., 2019). Such dressings provide an effective healing process by temporarily performing the basic physiological functions of the skin until the wound is completely healed. In recent years, innovations in wound dressings developed by combining plants and polymers have made great progress in wound care treatment (Rezvani Ghomi et al., 2019). This approach offers promising solutions in the field of wound healing by promoting the integration of natural resources into biomedical practices. Hydrogels, especially supported by plant extracts, contribute to the treatment process by accelerating wound healing, antibacterial and antioxidant properties (Ahmad et al., 2022; Pelin et al., 2023). Although the polymers used in cryogels are generally biocompatible, they do not show sufficient biological activity on their own. Therefore, it is important that they are combined with natural materials, such as plant extracts, to enhance antioxidant and antimicrobial properties (Singh et al., 2018; Liu and Jia, 2018). Cryogels obtained as a result of the combination of these polymers with herbal extracts are not only biodegradable and biocompatible, but also offer better mechanical resistance, antioxidant and microbial barrier properties. Considering the complexity of wound environments, new generation biopolymer-based wound dressings with breathable, immunomodulatory, exudate absorbing and microbial contamination inhibiting properties, which can be produced by simple synthesis processes, have the potential to offer innovative solutions in this field.

2.3. Properties of Hydrogels and Cryogels

Hydrogels are polymer-based materials that are widely used in the biomedical and engineering fields. Hydrogels; Thanks to its high-water-holding structure consisting of three-dimensional, cross-linked polymer networks, it plays an important role in many fields such as medicine, tissue engineering, drug delivery, wound dressings, and biosensors (Ho et al., 2022).

Cryogels, on the other hand, are a subclass of hydrogels, gel matrices that are synthesized by polymerization at low temperatures, forming porous and elastic structures (Razavi et al., 2019). Although the production of cryogels requires special synthesis conditions; Thanks to their advanced swelling capacity, superior mechanical strength and interconnected macroporous structures, they offer remarkable advantages, especially in wound care (Bereli et al., 2020).

This porous structure gives cryogels a high water absorption ability and volumetric recycling properties, while at the same time allowing gas exchange, creating a moist microenvironment in the wound area and ensuring the removal of excess exudate. In addition, the mechanical properties of cryogels similar to the extracellular matrix (ECM) are an important reason for preference in tissue engineering applications (Akin and Özmen, 2022). Hydrogels can be easily modified with biocompatible polymers and their chemical structure is open to functionalization, making them highly suitable for biomedical applications (Eggermont et al., 2019). Enriching these structures with natural components stands out as an important strategy in terms of increasing biological activity (Micale et al., 2020).

2.3.1. Combination with Biopolymers: PVA and Gelatin

Although the polymers used in cryogels are generally biocompatible, they do not show sufficient biological activity on their own. Therefore, it is important that they are combined with natural materials, such as plant extracts, to enhance antioxidant and antimicrobial properties (Singh et al., 2018; Liu and Jia, 2018). Synthetic polymers include PVA, polyglycolic acid, poly-l-lactic acid, and poly(ethylene glycol); Among natural polymers, structures such as alginate, Gel, silk fibroin, chitosan and hyaluronic acid (HA) are frequently used (Savina et al., 2021; Vishnoi et al., 2021). Cryogels obtained as a result of the combination of these polymers with herbal extracts are not only biodegradable and biocompatible, but also offer better mechanical resistance, antioxidant and microbial barrier properties. This method offers a

suitable platform for innovative designs involving the integration of AL extract with polymers such as PVA and Gel.

2.4. Biological Characteristics, Pharmacological Potential and Use in Wound Care of *A. lakoocha*

2.4.1. Usage of *A. lakoocha* in Wound Dressings

Traditional wound dressings do not respond to modern wound care needs due to their limiting properties such as strong adhesion, insufficient moisture holding capacity, and low gas permeability (Roy et al., 2010; Waring et al., 2001). At this point, the integration of natural resources such as *AL*, which has a high bioactive content, with wound dressings offers a promising alternative. However, scientific studies in this area are still limited. Even though it is an ideal wound dressing; it should be able to absorb excess exudate from the wound, provide a moist healing environment, offer effective protection against external factors, and allow gas exchange (He et al., 2022). It is also expected to be non-toxic, biocompatible, and biodegradable (Schoukens et al., 2019).

2.4.2. Usage of *A. lakoocha* in Traditional and Modern Medicine

The species *AL Roxb*. is a plant of economic and medicinal importance belonging to the Moraceae family. In traditional medicine, it has been widely used for malaria, diarrhea, abscesses, ulcers, snake bites, glandular swellings, expectorant and milk enhancer; at the same time, its leaves and fruit have been evaluated as medicinal against various diseases (Perry, 1980; Heyne, 1987; ICUC, 2003; Verheij and Coronel, 1992). Traditional knowledge such as the use of its leaves and flowers as fresh feed, its tonic effects, and its properties to increase milk yield are included in the literature (Pandey and Bhatnagar, 2009; Shailendra et al., 2010).

2.4.3. Phytochemical Profile and Bioactive Components

AL contains many bioactive components such as flavonoids, stilbenoids (especially oxyresveratrol), chalcones, lectins, and triterpenoids (Hakim et al., 2006; Kabir, 1998). These ingredients have been shown in various studies to have antioxidant, anti-inflammatory, antimicrobial, and anticancer effects (Likhitwitayawuid et al., 2006; Tsai et al., 2017). Oxyresveratrol stands out as the most dominant phenolic compound of this type and has a

stronger effect potential due to its one more hydroxyl group than resveratrol (Palanuvej et al., 2007).

2.4.4. Antimicrobial, Anti-Inflammatory Effects

In literature, it was observed that ethanol extract of *AL* provide bacterial inhibition against species such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Propionibacterium acnes*, *Pseudomonas aeruginosa*, *Corynebacterium* sp. with an average of 10–20 mm in the strong category in the disc diffusion assay (Senapong et al., 2014; Kaewkor et al., 2016; Teanpaisan 2014).

In a study conducted by Pertiwi et al., ethanolic extract of *AL* exhibited an inhibition zone with a diameter of 12.68 mm and 12.57 mm on *S. aureus* and 7.46 mm on *E. coli*, respectively, at a dose of 3000 µg/disc (Pertiwi et al., 2024). Methanolic extract, on the other hand, showed a strong bactericidal effect against both bacteria at a concentration of 100 mg/mL, with a 2.2 cm zone of inhibition, a minimum bactericidal concentration (MBC) of 312.5 µg/mL, and a minimum inhibitory concentration (MIC) of 625 µg/mL (Shailendra Kumar et al., 2010; Pandey and Bhatnagar, 2009). Besides bacteria, *AL* has been investigated for its ability to inhibit the growth of the Herpes simplex virus (HSV). The heartwood of *AL*, which contains large amounts of oxyresveratrol, has been reported to be an important source for the development of a new natural product as anti-HSV and anti-HIV agents (Likhitwitayawuid et al., 2005). In an in-vivo study, it significantly delayed the development of a herpetic skin lesion and protected animals from death (Chuanasa et al., 2008). Moreover, *AL* extract's anti-inflammatory effects were realized by suppression of cytokines such as IL-6, TNF-α, MCP-1 via PI3K/Akt and NF-κB pathways. These effects are also associated with the activation of mechanisms that promote wound healing. These antimicrobial and anti-inflammatory effects are thought to be demonstrated by the phytochemicals found in *AL* extract by disrupting bacterial cell wall and membrane integrity and inhibiting protein synthesis. The data obtained show that this extract has a high content of phenolics and flavonoids, is especially rich in oxyresveratrol, and exhibits strong antioxidant properties.

2.4.5. Contribution to Wound Healing and Literature Findings

The ethanolic form of *AL* extract promotes wound shrinkage, epithelialization, and matrix formation by increasing fibroblast proliferation, vascular endothelial growth factor-2 (VEGFR-2), transforming growth factor-β1 (TGF-β1), and platelet-derived growth factor-BB (PDGF-BB) gene expressions (Tanjung et al., 2022; Andriani et al., 2019; Hanafiah et al., 2024). It has also been reported that when used in combination with other herbal extracts, it shows synergistic effects and results in accelerating wound healing after tooth extraction (Satria et al., 2022).

2.4.6. Biomaterial Integration of *A. lakoocha* in the Literature

In the literature review, *a limited number of publications on the combination of AL* with biomaterials were identified. Only 2 publications have been found with the phrase "*Artocarpus lakoocha* biomaterials", and in most studies, gelatin has either not been used directly or has only been evaluated as a biomarker (Mishra et al., 2014; Charron et al., 2019; Brüningk et al., 2019). This suggests that the integration of *AL* in plant-added biomaterial designs is an innovative approach.

2.4.7. Development of Biomaterials with *A. lakoocha*

Within the scope of the project, gelatin and *AL* extract will be combined with PVA matrix to apply chemical crosslinking via glutaraldehyde and biologically enriched functional cryogels will be synthesized. Characterization of these biomaterials; swelling behavior will be carried out by methods such as porosity, FTIR, BET, biodegradation, MTT test, SEM, live/dead staining, agar disk diffusion test and qRT-PCR. In addition, the expression levels of *ITGB1, FAK and IL-1β* genes will be analyzed in detail (Hynes, 2002; Schaller, 2010; Dinarello, 2009).

2.4.8. Clinical Application Potential and Scientific Contribution

It has been determined that PVA/Gel/*A. lakoocha* based cryogels developed in line with the data obtained support proliferation in HaCaT cells, do not show cytotoxic effects and offer a biocompatible system that can be used as a wound dressing. In laryngeal cancer cell lines, it has demonstrated anti-tumor potential by showing proapoptotic effects (Kuran et al., 2024).

2.5. Gaps in Literature and Scientific Contribution

At this point, the integration of natural resources such as A. lakoocha with high bioactive content with wound dressings offers a promising alternative. However, scientific studies in this area are still limited. Considering the complexity of wound environments, new generation biopolymer-based wound dressings with breathable, immunomodulatory, exudate absorbing and microbial contamination inhibiting properties, which can be produced by simple synthesis processes, have the potential to offer innovative solutions in this field. This method offers a suitable platform for innovative designs involving the integration of *AL* extract with polymers such as PVA and Gel. The PVA and Arm-based biodegradable cryogel wound dressing enriched with *A. lakoocha* extract to be developed within the scope of this project is a strategic initiative that will both reduce Türkiye's technological dependence on foreign medical equipment production and increase its capacity to develop innovative products based on biomaterials.

