



**TÜRKİYE CUMHURİYETİ
ADANA ALPARSLAN TÜRKER SCIENCE AND TECHNOLOGY
UNIVERSITY**

**GRADUATE SCHOOL
BIOENGINEERING DEPARTMENT**

***PHYLLANTHUS EMBLICA* LOADED WOUND DRESSING
MATERIALS**

İPEK CANATAR

M.Sc.

ADANA 2023



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**THESIS ADVISOR
ASSOC. PROF. DR. SİBEL ÖZDAŞ**

**SECOND ADVISOR
PROF. DR. GÖZDE BAYDEMİR PEŞİNT**

ADANA, 2023

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.

13/07/2023

[Signature]

İpek CANATAR

ÖZET

Phyllanthus emblica Yüklü Yara Örtüsü Malzemeleri

İpek CANATAR

Yüksek Lisans, Biyomühendislik Anabilim Dalı

Danışman: Doç. Dr. Sibel ÖZDAŞ

II. Danışman: Prof. Dr. Gözde BAYDEMİR PEŞİNT

Temmuz 2023, 86 sayfa

Antik çağlardan beri bitkiler, çeşitli hastalık ve rahatsızlıklar için terapötik yaklaşımlarda hayati bir rol oynamıştır. *Phyllanthus emblica*'nın (*P. emblica*) birçok çalışmada çoklu terapötik aktivitelere sahip olduğu tanımlanmıştır ve Ayurvedik ve Unani tıbbi sistemlerinin önemli bir parçası olarak kabul edilir. Bu çalışma, sentezlenen *P. emblica* yüklü kriyojellerin yara iyileşmesi üzerindeki etkilerini ve yara pansuman uygulamalarındaki potansiyellerini incelemeyi amaçlamıştır.

Bu amaçla, %0,5 (PVA/Gel/*P.emblica*-0,5), %1 (PVA/Gel/*P.emblica*-1), %1,5 (PVA/Jel/*P.emblica*-1.5), %2 (PVA/Gel/*P.emblica*-2) *P. emblica* içeren polivinilalkol/jelatin (PVA/Gel) kriyojeller sentezlenmiştir. Karakterizasyon çalışmaları kapsamında şişme testleri, Brunauer-Emmett-Teller (BET) ve morfoloji analizleri yapılmıştır. Sentezlenen kriyojellerin yara pansuman malzemesi olarak uygunluğunu göstermek amacıyla HaCaT hücreleri kullanılarak in vitro hücre kültürü çalışmaları yapılmıştır.

Şişme testleri ve BET analizi sonuçları, *P. emblica* yüklü PVA/Jel kriyojellerinin *P. emblica* miktarlarının artmasıyla arttığını kanıtlamıştır. Morfolojik sonuçlar, kriyojellerin yoğun, birbirine bağlı gözenek morfolojisine ve makro gözenekli bir yapıya sahip olduğunu göstermiştir. 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolyum bromür (MTT), tripan mavisi deneyi ve canlı ölü boyama verileri, *P. emblica*'nın kriyojellere eklenmesinin hücre proliferasyonunun artmasına, hücre sayısının artışına ve hücre canlılığının yükselmesine yol açtığını göstermiştir. *P. emblica* yüklü kriyojeller üzerindeki HaCaT hücrelerinin faz kontrast mikroskop, taramalı elektron mikroskobu (SEM), immünofloresan ve giemsa görüntülerine dayanarak, *P. emblica*'nın hücre tutunmasını, çoğalmasını ve penetrasyonunu desteklediği

gözlendi. Sonuç olarak, PVA/Gel/*P.emblica* kriyojellerinin yara örtüsü malzemesi olarak kullanıma uygun olduğunu ve ileri çalışmalarla geliştirilebileceği gösterilmiştir.

Anahtar Kelimeler: Yara iyileşmesi, *Phyllanthus emblica*, kriyojel, yara örtüsü malzemesi



ABSTRACT

***Phyllanthus emblica* Loaded Wound Dressing Materials**

İpek CANATAR

M.Sc., Department of Bioengineering

Supervisor: Assoc. Prof. Dr. Sibel ÖZDAŞ

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

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Since ancient times, plants have played a vital role in therapeutic approaches for a variety of diseases and ailments. *Phyllanthus emblica* (*P. emblica*) has been described in many studies to have multiple therapeutic activities and is considered an important part of Ayurvedic and Unani medicinal systems. This study sought to examine the efficacy of synthesized *Phyllanthus emblica* loaded cryogels on wound healing and their potential in wound dressing applications.

For this purpose, polyvinylalcohol/gelatin (PVA/Gel) based cryogels were synthesized containing 0.5% (PVA/Gel/*P.emblica*-0.5), 1% (PVA/Gel/*P.emblica*-1), 1.5% (PVA/Gel/*P.emblica*-1.5), 2% (PVA/Gel/*P.emblica*-2) *P. emblica* extract. Swelling tests, Brunauer-Emmett-Teller (BET) and morphology analysis were conducted within the scope of characterization studies. In vitro cell culture studies were performed using HaCaT cells to illustrate the suitability of the synthesized cryogels as wound dressing material.

The swelling tests and BET results revealed that *P. emblica* loaded PVA/Gel cryogels was increased with increasing the amounts of *P. emblica*. Morphological results displayed that the cryogels had an dense, interconnected pore morphology and a macroporous structure. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), trypan blue exclusion and live-dead assay results revealed that adding of *P. emblica* into cryogels led to enhanced cell proliferation, increased cell number, and improved cell viability. Based on the phase contrast microscope, scanning electron microscope (SEM), immunofluorescence and giemsa images of the HaCaT cells on *P. emblica* loaded PVA/Gel cryogels, it was observed that *P. emblica* promoted cell attachment, proliferation and penetration. In conclusion, it has been shown that

PVA/Gel/*P.emblica* cryogels are suitable for use as wound dressing materials and can be developed with further studies.

Keywords: Wound healing, *Phyllanthus emblica*, cryogel, wound dressing materials



Dedication

To my beloved mother, who has always been there for me and makes sacrifices for my future...

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LIST OF ABBREVIATIONS

PVA	: Polyvinyl Alcohol
Gel	: Gelatin
<i>P. emblica</i>	: <i>Phyllanthus emblica</i> Linn
BET	: Brunauer-Emmett-Teller
EGF	: Epidermal 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 SYMBOLS

μL	: Microliter
mL	: Mililiter
mm	: Millimeter
m_d	: Weight of The Dry Cryogel
m_{sw}	: Weight Of The Swollen Cryogel
m_{sq}	: Weight Of The Squeezed Cryogel
m_t	: Total Amount Of Components In Cryogel
μM	: Micromolar

1. INTRODUCTION

Wound healing is a biological and dynamic process that results from multiple interactions between various systems to restore damaged tissues and maintain tissue integrity (Santoro and Gaudino, 2005). Appropriate wound care treatment assists to stimulate the healing process by preventing infection and other complications. Throughout history, wound care and healing have been supported by various types of materials and structures (Koyutürk and Soyaslan, 2016). Currently, wound dressings are mainly used to protect the wound from infection, and for the regeneration and maintenance of dermal and epidermal tissues during the wound healing process (Dabiri et al., 2016; Han and Ceilley, 2017).

Wound dressings are medical biomaterials that can be designed with many types and different functional properties for use in wound treatment, and their use is increasing through the healing process accelerating and restoring skin structural properties (Gaspar-Pintilieșcu et al., 2019). The properties of an ideal wound dressing should be listed as having a high water absorption capacity, providing a moist environment on the wound surface, allowing gas exchange, being non-toxic, biodegradable and biocompatible (Schoukens, 2019). Therefore, it is important to choose a suitable wound dressing material. Owing to their distinctive properties, such as adequate flexibility, hydrophilicity, superabsorbancy, and softness, hydrogels have become appealing candidates for use as wound dressings (Liang et al., 2021). However, a major drawback of hydrogels is their low mechanical stability at swollen state which complicates their usage (Kamoun et al., 2017; Akin and Ozmen, 2022). In contrast, cryogels which are macroporous structures offering superior physical properties compared to hydrogels are highly desirable for various biomedical applications, especially for wound dressings (Tripathi et al., 2013; Shirbin et al., 2016). Cryogels exhibit advanced swelling and mechanical properties as well as interconnected porous structures (Akin and Ozmen, 2022). Cryogel wound dressings are produced from polymeric materials that have a synthetic or natural origin (Hixon et al., 2017). Synthetic polymers that are used for the synthesis of cryogel wound dressings are poly(vinyl alcohol) (PVA), polyglycolic acid, poly-L-lactic acid (PLLA), poly(lactic acid) (PLA) and poly(ethylene glycol) (PEG) (Savina et al., 2021). Natural polymers such as alginate, gelatin, collagen, silk fibroin (SF), chitosan and hyaluronic acid (HA), are also frequently used (Vishnoi, 2021; Singh et al., 2018). The polymers used in the production of these materials generally do not possess any antioxidant and antimicrobial properties unless

they are combined with other materials that can improve their antioxidant and antimicrobial properties (Singh et al., 2018; Liu and Jia, 2018).

Plant-based therapies have been a source of medical treatment since ancient times. Nowadays, many different medicinal plants and their extracts are being studied and utilized to treat wounds because of their potential reparative and therapeutic effects on wounds (Maver et al., 2015). The medicinal value of plants depends on the presence of phytochemical components such as flavonoids, tannins, alkaloids and phenolic compounds. The wound healing process is supported and accelerated by bioactive substances found in plants. These herbal extracts are added to wound dressings to accelerate wound healing. Recent advances in wound dressings developed with the combination of plants with polymers enable great progress in wound care treatment (Rezvani et al., 2019).

Phyllanthus emblica Linn (*P. emblica*; *Emblica officinalis* Gaertn) is a medicinal plant from the Phyllanthaceae family, also known as the Indian gooseberry or amla. It is distributed throughout tropical and subtropical regions of India, Indonesia, Uzbekistan, Pakistan, Southeast Asia, China, Srilanka and Malaysia (Joseph et al., 2013; Khan, 2009). *P. emblica* has been used for the treatment of various diseases including asthma, cough, diabetes, diarrhea, cancer, hepatic disorders, peptic ulcer, inflammation, jaundice and heart diseases (Bhandri and Kamdod, 2012; Kulkarni and Ghurghure, 2018). Previous studies revealed that *P. emblica* extracts possess strong antioxidant, anti-microbial and anti-inflammatory properties. Antioxidants are thought to aid tissue repair during wound healing by helping to defend against oxidative stress and inflammation (Joseph et al., 2013). The activity of *P. emblica* extract on wound healing has also been investigated in various studies, and in vitro studies have shown that it exhibits significant wound healing activity through the proliferation and mobilization of fibroblasts and keratinocytes (Talekar et al., 2017; Ahmad et al., 2021).

In this thesis, for the first time in the literature, it is aimed to produce a wound dressing material with appropriate physical and mechanical properties that will provide rapid and effective wound healing by loading *P. emblica* extract into the cryogel alone, which has an effective wound healing potential. Polyvinyl alcohol (PVA) and gelatin (Gel) were chosen as the primary materials to prepare cryogels for use as wound dressing materials. The contribution of *P. emblica* to wound healing was evaluated, and its use as a wound dressing was investigated in cell culture studies. In order to find the most suitable doses of *P. emblica* in the cryogel, cryogels

were prepared with different ratios of *P. emblica* extract (0.5%, 1%, 1.5%, and 2%). Swelling tests, Brunauer-Emmett-Teller (BET) analysis and morphology analysis were performed within the scope of characterization studies of cryogels. The efficacy of cryogels on cell viability was determined by MTT assay, trypan blue exclusion assay and live/dead staining at 72 hours after cell cultivation. The interaction of the cells with the cryogels, such as attachment and spreading, was demonstrated using SEM and optical microscope images. Immunofluorescence (F-actin, DAPI) and giemsa staining were used to evaluate the impact of *P. emblica* loaded cryogels on cell morphology and nuclei.



2. THEORETICAL FOUNDATIONS AND LITERATURE REVIEW

2.1. Skin

The skin is one of the most important and largest organs of the body with a surface area of about 2 m² and 12-15% of the body weight. It surrounds the body and acts as a barrier. The skin is the binder of other systems because it contains blood vessels, nerves, sebaceous glands, sweat glands, hair and nails (Farley et al., 2012). While the primary function of the skin is to form a barrier that protects the body from dehydration, the skin is a multifunctional and complex organ that has numerous other functions (Voegeli, 2012). It prevents harmful liquids, bacteria, microbes and rays coming from the outside, as well as protects the body from mechanical impacts (Paz et al., 2014). Moreover, it plays a role in providing the body's heat balance and water, salt and urea-based fluid excretion owing to the sweat glands and blood vessels in its structure (Duplan and Nocera, 2018).

One of the most important functions of the skin is to synthesize vitamin D. Synthesis of vitamin D through the skin is facilitated by UV from the sun, particularly UV-B. It acts as a regulator of calcium metabolism; therefore it is vital to human life. Vitamin D deficiency is an important health concern worldwide (Nair and Maseeh, 2012). Vitamin D deficiency interrupts calcium metabolism and causes diseases such as rachitis (Bikle, 2011). The skin comprises three layers that perform these tasks. These layers are the epidermis, dermis, and hypodermis. All these layers are located on top of each other and have different functions and structures in the skin system. Detailed skin structure and layers are depicted in Figure 2.1.