The skin is the human body's first line of defense against external factors and acts as a protective barrier (Farley et al., 2012). Wounds that cause the deterioration of this barrier are defined as damage to tissues caused by external or internal reasons that impair physiological structure and function (Ramasastry and Sai, 2005). Inadequacy of wound care causes millions of people around the world to become ill or die due to infections (Dai et al., 2011). For example, it is known that approximately 30-40 out of every 1000 surgical interventions develop infections, and some of these infections are life-threatening. Chronic non-healing wounds, on the other hand, have become a major public health problem, affecting approximately 2% of the US population (Gaspar et al., 2019). For this reason, rapid closure of the wound is of great importance in terms of regaining the barrier function of the skin. The main objectives of wound care are; to prevent moisture loss, to protect against microorganisms and to minimize the damage of external factors (Gaspar-Pintiliescu et al., 2019). For all these reasons, the development of effective wound care materials is of great importance both in terms of preventing infections and shortening the healing time. For this purpose, various materials have been used to support wound care throughout history (Koyutürk and Soyaslan, 2016). Wound healing; Hemostasis is a dynamic and complex process consisting of stages such as inflammation, proliferation and restructuring. In order for this process to proceed healthily, it is of great importance that the materials used are biocompatible (Alven et al., 2020). Traditional

wound dressings – such as iodophore gauze, hydrocolloids and silver-containing materials – do not fully meet the basic requirements such as exudate absorption, moisture balance, infection prevention and air permeability. In addition, the lack of immunomodulatory properties of these dressings limits their contribution to the healing process and causes them to be ineffective, especially in complex or chronic wounds (Long et al., 2022). Understanding the contribution of plant-based substances to wound healing paves the way for innovations in this field. Its limiting properties, such as strong adhesion, insufficient moisture-holding capacity, and low gas permeability, mean that these traditional approaches are unable to respond to modern wound care needs (Roy et al., 2010; Waring et al., 2001). At this point, the integration of natural resources such as *A. lakoocha* with high bioactive content with wound dressings offers a promising alternative. However, scientific studies in this area are still limited. However, it is an ideal wound dressing; It should be able to absorb excess exudate from the wound, provide a moist healing environment, offer effective protection against external factors, and allow gas exchange (He et al., 2022). It is also expected to have non-toxic, biocompatible, and biodegradable qualities (Schoukens et al., 2019). Today, bandages, gauze bandages and various fixative materials are often used, especially in the treatment of superficial wounds. However, these materials; it does not adapt well to rough or hairy skin surfaces, increase the risk of infection by causing a gap between the wound and the dressing, and cannot provide adequate fluid absorption or deep wound coverage (Liu and Jia, 2018). In order to overcome such clinical limitations, a new generation of biopolymer-based, functional polymeric membranes is being developed using advanced tissue engineering techniques (White et al., 2012). Modern wound dressings promote not only protection from infection, but also the regeneration of dermal and epidermal tissues. These versatile biomaterials, designed with different functional properties, play an important role in accelerating the healing process and restoring the structural integrity of the skin (Gaspar-Pintiliescu et al., 2019). Such dressings provide an effective healing process by temporarily performing the basic physiological functions of the skin until the wound is completely healed.

Plant-based therapies have been an important source of medical treatment since ancient times. Today, many different medicinal plants and their extracts are studied and used for their potential restorative and therapeutic effects on wound healing (Maver et al., 2015). The medicinal value of plants is based on the presence of phytochemical components such as flavonoids, tannins,

alkaloids and phenolic compounds. These bioactive substances found in plants support and accelerate the wound healing process. These herbal extracts promote wound shrinkage, epithelialization, and vascularization by being added to wound dressing materials, thereby accelerating the wound healing process (Gaspar-Pintiliescu, 2015). In recent years, innovations in wound dressings developed by combining plants and polymers have made great progress in wound care treatment (Rezvani Ghomi et al., 2019). This approach offers promising solutions in the field of wound healing by promoting the integration of natural resources into biomedical practices. Hydrogels are polymer-based materials that are widely used in the biomedical and engineering fields. Hydrogels; Thanks to its high-water-holding structure consisting of three dimensional, cross-linked polymer networks, it plays an important role in many fields such as medicine, tissue engineering, drug delivery, wound dressings, and biosensors (Ho et al., 2022). Cryogels, on the other hand, are a subclass of hydrogels, gel matrices that are synthesized by polymerization at low temperatures, forming porous and elastic structures (Razavi et al., 2019). Although the production of cryogels requires special synthesis conditions; Thanks to their advanced swelling capacity, superior mechanical strength and interconnected macroporous structures, they offer remarkable advantages, especially in wound care (Bereli et al., 2020). This porous structure gives cryogels a high water absorption ability and volumetric recycling properties, while at the same time allowing gas exchange, creating a moist microenvironment in the wound area and ensuring the removal of excess exudate. In addition, the mechanical properties of cryogels similar to the extracellular matrix (ECM) are an important reason for preference in tissue engineering applications (Akin and Özmen, 2022). Hydrogels can be easily modified with biocompatible polymers and their chemical structure is open to functionalization, making them highly suitable for biomedical applications (Eggermont et al., 2019). Enriching these structures with natural components stands out as an important strategy in terms of increasing biological activity (Micale et al., 2020). Hydrogels, especially supported by plant extracts, contribute to the treatment process by accelerating wound healing, antibacterial and antioxidant properties (Ahmad et al., 2022; Pelin et al., 2023). Although the polymers used in cryogels are generally biocompatible, they do not show sufficient biological activity on their own. Therefore, it is important that they are combined with natural materials, such as plant extracts, to enhance antioxidant and antimicrobial properties (Singh et al., 2018; Liu and Jia, 2018). Synthetic polymers include PVA, polyglycolic acid, poly-l-lactic acid, and poly(ethylene

glycol); Among natural polymers, structures such as alginate, Gel, silk fibroin, chitosan and hyaluronic acid (HA) are frequently used (Savina et al., 2021; Vishnoi et al., 2021). Cryogels obtained as a result of the combination of these polymers with herbal extracts are not only biodegradable and biocompatible, but also offer better mechanical resistance, antioxidant and microbial barrier properties. Considering the complexity of wound environments, new generation biopolymer-based wound dressings with breathable, immunomodulatory, exudate absorbing and microbial contamination inhibiting properties, which can be produced by simple synthesis processes, have the potential to offer innovative solutions in this field. This method offers a suitable platform for innovative designs involving the integration of *A. lakoocha* extract with polymers such as PVA and Gel.

The prevalence of diabetes in Türkiye is 15.9% (approximately 12 million people) and the prevalence of chronic wounds varies between 0.18-1.3%. In addition, the fact that infection is the cause of amputation in 60% of patients with diabetic foot ulcers clearly reveals the need for anti-infection and wound-healing bioactive materials. However, the vast majority of advanced wound care products used in Turkey are imported and domestic production remains limited. The Turkish medical device market is estimated at TL 17 billion in 2022 and is expected to reach TL 25.3 billion in 2026; One of the biggest items in this increase is wound care products (SEİS, 2022). In the first quarter of 2024, there is a high demand for products such as silver hydrophilic wound dressing (118,574 units) and arm-based wound dressings (11,990 units) in public procurement, indicating a high-volume commercial potential in this field (DMO, 2024). In this context, the cryogel-based dressing to be developed will offer antioxidant and antimicrobial properties with its innovative content (*A. lakoocha* extract), It will be both an environmentally friendly and biocompatible alternative with its PVA/Armbased biodegradable structure. Successful development of this product; it will encourage domestic innovation in the tissue engineering, regenerative medicine, medical textiles and wound care products sectors, and increase the export potential of biomedical materials. As a result, this thesis is a national strategic achievement that can be the basis for the nationalization of high value-added medical products that cannot be produced domestically, reducing foreign exchange outflow, increasing competitiveness in international markets and developing bioactive, sustainable, environmentally friendly medicinal products. This project has the potential to make a partial and focused contribution to the reduction of foreign dependency in the relevant category in the

import of medical equipment by targeting the use of biomaterials of natural origin in wound care. By creating an alternative to imported products in health areas such as chronic wound treatment, it will contribute to the development of a potential biomaterial that will both contribute to the health economy and increase national competitiveness. The consumption of medicines of herbal origin is quite common in both traditional and modern medicine and is increasing significantly. According to the World Health Organization, more than 80% of the world's population in developing countries is primarily dependent on plant-based medicines for their basic health needs (Canter et al., 2005). A few of these breeds are viz. Morus, Ficus, and Artocarpus are economical food sources and are widely used in traditional medicine, agriculture, and industry (Jagtap and Bapat, 2010). These genera have received a great deal of scientific attention, as they contain medically important secondary metabolites with beneficial biological activities. Many species of Artocarpus are used for food and traditional folk remedies in Southeast Asia, Indonesia, the western part of Java and India. As a group among forest tree plants, Artocarpus species are known to occupy various ecological niches in different habitats and are located in various forest ecosystems. The Artocarpus (Moraceae) family consists of 60 genera, consisting of 1400 species distributed in the tropical and subtropical regions of Asia. The genus Artocarpus J.R. & G. Foster (Moraceae) consists mainly of breadfruit and jackfruit trees. 50 species of this genus are widely observed in South and Southeast Asia, New Guinea and the South Pacific. These are confined to evergreen forests in the humid tropics and are usually found in habitats below 1000 m altitude. Artocarpus species are evergreen or deciduous small to large monoecious trees; All parts contain white latex. The leaves can be spirally arranged or alternate and distinct, simple and whole pubescent or pubescent, coriaceous, glabrous or pubescent. The medicinal value of the Artocarpus family has been known for a long time and includes Artocarpus heterophyllus (jackfruit), Artocarpus altilis (breadfruit), Artocarpus chempeden (Chempedak) *Artocarpus lakoocha* Roxb. (Monkeyjack, Species such as *A. lakoocha*, *Artocarpus lacucha* Buch.-Ham, *A. lakoocha*) are of considerable economic importance as a source of edible aggregate fruit, yield very good timber and are widely used in folk remedies (Verheij and Coronel, 1992). Many members of the genus Artocarpus have also been used in Southeast Asia as traditional folk remedies for the treatment of inflammation, malarial fever, abscesses, and diarrhea (Perry, 1980; Heyne, 1987). Some fruits of the species are used as a cooling tonic and pectoral, to increase milk in women and animals, and the leaves

are used as a healer for ulcerative wounds. Plant latex mixed with vinegar promoted the healing of abscesses, snake bites and glandular swellings. The leaves and bark of the stems have been used to treat anemia, asthma, dermatitis, diarrhea, cough, and as an expectorant. In addition, extracts from underground and aboveground plant parts of Artocarpus species have been used in traditional medicine as expectorants and in the treatment of diabetes, tapeworm infection, anemia, asthma, dermatitis (Balbach and Boarim, 1992; ICUC, 2003). However, Artocarpus species, especially extracts from leaves, bark, trunk and fruit, and metabolites obtained have been shown to have antisyphilic, antibacterial, antitubercular, antiviral, antifungal, antiplatelet, antiarthritic, antidiarrheal, cytotoxic, anticancer, tyrosinase inhibitor, antihelminthic, antiinflammatory, antioxidant, tyrosinase, 5-lipoxygenase activities (Jagtap and Bapat, 2010). Fruits, seeds and trunk wood contain chemical compounds that have aphrodisiac properties and a soothing effect in contractions (Ferrao, 1999). Some flavonoids in Artocarpus species have been reported to have anti-cancer functions against reactive oxygen species (Tsai et al., 2017). In previous studies, anticancer activities of Artocarpus species have been reported in various cancer cell lines, including melanoma (Arung et al., 2010), hepatocellular carcinoma (Tzeng et al., 2015), gastric carcinoma (Ma et al., 2010) and colorectal carcinoma (Soo et al., 2017), liposarcoma (Fang et al., 2008), leukemia (Chatterjee et al., 2008), breast cancer, and nasopharyngeal carcinoma (Puntumchai et al., 2004). The fruits of *A. lakoocha* or Ma-haad are usually eaten fresh. It is known as "lakuchi" in India, "lokhat" in Thailand, and "tampang" in Malaysia. It is mostly known for its wood and edible fruits due to its excellent nutraceutical properties (Shailendra et al., 2010). In the foothills of the Himalayas in Nepal, the leaves and flowers of this tree serve as fresh feed for lactating animals due to its excellent source of bioactive compounds (Pandey and Bhatnagar, 2009). It is believed that the pulp of the edible fruit acts as a tonic for the liver. Raw fruits and their flowers (acidic and astringent) are used in pickles and chutneys (Shailendra et al., 2010). Wood and its roots are used as dyestuffs (Joshee et al., 2002). It has been used in Thai traditional medicine and the extract and bioactive compounds from the skin, leaves, seeds, and pericarps of the *AL* fruit have been shown to have exceptional phytochemical, nutrient, and valuable pharmacological properties. The main active ingredient is oxyresveratrol (trans-2,3′,4,5′-tetrahydroxystilbene), a resveratrol analogue that is a polyphenolic stilbene predominant in the heartwood of the plant and carries an extra hydroxyl group relative to resveratrol (Palanuvej et al., 2007; Likhitwitayawuid et al., 2006). In addition,

apart from stilbene, lectins and a flavonol are found in glycosides (Puntumchai et al., 2004). Due to its high oxyresveratrol content and low toxicity, *A. lakoocha* is a rationally good candidate for pharmacological treatments (Nilvises et al., 1985). Brown powder, called PuagHaad in Thailand, is a product of the aqueous extraction of *A. lakoocha*, which is prepared by boiling wood chips and then evaporating water. This preparation has been used in Thailand as a traditional anthelmintic medicine for the treatment of tapeworm infection (Salguero, 2003). *A. lakoocha* was evaluated for in-vitro tyrosinase-inhibiting activity and in-vivo melanin-reducing activity in human volunteers. It has been reported that *A. lakoocha* may have a promising potential with its melanogenesis inhibitor and antioxidant activity as an effective and economical skin whitening agent (Tengamnuay et al., 2006). *A. lakoocha* has been reported to exert fasciolicidal action against adult Fasciola gigantica by causing tegumental damage, and has been used as an anthelmintic by indigenous people in Thailand and Laos (Saowakon et al., 2009; Salguero, 2003). Recent studies have shown that *A. lakoocha* has many pharmacological activities such as anti-cancer, anti-diabetic, anti-tyrosinase, anti-tubercular, anti-fungal, anti-platelet, anti-arthritic, antibacterial, anti-oxidant, hepatoprotective, neuroprotective, cytotoxicity and H_2O_2 clearance anti-inflammatory (Likhitwitayawuid et al., 2006; Hankittichai et al., 2020; Burlando et al., 2017; Puntumchai et al., 2004; Wongon and Limpeanchob, 2020; Peng et al., 2018). All these effects make it logical to integrate *A. lakoocha* into wound dressings and use it in functional biomaterial designs. In an in-vivo study, hydro-methanolic extract of *A. lakoocha* was shown to have a strong analgesic effect and did not produce any toxic effects on experimental animals (Nesa et al., 2015). This plant also has pharmacological properties that protect nerve cells and the liver. It has been reported that it supports cell survival, reduces reactive oxygen species (ROS) levels and shows neuroprotective effects by suppressing lipid peroxidation, especially thanks to the oxyresveratrol compound in its aqueous extract (Hasriadi et al., 2017). In addition, it is reported that it may protect against neurodegenerative diseases through its regulatory effects on the expression and pharmacokinetics of redox-sensitive antioxidant enzymes. *A. lakoocha*, which also draws attention from a metabolic point of *view*, significantly reduces serum total cholesterol, triglyceride, and low-density lipoprotein (LDL) levels, while showing effects that increase high-density lipoprotein (HDL) levels (Shafaq et al., 2022). On the other hand, ethanolic extract has been observed to promote cell proliferation and migration, as well as accelerate wound healing in NIH-2T3 cell lines by increasing VEGFR-3

gene expression (Nazliniwaty et al., 2022). *A. lakoocha* has been shown to have anti-aging effects in relation to its antioxidant and antiglycation activities, with the phytooxyresveratrol compound it contains (Suwannalert et al., 2012). Furthermore, in studies conducted on the RAW 264.7 mouse macrophage cell line, it was determined that the plant's ethanol extract significantly reduced the production and secretion of cytokines and chemokines such as IL-6, TNF-α and MCP-1, which are stimulated by lipopolysaccharide (LPS). This anti-inflammatory effect has been reported to be through inhibition of PI3K/Akt and NF-κB signal transduction pathways. In one study, the healing effect of nanoemulgel containing ethanol extract of *A. lakoocha* leaf on burn wounds was evaluated. In this study, it was found that the formulation of nanoemulgel provided significant improvement in wound diameter reduction and fibroblast proliferation in rats treated for burns; It has been shown to promote wound healing by increasing TGF-β1 and PDGF-BB gene expression (Tanjung et al., 2022). In addition, extracts of *A. lakoocha* leaves have been reported to provide baseline data for excisional wound healing and potency in rats, supporting traditional claims (Andriani et al., 2019). In this study, the wound-healing effect of the ointment formulated using a combination of ethanol extract of *A. lakoocha* leaves and pure coconut oil (VCO) was evaluated. It has been shown that VCO enriched *A. lakoocha* extract shortens epithelialization time and significantly increases wound contraction in excisional wound type (Hanafiah et al., 2024). In an in-vivo study, the effect of the gel form combination of ethanolic extracts of *A. lakoocha* and *Anredera cordifolia* leaves on fibroblast and osteocyte proliferation in socket wounds after tooth extraction was evaluated and it was shown to have the potential to accelerate wound healing (Satria et al., 2022*). A. lakoocha* evaluated the in-vitro tyrosinase inhibition and in-vivo melanin-reducing effects of heartwood extract. In addition, it has been observed that the whitening effect appears earlier and stronger with the use of the extract in lotion formulation, indicating that it can be an effective and economical skin lightening agent (Tengamnuay et al., 2006). Other studies have shown *that A. lakoocha* heartwood hydroglycolic extract shows strong antioxidant and tyrosinase inhibitory effects. Encapsulation of this extract with liposomes both increased skin permeability and increased efficiency by providing controlled release. Furthermore, in vivo studies, the skin whitening effect of liposome-laden lotion was found to be more pronounced in human volunteers than in the non-encapsulated formulation (Teeranachaideekul et al., 2013). These studies have revealed that *A. lakoocha* is a valuable vegetable resource that can be used in the

production of medicines and medical equipment in the fields of pharmaceuticals, cosmetics and health (Xu et al., 2014). Thanks to its high oxyresveratrol content, this plant, which has anticancer, antimicrobial, antibacterial and antiviral effects, offers a remarkable potential especially for wound healing and tissue engineering applications. Despite its strong antioxidant and antimicrobial properties, there are no direct studies in the literature on the in-vitro wound healing supportive effects of *A. lakoocha* by integrating it into polymeric biomaterials. In line with the findings of the literature, an innovative approach including the synergistic use of gelatin and herbal additives has been developed within the scope of our project. Gel and *A. lakoocha* extract will be integrated into the PVA matrix to chemically cross-link with glutaraldehyde, resulting in biologically enriched functional cryogels. This makes the idea of developing a functional wound dressing by combining *A. lakoocha* with the PVA/Arm matrix scientifically valuable in our project. On the other hand, the literature review on the "Moraceae" family identified 5,299 publications, 1,653 publications with the keyword "Wound Healing Medicinal Plant", and only 49 publications with the phrase "Moraceae Wound Healing". This suggests that species belonging to the Moraceae family have high medicinal and wound healing potential, but there are still limited studies in this area. In the scan conducted on the "*A. lakoocha*" species, which belongs to the Moraceae family, it was determined that there were 67 general publications and only 2 publications with the expression *"Artocarpus lakoocha* biomaterials". In the available literature, it has been reported that oxidative stress is reduced in human monocytes and the release of proinflammatory cytokines such as R848-stimulated IL-12, IL-6, TNF-α is suppressed in dendritic cells in studies in which A. lakoocha-derived oxyresveratrol is loaded into PLGA nanoparticles (Donini et al., 2021; Gaglio et al., 2021). These effects indicate wound healing, inflammation-reducing and antimicrobial properties. Likewise, although data on the use of *A. lakoocha* in biomaterials reveal its potential, the number of studies on its integration into polymeric materials is very limited. This makes the idea of developing a functional wound dressing by combining *A. lakoocha* with the PVA/Arm matrix scientifically valuable in our project. In in vitro tests performed on the HaCaT cell line, cytotoxic effects were observed at high doses, while increasing cell proliferation at low concentrations. In laryngeal carcinoma cell lines, selective cytotoxic and proapoptotic (Bax, BCL-2) effects of the extract were determined. In addition, PVA/Gel-based cryogels containing 1.5% *A. lakoocha* increased cell viability and proliferation and provided better adhesion of the cells to the matrix in culture

studies with HaCaT cells. These findings suggest that *A. lakoocha* can be evaluated as a potential biomaterial in wound healing processes. Therefore, within the scope of the thesis, it is aimed to synthesize PVA and Gel-based cryogels containing different concentrations of *A. lakoocha* through physical mixing, chemical crosslinking and freeze drying, and to comprehensively characterize the physical, chemical, morphological and bioactive properties of these biomaterials with further studies. For this purpose, *A. lakoocha* extract and monomer solution at different mixing ratios will be used to design polymeric macroporous scaffolds. For the characterization of the obtained biomaterials, swelling behavior with "Swelling test", polymer formation process with "Polymerization efficiency", porosity structure with "macroporosity", pore homogeneity with "surface morphology", chemical structure with "Fourier Transform Infrared Spectroscopy (FTIR) analysis", degradation process with "Biodegradation profile", surface area properties with "Brunauer-Emmett-Teller (BET)" will be characterized. However, it will evaluate the antibacterial activity with the "Agar disc diffusion test". In addition, by culturing cells in biomaterials, "MTT assay", "Live/dead cell staining" for cell viability, effect on biocompatibility; "Scanning Electron Microscope (SEM) analysis", "Immunofluorescence staining (F-actin, DAPI)" methods will be applied to evaluate its effect on cell adhesion, body and nucleus morphology. As a result, an important step will be taken towards the development of an innovative and effective dressing material that can be used in wound care. This study will allow us to better understand the contribution of plant-based bioactive components to the wound healing process and strengthen the role of naturally sourced materials in modern biomaterial technologies. At the same time, it has the potential to contribute to the development of effective solutions that can be used in the treatment of chronic and infected wounds by offering a complementary alternative to existing treatment approaches. The outputs of the project are expected to pave the way for advanced studies to investigate the evaluability of the developed biomaterial in pre-clinical applications. Thus, this study will reveal an innovative approach that will both contribute to the scientific literature in the field of wound healing and serve as a basis for future multidisciplinary research.

a) Patent/Utility Model Screening: Cryogels are biomaterials with wide application potential in biomedical fields such as tissue engineering, controlled drug release, and regenerative medicine. Cryogels, especially developed with combinations of natural and synthetic polymers, attract attention due to their superior properties such as mechanical

durability, biocompatibility and controlled release. As a result of the scans in national and international patent databases, "*Artocarpus lakoocha*" and "*Artocarpus lakoocha* wound dressing" were not found in the national database, but some important applications and registered patents were detected in international databases. It includes water-based extraction of oxyresveratrol from heart wood and purification by membrane technology. This method offers an environmentally friendly and energy-efficient process. This invention enables the purification of oxyresveratrol from *A. lakoocha* extract by water-based extraction and nanofiltration membrane with high efficiency. The process only acquires the trans isomer while not causing cis isomerization. This patent provides a method for the extraction of oxyresveratrol from *A. hirsutus* (a different species of the same genus). This method provides a highly efficient extraction process. This invention describes cosmetic and pharmaceutical compositions with skin whitening effects containing resveratrol derivatives (in acetylated or hydroxy form) or their cosmetically or pharmaceutically acceptable salts from *A. lakoocha*. This invention describes cosmetic formulations with nourishing and restorative effects for skin care, containing natural extracts such as *Centella asiatica*, ginseng, turmeric, artemia, silk protein and white tea oil, including *A. lakoocha*. One of the inventions describes a solution and manufacturing process that promotes hair growth. Herbal raw materials such as Mahad (*A. lakoocha*) and coffee, which are obtained from plants grown in countries of natural origin, are used as active ingredients that promote hair growth, reduce hair loss and support new hair growth. This formulation is a patented drug, focused on the extraction and purification processes of oxyresveratrol from *A. lakoocha* and similar species, and these compounds are not directly related to wound dressing or biomaterial applications. Similarly, patents describe the use of ingredients derived from *A. lakoocha* in cosmetic or pharmaceutical fields, particularly in skin and hair care products, but do not include the use of these compounds as wound dressings or biomaterials. Although extensive searches have found some wound dressing patents containing plant extracts, there are no specific applications for wound dressing materials enriched with *A. lakoocha* or its extract. As a result, the current patents focus only on obtaining bioactive compounds of *A. lakoocha* and do not provide any information on the use of these compounds in cryogel wound dressings containing PVA and/or Gel. Therefore, the PVA and Arm-based cryogel wound dressing enriched with A. lakoocha extract, which we proposed in our study, represents an innovative and original application that is not included in the literature and current patent applications.