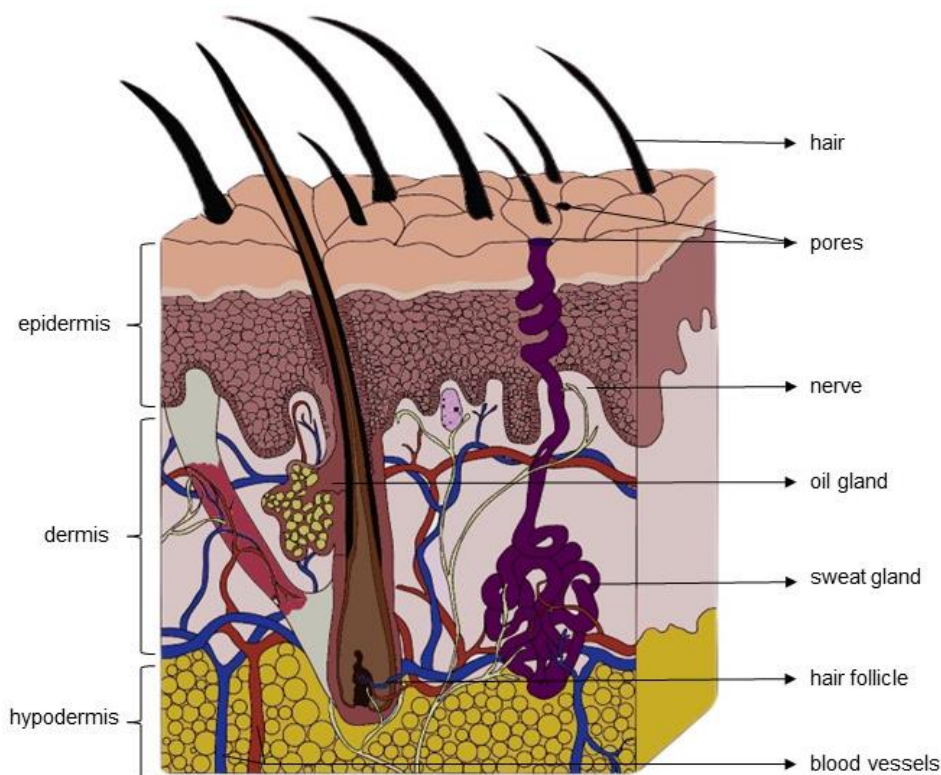


Figure 2.1. Diagram of skin layers

2.1.1. Epidermis

The epidermis is the uppermost and thinnest layer of the three layers in the skin. It is responsible for the moisture level, color and texture of the skin. Since it is at the top, it is exposed to external factors before other layers. Accordingly, its function is to protect people directly from external influences and factors. It is a continuously regenerative layer, so it contains sweat glands, pilosebaceous units and nails.

The epidermis layer consists of 4 layers within itself. These layers are as follows, from the outside to the inside; stratum corneum, stratum granulosum, stratum spinosum, stratum basale (Voegeli, 2012). The stratum corneum layer is formed when the organelles in the uppermost layer of the epidermis are lost over time and become more compact. The stratum granulosum layer comes after the stratum corneum layer and contains keratinocytes in its structure. These keratinocytes are mature enough to produce keratin. The stratum spinosum is the layer above the stratum basale and is typically 5-10 cell layers thick. They have limited cellular proliferation capacity and consist of keratinocytes. Keratinocytes adhere to each other in this

layer by desmosomes. The epidermis's deepest layer, located just above the dermis, is known as the stratum basale. There is a structure called the basement membrane between the epidermis and dermis, which plays various roles in the structure and support of the skin. This layer contains melanocytes and mitotically active stem cells that produce new keratinocytes (Kolarsick et al., 2011).

2.1.2. Dermis

The dermis, the second layer of the skin, is located below the epidermis (Sundberg et al., 2018). The dermis is a system of fibrous, filamentous and amorphous connective tissue. It contains stimulus-induced inputs by epidermally derived appendages, neural and vascular networks, macrophages, fibroblasts, and mast cells in its structure. The dermis forms the skin's resistance system to mechanical effects and provides flexibility, tensile strength and elasticity to the skin. It protects the body from injuries with the mechanical strength it provides to the skin. It is involved in the collection and transmission of senses through the neural networks in its structure. It also acts on water binding and thermal regulation. Its most important component is collagen. Collagen is a significant protein that increases the stress resistance of the skin, and it is also known to be associated with skin brightness, health and aging. The dermis cooperates with the epidermis to repair and reshape the skin in skin injuries (Chu, 2008; Kolarsick et al., 2011).

2.1.3. Hypodermis

The hypodermis, also known as subcutaneous adipose tissue, is the third and deepest layer of the skin, consisting of loose connective tissue. It contains sensory neurons, blood vessels, hair follicles and adipose tissue (fat cells) in its structure. Adipose tissue both contributes to thermal insulation and provides energy storage. It is very effective in insulating the body. It establishes the connection between the dermis and the muscles and bones, thus protecting tendons, joints and bones from external impacts (Bahri et al., 2019; Sundberg et al., 2018).

2.2. Wound and Wound Dressings

2.2.1. Wound

A wound is the deterioration of the physiological structure and functions of the tissues as a result of internal or external damage (Broughton et al., 2006). Depending on their underlying

causes and outcomes, these injuries are divided into two groups as chronic wounds and acute wounds. Acute wounds heal within the expected time and in which the tissue can recover physiologically and functionally as a result of healing. These wounds involve a normal wound healing process (Tottoli et al., 2020). In this type of wounds, the number of negative factors affecting the healing process is low. There are different types of acute wounds such as surgical, traumatic and burn diseases (Ramasastry, 2005; Broughton et al., 2006). On the other hand, chronic wounds do not heal in a reasonable time and pass through a disordered healing process. Since the factor is continuous in chronic wounds, these wounds have a slow healing process and even do not heal depending on the condition of the wound. It is estimated that 30 to 40 patients per 1000 surgical operations develop infections in the body after surgery, and some of these infections are life-threatening. The prevalence rate of chronic non-healing wounds in the US population is 2% (Gaspar-Pintilieșcu et al., 2019). There are many factors that affect the healing process in chronic wounds. Venous wounds, ischemic wounds, pressure sore and diabetic wounds are the major types of chronic wounds (Wolcott et al., 2010).

2.2.2. Wound healing process

The wound is the deterioration of the integrity and functioning of tissues. Wound healing is a complex process that is the result of coordinated interactions between various systems to restore damaged tissue and regain tissue integrity. When tissue damage occurs, a cellular process begins, involving keratinocytes, fibroblasts, endothelium, macrophages, and platelets. In this process, migration, proliferation, differentiation of cells and also tissue regeneration occur. Cells gradually synthesize proteins necessary for proliferation and migration. Many growth hormones, cytokines and chemokines play a role in this healing process (Barrientos et al., 2008). It has been reported that wound healing stages in all mammals have a similar process. The healing process is classified into 4 different interlinked phases depending on time including hemostasis, inflammation, proliferation, and remodeling as shown in Figure 2.2. (Öztopalan et al., 2017).

Hemostasis: The hemostasis phase begins immediately after wound formation and lasts for approximately 1-3 days. The hemostasis phase in wound healing aims to abolish the cells that have lost their function, dead tissues or foreign substances in the environment, and also to prepare the appropriate environment for the healing phase (Johnstone et al., 2005). This phase protects the wound from dehydration and infection. In addition, it prevents excessive

exsanguination. Hemostasis occurs in 3 stages including vascular endothelial response, platelet plug formation, and coagulation. Vasospasm promotes the release of coagulation factors at the site of a wound. Following vasoconstriction, platelets aggregate and trigger coagulation. Platelets form a clot and bind to each other with fibrin. The clot contains collagen, vitronectin, fibronectin, von Willebrand factor, thrombospondin and thrombin. This fibrin clot creates a temporary seal over the wound area and protects the wound from contamination. It forms a basic matrix for cell migration and attachment (Atalan, 2013; Kumar et al., 2007). Aggregated platelets release several growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor- β (TGF- β), insulin-like growth factor-1 (IGF) and platelet factor-IV. After that, these growth factors initiate the wound healing cascade by attracting neutrophils, fibroblasts, macrophages and endothelial cells (Enoch and Leaper, 2008).

Inflammation: The second phase of wound healing is inflammation. Inflammation can be divided into early and late stages. Neutrophils are leukocytes that first reach the wound site and initiate wound repair by stimulating epithelial cells and fibroblasts. Neutrophils secrete proteases such as elastase and collagenase, eliminating foreign residues and bacteria and ensuring the removal of dead tissues from the wound. Neutrophils initiate to adhere endothelial cells in adjacent blood vessels and move on the vessel wall (diapedesis) (Strodtbeck, 2001; Diegelmann and Evans, 2004). Interleukin-1 (IL-1) and tumor necrosis factor- α (TNF- α) secretion by neutrophils are activators of growth factors to be secreted from macrophages, keratinocytes and fibroblasts in the following periods. When neutrophils complete their task, they are destroyed by apoptosis or phagocytosed by macrophages.

In the late inflammation phase, monocytes follow neutrophils during wound healing. In the absence of infection, the existing monocytes differentiate into macrophages. Factors released from platelets are the first and most potent stimulator of macrophage activation (Strodtbeck, 2001). When macrophages are activated, they release cytokines that promote angiogenesis and fibroplasia. Also, activation of wound macrophages results in the synthesis of nitric oxide, which serves a variety of functions, including killing pathogens. Lymphocytes appear in the wound on the 5th and 7th days of the injury. During the late inflammatory phase, epithelial cell proliferation continues to form a thickened epidermal cover layer (Young and McNaught, 2011).

Proliferation: The proliferation phase follows the inflammatory phase in the wound healing process. This stage lasts from 4 days up to 2 weeks after injury (Topalan and Önel, 2010). The proliferation phase is characterized by cellular proliferation, angiogenesis, deposition of the extracellular matrix and granulation tissue formation. The main task of the proliferative phase is to reduce damaged tissue area by fibroplasia and contraction and to form an appropriate epithelial barrier to activate keratinocytes. It is the process in which epithelialization is completed with the development of granulation tissue. Growth factors and cytokines secreted by platelets and activated macrophages are responsible for the proliferation of fibroblasts and endothelial cells. In the proliferation phase, fibroblasts begin to proliferate and synthesize the matrix proteins hyaluronan, fibronectin, proteoglycans and collagen that constitute granulation tissue. These compounds enable the formation of a new extracellular matrix necessary for the repair process. Fibroblasts are the most significant mesenchymal cells involved in wound healing. Fibroblasts migrate towards the granulation tissue in this phase. They produce collagen fibers, alter the fibrin-rich matrix and differentiate into myofibroblasts. Myofibroblasts initiate wound contraction, thereby reducing wound size (Öztaş, 2011; Young and McNaught, 2011). The final stage of the proliferative phase is the re-epithelialization process which begins with chemotactic and mitogenic stimulation of keratinocytes by EGF and TGF- β (Mendonça and Coutinho-Netto, 2009). Over the granulation tissue, epithelial cells migrate and proliferate throughout this phase. Endothelial cells migrate from intact venules near the wound and form new capillary tubules by angiogenesis. It also includes the migration and proliferation of keratinocytes to coat the peeled dermal surface. When granulation tissue is formed and epithelialization is completed, the proliferation phase ends.

Remodeling: Remodeling is the last stage of wound healing that starts two to three weeks after injury and can take up to a year or more. The main objective of the remodeling step is to return the damaged area to its pre-wound position both functionally and anatomically (Gonzalez et al., 2016). Remodeling is characterized by wound contraction as well as collagen remodeling. Wound myofibroblasts are responsible for wound contraction (Thiruvoth et al., 2015). Type III collagen of the granulation tissue is degraded and replaced by Type I collagen, which is rearranged into parallel fibrils. As a result of the slowing and eventually stopping of angiogenesis, blood flow and production/destruction processes also end. Finally, a low cellularity scar is formed (Cañedo-Dorantes and Cañedo-Ayala, 2019; Enoch and Leaper,

2008). The tensile strength of the wound increases during remodeling. This phase results in normal epithelial development and maturation of scar tissue (Singh et al., 2017).

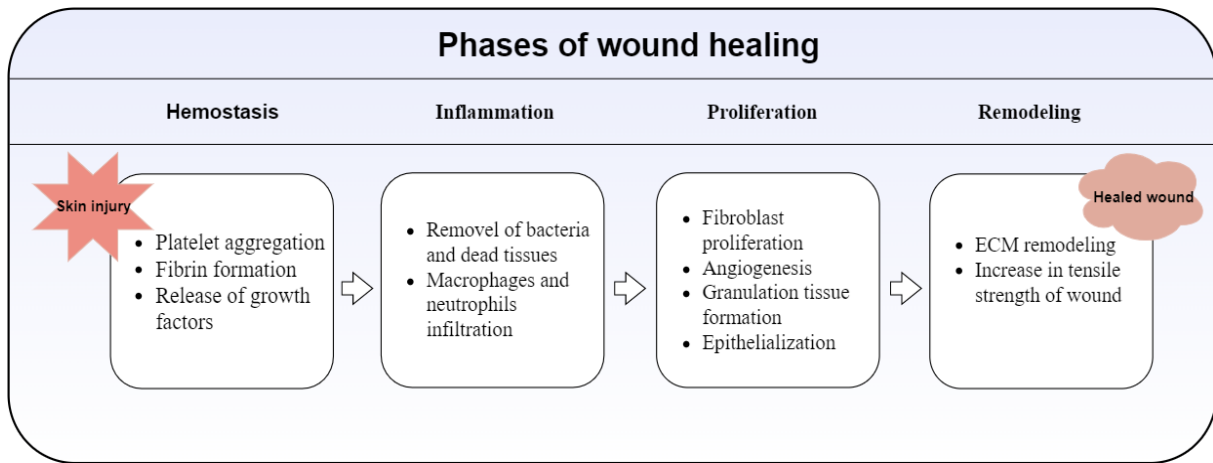


Figure 2.2. Mechanism of wound healing

2.2.3. Factors affecting wound healing

Wound healing is a highly complex process influenced by various local and systemic factors. While local factors directly affect the properties of the wound, systemic factors are the general state of health that affects the healing ability of the person (Robson et al., 2001). Local factors include vascular disruption, infection, trauma, tissue neurosis, overstretching of tissues, poor venous drainage, oxygenation, mobility of the wound area and various malignant structures (Mir et al., 2018). Systemic factors are age, obesity, diabetes, inactivity, various medications used, insufficient nutrition, protein and vitamin deficiencies, chronic renal failure, and radiotherapy/chemotherapy (Robson et al., 2001).

2.2.4. Connexin 43

The role of gap junction channels and the function of connexins are known to have crucial functions in almost all stages of the skin wound healing process. This includes coordinating the inflammatory response, transmitting injury signals between cells, facilitating wound closure, promoting the formation of granulation tissue, and facilitating scar remodeling following injury (Montgomery et al., 2018). Connexins can be found in both the dermis and epidermis, except for the outermost layer of the epidermis known as the stratum corneum (Scott et al., 2012; Yamada et al., 2021). A number of connexin gene family members reported to be expressed in the skin, and the most abundant among them is connexin 43 (Cx43). Cx43 has been reported to

be an attractive target for modulating the wound healing response (Montgomery et al., 2018). According to previous studies, after injuries in humans, Cx43 expression decreased in the wound area and surrounding areas in the epidermis, and Cx43 remained low throughout the healing process. In conclusion, Cx43 downregulation is required for effective wound re-epithelialization (Scott et al., 2012).

2.2.5. Importance of wound care

A wound is the deterioration of the anatomical integrity and function of a tissue or organ due to internal or external injury (Topalan and Önel, 2010). Traumas, burns and chronic wounds on the skin can cause serious health problems and hence wound care is essential. Appropriate wound care is vital in terms of accelerating the healing process, prevention of infection, pain and external impact, absence of edema and discharge that may occur in the injured area, and restoration of the aesthetics and functionality of the deteriorated structure. Finding the reasons that prevent the healing of wounds in treatments and eliminating these causes accelerate the healing process. Furthermore, covering the wound during the healing process promotes the healing process. In recent years, innovative technologies have been employed to facilitate the evaluation, diagnosis and treatment of these wounds (Baktır, 2019). Wound dressings both protect the wound from infection and support the wound healing process (Han and Ceilley, 2017).

2.2.6. Wound dressings

Throughout the history of humanity, wound care and healing have been supported by various types and structures of materials. Wound healing is a physiological process consisting of a complex interaction between many regulated factors to restore injured skin to its repaired barrier function. Considering the complex nature of the wound healing process, it is of great importance to choose the appropriate treatment method (Koyutürk and Soyaslan, 2016). Currently, biocompatible and reliable wound dressings that support and accelerate the healing process and have a regenerative capacity in to repair the structure of the skin are mainly used in the wound treatment process (Gaspar-Pintilieșcu et al., 2019).

Wound dressings should provide a barrier to the wound area against external factors, keep the wound area moist, and permit gas exchange to ensure optimal healing. In addition to these properties, it should be non-toxic, non-adherent, non-allergenic, non-inflammable, comfortable,

biocompatible and biodegradable (Schoukens, 2019). Since the wound dressings are in direct contact with the wound, their structure is significant and many different types of dressings have been used for years (Figure 2.3.). The wound dressing to be used is selected according to the characteristics of the wound (Schoukens, 2019; Tahmakar et al., 2020; Rani Raju et al., 2022).

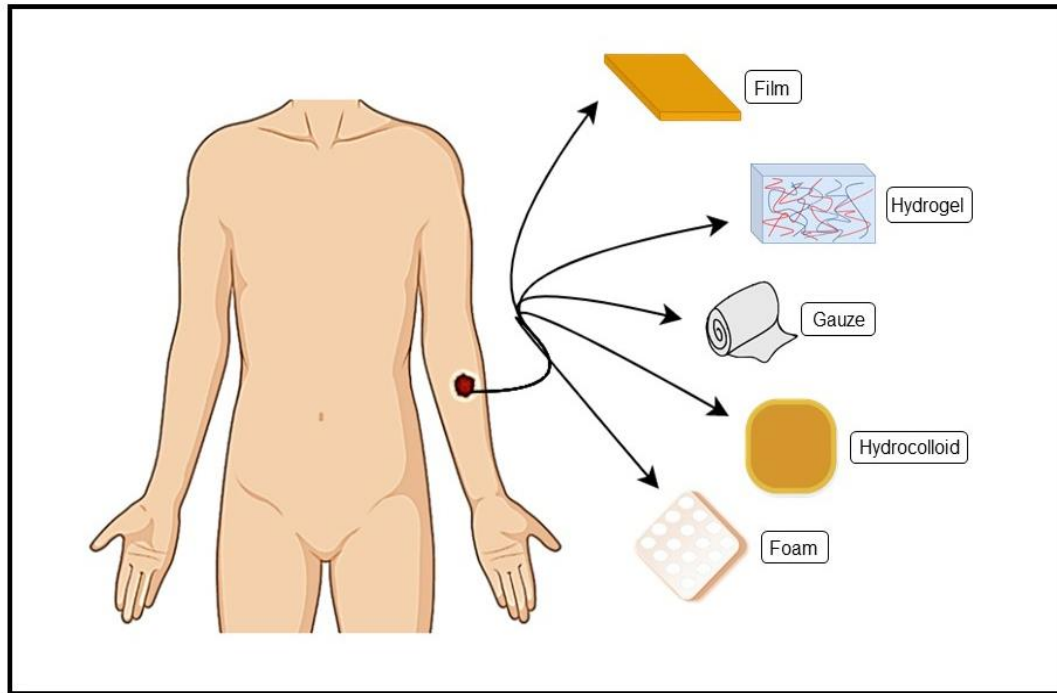


Figure 2.3. Various wound dressing materials

Traditional dressings including gauze, bandages and cotton pads are the most commonly used dressings due to their simple manufacturing process and low cost (Broughton et al., 2006). However, some shortcomings such as the difficulty of keeping the wound bed moist, leaving residue in the wound area and creating a problem when it is removed from the applied area by sticking to the wound. Gauze is one of the oldest wound care products known to be used in wound care. With the application of the gauze, it is aimed to fix the dressing to be applied to the injured area, to protect the wound from external impacts and to prevent infection. Although they are simple wound care materials, they are frequently used strategically in the care of various wounds (Jones, 2006; Daunton et al., 2012).

Modern dressings are preferred over traditional dressings as they have better degradability, biocompatibility, and moisture retention, and provide a moist environment for wound healing.

Hydrogels, foams, hydrocolloids, alginates, and films are the most commonly used modern wound dressings.

Hydrocolloid dressings are waterproof, adhesive and occlusive dressings (Stoica et al., 2020). They provide thermal insulation and thus keep the wound bed close to body temperature. Although hydrocolloids are suitable for dressing both acute and chronic wounds, they should not be used on infected wounds (Liang et al., 2019).

Foam dressings are semi-permeable and can be hydrophobic or hydrophilic. They contain polyurethane and may contain substances that may play a role in wound healing and care in their structures. They can be used alone or in combination with a different dressing for wound care. They provide a moist environment, constant heat and absorption of wound currents to the wound area (Jones et al., 2006). At the same time, since they are non-adhesive, they can be separated from the wound area without causing traumatic damage. Foam dressings maintain moisture in the wound and provide thermal insulation (Davies et al., 2017).

Alginate dressings are obtained from seaweed and are used in wound care. It is applied with a secondary dressing that will fix them. The most important use of alginate dressings is to provide absorption of wound drainage. Thanks to its structures, it absorbs the wound fluid and traps it in its body. In this way, it both prevents the external contact of the wound and ensures the correct management of the care of the bleeding wounds. It is not recommended for use on non-draining wounds (Mirasoğlu, 2015).

Film dressings consist of porous, adhesive, and thin transparent polyurethane. While liquids and bacteria are effectively contained, the wound dressing allows for the passage of carbon dioxide, oxygen, and water vapor from the wound. On the other hand, film dressings should be removed carefully, as their stickiness can lead to damage to the surrounding skin (Stoica et al., 2020).

Considering the materials used, the hydrogels have a similar structure to extracellular matrix (ECM) and a network structure that supports cell growth and viability, making them highly preferred in wound dressing studies. Hydrogel dressings are composed of hydrophilic polymers and approximately 90% water. Hydrogel dressings are suitable for dry and necrotic wounds due to their high water content (Sahiner et al., 2016). They swell when they come in contact with

water and, as they are usually transparent, the healing process of the wound can be observed without removing the dressing. One of their best features is that they give a moist environment to the wound area. They can also provide the release of antibiotics, antimicrobials and derivatives through their structure that will accelerate the healing of the wound area. They are generally used in the care and treatment of ulcers, surgical wounds, burns and skin tears (Vowden and Vowden, 2017). The main disadvantages of hydrogels are non-adherent and have poor mechanical strength (Singh et al., 2014). On the other hand, cryogels are a type of hydrogel that are interconnected macroporous materials with superior physical properties.

2.3. Cryogel

Cryogels, which have a 50-year history, are gel matrices synthesized by polymerization using monomeric or polymeric precursors at sub-zero temperatures. The origin of word cryogel is derived from the Greek word kri'os (kryos), meaning ice or freezing (Lozinsky et al., 2003). The superior properties of cryogels such as elasticity, physical resistance, swelling and interconnected pore structures make them highly preferred in biomedical applications. The supermacropores ensure excellent spongy structure and water absorption ability to the cryogels. In addition, the interconnected macroporous structure allows the effective removal of wound exudate and maintains moisture in the wound area (Akin and Ozmen, 2022). Cryogels are also very stable, economical and easy to synthesize (Bakhshpour et al., 2018). The type of monomers or polymers, initial concentration of monomers, temperature, reaction time, and the ratio of precursors affect cryogel properties (Baimenov et al., 2020). Since various parameters can be easily changed, cryogels can be synthesized in many different shapes and properties, which allows them to be used in various applications (Türkmen et al., 2022). Thanks to these unique features, cryogels are extensively used in a variety of processes, including in biosensors, cosmetic and pharmaceutical applications, removal of heavy metals, drug delivery systems, tissue engineering, separation and purification techniques and so on (Bakhshpour et al., 2018; Bereli et al., 2020).

2.3.1. Synthesis of cryogels

The synthesis of cryogels occurs in three steps as seen in Figure 2.4. In the first step, the appropriate monomers/polymer precursors are dissolved in the solvent and the polymer solution containing the crosslinkers and initiators is prepared. In the next step, the prepared polymer

solution is frozen under suitable conditions and sub-zero temperatures for pore formation. At this stage, cryogelation occurs in the unfrozen liquid part due to the rapid freezing of the solvent in the solution. In the final step, large interconnected macropores of the cryogel are formed when the ice crystals melt at room temperature. Thus, sponge-like, mechanically stable and flexible cryogels are obtained (Kumar et al., 2010; Razavi et al., 2019).

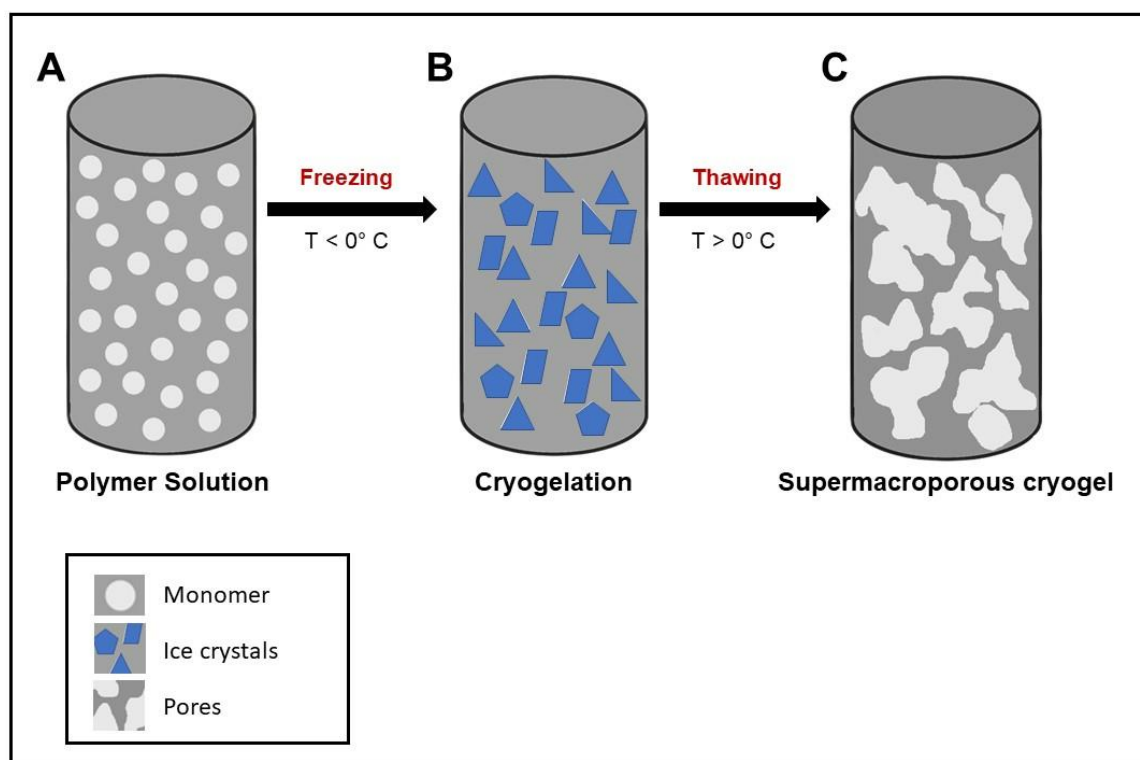


Figure 2.4. Schematic representation of the synthesis of cryogel. A) Solution of monomer and an aqueous solvent; B) cryogelation achieved after sub-zero incubation and ice crystal formation. C) formation of mature super-macroporous cryogel.

The polymers are used to synthesize cryogels as wound dressing materials. In this context, both natural and synthetic polymers can be used. These materials are preferred for cryogel production considering their suitability for use in tissue engineering applications (Hixon et al., 2017). Natural polymers including collagen, gelatin, laminin, hyaluronic acid, chitosan and alginate have been widely used for the synthesis of cryogels. The appealing advantages of natural polymers include being economical, biodegradable, biocompatible, accessible, nontoxic, and having suitable surface properties for cell adhesion (Guo et al., 2021). Natural polymers mimic the physical and chemical properties of the ECM, thereby promoting the

wound healing process (Vishnoi, 2021; Mogoşanu and Grumezescu, 2014). On the other hand, the low mechanical strength of natural polymers may cause the biomaterial to have insufficient mechanical performance (Akin and Ozmen, 2022).

Synthetic polymers such as poly(vinyl alcohol) (PVA), polyglycolic acid, poly-L-lactic acid (PLLA) and polyethylene glycol (PEG) have been extensively used for the fabrication of cryogels. Synthetic polymers have been used to reveal the desired properties in tissue engineering (Akgönüllü et al., 2020). Large-scale reproduction of synthetic materials can be achieved by controlling parameters such as degradation time and molecular weight (Tottoli et al., 2020). The major drawbacks of synthetic polymers are the lack of bioadhesive and bioactivity properties (Türkmen et al., 2022).

Synthetic and natural materials can be used together to manufacture cryogels with better properties in tissue engineering applications. In this context, a mixture of Gel and PVA can enhance the mechanical properties of the cryogel (Zulkiflee and Fauzi, 2021).

2.3.2. Polyvinyl alcohol

PVA is a water-soluble synthetic polymer. It was discovered by W.O. Herrmann and W. Haehnel in 1924 via saponifying polyvinyl ester with sodium hydroxide solution. PVA is a tasteless, odorless, creamy to whitish polymer. It is used in a wide range of biomedical fields due to its biodegradable, non-carcinogenicity, nontoxic, biocompatible, strength, elastic structure in the swollen state, chemical resistance and high water solubility properties (Muppalaneni and Omidian, 2013). Besides these features, the use of this material is increasing day by day due to the high water retention capacity and compatibility with living tissue. PVA crystallization occurs due to the hydrogen bonding between PVA chains. The structure of PVA is chemically inert and highly stable due to its crystallization (Kantoğlu, 2006). Due to the compatible structure and hydrophilic properties of PVA, it is commonly mixed with other compounds to assist in the improvement of mechanical properties. The chemical structure of PVA is shown in Figure 2.5.