In the patent database of the Turkish Patent and Trademark Office, only 6 patent applications were detected in the search with the keyword "cryogel". On the other hand, while there were 34 applications and registered patents in the search made with the keyword "wound dressing", only 1 application was found in the query made with the expression "cryogel wound dressing". This patent covers the development of a hybrid biomaterial that can be used in biomedical applications such as bone regeneration by combining cryogel and nanofiber technologies. This invention aims to use porous cryogels produced from natural biomaterials such as LBG, XG and MG as a tissue scaffold to which cells can attach and grow, or as a delivery system for the controlled release of drugs, hormones and growth factors. One of the inventions relates to the first photopolymerization of the cryotropic gelation of an acrylamide-based monomer system in a frozen state at a certain temperature and under isothermal conditions. The invention is also related to the prevention of hydrogel products formed as a result of premature polymerization by means of this new initiator system. This invention was developed for use in the treatment of melanoma; It describes a nanofiber wound dressing made of polymers such as PVA, PLA, PLGA, containing photodynamic therapy agent (graphene quantum dots), chemotherapeutic drug (doxorubicin) and Gel-based hydrogel, and its production method. It is related to the cryogel wound dressing, which protects the wound from bacterial infections by releasing *Origanum Minutiflorum* L. (Thyme) essential oil, which can be used in acute or chronic wounds, accelerates the regeneration of cells in the tissue, heals wounds quickly and has liquid absorption properties. Applications which were detected in the scans made in the national patent database, generally focus on cryogel synthesis methods and are not directly related to the use of biopolymeric materials containing PVA and/or Arm as wound dressings. In our study, we proposed a porous cryogel biomaterial enriched with *AL*, based on PVA and Gel; From the photodynamic therapy-based wound dressing containing nanofiber PVA and Arm; the cryogel that releases oregano essential oil is markedly different from the wound dressing in terms of content and intended use. As a result, there are no patent or utility model applications in the existing national databases for the use of PVA and gel-based cryogel structures enriched with *AL* extract as wound dressings. This makes it clear that the project we propose aims at both an innovative and original application and shows that it differs from the literature and existing patents.

There are some patent and utility model applications in the fields of "cryogel", "polyvinyl alcohol-based cryogel", "gelatin-based cryogel" and "polyvinyl alcohol-gelatin-based hydrogel" in international patent databases. Below are summarized some of the important applications and registered patents in this field. First invention presents a two-layer skin skeleton; The epidermal layer consists of a lyophilized film containing chitosan and gelatin, while the dermal layer consists of a macroporous cryogel containing chitosan, gelatin, egg membrane membrane, and rutin nanoparticles. It is designed for skeletal, skin substitutes, wound dressings, tissue engineering products and devices for therapeutic purposes. In addition, the production process of this two-layer structure is described in detail. One of the patents covers the biomedical uses of cryogel scaffolds with bioadhesive properties that can be administered by injection. Another patent focuses on the production of three-dimensional, porous and cell-free skeletal structures obtained by cryogelation method and the use of these structures in tissue engineering applications. In cryogel formation, highly porous, elastic and compressible biomaterials are obtained by using freezing and thawing processes at low temperatures. These skeletons can be applied to the body with minimally invasive methods and have potential in areas such as nerve, bone and cartilage tissue repair. The other invention describes a PVA-based cryogel structure containing therapeutic agents that provides controlled release. The bandage may contain ion exchange resins and hydrophobic particles to balance the release and plasticizers to provide softness. One another patent focuses on the preparation and use of PVA-based cryogels for biomedical applications (such as tissue substitutes, implants, artificial organ supports). Patent deals with the use of highly porous, elastic and compressible 3D skeletal structures produced by cryogelation with natural polymers such as chitosan, alginate, gelatin and gelatin in tissue engineering, especially nerve, bone and cartilage repair. One another patent describes the production of gelatin-based cryogels obtained by freezing animal gelatin at low temperature by crosslinking it with modified polysaccharides; these structures can be used in biomedical applications such as biological skeleton, cell culture, drug delivery and dressing. This patent focuses on the preparation of crosslinked hydrogel compositions containing Arm and hyaluronic acid, these gels have been developed for use in soft tissue repair and implant applications. These gels, which are biodegradable and promote cellular growth, have significant potential, especially in the fields of aesthetic medicine and regenerative medicine. Another invention presents a method for preparing a cross-linked PVA-

Arm IV hydrogel skeleton for cell culture. The method provides a biocompatible skeleton with a strong gel structure by mixing PVA and Gel IV in certain proportions and then applying radiation in the range of 25–50 kGy. Cryogels are biomaterials with wide application potential in fields such as tissue engineering, controlled drug release, and regenerative medicine. In particular, cryogels, which are obtained by combinations of natural and synthetic polymers, stand out with their mechanical durability, biocompatibility and controlled release properties. The applications which were detected in the scans made in international patent databases, are about cryogel skeletal systems that can be applied therapeutically based on natural components; however, it does not directly include the use of cryogels containing PVA and/or Arm as wound dressings. Similarly, some of the patents focuses on the general biomedical use of PVA-based cryogel structures in controlled release, tissue repair, and implant applications, but do not include biologically active wound dressing applications enriched with plant extracts. In addition, patents deal with the production of arm-based cryogels and their use in biomedical fields; one of them deals with cryogel skeletal structures obtained with natural polymers, and one another deals with the conversion of animal arm into cryogel by cross-linking. However, these patents do not contain any information about *AL* or its extract, as well as PVA-containing and its use as a wound dressing. In addition, patent which was detected in scans with the keyword "polyvinyl alcohol-gelatin-based hydrogel", covers the use of hydrogels containing arm and hyaluronic acid for soft tissue repair; another patent deals with the preparation of PVA and Arm IV-based hydrogel skeletons in accordance with cell culture. In conclusion, our proposed study focuses on the development of PVA and gel-based porous cryogel structure enriched with AL extract and investigates the potential of this structure for use as a wound dressing material. This approach, which is unique in national and international patent databases in terms of formulation, synthesis method, biological content and purpose of application, clearly reveals both the innovative and original aspect of our study.

b) Innovative aspect: *AL* is a biologically rich plant species native to South and Southeast Asia, which grows naturally in monsoon climates such as India, Thailand, Nepal, Malaysia, Indonesia, Bangladesh and Myanmar, and has no natural population in Türkiye. Thanks to its oxyresveratrol content, it has a high value, especially in the medical and cosmetic fields. Although the plant itself is not included in the flora of Türkiye, its extract is widely offered for sale in commercial packages (50–500 g) by international suppliers and there are no restrictions

on its supply. In this context, 500 g of *ALa* extract to be used for our project has already been procured from a reliable international supplier for the 2024 production year. In addition, literature reviews and commercial records show that this plant or its extract has not been used in Turkey before, which supports the originality of our project on both a domestic and a field basis. PVA/gelatin-based cryogel wound dressing enriched with *ALa* extract to be developed within the scope of the project is an innovative form of biomaterial that has no equivalent in the existing literature and patents in terms of both content and functional properties. Searches of national and international patent databases have found that applications for the plant *AL* are limited to the extraction of compounds such as oxyresveratrol and cosmetic applications . However, there has been no registration or patent application indicating that this extract is used as a biomaterial in the form of wound dressing in PVA and gelatin matrix. Similarly, most of the commercially available wound dressing biomaterials are either entirely synthetic-based or *ALa*, even if it contains plant extract. In the national patent database, there is only one application under the heading "cryogel wound dressing", and this application is for a product that releases oregano oil. Thus, it appears that there is an important gap in the development of bioactive cryogel structures containing *AL* as wound dressings. In the literature, the potent antioxidant and antibacterial effects of AL extract have been associated with its potential to accelerate free radical scavenging and tissue regeneration, especially thanks to its oxyresveratrol content (Shailendra Kumar et al., 2010; Kamble et al., 2022; Pertiwivd., 2024). Thanks to the cryogel structure in which these features will be combined with a controlled release system, it is aimed to reduce the risk of infection and accelerate healing in the wound healing process. In addition, the porous matrix based on biodegradable PVA and gelatin supports cell infiltration, moisture balance and tissue adaptation, providing a platform that is compatible with the wound microenvironment. As a result, the bioactive wound dressing to be developed with this project; It offers an innovative and original solution both scientifically and industrially with its plant extract-supported cryogel structure, which is not included in the literature and patents, the combination of antioxidant and antibacterial effects, and the use of *AL* extract for the first time in the PVA-Gel matrix.

3. METHODOLOGY

3.1 Preparation of *Artocarpus lakoocha* Extract

A commercial ethanolic extract of *AL* heartwood (ARTONOX®) was used in this study. The extract (Product Code: 0102) was supplied by Sabinsa Corporation/Sami Labs Limited and standardized to contain 95.1% oxyresveratrol (w/w, HPLC). *AL* powder was dissolved in a 2% (v/v) DMSO solution and subsequently filtered through sterile Millex® syringe filters (0.22 μm). The *AL* extract was stored at 4 °C and kept at this temperature until used for the cryogel preparation.

3.2 Preparation of Cryogels

For the preparation of cryogel matrices, a 10% (w/v) poly(vinyl alcohol) (PVA, Sigma-Aldrich, USA) solution was prepared by dissolving PVA in ultrapure water at 90 °C under continuous stirring, while a 3% (w/v) gelatin (bovine skin, Sigma-Aldrich, USA) solution was obtained at 40 °C. These two polymeric solutions were then mixed at a 1:2 (v/v) ratio to obtain a homogeneous blend. The *AL* extract was subsequently incorporated into this polymer mixture to reach final concentrations of 0.5% (w/v) and 1% (w/v). Glutaraldehyde (Merck, Germany) was employed as the cross-linking agent, and the mixtures were gently homogenized and immediately dispensed into 24-well plates. A control cryogel, prepared without the addition of *AL* extract, was also synthesized under identical experimental conditions. All cryogel formulations were frozen at −16 °C for 12 h to initiate the cryogelation process, followed by thawing at room temperature and repeated washing with distilled water to remove unreacted monomers and residual reagents.

Thus, three experimental groups were formed:

(i) **Blank Cryogel** (without *AL* extract),

(ii) **AL-0.5** (cryogel containing 0.5% *AL* extract),

(iii) **AL-1** (cryogel containing 1% *AL* extract).

3.3 Characterization Studies

The surface morphology of the synthesized cryogel samples, namely Blank, AL-0.5, and AL-1, was investigated by Scanning Electron Microscopy (SEM) in order to examine the internal architecture and porosity of the structures. Prior to SEM analysis, the cryogel samples were first equilibrated in distilled water to ensure complete swelling. Following this step, excess surface water was gently removed, and the swollen cryogels were frozen at −20 °C to preserve their internal pore structure. Subsequently, the samples were subjected to freeze-drying using a lyophilizer (BK-FD10P, Biobase, China) to maintain the porous framework during the drying process and ensure optimal visualization. The dried cryogels were then sputter-coated with a gold–palladium (40/60) alloy and examined using a JEOL JSM-5600 SEM (Tokyo, Japan) for morphological assessment (Çimen et al., 2021; Eren et al., 2023).

3.3.1 Swelling tests

The swelling behavior of the synthesized cryogel samples was investigated to evaluate their water absorption capacity and network flexibility, which are directly related to their structural porosity and cross-linking density. Prior to the swelling analysis, the cryogels were completely dried using a lyophilizer (BK-FD10, Biobase, China) at −55 °C for 2 hours to ensure the removal of all residual moisture. The dried cryogels were accurately weighed to determine their dry mass (*md*).

Subsequently, the samples were immersed in phosphate-buffered saline (PBS, pH 7.4) and incubated at 37 °C to simulate physiological conditions. At predetermined time intervals, the cryogels were gently removed from the solution, the surface water was carefully blotted with filter paper, and the swollen mass (*msw*) was recorded. The experiment continued until the samples reached equilibrium swelling (Canatar et al., 2024; Canatar et al., 2023).

The swelling ratio (SR) or swelling degree (S), which reflects the cryogel's ability to retain water within its polymeric network, was calculated using Equation (1):

$$S(\%) = \frac{m_{sw} - m_d}{m_d} \times 100 \tag{3.1}$$

In this equation, *msw* and *md* represent the masses of the swollen and dry cryogel samples, respectively. A higher swelling ratio indicates a more open and hydrophilic polymeric structure with greater pore interconnectivity, whereas a lower swelling ratio suggests tighter cross-linking and reduced water uptake (Demir Karakuş et al., 2023; Çimen et al., 2021).

The polymerization yield (Y) of the cryogels was subsequently determined to assess the overall efficiency of the gel formation process. The yield was calculated by comparing the dry weight of the final cryogel (*md*) to the total initial mass of all components (*mt*) used during the synthesis, as given in Equation (2):

$$Y(\%) = \frac{m_d}{m_t} \times 100 \tag{3.2}$$

This value provides an estimate of the degree of monomer conversion and cross-linking efficiency during cryogelation (Çimen et al., 2021).

The microporosity (M) of the cryogels was evaluated to determine the fraction of water retained within the interconnected pore system. For this measurement, the cryogels were first weighed in their fully swollen state (*msw*). They were then gently squeezed to remove the water contained in the macropores and weighed again (*msq*). The microporosity was calculated according to Equation (3):

$$M(\%) = \frac{m_{sw} - m_{sq}}{m_{sw}} \times 100 \tag{3.3}$$

Here, *msq* corresponds to the mass of the cryogel after the expulsion of loosely bound water, while *msw* represents the mass before squeezing. The resulting microporosity value reflects the proportion of water confined within the fine pores of the cryogel network, serving as an indicator of its internal architecture and fluid-retention capacity (Eren et al., 2023).

3.4 Determination of Antibacterial Activity of Membranes

The antibacterial effects of membranes were determined with two different antimicrobial assay against Gr (+) *Staphyloccoccus aur*eus (ATCC 29213) and Gr (-) *Escherichia coli* (ATCC 25922).

3.4.1 Preparation of bacterial suspension

A loop of each bacteria from fresh growing bacterial colonies on nutrient agar (37 ℃ 24 h) were transferred to nutrient broth and incubated at 37 ℃ 24h. Afterwards bacterial cultures were centrifuged at 4000 rpm for 5 min, supernatant removed and pellet dissolved in phosphate buffer saline (PBS, pH 7.4). This washing process was repeated 3 times for each microorganism to remove residues. Finally the concentration of bacterial suspensions was adjusted to 0.08-0.1 optical density (OD) at 600 nm by UV-VIS spectrophotometer according to the McFarland to McFarland turbidity standard. This OD value corresponds to a bacteria concentration of 1.5×10^8 CFU/ml. Before antibacterial analysis, in order to prevent contamination each surface of cylindrically cut membranes were sterilized with UV light for 10 min. All membranes were cut cylindrically with a diameter of 6 mm. Each experiment was repeated 4 times.

3.4.2 Disc diffusion analysis

100 ul of bacteria solution was dispersed on to the Muller Hinton agar (MHA) plates for each microorganism. Then membranes containing *AL* 0.5% and %1 and without extract disks used as a were placed on the agar. The plates were incubated at 37 ℃ for a period of 24 h. After completing the incubation period, the diameter of inhibition zones were recorded.

3.4.3 Total viability counts

Membranes were added into glass tubes containing 15 ml of NB inoculated with a 100 ul of bacterial suspension either *S. aureus* or *E. coli*. In order to perform control experiments, membranes without extract were used. Tubes were incubated in a shaking incubator (150 rpm) at 37℃ for 24 h. Samples were taken at 0., 1., 3., 5., 8., 12. and 24., hours of the incubation and the optical density (OD) was recorded using a spectrophotometer at 600 nm. In addition, to determine the viable cell count serial dilutions were prepared up to 10^{-8} plated on a Nurient Agar medium and following 18-24 hour incubation at 37℃ colony counting (CFU) was performed.

3.5 In Vitro Studies

3.5.1 Cell culture

Spontaneously immortalized human keratinocyte cell line (HaCaT) was used for the in vitro studies. All the sterile enviroment required cell culture studies were performed in a laminar flow cabinet (DEMAIR, MSC-II A, Turkey Cells were cultured in Roswell Park Memorial Institute (RPMI-1640) medium (Sigma-Aldrich, Germany). The medium was supplemented with 10% fetal bovine serum (FBS), 1% L-Glutamine (Biowest, Nuaillé, France), and 100 U/mL Penicillin/Streptomycin (Hyclone, GE Healthcare, USA) to maintain optimal proliferation and metabolic activity of the cells. The incubations were performed in a 5% CO_2 incubator (ILD-CI-80, İldam, Turkey) at 37°C. Cells were observed at regular intervals throughout the culture process, and their morphological integrity was assessed using an inverted phase-contrast microscope (Dmil Led Fluo, Leica, Germany). After reaching confluence, cells were detached from the surface with trypsin-EDTA solution and centrifuged for use in experimental studies. Before use in experiments, the cells were confirmed to retain their typical morphological characteristics and proliferative capacity.

3.5.2 Cultivation of HaCaT cells on cryogels

In vitro studies were conducted to evaluate the suitability of cryogel materials for use as wound dressings. Cryogels of the same dimensions (15 mm diameter, 2 mm thickness) were used in all in vitro analyses. Before proceeding to cell culture applications, cryogel samples were carefully sterilized. The sterilization process was performed to completely eliminate microbial contamination and ensure appropriate biosafety conditions for cell culture. In this context, cryogels were subjected to a stepwise sterilization protocol by immersing them in increasing concentrations of ethanol (50%, 70%, and 90%) for 30 minutes (Canatar et al., 2024).

Sterilized cryogels were placed into 24-well plates before seeding cells on cryogels. HaCaT cells were trypsinized once they reached about 80–90% confluence to detach them from the culture surface. After that, a total of 1×10^6 viable cells were seeded onto each cryogel. After seeding, the cryogels were incubated at 37°C with 5% CO_2 for 3 hours to allow proper cell attachment and growth. Finally, 1 mL of cell culture medium was added to the cryogels.

3.5.3 Cell viability

The MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) is a colorimetric technique that determines cell viability based on the reduction of the MTT reagent by metabolically active mitochondria in viable cells (Koc vd., 2023). This method is based on the principle that the mitochondrial enzymes of metabolically active cells reduce the MTT salt to insoluble purple formazan crystals. Thus, the amount of formazan formed is directly proportional to the viability of the cells, providing quantitative information about the biocompatibility of the material. In this context, the effect of the amount of *A.lakoocha* loaded into the cryogel on cell viability and the potential usability of these materials as wound dressings were investigated. Sterilized cryogel samples were placed in 24-well cell culture plates and HaCaT cells were seeded into each well at a density of 1×10^6 cells/mL. Samples were incubated in an incubator at 37°C and 5% CO_2. No cells were seeded into the cryogels, which were allocated as the blank control group. In addition, wells containing only cells and no cryogel were considered negative controls to represent the natural proliferation and metabolic activities of the cells. The absorbance value of this group was used as a reference for 100% cell viability. MTT assay was performed at 24,48 and 72 hours to assess the cytotoxic and biocompatibility properties of cells. MTT solution (0.5 mg/mL in RPMI) was added to each well and incubated for approximately 4–5 hours at 37 °C. After incubation, the medium was removed and dimethylsulfoxide (DMSO) was added to each well to dissolve the crystals. The optical density of the solutions was measured using a UV-Vis spectrophotometer (Uvmini-1240, Shimadzu, Japan) at a wavelength of 570 nm. The percentage of cell viability was calculated using the following formula:

$$Cell\ Viability\ (\%) = \frac{Average\ OD570\ of\ control\ cells}{Average\ OD570\ of\ test\ cells}\ x\ 100 \qquad (3.4)$$

In this equation, "test cells" represent the viability of cells cultured on the cryogel surface, while "control cells" represent the viability of cells grown only in the wells.

3.5.4. Phase contrast microscopy

The synthesized cryogels were imaged using a phase-contrast microscope to examine the interaction of cryogels with cells and their microscopic morphology. For this purpose, cryogels were synthesized to a thickness of approximately 0.5 mm. Microscopic imaging was performed

at the end of the 48-hour incubation period to monitor the cell interactions with the cryogel matrix. Imaging was performed using a phase-contrast microscope (Leica Dmil Led Fluo, Germany) to observe the structural properties of cells.

3.5.5. SEM analysis

Scanning electron microscopy (SEM) analysis is a powerful and comprehensive method for examining the microscopic interactions between cryogels and cells. This analysis was used specifically to assess the adhesion behavior of cells to the cryogel surface, their morphological properties and the potential for cell spreading within the cryogel matrix (Fan et al., 2019).

In this study, SEM analysis was performed to determine the adhesion and morphological changes of HaCaT cells to the cryogel surface. HaCaT cells were seeded onto cryogel samples under sterile conditions and cultured for 48 hours. After the incubation period was completed, the culture medium on the cryogels was carefully removed and the cryogels were washed twice with PBS to remove residual medium components. After that, cryogels were fixed in a 2.5% glutaraldehyde solution in PBS. Cells were fixed for 3 hours at room temperature in order to preserve cell morphology and stabilize surface interactions. Following fixation, cryogels were rinsed several times with PBS and then dehydrated through a graded ethanol series (30%, 50%, 70% and 90%). The dehydrated samples were dried using a freeze-dryer to maintain structural integrity and completely remove moisture. Following drying, the surface of the samples was coated with a thin layer of a gold-palladium mixture (2:3 ratio) to impart conductivity for high-resolution imaging. The coated samples were then placed in the sample chamber of the SEM device and imaged at 1000× and 2500× magnifications.

3.5.6. Immunofluorescence staining

Immunofluorescence staining studies were performed to investigate the effects of *AL* loaded cryogels on keratinocyte cell morphology, adhesion, infiltration, and cell-cell and cell-matrix interactions. This method allows high resolution observation of cell adhesion patterns, cytoskeletal organization, and nuclear positioning on the cryogel surface.

First, sterilized cryogels were placed in 24-well cell culture plates and seeded with HaCaT cells. The cells were incubated at 37°C in a 5% CO_2 atmosphere for 48 hours. At the end of the incubation period, the culture medium was carefully removed, and each sample was washed twice with PBS. PBS solution containing 4% (v/v) paraformaldehyde was added to each cryogel and fixation was performed for 30 minutes at room temperature. After fixation, the samples were rinsed several times with PBS. Phalloidin dye labeled with Alexa Fluor 594 (ThermoFisher Scientific) was used to highlight F-actin structures. 1% Bovine Serum Albumin (BSA; w/v) in PBS-T (PBS with Tween 20) at a 1/1000 ratio (v/v) was used to prepare Alexa Fluor 594 phalloidin dye. Furthermore, 4′,6-diamidino-2-phenylindole (DAPI) was used to detect cell nucles. The DAPI solution was prepared at a 1:1000 (v/v) ratio in PBS. For simultaneous staining of actin filaments and nucleus, the mixture containing Alexa Fluor 594 and DAPI was added to the fixed cells and then incubated for 100 minutes at room temperature in the dark. After that, the solutions were removed, and then the cells were rinsed with PBS to fully remove the solutions. Finally, imaging was performed using a fluorescence microscope (DMIL LED Fluo, Leica, Germany) at 20X magnification.