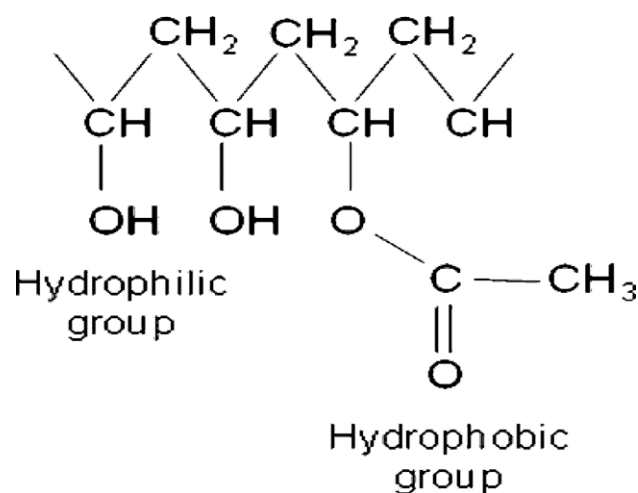


Figure 2.5. Chemical structure of polyvinyl alcohol (Lamminmäki et al., 2010).

2.3.3. Gelatin

Gelatin (Gel) is a natural polymer obtained from native, insoluble collagen via partial hydrolysis. It contains proteins (85–92%), water, minerals and salts. Farris et al. determined the amino acid compositions in Gel. It has been reported that all amino acids except tryptophan are present in the structure of gelatin (Farris et al., 2010). Gel is primarily produced from the skins and bones of pigs, bovine and cows by a process that begins with chemical hydrolysis and continues with thermal denaturation (Noor et al., 2021). Gel has high biocompatibility, gel-forming ability, and thereby it is one of the highly suitable candidates for cryogel production. Moreover, it is biodegradable and enhances cell adhesion (He et al., 2021). However, it is typically employed as a gel form with another polymer due to thermal stability and its low mechanical features (Mogoşanu and Grumezescu, 2014). Crosslinkers help improve mechanical properties. In some studies, crosslinker was used to strengthen the bond between Gel and PVA. Chaibi et al. conducted a study using glutaraldehyde as a crosslinker in PVA and gelatin-based cryogels (Chaibi et al., 2015). In Figure 2.6., the chemical structure of Gel is shown.

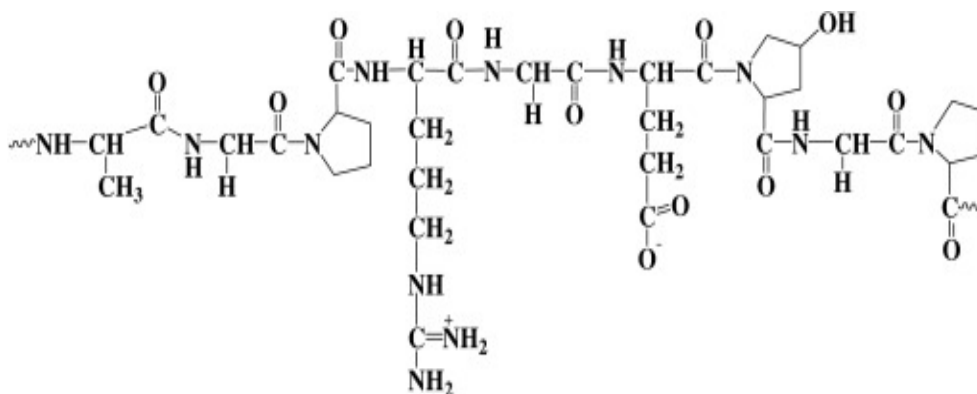


Figure 2.6. Chemical structure of gelatin (Deshmukh et al., 2017).

2.4. In vitro studies

In vitro models can provide a suitable environment for the study of the mechanism of action of a compound or a pharmacological agent as they are less expensive, more efficient and practical compared to in vivo models. Moreover, in vitro tests can be used to screen a wide variety of conditions or samples simultaneously (Thakur et al., 2011). In vitro models based on human skin cells allow the generation of large numbers of cells enabling the application of substances in a wide concentration range for toxicity screening. Cellular interaction and biocompatibility of cryogels can be evaluated more comprehensively with cytotoxicity, genotoxicity and proliferation tests in cells (Poniec, 1992).

To assess the effect of wound dressing materials on cell proliferation, the potential effect is evaluated by in vitro cell culture studies with cells. The wound healing properties of any dressing can be screened using in vitro experiments, avoiding the redundant use of experimental animals. It is necessary to construct a suitable tissue scaffold on which cells can attach, proliferate, grow and differentiate in vitro for tissue engineering applications (Shakya et al., 2014; Kathuria et al., 2009). Since keratinocyte and fibroblast cell lines accomplish various stages of wound healing and are easily available, they are widely used in skin tissue engineering and wound dressing applications. 3T3, L929 and HaCaT cell lines are frequently used in skin tissue engineering studies to observe cell-polymeric matrix interactions (Krishan, 2006).

2.4.1. Keratinocytes

Keratinocytes are epidermal cells found in the human epidermis. The main function of keratinocytes is to protect the body against external injuries by maintaining the structural and

physical integrity of the skin. In addition, they contribute to the initiation and maintenance of skin inflammatory and immunological responses through secreting cytokines, chemokines and growth factors. In addition to these properties, it performs wound repair processes in the re-epithelialization step. The growth factors secreted by keratinocytes enhance wound healing. Considering all these properties, keratinocytes have played an important role in wound treatment studies for many years. In 1981, cultured autologous keratinocytes were administered to a patient with a burn wound. After this treatment, keratinocytes were used as allografts and autografts to cover wounds (Bozkaya, 2021).

HaCaT is an immortalized human keratinocyte line transformed from adult human skin (Schoop et al., 1999). HaCaT cells are generally characterized by their polygonal shape, with a diameter of approximately 20-30 μm (Wilson, 2014). HaCaT cells have been widely used for the assessment of skin cytotoxicity of various substances. HaCaT cells can be seeded onto the cryogel materials to evaluate the potential cytotoxicity of cryogels as wound dressing material (Kempf et al., 2011). Several studies have utilized HaCaT cells for in vitro assessment of cryogels. Shiekh et al. (2020) reported the evaluation of a wound dressing developed to promote wound closure and skin regeneration in diabetic wounds using HaCaT cells (Shiekh et al., 2020). A study by Varlık et al. (2023) performed the viability analysis of cryogels developed for use in wound healing applications and skin injury using HaCaT cells (Varlık et al., 2023).

2.5. Medical plants used in wound healing

Nature has provided us with many plant species that grow in various parts of the world. Plants are represented as an important source of traditional medicine worldwide for thousands of years. The medicinal value of plants depends on the presence of phytochemical constituents such as flavonoids, tannins and phenolic compounds (Bektaş et al., 2018). These natural agents have been used to treat wounds, burns, diseases and ailments in many countries over the years (Ahmad et al., 2021). Owing to the potential reparative and therapeutic effects of plants on wounds, various plants have been used in wound treatment. These natural agents stimulate healing and regeneration of lost tissues through multiple mechanisms. They generally affect one or more stages of the healing process (Sumitra et al., 2009). Extensive research on the anti-inflammatory, antibacterial and wound-healing activities of various plants has been published in the literature, but most of them have not been studied yet. In recent years, several pharmacological studies have been conducted to determine the potential wound healing

activities and active constituents of plants (Thakur et al., 2011; Maver et al., 2015). Although synthetic compounds cause side effects and misuse leads to resistance and complications, plants and their extracts generally show fewer side effects (Ahmad et al., 2021). Accordingly, the use of plant extracts in tissue engineering not only supports wound healing, but also has properties that accelerate the wound healing process through its antioxidant, antimicrobial and anti-inflammatory effects.

In recent years, the synthesis of alternative wound dressing materials with the combination of polymers and different plant extracts provides an advantage in wound care treatment. The included plant-derived compounds impart antioxidant and antimicrobial activities to wound dressings as well as aid wound shrinkage, epithelialization and vascularization, thereby accelerating the wound healing process (Gaspar-Pintilieșcu et al., 2019).

Many studies have been conducted to examine herbal extracts from different plant species for their wound healing effects. In these studies, medicinal plants that affect various stages of wound healing such as coagulation, inflammation, collagen production and epithelial formation were examined. For instance, wound healing effects of *Butea monosperma*, *Ageratina pichinchensis*, *Andrographis paniculata*, *Annona squamosa* and *Calendula* extracts have been proven by in vivo and in vitro experiments (Jin et al., 2013). Yousefi et al. synthesized chitosan nanofibers loaded with henna extract that has been proven to accelerate wound healing with antimicrobial activity (Yousefi et al., 2017). Moreover, in vivo studies have shown that the *Centella asiatica* (*C. asiatica*) plant also has wound-healing effects on burn wounds (Liu et al., 2008). Bharti et al. applied aqueous and methanolic extracts of the *Cissus quadrangularis* to a rat model, and extracts have been proven to stimulate cell proliferation and support the wound healing process (Bharti et al., 2014). Dhurai et al. synthesized curcumin-loaded chitosan/polylactic acid nanofibers, and in vivo studies in the rat model confirmed that curcumin-loaded nanofibers promote wound healing (Dhurai et al., 2013). As a result, most herbal medicines are an alternative option, especially in wound treatments, with the effect of wound healing function of various phytochemicals.

2.5.1. *Phyllanthus emblica*

P. emblica is also named as the Indian gooseberry in English or amla in Hindi and belongs to genus *Phyllanthus* and family *Phyllanthaceae* (*Euphorbiaceae*) (Joseph et al., 2013).

Phyllanthus is a very large genus that contains over 1000 species. This genus was described for the first time by Swedish naturalist Carl Linnaeus in 1737 (Mao et al., 2016). *P. emblica* has distributed throughout tropical and subtropical regions of India, Indonesia, Uzbekistan, Pakistan, Southeast Asia, China, Srilanka and Malaysia (Khan, 2009). The plant species are mostly cultivated in hill slopes up to 200 meters, in plain areas and throughout coastal districts (Kumar et al., 2012). In accordance with the belief in traditional Indian mythology, *P. emblica* is considered as the first tree to be created in the universe. Hence, it has been considered as a sacred tree in India (Jayaweera, 1980).

P. emblica has been used in medicinal systems including Ayurveda for centuries due to its unique restorative and rejuvenating potential. Moreover, it is considered as "the King of Rasyana" In Ayurveda because of its amazing rejunavation and reconstructive abilities. It is one of the traditional herbal remedies that has been utilized to treat and manage ailments since ancient times (Ahmad et al., 2021). The taxonomic classification of *P. emblica* is summarized in Table 2.1. (Rao, 2011).

Table 2.1. Taxonomic classification of *P. emblica*.

Kingdom	Plantae
Division	Angiospermae
Class	Magnoliopsida
Order	Malpighiales
Family	Phyllanthaceae
Tribe	Phyllanthae
Sub tribe	Flueggeinae
Genus	<i>Phyllanthus</i>
Species	<i>Phyllanthus emblica</i> L.

2.5.1.1. Botanical description of *P. emblica*

The tree of *P. emblica* is small to medium in size and grows 8 to 18 meters in height. It is a slightly pubescent or glabrous deciduous tree. It has a crooked trunk with irregularly spreading branches. The branchlets of the tree are 10–20 cm long. The leaves have oblong shapes that are simple, feathery, subsessile, bright green and closely set along the branchlets to resemble pinnate leaves. The flowers are chartreuse yellow in appearance (Mirunalini et al., 2010; Habibur-Rehman et al., 2007). The fruits are almost globular in shape, 1–2.5 cm in diameter and quite smooth, with six vertical stripes (Figure 2.7.). The fruit which is bright green at first, changes into greenish yellow or orange after maturity (Vimala et al., 2011). The fruit weighs 60 to 70 g on average. Flowering begins to appear at the beginning of the spring season while the fruits ripen in the fall. The fruits are usually harvested by hand after climbing to higher branches bearing the fruits (Kumar et al., 2012).



Figure 2.7. *Phyllanthus emblica* (Bhagat, 2014).

2.5.1.2. Pharmacological investigations of *P. emblica*

P. emblica extracts are extensively researched for different biological activities. Pharmacological researches revealed that *P. emblica* extracts possess many properties such as anticancer, antifungal, antimicrobial, antiviral, antibacterial, hypolipidemic, anti-ulcerogenic, antimutagenic, anti-inflammatory, anti-hypercholesterolemic, anti-atherogenic, anti-anemic, antitussive, anti-diarrheal, anti-atherosclerotic, wound healing, restorative free radical scavenging, antipyretic, immunostimulatory, immunomodulatory, analgesic, adaptogenic, snake venom neutralizing, gastroprotective, radioprotective, cardioprotective, neuroprotective and nephroprotective activities (Joseph et al., 2013; Kulkarni and Ghurghure, 2018; Middha et al., 2015; Bhandari and Kamdod, 2012; Saini et al., 2022). Moreover, in vitro studies have shown that *P. emblica* possesses antioxidant, antidiabetic, anti-inflammatory, antiaging, cryoprotective and hepatoprotective properties (Khan et al., 2019).

The use of *P. emblica* especially as an antioxidant has been studied by many researchers. These characteristics are effective in the prevention and treatment of various diseases (Kulkarni and Ghurghure, 2018).

2.5.1.3. Chemical Constituents of *P. Emblica*

Studies on the chemical composition of *P. emblica* have been extensively investigated recently. These studies revealed phytochemicals present in *P. emblica*. Different parts of the *P. emblica* plant including root, leaves, branches and fruit have rich phytochemicals. Phytochemical investigations reported that *P. emblica* primarily contains phenolic compounds, tannins, alkaloids and many other compounds (Hasan et al., 2016).

Polyphenols are one of the major groups of secondary metabolites. Polyphenolic content of *P. emblica* are gallic acid, chebulagic acid, phyllembelic acid, kaempferol, ellagic acid, curcuminoids, methyl gallate, phyllanemblinin, emblicol, corilagin, isostrictiniin, furosin, geraniin and punicafolin (Ahmad et al., 2021).

Additionally, 2,4-di-tert-butylphenol and phenol, 3,5-bis (1,1-dimethylethyl) were phenolics that were present in *P. emblica* fruit (Khan, 2009). The presence of hydroxybenzoic acids, which are phenolic acids, has been determined in the fruit. However, only gallic acid was identified in leaves and branches among hydroxybenzoic acids.

The plant was also analyzed for its tannin content. In this context, *P. emblica* contains tannins like ellagic acid, chebulagic acid, chebularin, chebulinic acid, nitric acid, 3-ethoxy-4,5-dihydroxybenzoic acid, emblicanin A, emblicanin B, ellagitannin, 1-O-galloyl- β -D-glucose (β -glucogallin), punigluconin, pedunculagin, trigallayl glucose, corilagin, 1-O-galloyl- β -D-glucose, 1-O-galloyl- β -D-glucose and 1,6-di-O-galloyl- β -D-glucose (Puppala et al., 2012; Ahmad et al., 2021). The bark, fruits, and leaves of plant are rich in tannins. Furthermore, alkaloids such as phyllantine and phyllantidine were also found in *P. emblica* (Bhandari and Kamdod, 2012).

It is reported that *P. emblica* includes flavonoids such as rutin, quercetin, apigenin 7-glucoside, luteolin 4'-neohesperidoside, kaempferol-3-O- α -L(6''ethyl)rhamnopyranoside and kaempferol-3-O- α -L-(6''-methyl)-rhamnopyranoside (Habib-ur-Rehman et al., 2007; El-Desouky et al., 2008, Bhandri and Kamdod, 2012; Ahmad et al., 2021). Quercetin and rutin are found in leaves, branches and fruits. Also, kaempferol-3-O- α -L(6''ethyl)rhamnopyranoside, and kaempferol-3-O- α -L-(6''-methyl)-rhamnopyranoside are distributed in shoots, branches, leaves and fruits (Gul et al., 2022; Joseph et al., 2013).

P. emblica fruit is known as an important reservoir of vitamins including vitamin C, thiamine, riboflavin, niacin and carotene, minerals such as copper, magnesium, iron, phosphorus, potassium, sodium, zinc, and calcium, carbohydrates like pectins, amino acids such as glutamic acid, cysteine lysine, proline, alanine, and aspartic acid, fatty acids such as palmitic acid, stearic acid, linolenic acid, myristic acid, and oleic acid and other biochemical compounds (Bhandari and Kamdod, 2012; Ahmad et al., 2021).

It is known that *P. emblica* has potent antioxidant activity, mainly due to the presence of phenolics, flavonoids and various gallic acid derivatives. In a study, phenolic and flavonoid contents related to antioxidant activities of methanolic extracts of *P. emblica* fruit collected from six diverse regions in China were analyzed. These methanolic extracts exhibited stronger antioxidant activities (Liu et al., 2008). Consequently, *P. emblica* is a valuable medicinal plant due to the existence of these compounds (Ahmad et al., 2021). Some of the chemical components found in *P. emblica* are shown in Figure 2.8.

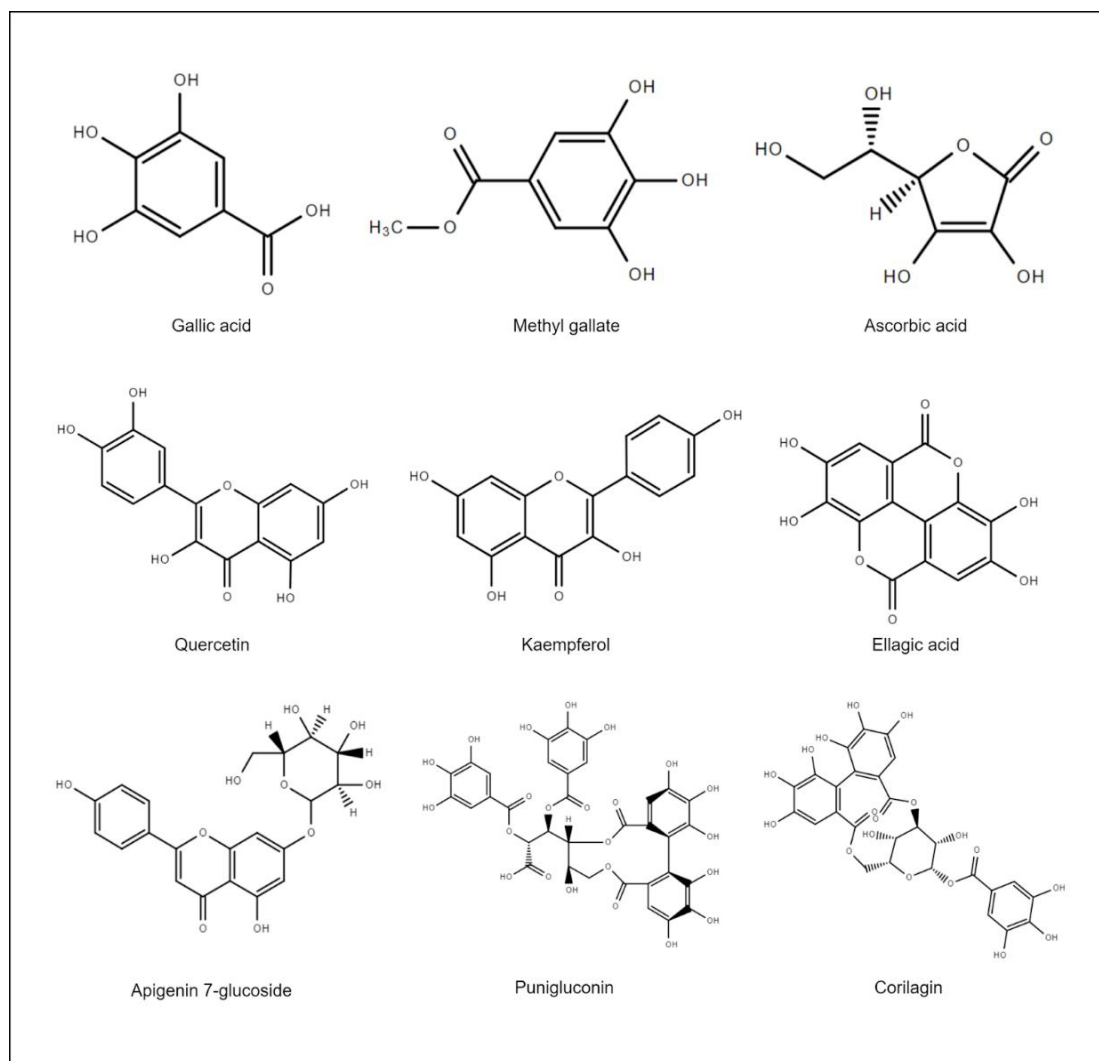


Figure 2.8. Chemical components present in *P. emblica*

2.5.1.4. *Traditional uses*

Various methods containing *P. emblica* have been used in several medical systems to treat different diseases. The medicinal use of *P. emblica* has been particularly reported in the Unani Tibet, Sri Lanka, Siddha and Chinese medicinal systems.

All parts of the *P. emblica* are used for a variety of purposes. Notably, the fruit is widely used in traditional medicine to treat cold, fever, diabetes, diarrhea, cancer, hepatic disorders, peptic ulcer, inflammation, jaundice and heart diseases (Bhandari and Kamdod, 2012). In addition, the juice of the bark along with turmeric and honey is used to treat gonorrhea. *P. emblica* seeds were used to treat itch, scabies, asthma, and bronchitis. Additionally, the dried fruits of plant in coconut oil was used to delay graying hair (Joseph et al., 2013). This plant is stated to possess anti-aging, anti-melanogenic and anti-wrinkling effects. Chaikul et al. evaluated the anti-skin

aging properties of *P. emblica* branches. As a result of the study, they revealed that the *P. emblica* branch can be a promising component for use in anti-skin aging products (Chaikul et al., 2021). Also, *P. emblica* is used as a hair tonic for hair growth (Saini et al., 2022).

According to the ayurvedic medical system, it helps to reduce cough, treat respiratory infections, cleanse the blood, diminish fever, relieve asthma, treat skin disorders, benefit the eyes, rejuvenate entire body organ systems promote hair growth, prevent acne, strengthen the heart, maintain the health of eyes, enhance the immune system, boost the intellect, protect cells from free radical damage, stimulate the body, and so forth (Bhandari and Kamdod, 2012). In Ayurveda, *P. emblica* is used as rasayana. Besides being used as rasayana, *P. emblica* is also used in culinary in many countries. It is used to make chutney, pickles and murabbah (Saini et al., 2022).

P. emblica is useful against various diseases including asthma, bronchitis, peptic ulcer, ophthalmopathy, dyspepsia, cephalalgia, diabetes, cardiac disorders, colic, flatulence, high acidity, leprosy, anemia, erysipelas, hepatopathy, haematogenesis, inflammations, emaciation, jaundice, dysentery, strangury, diarrhoea, hemorrhage, menorrhagia, leucorrhoea, intermittent fevers and graying of hair (Kulkarni and Ghurghure, 2018).

2.6. *P. emblica* as an Extract in Wound Dressing

Botanicals with free radical scavenging activity and antioxidants are substantial in wound healing. (Datta et al., 2011). Researches confirmed that *P. emblica* promotes wound healing due to possess anti-oxidant, antimicrobial and free radical scavenging activities. The activity of *P. emblica* extract on wound healing has been investigated in several studies. *P. emblica* extract exhibited significant wound healing activity by proliferation and mobilization of fibroblasts and keratinocytes in in vitro studies (Talekar et al., 2017; Ahmad et al., 2021). Also, it was shown to possess different functions at various stages of wound repair. Another research revealed that *P. emblica* accelerates the wound healing process which may be explained by the presence of compounds such as ascorbic acid, emblicanin A and emblicanin B (Sumitra et al., 2009). Hence, *P. emblica* has significant potential for wound treatments. Chularojmontri et al. documented that *P. emblica* has a high antioxidant capacity and improved endothelial wound healing at low concentrations. However, it was observed to have an adverse effect on wound healing at high concentrations (Chularojmontri et al., 2013).

The wound healing model of *P. emblica* in the Wistar albino rat was investigated. This study demonstrated 95% regeneration of the dermis and epidermis in *P. emblica* treated rats over a 16-day period. Treated rats showed higher amounts of type 1 collagen along with better maturation and crosslinking, thus verifying the role of *P. emblica* in wound healing (Klimis-Zacas and Rodriguez-Mateos, 2022). In conclusion, *P. emblica* can enhance the biological properties of cryogels, such as their ability to support cell growth, tissue regeneration and wound healing.



3. METHODOLOGY

The methods of this thesis study were schematized in Figure 3.1.

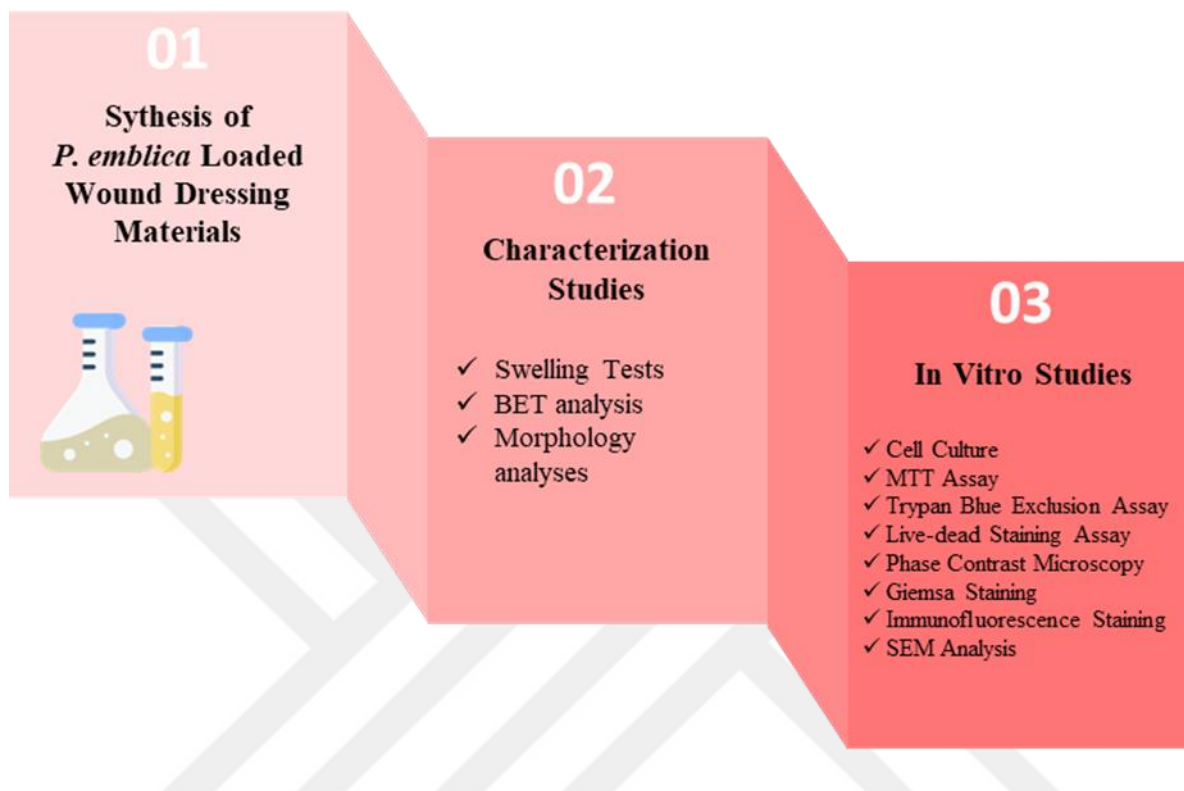


Figure 3.1. Diagram of the experimental protocol

3.1. Synthesis of *P. emblica* Loaded Wound Dressing Materials

PVA solution (10%; w/v) was dissolved in ultrapure water under slow stirring and boiled until the solution becomes clear. Also, Gel solution was prepared in ultrapure water with a final concentration of 3% (w/v) at 40 °C in a water bath. After that, PVA and Gel were mixed at a ratio of 1:2. *P. emblica* in powder form was obtained from Sabinsa Corporation, USA. *P. emblica* powder was dissolved in 5% DMSO (v/v) solution and then it was filtered through sterile Millex® Syringe Filters (0,22 µm). This extract was added into the polymeric solution at final concentrations of 0.5%, 1%, 1.5% and 2%. Finally, glutaraldehyde was added as a crosslinker to the final polymer solution and vortexed. For comparison, cryogel without *P. emblica* was also synthesized according to the same protocol. Finally, the mixture was poured into 24 well plates and cooled in the freezer at -16 °C for 12 hours. After the polymerization process, the cryogels were taken out of the freezer and thawed at room temperature and so supermacropores structure were obtained. After the melting phase, the cryogels were washed

several times with distilled water to remove the polymer precursors and crosslinkers that could not enter the structure. In this thesis study, five different cryogels (PVA/Gel, PVA/Gel/*P.emblica*-0.5, PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5, PVA/Gel/*P.emblica*-2) was synthesized. These cryogels were named according to the amount of *P. emblica* used.

3.2. Charazterization Studies

Swelling properties, surface properties and structural properties of cryogels were investigated by characterization studies.

3.2.1. Swelling tests

The swelling ratio (%), polymerization yield (%) and macroporosity (%) of the cryogels were revealed within the scope of swelling tests.