3.5.7. Statistical analysis

All statistical analyses were performed using SPSS software (version 20.0; IBM, Chicago, IL, USA). Data were expressed as mean ± standard deviation (SD) from three independent replicates. Additional statistical evaluations were carried out with GraphPad Prism (version 8.4.3; GraphPad Software, La Jolla, CA, USA). Fluorescence intensity measurements were quantified using ImageJ software. For comparisons involving three or more groups, two-way ANOVA followed by Tukey's post-hoc test was applied. A p-value of less than 0.05 was considered statistically significant.

4. ANALYSIS AND DISCUSSION

4.1. Synthesis of *Artocarpus lakoocha* Extract Loaded Cryogels

The *AL* extract-loaded cryogels were successfully synthesized by the cryogelation method under subzero conditions. During the freezing process, ice crystals acted as porogens and created a highly porous, sponge-like structure that was maintained after thawing and freeze-drying. All

cryogel formulations exhibited good mechanical stability and elasticity, indicating that the cross-linking process occurred efficiently within the polymeric matrix.

The incorporation of *AL* extract caused a slight color change in the cryogels compared with the blank formulation, confirming that the extract was effectively embedded within the polymer network. The homogeneous appearance and intact structure of the gels demonstrated that the presence of the extract did not interfere with the cryogelation process. Both extract-loaded and control cryogels retained their cylindrical shape and flexibility after thawing and drying (Canatar et al., 2024; Canatar et al., 2023).

The formation of interconnected pores is an essential feature of cryogels, enabling water diffusion and molecular transport. The obtained *AL*-loaded cryogels exhibited a visibly open and continuous porous structure, suggesting that the ice templating and cross-linking reactions were well balanced. The addition of the extract may have slightly influenced the pore size and density, as plant-derived molecules can interact with polymer chains during gelation; however, no structural collapse or irregularity was observed.

Overall, these results indicate that *AL* extract was successfully incorporated into the cryogel matrix without compromising the structural integrity of the material. The produced cryogels displayed uniform porosity, flexibility, and stability, making them suitable for further physicochemical characterization and functional testing (Canatar et al., 2023; Eren et al., 2023).

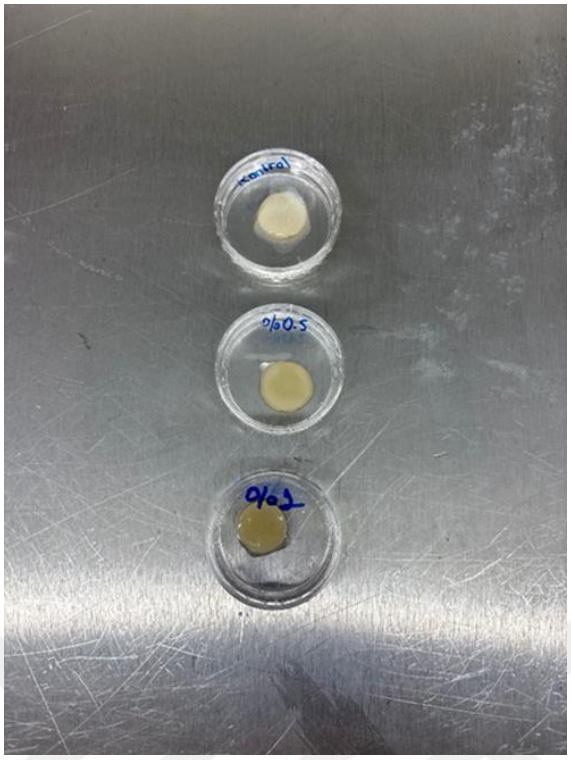

Figure 4.1. The real image of synthesized cryogels

4.2. Characterization Studies

4.2.1. Morphology analysis

The SEM images showed that the structural properties of the cryogels changed noticeably with the addition of *Artocarpus lakoocha* extract. The control sample exhibited a relatively compact and dense structure. The pore openings were small and irregular, and the pore walls appeared thick and continuous. This morphology indicates that the polymer network formed in a more uniform way during cryogelation, resulting in a tighter internal structure.

In the cryogel containing 0.5% extract, a more open and interconnected porous structure was observed. Compared to the control, the pores were better defined and the polymeric walls were noticeably thinner. This suggests that the presence of the extract affected the freezing and phase-separation steps, leading to improved pore formation and an increased internal surface area. When the extract concentration was increased to 1%, the structure became more irregular. The pores appeared larger and less uniform, and some areas showed thinner or partially disrupted pore walls.

This indicates that a higher amount of extract may interfere with the stability of the cryogel network, reducing crosslinking efficiency and resulting in a looser and more heterogeneous structure.

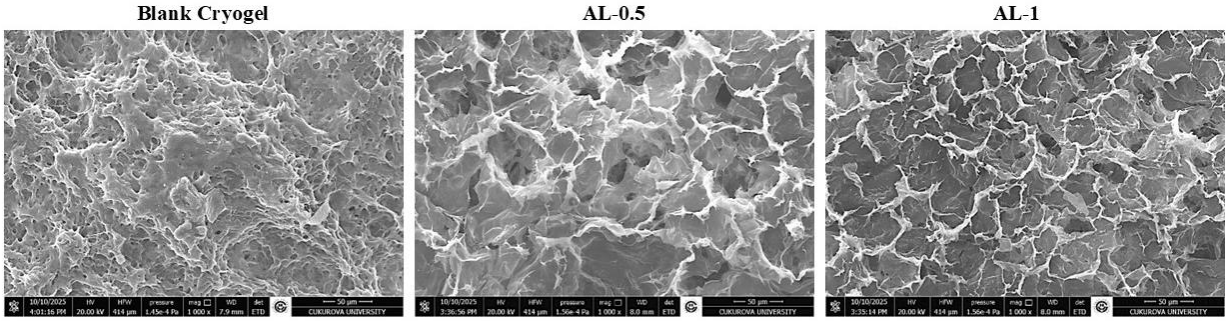

Figure 4.2. The surface and structures of cryogels without cells by scanning electron microscopy (SEM). SEM images images taken at 1000X magnification, scale bar: 50 μm

4.2.2. Swelling tests

After the swelling experiments, the obtained data were analyzed to understand how the addition of *AL* extract affects the water absorption behavior of the cryogels. The swelling degree is an important parameter that shows how much water the cryogel can absorb, and it is closely related to the network structure, pore size, and hydrophilicity of the material. A higher swelling degree usually indicates a more open and flexible structure that allows more water to enter the network. The calculated swelling degree values for all cryogel formulations are summarized in Table 4.1 and the results are discussed in the following section.

Table 4.1. Swelling Degree (S), Polymerization Yield (Y), and Microporosity (M) Values of Cryogels Containing Different Concentrations of *AL* Extract

Sample	S Value	Y Value	M Value
Blank Cryogel (Control)	1777.945	34.43709	75.33051
0.5% *AL* Cryogel (AL-0.5)	1778.566	41.68656	72.69178
1% *AL* Cryogel (AL-1)	1270.482	27.6578	69.64557

The swelling degree (S) of the cryogel formulations was calculated as 1777.95% for the Blank Cryogel (control), 1778.57% for the 0.5% AL-0.5 and 1270.48% for the 1% AL-1. The swelling ratios of the control and AL-0.5 groups were found to be remarkably similar, indicating that the incorporation of a low concentration (0.5%) of *AL* extract did not significantly affect the water absorption capacity of the polymeric network. In other words, this concentration of extract did not substantially alter the cross-linking density or the hydrophilic–hydrophobic balance within the polymer matrix (Lozinsky, 2021).

In contrast, the swelling ratio of the AL-1 cryogel decreased by approximately 30% compared to the control. This reduction can be attributed to the higher extract concentration, which may have partially replaced hydrophilic regions with more hydrophobic moieties or induced the formation of a denser cross-linked structure through interactions with glutaraldehyde. As a result, the diffusion of water molecules into the polymeric network was restricted, leading to lower swelling capacity (Plieva et al., 2008). Therefore, it can be concluded that increasing the *AL* concentration beyond a certain threshold negatively influences the swelling behavior of the cryogels.

The polymerization yield (Y) was calculated as 34.44% for the control cryogel, 41.69% for AL-0.5, and 27.66% for AL-1. These results indicate that the addition of a small amount of *AL* extract (0.5%) enhanced the overall polymerization efficiency of the system. This enhancement could be due to the facilitation of bond formation between polymer chains, promoting more effective network formation and higher cross-linking efficiency (Park et al., 2018).

However, when the extract concentration was increased to 1%, a marked reduction in polymerization yield was observed. This decrease might be associated with the blocking of reactive functional groups or interference with monomer interactions due to excessive extract molecules. Furthermore, at higher concentrations, the extract may introduce heterogeneity within the polymer matrix, reducing cross-linking uniformity and overall network formation efficiency (Lozinsky, 2021; Eren et al., 2023). Consequently, it can be inferred that the optimal *AL* content for effective cryogel formation is around 0.5%, while higher concentrations lead to a decline in polymerization efficiency.

The microporosity (M) values of the control, AL-0.5, and AL-1 cryogels were determined as 75.33%, 72.69%, and 69.65%, respectively. The high porosity values observed across all groups confirm that the cryogel matrices possess the expected highly open and interconnected pore architecture typical of cryogel structures. However, a gradual decline in microporosity was observed with increasing *AL* concentration (Plieva et al., 2008; Lozinsky, 2021).

This trend can be explained by the partial filling of void spaces within the polymer matrix or the formation of additional cross-linking sites due to interactions between the extract and polymer chains. Such structural modifications may lead to a reduction in the total void volume within the cryogel, thereby decreasing the overall porosity. Moreover, the decrease in porosity is consistent with the observed reduction in swelling capacity, as less porous structures inherently restrict water diffusion. In summary, increasing the amount of *AL* extract reduces both the porosity and water retention capacity of the cryogels, indicating a denser and more compact internal structure at higher extract concentrations (Eren et al., 2023).

When the obtained data are evaluated together, it is observed that the addition of a low level (0.5%) of *AL* has positive effects on the cryogel structure, increasing both polymerization efficiency and preserving structural integrity. In contrast, the addition of a higher concentration of extract (1%) reduced both swelling capacity and porosity, limiting the cryogel's ability to retain water. These findings suggest that the *AL* content has a dose-dependent effect on cryogel properties (Lozinsky, 2021; Eren et al., 2023).

4.3. Antimicrobial Activity

Synthesized membranes loaded with AL extract showed antibacterial effects against both Gr (+) and Gr (-) microorganisms. Although the antimicrobial activity against *S. aureus* (16 mm of inhibition zone) was higher than against *E. coli* (13 mm of inhibition zone), it was determined that increasing the *AL* concentration in the membrane from 0.5% to 1% did not result in a significant difference ($p<0.05$). As expected any inhibition zone was observed with control membranes (Figure 4.3). In the literature, there are no studies reporting the use of *AL* in the synthesis of cryogel membranes as wound dressings, nor its evaluation for antimicrobial activity in this context. However, The antimicrobial potential of *A. lakoocha* extract has been previously demonstrated in several studies. *AL* extracts have been shown to show significant inhibition against *S. aureus, E. coli, P. acnes* and other pathogenic bacteria, with inhibition zones ranging from 7–20 mm and effective with low MIC/MBC values (Senapong et al., 2014; Pertiwi et al., 2024; Kaewkor et al., 2016). Consistent with our findings, Pertiwi et al. (2024) reported that the ethanolic extract of AL displayed a markedly stronger inhibitory effect against *S. aureus* (12.68 mm and 12.57 mm) compared to *E. coli* (7.46 mm) at a concentration of 3000 μg/disc, indicating that Gram-positive bacteria are more susceptible to *AL* extract. It was explained in some studies that antimicrobial activity is related to its high content of oxyresveratrol.