Before the test, cryogels were dried by lyophilizer (BK-FD10, Biobase) (Figure 3.2.) for 2 h and then dry samples (m_d) were weighed. The swelling study of cryogels was carried out gravimetrically by immersion in ultrapure water for 2 hours. Excess water was wiped off from the swollen cryogels before weighing them. The swollen cryogels were weighed (m_{sw}) regularly every 5 minutes until equilibrium was achieved. The swelling ratio (SR %) was determined with equation 3.1.

$$SR (\%) = (m_{sw} - m_d) / m_d \times 100\% \quad (3.1)$$

Where m_d are the weight of the dry cryogel before swelling, m_{sw} is the weight of the swollen cryogel.

The polymerization yield of cryogels was also determined. For this purpose, the total amount of components added to the polymer solution was recorded (m_t). Cryogels were dried at 60°C and dry cryogel samples were weighed (m_d). The polymerization yield was calculated according to equation 3.2.

$$\text{Polymerization yield } (\%) = (m_d / m_t) \times 100\% \quad (3.2)$$

The swollen and squeezed cryogel weights were recorded to determine the macroporosity of cryogels. In this regard, cryogels were immersed in ultrapure water until swelling. The swollen cryogels (m_{sw}) was weighted. After the swelling process was completed, swollen cryogels were

squeezed and weighed (m_{sq}). The macroporosity of cryogels were calculated according to equation 3.3.

$$\text{Macroporosity (\%)} = (m_{sw} - m_{sq})/m_{sw} \times 100\% \quad (3.3)$$

The same calculations were applied for all cryogels. The experiments were repeated three times. The mean values were calculated and recorded.



Figure 3.2. Lyophilizer

3.2.2. BET analysis

The specific surface area is one of the most important properties of porous materials to be detected. BET analysis was carried out to investigate the surface areas of the prepared PVA/Gel and PVA/Gel/*P.emblica* cryogels. BET analysis enables the determination of the surface area of the material through the nitrogens' strong interaction with solids and its availability of high

purity. Nitrogen adsorption of the vacuum-dried samples was carried out at 77 K (196°C). Finally, the specific surface area of the sample was calculated with utilization of the amount of adsorbed nitrogen, as a unit of m²/g.

3.2.3. Morphology analysis

The surface morphology and bulk structures of cryogels were assessed with a Scanning Electron Microscope (SEM) (Quanta FEG 650, Thermofisher, Massachusetts, USA). SEM is an effective imaging method utilized to display the morphology of cryogels (Gun'ko et al., 2013). The cryogels were frozen at -20°C. The frozen cryogels were dried at -55°C via lyophilizer to remove water from the sample without causing shrinkage. After cryogels were completely dried, they were coated with gold-palladium (2:3) and viewed under an SEM. Image J software was employed to determine the pore size and distribution of these pores of the cryogels.

In addition, a camera optical microscope was used to study the morphology of the cryogel samples. Samples were placed on a glass slide and images were recorded at 20X and 40X magnification (Górska et al., 2021).

3.3. The In Vitro Studies

In vitro studies were conducted to illustrate the suitability of the synthesized cryogel materials for use as wound dressings. For all in vitro analyses, the cryogels of identical dimensions (13 mm in diameter 0,5 mm in thickness) and dry weight (20 mg) were utilized (Nikhil and Kumar, 2022). In vitro cell experiments were performed by culturing HaCaT cell lines on the cryogels. HaCaT cells were seeded on the cryogels with a density of 1×10^6 cells/sample (Xu et al., 2022). The experimental groups include (i) control (only polystyrene-culture plate), (ii) PVA/Gel (*P. emblica* free cryogel), (iii) PVA/Gel/*P.emblica*-0.5 (0.5% of *P. emblica* loaded cryogel) (iv) PVA/Gel/*P.emblica*-1 (1% of *P. emblica* loaded cryogel), (v) PVA/Gel/*P.emblica*-1.5 (1.5% of *P. emblica* loaded cryogel) and (vi) PVA/Gel/*P.emblica*-2 (2% of *P. emblica* loaded cryogel).

3.3.1. Cell culture

The in vitro studies were conducted with spontaneously immortalized human keratinocyte cell line (HaCaT) (at the 20th-25th passages). HaCaT cell lines were grown in Roswell Park Memorial Institute (RPMI) (Sigma-Aldrich, Germany). The culture 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). The cells were incubated at 37°C in a 5% CO₂ incubator (IN55, Memmert, Germany). During the cell culture studies, all experiments requiring a sterile environment were carried out in a laminar flow cabinet (DEMAIR, MSC-II A, Turkey). Cells were observed regularly with an inverted phase-contrast microscope (Dmil Led Fluo, Leica, Germany) until they were confluent. Experimental studies began by confirming that cells carry ancestral morphological and molecular characteristics.

3.3.2. Thawing and passaging of HaCaT cells

For the experiments to be carried out, the cells which had been frozen before were removed from the -80°C refrigerator. The cell lines were thawed at 37°C for approximately 2 minutes. Subsequently, the cells in the cryovial were added to a falcon tube containing culture medium and centrifuged at 1000 rpm for 5 minutes. Afterward, the supernatant was ejected and the pellet was reconstituted in a culture medium. The cells were seeded in T-25 flasks. The proliferation processes of the cells were followed and the medium was renewed every 2 to 3 days.

Cells that became 70-80% confluent in flasks were washed with PBS three times. Cells were exposed to 0.25% trypsin-EDTA for 5 minutes in the incubator. 0.25% trypsin-EDTA Solution was inactivated by medium and then the solution was taken into a falcon tube. After centrifugation for 5 minutes, the supernatant was discarded. The pellet was dissolved with a clean medium. Finally, the cell suspension was seeded into the new T-25 culture flasks at a concentration of 5×10^4 cells/cm².

3.3.3. Cryogel sterilization

Before going through the cultivation of cells on cryogels, they were sterilized by immersing them into increasing concentrations of ethanol (60–100%) (v/v) for 60 min. Afterward, they were washed with sterile ultrapure water several times to remove ethanol. Thereby, cryogels were sterilized and became ready to use in cell culture studies.

3.3.4. Cultivation of HaCaT cells on cryogels

Prior to culturing the cells, sterile cryogel samples were kept in the completed medium at 37 °C for 3 h. After that, the medium was removed from the cryogels. Cryogels were placed into the wells of 24-well plates before seeding cells on cryogels.

For the cultivation of cells on wound dressing materials, HaCaT cells were cultured on a T-25 flask in a humidified incubator. Cells that reach 80-90% confluency were trypsinized and centrifuged. The cell pellet was diluted with culture media. Cell cultivation was carried out in two stages in order to facilitate the uptake of cells into the gels. For this purpose, firstly, 20 μ L of cell suspension was cultured at a seeding density of 1×10^6 cells per cryogel on the surface of the cryogel. After that, cryogels were left in the incubator at 37°C and 5% CO₂ for 3 hours without adding medium to allow time for promoting cell adhesion. After 3 hours of incubation, 1 ml of medium was added to the cryogels and cells were incubated in the incubator.

3.3.5. MTT Assay

The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay is a common and rapid method to assess the viability and cytotoxicity of cells on cryogels. It relies on the reduction of a MTT to formazan product by metabolically active cells. The effect of *P. emblica* amounts in the cryogel on cell viability and the usability of these cryogels as wound dressings were examined. The sterilized cryogels were placed into 24 well plates, respectively as follows: PVA/Gel, PVA/Gel/*P.emblica*-0.5, PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5, PVA/Gel/*P.emblica*-1.5, PVA/Gel/*P.emblica*-2. Cells were seeded into the cryogels at 1×10^6 density per well. The seeded cryogels were incubated at 37°C for cell attachment and growth. The first well was not seeded with cells. MTT assay was done in 24, 48 and 72 h intervals. After the cells were inoculated on the cryogels and the incubation period was completed, the medium was aspirated, followed by washing with phosphate-buffered saline (PBS). MTT reagent (0.5 mg/mL in RPMI) was applied to each well. The cryogels, along with the MTT solution were incubated for nearly 4 h at 37 °C to allow MTT to be taken up by living cells and converted into insoluble formazan crystals within the cells. Subsequently, DMSO was supplied to the wells to dissolve formazan crystals and placed on a shaker (Everlast Rocker 247, Benchmark, USA) for 30 min (Figure 3.3.).



Figure 3.3. 24-well plate for MTT assay

The absorbance value of each well was measured using a spectrophotometer (Uvmini-1240, Shimadzu, Japan) at 570 nm. The formula of the percentage of cell viability was:

$$\text{Cell viability (\%)} = \frac{\text{Average OD570 of test cells}}{\text{Average OD570 of control cells}} \times 100 \quad (3.4)$$

In this Equation, test cells indicate the viability of cells cultured on the cryogels, while control cells indicate the viability of cells cultured on culture plate.

3.3.6. Trypan blue exclusion assay

The effect of the *P. emblica* loaded cryogels on cell viability was evaluated by the trypan blue exclusion assay. Viable cells with intact membranes exclude trypan blue and appear unstained while non-viable cells are stained with trypan blue. First, cells were plated onto the cryogels. After the incubation time, the cryogels were carefully scraped using scissors, and the cells were subsequently detached from the surface using trypsin. By dissolving the medium with the pellet, the cells and 0.4% trypan blue dye were mixed in ependorf at a ratio of 1:1. 10 μ l of the mixture was taken and loaded on the hemocytometer. The number of viable cells was then counted by inverted microscope at 10X magnification. The cell viability were calculated using equation 3.5.

$$\text{Cell viability (\%)} = \frac{\text{Average viable cell count of test cells}}{\text{Average viable cell count of control cells}} \times 100 \quad (3.5)$$

In equation 3.5, the term "test cells" refers to the viable cells that were cultured on cryogels, whereas the term "control cells" refers to the viable cells that were cultured on a culture plate.

3.3.7. Live-dead staining assay

The live/dead staining assay is a widely used technique for distinguishing live cells from dead cells within a population. It was performed to assess the activity of *P. emblica* loaded wound dressing materials on cell viability. For the live/dead cell assay, Calcein-AM (C-AM) and Ethidium homodimer-1 (Eth-1) dyes were used. Eth-1 binds to the DNA of cells with impaired cell membrane integrity, showing dead cells with red fluorescence. C-AM, on the other hand, is a non-fluorescent acetomethoxy-derived compound, but it is broken down by esterase enzymes in the cytoplasm and turns into Calcein, which makes living cells appear green with the effect of fluorescence. After 72 hour cell were seeded on cryogels, cells were washed twice with PBS. For the continuation of the staining process, 1 μM Eth-1 and 1 μM C-AM dyes were loaded onto the surface of the cryogels. Following 30 minutes of incubation which is under room temperature and shielded from light, staining solutions were discarded from the wells and then rinsed with PBS to remove non-specific staining and excess fluorescence dye. The stained cells on cryogel samples were viewed under a microscope at 20X magnification.

The cell viability of cryogels were calculated using equation 3.6.

$$\text{Cell viability of cryogels (\%)} = \text{Live cells} / (\text{Live cells} + \text{dead cells}) \times 100 \quad (3.6)$$

3.3.8. Phase Contrast Microscopy

In order to monitorize the microscopic images of the synthesized cryogels, 13 mm diameter Thermanox coverslips (Thermo Fisher Scientific, Waltham, USA) were placed in the 24-well plate and then cryogels were synthesized onto them with a thickness of 0,5 mm. The cryogels were imaged 72 hours after cell cultivation. Images of the cells cultured in polystyrene-culture plate was used as a control to compare and evaluate the cells. The images of cryogels were obtained with phase contrast microscope (Leica, Dmil Led Fluo, Germany) (Figure 3.4.).

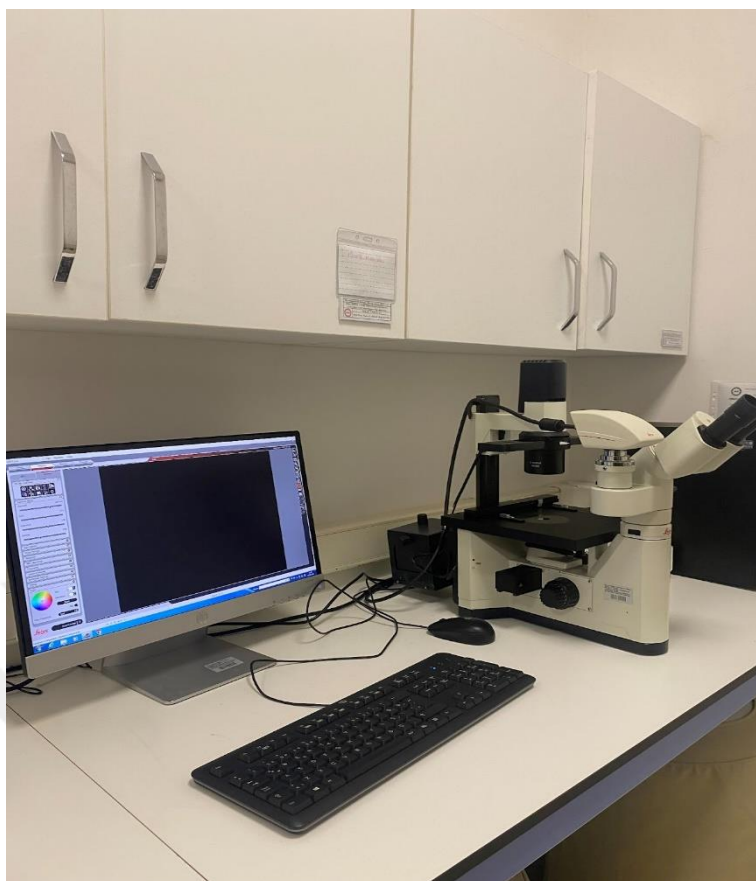


Figure 3.4. Phase contrast microscope

3.3.9. Giemsa staining

Giemsa staining was performed to investigate the efficacy of *P. emblica* on HaCaT cell morphology. The coverslips were placed in a 24-well cell culture plate. Then, cryogels were synthesized to form a thin layer on coverslips and cells were cultured on cryogels. After 72 hours of incubation, the medium was removed and cells were treated with methanol for 15 minutes. Fixation plays a crucial role in maintaining the integrity of cellular and tissue structures and prevents degradation during subsequent staining steps (Hobro and Smith, 2017). The cryogels were immersed in a 5% Giemsa staining solution for 30 minutes. Following the staining step, the cryogels were thoroughly washed with water to remove excess stain. The images of cells on cryogels were captured using an inverted microscope at a magnification of 20X.

3.3.10. Immunofluorescence staining

Immunofluorescence staining was carried out to determine the effectiveness of the *P. emblica* loaded cryogels on keratinocyte morphology, adhesion, infiltration, cell-cell and cell-matrix interactions. The cells were then seeded on the cryogels in a 24-well cell culture plate for 72 hours. The medium in the wells was discarded and the cells were rinsed with PBS. For cell fixation, 4% (v/v) paraformaldehyde in PBS was added and incubated at room temperature for 30 minutes followed by washing with PBS. The samples were permeabilized with Triton-X 100 in PBS (0.1%; v/v) for at least 10 minutes. Alexa Fluor 594 phalloidin dye was prepared with 1% Bovine Serum Albumin (BSA; w/v) in PBS-T (PBS with Tween 20) at a 1/1000 ratio (v/v). Phalloidin is a toxin derived from the dangerous *Amanita phalloides* mushroom that is used to stain actin filaments (F-actin) when labeled with fluorescent dyes. Gap junctional protein (Connexin 43, Cx43) was detected under fluorescence microscope using a primary rabbit anti-Cx43 antibody (ab230537, 1:500 dilution) with a secondary goat anti-rabbit IgG H&L antibody (Alexa Fluor® 488) (ab150077, 1:1000 dilution) (Zhang et al., 2021). In addition to staining the actin filaments of the cells, DAPI (4',6-diamidino-2-phenylindole) dye was employed to stain the cell nucleus. For this purpose, DAPI dye was prepared in PBS at a ratio of 1/1000 (v/v). For microfilament and nucleus staining, Alexa Fluor 594 and DAPI staining solution were added to the fixed cells. The incubation process was carried out for 90 minutes in the dark at room temperature. After the incubation period, the staining solution was removed by aspiration and rinsed with PBS to remove the excess stain. Finally, the cryogels were visualized at 20X magnification under a fluorescence microscope (Dmi1 Led Fluo, Leica, Germany). Also, imageJ software was used to analyses fluorescein signals in Cx43 images (imagej.nih.gov) (Gülner et al., 2023).

3.3.11. SEM analysis

SEM analysis is a powerful method that can provide valuable insights into the detailed interactions between cells and cryogels. It was performed to assess the effect of cryogels on HaCaT morphology. It was evaluated whether the cells adhered to the surface of the cryogels and spreading potential within the cryogel.

HaCaT cells were seeded on sterilized cryogels and incubated for 72 hours. 72 hours after cell seeding, the culture medium on the cryogels was discarded and the cryogels were washed twice with PBS. After that, cells were fixed using 2.5% glutaraldehyde solution in PBS for 3 h at

room temperature to preserve the cellular structures. The cryogel samples were rinsed with PBS and dehydrated with increasing concentrations of alcohol (30% to 100%). Cryogel samples were dried with lyophilizer to remove moisture without causing structural damage and then coated with gold-palladium (2:3) prior to SEM imaging. The coated samples were loaded into the SEM chamber and imaged at 1000X and 2500X.

3.3.12. Statistical analysis

All data were accomplished by Statistical Package for Social Sciences v20.0 software (SPSS, Chicago, IL, USA). Results were presented as mean $\pm$ standard deviation (SD) of values from three independent experiments. Statistical tests were conducted using GraphPad Prism software 8.4.3 version (GraphPad Software, La Jolla, CA, USA). ImageJ software was used to analyses fluorescein signals. A two-way analysis of variance (ANOVA) and Tukey's test were utilized to compare data when three or more variables were present. Statistical differences were considered significant at $P < 0.05$.

4. ANALYSIS AND DISCUSSION

4.1. Characterization Studies

Swelling tests, BET analysis and morphological analyses were performed for the characterization of the synthesized cryogels.

4.1.1. Synthesis of cryogels

Polymerization of PVA/Gel based cryogels synthesized within the scope of the thesis study was carried out at -16°C. During this 12 hours freezing period, ice crystals form and grow within the material, forming a network of channels and voids. At the end of the freezing period, the cryogels were allowed to melt at room temperature. During the thawing process, these ice crystals melt and leave behind a network of interconnected pores throughout the material. The use of both synthetic and natural materials in the cryogel composition provides a balance between mechanical strength and biocompatibility, making them suitable for a wound dressing applications (Canatar et al., 2023).

The properties of five different PVA/Gel-based cryogels containing different amounts of *P. emblica* were investigated. When the *P. emblica* ratio in the cryogel was increased above 2%, it was observed that there was a deterioration in the structure of the cryogel. Therefore, *P. emblica* concentrations in the cryogel were selected as 0.5%, 1%, 1.5% and 2% final concentrations to ensure the stability and functionality of the cryogel. The cryogels synthesized in a 24-well plate had a diameter of 13 mm. As a result of the morphological examinations, it was observed that the cryogels were flexible, good mechanical strength porous and sponge-like structures. Optical images of cryogels synthesized within the scope of the study were given in Figure 4.1. It was observed that as the amount of *P. emblica* in the cryogels increased, it became slightly whiter.

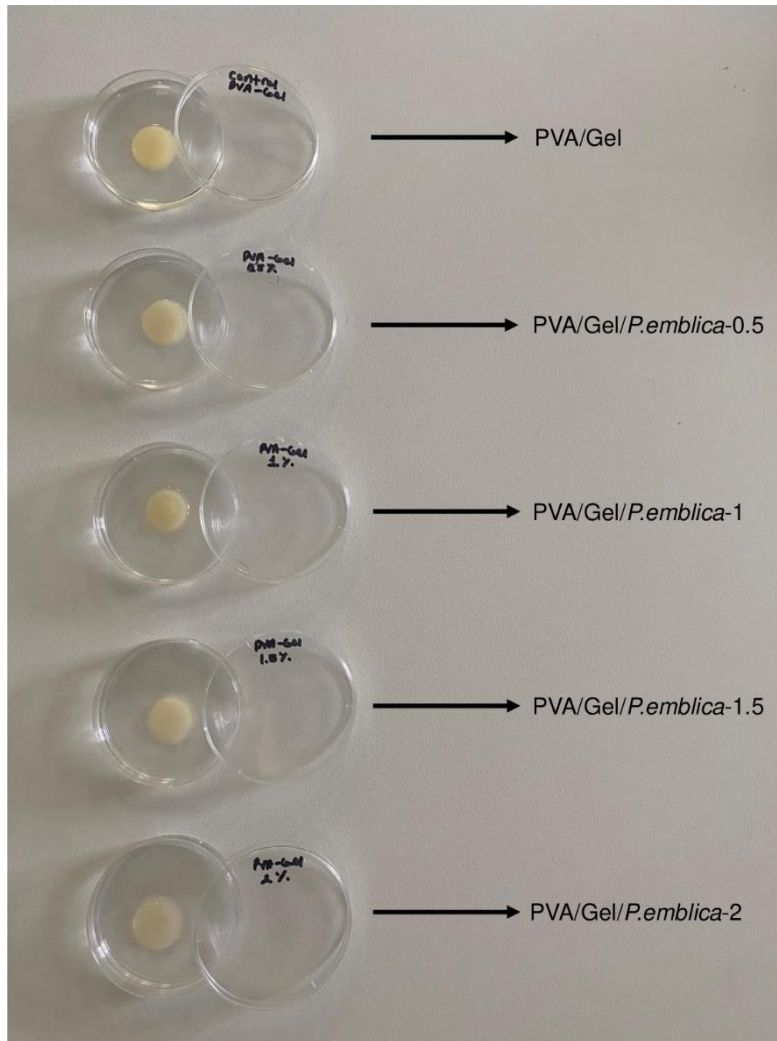


Figure 4.1. Images of synthesized cryogels.

4.1.2. Swelling studies

The effect of *P. emblica* amounts on swelling rate, polymerization yield and porosity of cryogels were assessed by swelling tests (Table 4.1.).

The swelling process is defined as the penetration of liquid molecules into the polymer network (Gugliuzza, 2016). Owing to the porous structure of the cryogels, the liquid enters the pores and causes swelling. The high and rapid swelling capabilities of cryogels allow them to maintain a moist environment at the wound surface, prevent leakage of body fluids and absorb wound fluids; therefore, they are preferable for wound dressings. The synthesized cryogels exhibited a rapid and high swelling response when immersed in a liquid solution which is in line with the literature (Shetty and Rao, 2022; Choi et al., 2013).

Within the first 15 minutes, the synthesized cryogels achieved the maximum swelling level and thus no change was observed in subsequent measurements and equilibrium was attained. Figure 4.2 showed the image of the cryogels dried with a lyophilizer.



Figure 4.2. Dried cryogels.

The swelling rate, polymerization yield and macroporosity of the cryogels were obtained from swelling tests and the results were summarized in Table 4.1.

Table 4.1. Swelling properties of cryogels

	Swelling Rate (%)	Polymerization Yield (%)	Macroporosity (%)
PVA/Gel	450	92	82
PVA/Gel/ <i>P.emblica</i> -0.5	452	93	82.2
PVA/Gel/ <i>P.emblica</i> -1	457	92	82.4
PVA/Gel/ <i>P.emblica</i> -1.5	458	93	82.5
PVA/Gel/ <i>P.emblica</i> -2	446	91	81.4

The swelling ratio of cryogel samples is an important parameter that indicates the extent to which they absorb water or swell when immersed in a liquid medium. As can be seen in Table 4.1, all cryogel samples, including the control, exhibited high swelling ratios ranging from 446% to 458%. The swelling ratio of PVA/Gel, PVA/Gel/*P.emblica*-0.5, PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5, PVA/Gel/*P.emblica*-1.5, PVA/Gel/*P.emblica*-2 cryogels were calculated as 450%, 452%, 457%, 458% and 446% respectively. According to these results, there were slight changes in swelling rate with the addition of *P. emblica* extract at different concentrations. Changing the *P. emblica* amount from 0% to 1.5% increased water uptake. Cryogel containing 1.5% *P.emblica* extract (PVA/Gel/*P.emblica*-1.5) displayed the highest swelling ratio. On the other hand, cryogel containing 2% *P. emblica* showed the lowest swelling rate. This may be due to excessive amounts of *P. emblica* clogging the pores within the cryogels, resulting in a reduction in pore size.

The polymerization yield is a measure of the efficiency of the polymerization process and indicates how much of the monomer material is converted into the final cryogel. It was calculated with equation 3.2. The yield of the polymerization of PVA/Gel, PVA/Gel/*P.emblica*-

0.5, PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5, PVA/Gel/*P.emblica*-1.5, PVA/Gel/*P.emblica*-2 cryogels was calculated as 92%, 93%, 92%, 93% and 91%, respectively. Polymerization yields were found to be high in compatible with the other studies (Derazshamshir et al., 2010; Huang, et al., 2000). The data show that a slightly higher yield was achieved when the amount of *P. emblica* was increased.

The porosity of cryogels is one of their most important features for tissue engineering applications. The interconnected pores provide a large surface area for cell adhesion, proliferation, and nutrient diffusion. The synthesized cryogels showed a high amount of porous structure in accordance with the literature (Du Toit and Pott, 2020). The macroporosities were calculated as 82%, 82.2%, 82.4%, 82.5% and 81.4% for PVA/Gel, PVA/Gel/*P.emblica*-0.5, PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5, PVA/Gel/*P.emblica*-1.5, PVA/Gel/*P.emblica*-2, respectively. As with swelling properties and polymerization yield results, a slight increase was observed in the macroporosity was observed as the amount of *P. emblica* increased, except for the PVA/Gel/*P.emblica*-2 cryogel.

Based on the results of the swelling tests, the synthesized cryogels exhibit excellent macroporosity and swelling rate. The swelling properties of cryogels are associated with mimicking the ECM with features such as oxygen migration, diffusion of molecules, cell adhesion that the wound dressing materials meet. Accordingly, it was concluded that *P. emblica* loaded cryogels are suitable as wound dressings (Liu et al., 2009; Choi et al., 2013).

4.1.3. BET analysis

The BET analysis was performed to utilize for determining the surface area of cryogels. The BET analysis results of PVA/Gel, PVA/Gel/*P.emblica*-0.5, PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5, PVA/Gel/*P.emblica*-2 cryogels were determined as $18 \text{ m}^2/\text{g} \pm 0.74 \text{ m}^2/\text{g}$, $18 \text{ m}^2/\text{g} \pm 0.52 \text{ m}^2/\text{g}$, $20 \text{ m}^2/\text{g} \pm 0.63 \text{ m}^2/\text{g}$, $21 \text{ m}^2/\text{g} \pm 0.81 \text{ m}^2/\text{g}$, $20 \text{ m}^2/\text{g} \pm 0.66 \text{ m}^2/\text{g}$. BET analysis obtained in three replicates are provided with their standard deviations. Overall, the results showed that the addition of *P. emblica* extract to cryogels slightly increased their specific surface area.

Cryogels with higher surface area offer more attachment for cells, promoting cell adhesion and proliferation. This can result in better compatibility and combination with living tissue (Canatar et al., 2023).

4.1.4. Morphology analysis

In biomaterial applications, a porous structure is required for the polymeric material to be compatible with cell growth (Razavi et al., 2019). The macroporous structure of the cryogels were evaluated using SEM and optical microscopy. In this thesis, cryogels used for SEM analyzes were first frozen at -20°C and then dried with a lyophilizer. In Figure 4.3., the internal morphology of each cryogel sample was visualized by SEM. The PVA/Gel and *P. emblica* loaded PVA/Gel cryogels possess similar structures and pore sizes of 30-120 μm and 30-100 μm , respectively. Moreover, SEM results displayed that the synthesized cryogels had an dense, thin and interconnected pore morphology and a macroporous structure. This macroporous structure makes the cryogel appear opaque. The macroporous polymeric structure of the cryogel is suitable to serve as a scaffold for cell adhesion, proliferation, and migration (Shakya et al., 2014; Kathuria et al., 2009; Kao et al., 2019). In addition, the morphological surface properties of the synthesized cryogels were visualized using optical microscope images (Figure 4.4.). Although increasing amounts of four different *P. emblica* concentrations were used to synthesize the cryogels, *P. emblica* did not significantly affect the macroporous morphological structure and surface matrix of the cryogels.