In a study conducted by Teanpaisan et al., (2014) the extract was evaluated against a various oral pathogens, including five Gram-positive cariogenic bacteria (*S. mutans*, *S. sobrinus*, *E. faecalis*, *L. fermentum*, and *L. salivarius*) and six Gram-negative periodontopathogens (*A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *P. nigrescens*, *F. nucleatum*, and *T. forsythia*). The agar diffusion assay results demonstrated that all tested microorganisms were susceptible to *A. lakoocha* extract, although the inhibition zones varied among species. Notably, the extract exhibited broad-spectrum antimicrobial activity, being effective against both Gram-positive and Gram-negative strains, while the solvent control (10% DMSO) showed no inhibitory effect, confirming that the antimicrobial activity was solely attributable to the plant extract. Moreover, the microdilution assay revealed that *S. mutans*, *P. gingivalis*, *F. nucleatum*, and *T. forsythia* were the most sensitive species, with MIC values of approximately 0.1 mg/mL and MBC values of 0.2 mg/mL (Teanpaisan et al., 2014).

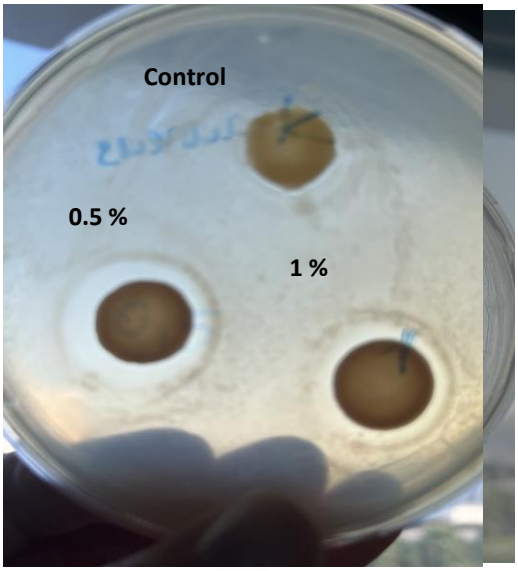

Figure 4.3. Inhibition zones of **A.** *E.coli*, **B.** *S. aureus*

4.4. In vitro Evaluation of Cryogels

4.4.1 Assessment of cell viability of cryogels by MTT assay

The viability levels of keratinocyte cells were evaluated at 24, 48 and 72 hours (Figure 4.4). At 24 hours, cell viability was 96.2% in the blank cryogel group, 101.2% in the AL-0.5 group, and 80.9% in the AL-1 group. At 48 hours, cell viability was 93.1% in the blank cryogel, 102.0% in the AL-0.5 group, and 93.5% in the AL-1 group. At 72 hours, cell viability was 95.2% in the blank cryogel, but decreased to 89.1% in the AL-0.5 group and 82.0% in the AL-1 group. According to the MTT assay, keratinocyte viability in the Blank Cryogel group remained close to the control at all time points, indicating that the PVA/Gel matrix itself exhibited no cytotoxic effect. Incorporation of the *AL* extract notably influenced cell behavior. The AL-0.5 cryogel enhanced keratinocyte viability by 1.18% at 24 hours and 2.05% at 48 hours compared to the control. However, cell viability decreased to 89.15% at 72 hours, indicating a possible decline in proliferative stimulation or mild cytotoxicity at longer exposure. When compared with the Blank Cryogel, AL-0.5 increased viability by 5.24% and 9.63% at 24 and 48 hours, respectively. In contrast, the AL-1 formulation showed reduced cell viability at all time points, with decreases of 19.15%, 6.47%, and 18.01% at 24, 48, and 72 hours, respectively, compared to the control. Overall, these findings indicate a **c**oncentration-dependent cellular response to *AL* loaded cryogels. While 0.5% *AL* promotes early stage keratinocyte proliferation, 1% *AL* demonstrates a

measurable reduction in viability, which may reflect cytotoxic or metabolic stress at elevated doses.

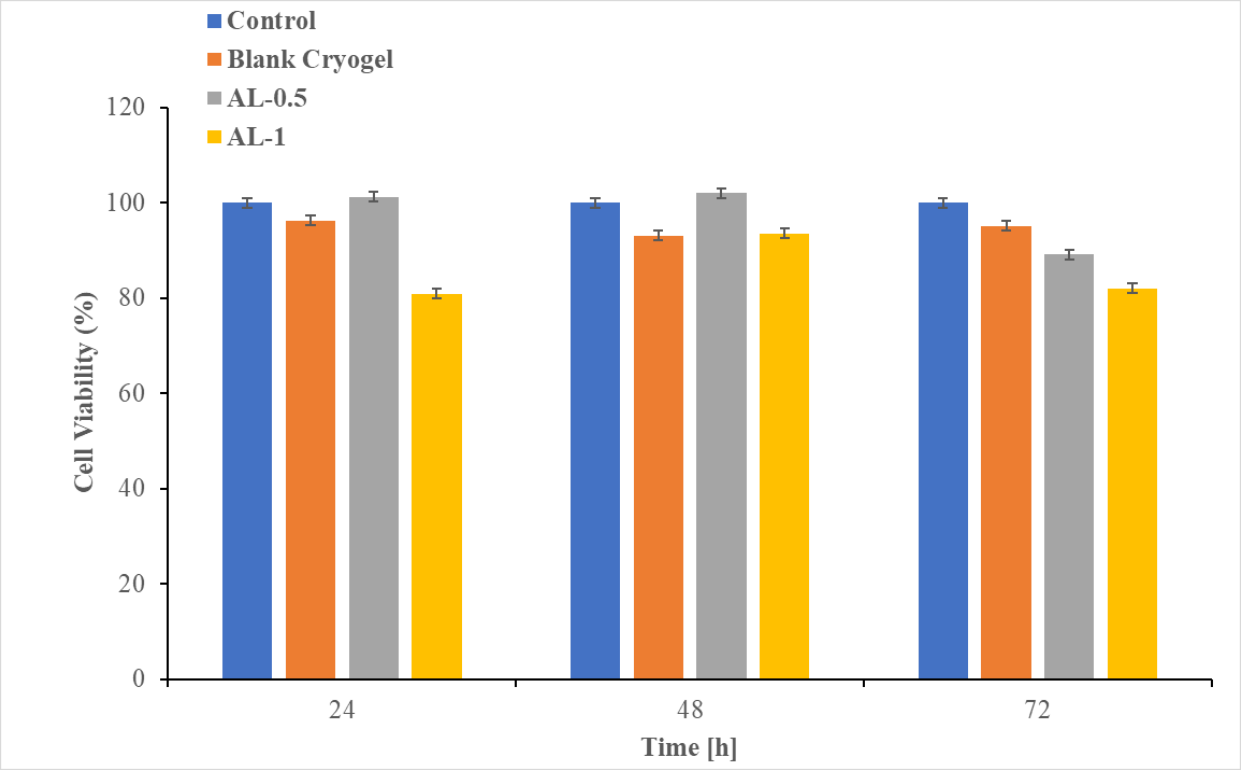

Figure 4.4. Cell viability (%) of keratinocytes on cryogels compared with the control. The control cells were considered to have 100% survival. The histograms illustrate the percentage of viable cells after the incubation, relative to the control cells

4.4.2. The effect of cryogels on cells morphology

In the study, phase contrast microscopy analysis performed on three different cryogels, namely Blank Cryogel, AL-0.5, AL-1, provided important information in terms of cell attachment, morphology and distribution (Figure 4.5).

Phase contrast microscopy images showed that HaCaT cells in the blank cryogel group adhered to the surface and formed more dispersed cell clusters. In contrast, cells in the AL-0.5 and AL-1 cryogels were more densely packed, forming distinct colonies and spheroid structures. Cells were observed to make tighter contact with each other, exhibiting a more three-dimensional organization and increased structural integrity. Furthermore, a more widespread and uniform cell

morphology was observed in cryogels loaded with *AL* extract, suggesting that the biological activity of the extract supports cell attachment and proliferation.

It has been reported in the literature that *AL* extract and its main phenolic compound, oxyresveratrol, promote cellular proliferation and wound healing. These findings suggest that the cell colonization observed in our study may be related to the phenolic compounds in *AL* extract, particularly oxyresveratrol, supporting cytoskeletal organization and cell-matrix interactions. Furthermore, the antioxidant properties of the extract are thought to make cells more resistant to oxidative stress on the cryogel surface, thus increasing cell density.

The findings are consistent with studies in the literature indicating that macroporous cryogel structures provide advantages in terms of cell attachement, infiltration, and distribution (Carriero et al., 2023). In conclusion, phase contrast microscopy analyses revealed that AL extract-loaded cryogels promoted the adhesion and proliferation of HaCaT cells, thus suggesting that these biomaterials could be considered a potential carrier system in wound healing processes.

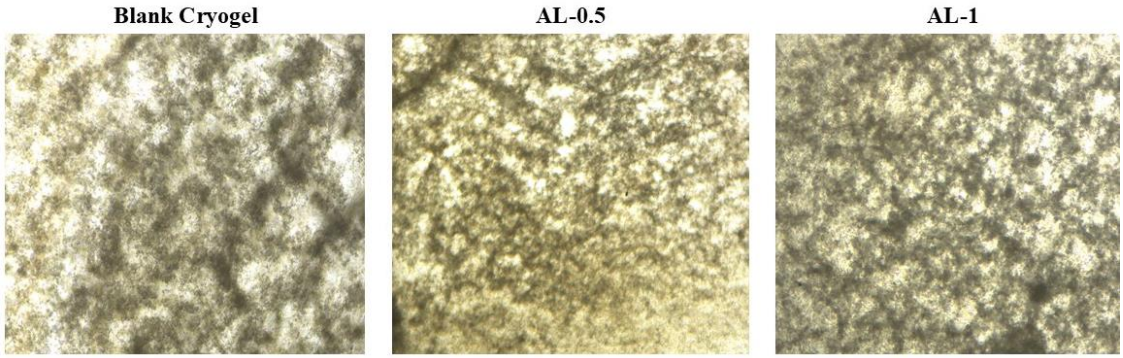

Figure 4.5. Phase contrast microscopy images illustrating the morphology and distribution of cells cultured on cryogels after 48 h of incubation (at 10X magnification).

4.4.3. SEM analysis

The main purpose of biomaterial production is to create suitable cell attachment surfaces that will regulate cell migration, proliferation, differentiation and extracellular matrix (ECM) synthesis. In this study, SEM analyses performed after 48 hours of cell cultivation on three different cryogels provided information about the biological side of cell-matrix interaction, cell spreading,

colony/spheroid formation and macroporous skeletal structure (Iandolo et al., 2019) (Figure 4.6). SEM images showed that cells in the Blank Cryogel had limited adhesion to the scaffold surface, with more limited spread within the pores and polymer walls. Cell boundaries were relatively distinct, and colonization or dense ECM formation was less frequent than in the other groups. In contrast, AL-0.5 and AL-1 cryogels, cells spread almost entirely over the scaffold surface, clustering into dense, contiguous colonies and spheroids that enveloped the pore walls and covered the entire macropore structure.

Cell-matrix and cell-cell interactions were observed to be quite high. Furthermore, ECM-like structures were significantly developed, and cells were observed to surround the interpore spaces, forming a 3D network structure. These results suggest that the biologically active components of *AL* extract promote cell adhesion, spreading, and colony formation. *AL* has been reported in the literature to have antioxidant, anti-inflammatory, and cytokine-regulating effects (Hankittichai et al., 2020; Gupta et al., 2020). This biological activity may be mediated by stimulation of cell-surface receptors, activation of ECM synthesis genes, or promotion of extracellular binding ligands in cytoskeletal interactions. In the context of the macroporous structure, it is possible to say that cells can infiltrate into the skeleton and nutrient flow occurs under adequate conditions thanks to the three-dimensional, interconnected porous network of the cryogel.

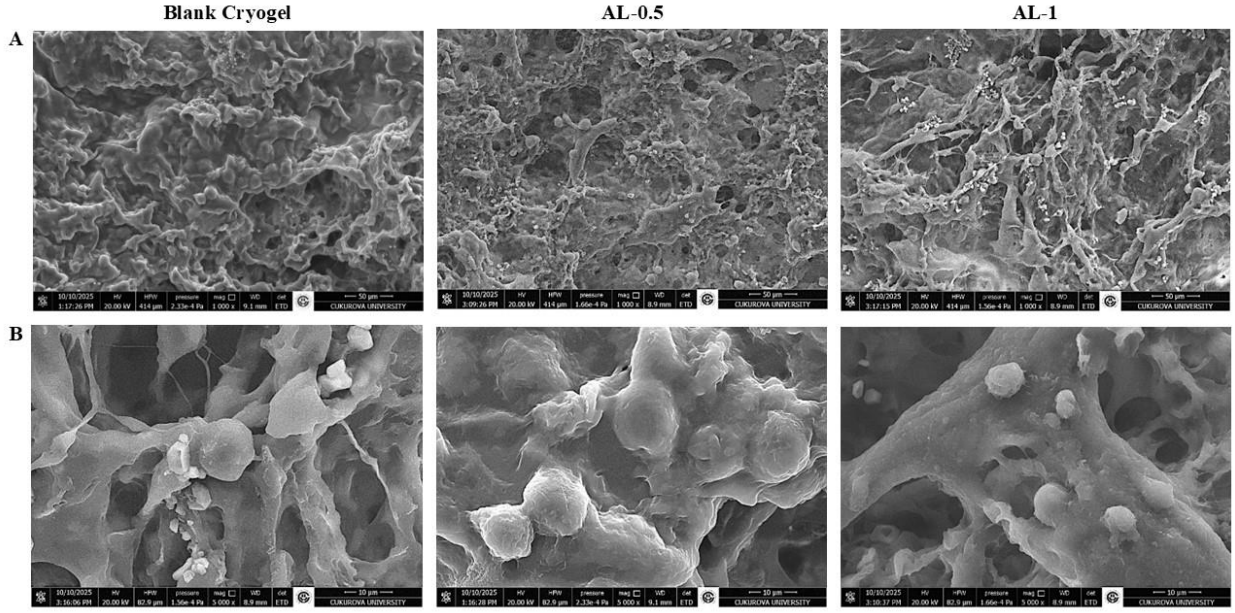

Figure 4.6. SEM images of cell seeded cryogels. Representative 48th h SEM images of cell

attachment to control, AL-0.5 and AL-1 cryogels (**A)** at 1000X magnification, scale bar: 50 μm and **(B)** at magnification 5000X, scale bar: 30 μm.

4.4.4. Cell adhesion and infiltration on cryogels assessed by DAPI and F-Actin staining

In tissue engineering applications, the structural and chemical properties of the biomaterial used are extremely important for the cells to be able to adhere properly, distrubition on the surface, proliferate and differentiate (Shakya vd., 2014; Kathuria vd., 2009). In this context, fluorescence microscopy analyses were performed using DAPI and F-Actin staining of the nuclear and cytoplasmic filaments, respectively to assess the cell adhesion and infiltration capabilities of the cryogel matrix.

The images obtained were taken after 48 hours of incubation on Blank Cryogel, AL-0.5, and AL-1 cryogels (Figure 4.7). Nuclei showing blue fluorescence with DAPI staining and cytoplasmic filaments showing red fluorescence with F-Actin staining provided information about the morphology of the cells on the matrix. DAPI staining (blue) demonstrated a uniform distribution of nuclei throughout the cryogel matrix, indicating a high density of viable cells.

The images show that cells, especially in the AL-0.5 and AL-1 cryogels, spread more homogeneously throughout the matrix and are densely packed within interconnected pore structures. This suggests that the *AL* containing cryogels promote cell infiltration and attachment to the matrix. Moreover, Blank Cryogel exhibited weaker and more limited fluorescence intensity, with cells more clustered and dispersed on the surface. This result suggests that formulations containing *AL* extract provide a more biologically active surface and enhance cell interaction.

The interconnected pores forming the structure of the cryogel allowed the cells to settle. PVA and gelatin scaffolds are widely used for tissue engineering applications with their biocompatible nature (He et al., 2021). It has been reported that vascularization, epithelialization, cell proliferation, adhesion and migration can be stimulated by loading different bioactive compounds into the cryogel (Vrana et al., 2010).

AL is a plant rich in phytochemicals and is known to contain bioactive molecules, particularly morin, oxyresveratrol, flavonoids, and phenolic compounds. These components exhibit high free

radical scavenging capacity and antioxidant activity, and they support wound healing by promoting cell proliferation, migration and collagen synthesis (Talekar et al., 2017). In this study, the release of biomolecules from *AL* extract into the matrix was not specifically investigated. However, considering the literature, it is thought that the active compounds in *AL* remain stable within the cryogel, thereby increasing the bioactivity of the material and supporting the attachment, spreading, and migration of keratinocytes to the surface. These findings suggest that *AL* loaded cryogels support keratinocyte attachment, proliferation, and cytoskeletal organization, which are critical parameters for wound healing applications.

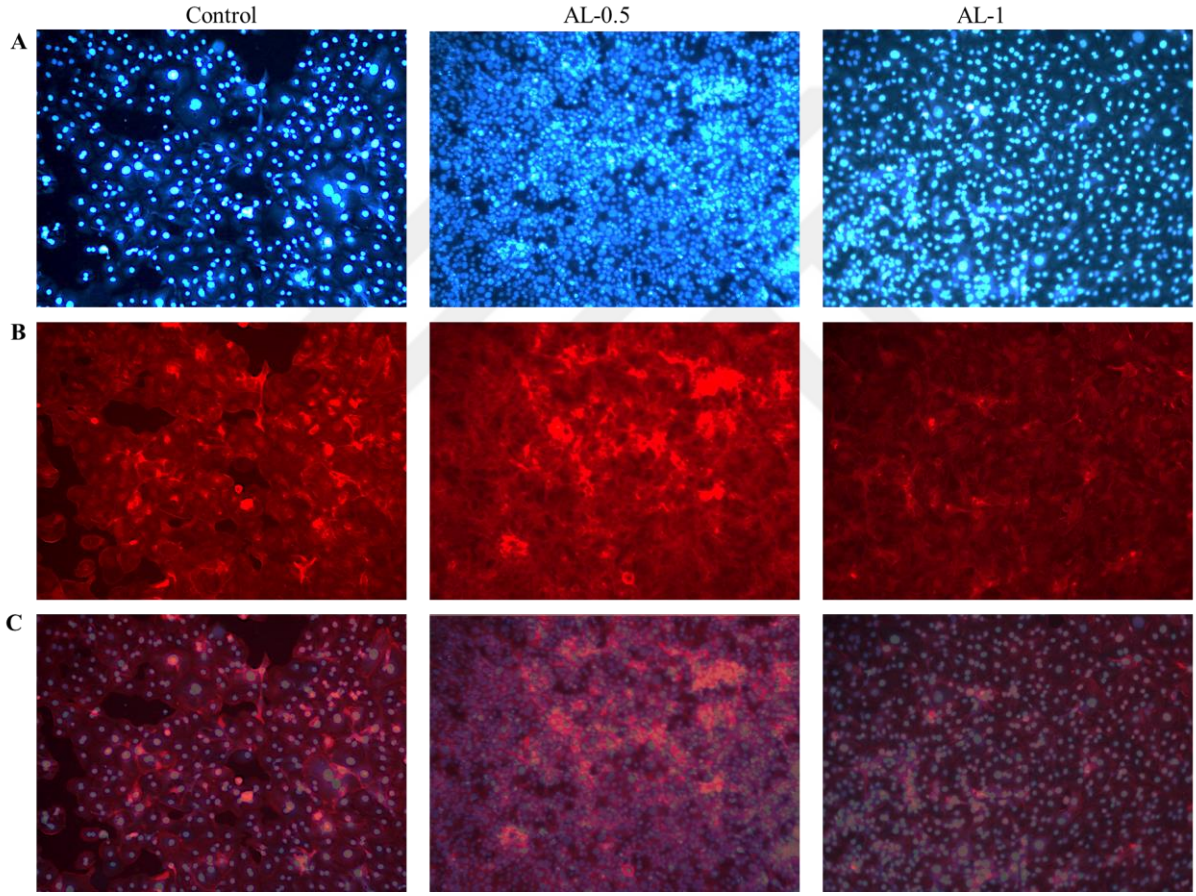

Figure 4.7. Representative images showing cell–matrix interactions on cryogels assessed by **(A)** DAPI, **(B)** F-actin staining at 48 h, and **(C)** merge immunofluorescence of F-Actin (red)/ DAPI (blue) stainings (at 10X magnification). (Cells cultured on polystyrene plates served as control.

5. CONCLUSION

In this study, the potential of the plant-derived extract *AL* to be incorporated into biopolymer-based cryogel systems for wound dressing applications was investigated. The findings demonstrated notable physicochemical and biological properties, indicating its promise as a multifunctional biomaterial.

Consistent with previous studies highlighting the antioxidant and antibacterial properties of *AL* (Gaspar-Pintiliescu et al., 2019; Ahmad et al., 2022), the current study confirmed that the bioactivity of the extract was retained and even supported when integrated into cryogel structures. This integration enables the development of more functional and versatile wound dressings compared to traditional approaches.

Hydrogels and cryogels have gained increasing attention in wound management due to their favorable properties. It is well-established in the literature that polymers such as PVA and gelatin are frequently used in wound care due to their high biocompatibility, although they exhibit limited biological activity on their own (Savina et al., 2021; Vishnoi et al., 2021). In the present study, these polymers were combined with *AL* extract to formulate a system that not only demonstrated mechanical stability and water retention capacity but also provided antioxidant and antimicrobial protection. These results are in line with prior findings indicating that the integration of natural extracts into biopolymer matrices enhances biological performance (Singh et al., 2018; Liu and Jia, 2018).

The developed cryogel structures exhibited porous morphology, which facilitated exudate absorption and helped maintain a moist microenvironment favorable for wound healing. This observation parallels the reported advantages of cryogels in tissue engineering applications (Bereli et al., 2020; Akin and Özmen, 2022). Furthermore, the formulation approach used in

this study presents a low-cost and scalable production process, enhancing the translational potential of the material for clinical applications.

To date, research on the use of *AL* in wound healing remains limited. Therefore, this study offers a novel perspective by incorporating this natural resource into biopolymer-based systems for wound care. The lack of previous studies in Turkey on the biomedical use of this plant also highlights the strategic and original value of this research on a national level.

In conclusion, this study contributes to the development of a multifunctional wound dressing system by promoting the application of plant-based bioactives in wound management. It addresses a notable gap in the literature regarding bioactive-integrated biomaterials, while also establishing a multidisciplinary framework for future research in the field.

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