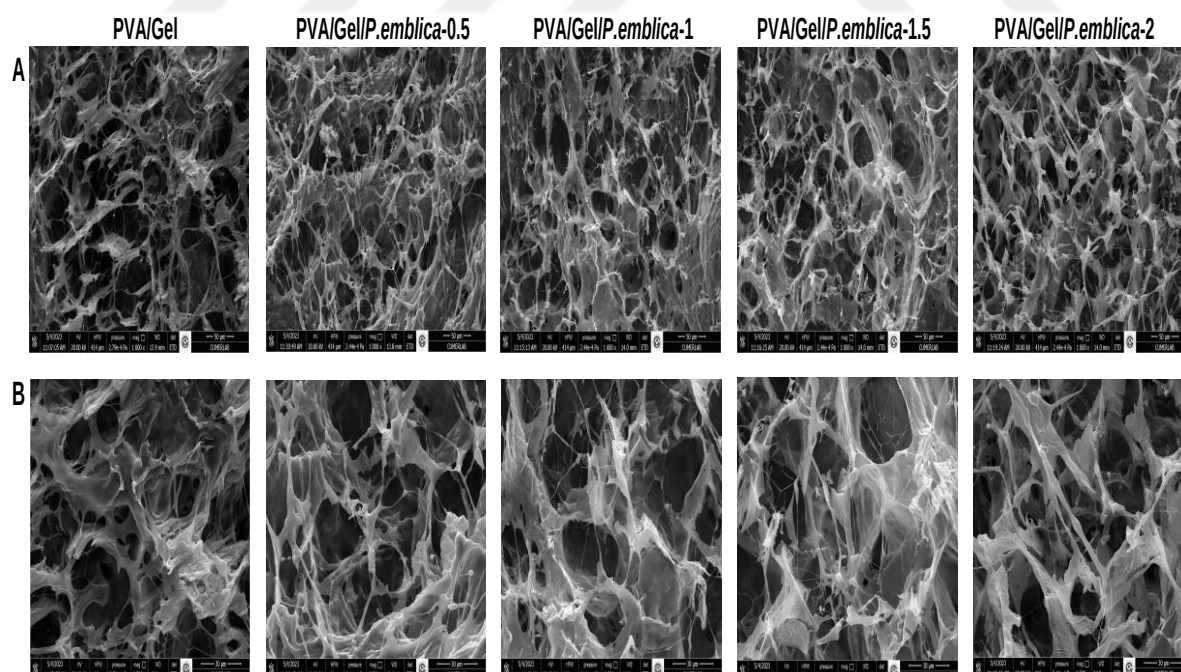


Figure 4.3. The surface and structures of cryogels without cells by scanning electron microscopy (SEM). SEM images taken **(A)** at 1000X magnification, scale bar: 50 μm and **(B)** at magnification 2500X, scale bar: 30 μm .

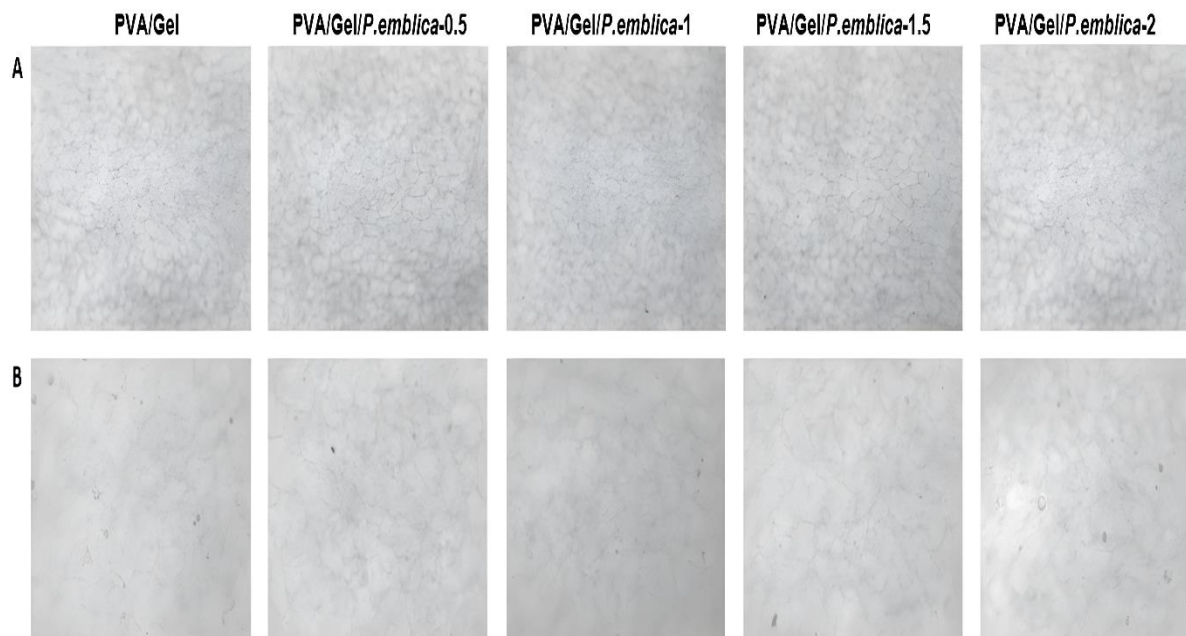


Figure 4.4. The surface morphology of PVA/Gel, PVA/Gel/*P.emblica*-0.5, PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5 and PVA/Gel/*P.emblica*-2 cryogels without cells by optical microscope. Optical microscope images taken **(A)** at 20X magnification, scale bar: 100 μm and **(B)** at magnification 40X, scale bar: 50 μm . PVA/Gel: Poly (vinyl alcohol)/Gelatin cryogels; PVA/Gel/*P.emblica*: *P. emblica* loaded PVA/Gel cryogels; *P. emblica*: *Phyllanthus emblica*.

4.2. In Vitro Assessment of The Effect of Cryogels

4.2.1. Cell culture

In this study, HaCaT cells were used for in vitro cell culture experiments. The inverted phase-contrast microscope images of HaCaT cells was illustrated in Figure 4.5.

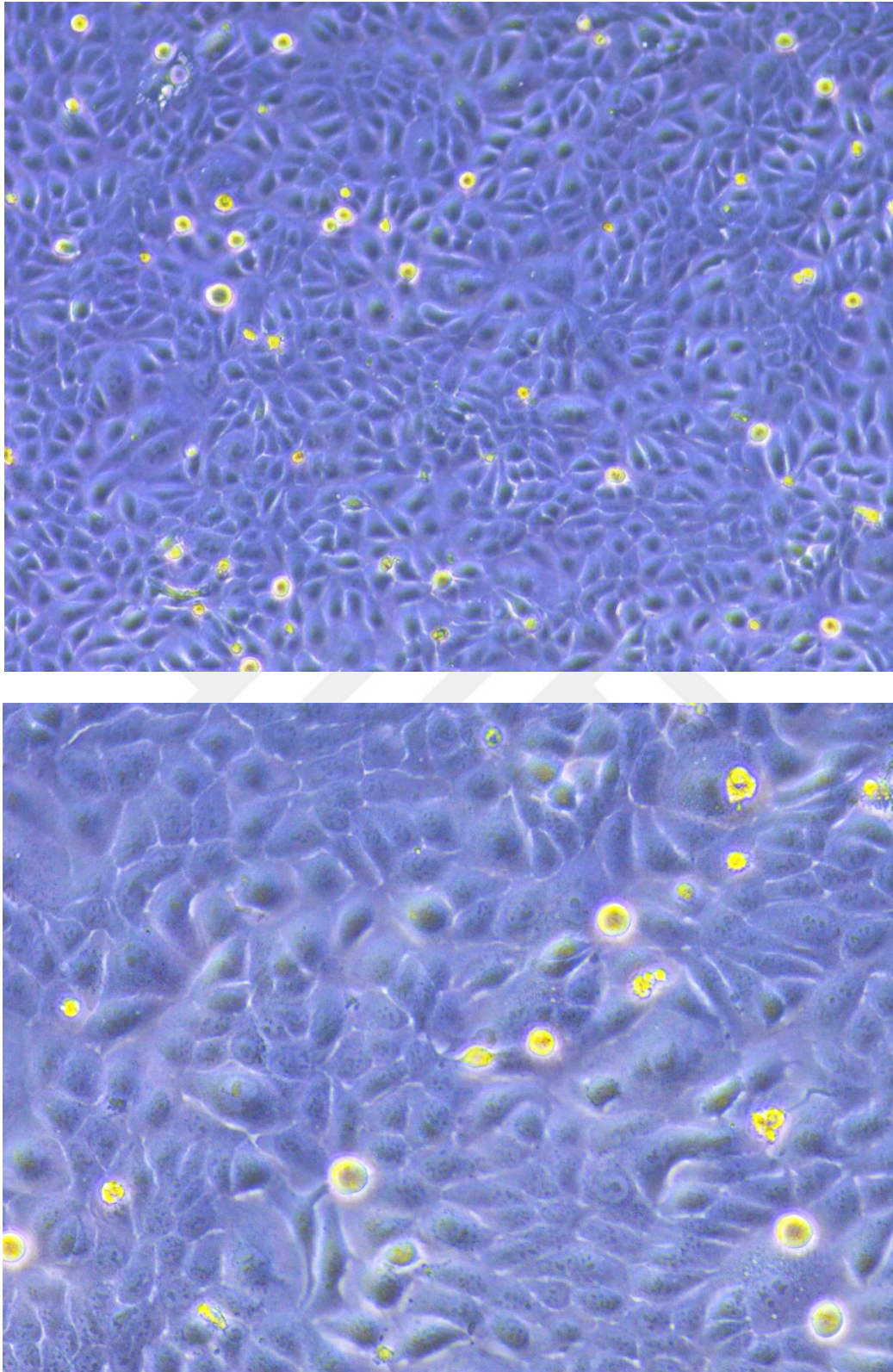


Figure 4.5. Inverted phase-contrast microscope images of HaCaT cells at 10X and 20X magnification.

4.2.2. Assessment of cell viability of PVA/Gel and PVA/Gel/*P.emblica* cryogels by MTT assay, trypan blue exclusion assay and live/dead staining

The cytotoxic impact of PVA/Gel, PVA/Gel/*P.emblica*-0.5, PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5 and PVA/Gel/*P.emblica*-2 cryogels on keratinocytes was investigated by the MTT assay at 24th, 48th and 72nd hour (Figure 4.6.). The cell viability in the control significantly increased by 40.7% at 48th hour compared to 24th hour, but decreased by 4.22% at 72nd hour as compared to the 48th hour ($P < 0.0001$, $P = 0.0663$). Also, the cell viability in PVA/Gel cryogel significantly increased by 29.78% and decreased by 6.99%, respectively ($P < 0.0001$, $P = 0.0006$). In PVA/Gel/*P.emblica*-0.5 cryogel, it increased significantly by 46.98% and 15.53%, respectively. ($P < 0.0001$, $P = 0.0006$). In PVA/Gel/*P.emblica*-1 cryogel it increased significantly by 48.26% and 17.73%, respectively ($P < 0.0001$, $P = 0.0133$). In PVA/Gel/*P.emblica*-1.5 cryogel, it increased significantly as 52.33% and 14.47%, respectively ($P < 0.0001$, $P = 0.0049$). In PVA/Gel/*P.emblica*-2 cryogel, it increased significantly as 34.32% and 6.92%, respectively ($P < 0.0001$, $P = 0.0272$) (Figure 4.6A).

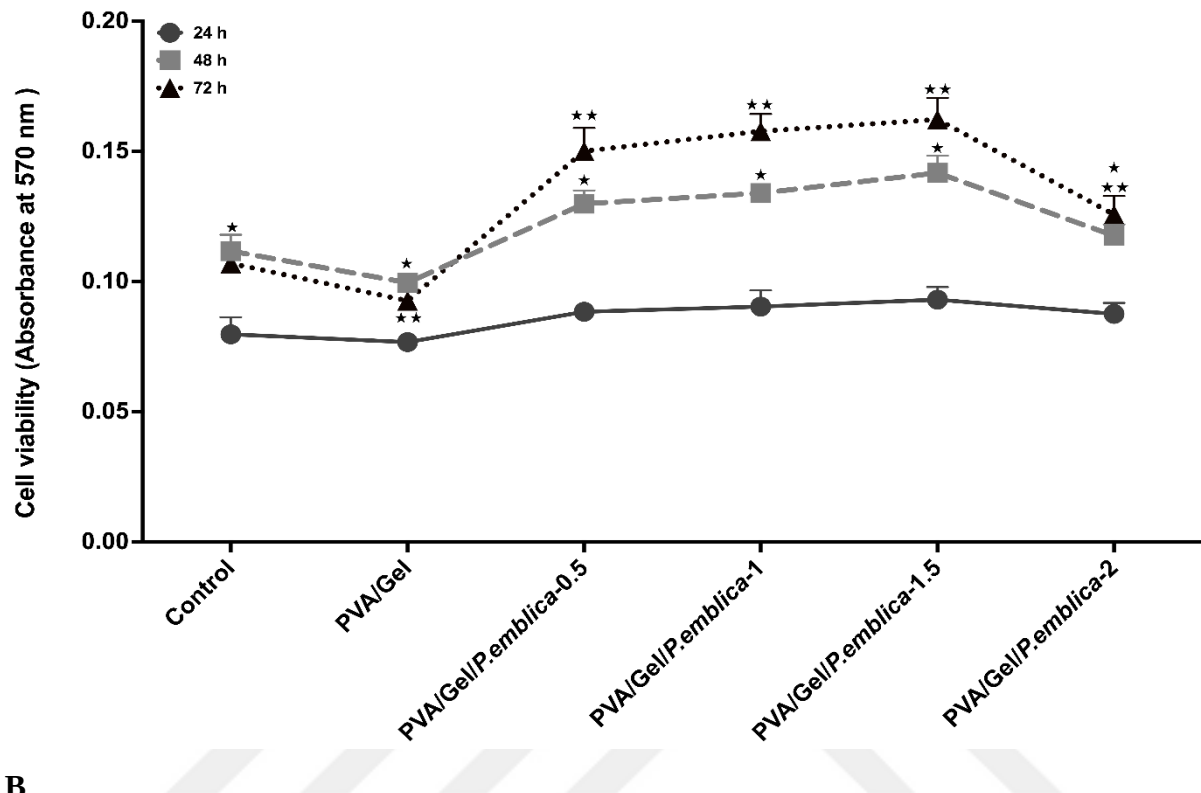
In comparison with the control, cell proliferation was decreased in the PVA/Gel cryogel by $3.77\% \pm 2.05\%$, $10.90\% \pm 0.75\%$, and $13.47\% \pm 1.86\%$ at 24th, 48th and 72nd, respectively ($P = 0.2530$, $P = 0.0049$, $P = 0.0001$, respectively). On the other hand, cell proliferation in PVA/Gel/*P.emblica*-0.5 showed an increase of $10.88\% \pm 2.20\%$, $10.88\% \pm 2.20\%$, and $40.25 \pm 8.36\%$, respectively, compared to control ($P = 0.0380$, $P = 0.0076$, $P < 0.0001$, respectively). The cell proliferation was higher in PVA/Gel/*P.emblica*-1 cryogel by $13.39\% \pm 7.85\%$, $19.93\% \pm 3.10\%$, and $47.42 \pm 6.16\%$, respectively, compared to control ($P = 0.0185$, $P = 0.0008$, $P < 0.0001$, respectively). The cell proliferation was increased in PVA/Gel/*P.emblica*-1.5 cryogel by $16.74\% \pm 6.10\%$, $26.87\% \pm 5.96\%$ and $51.63 \pm 7.74\%$, respectively, compared to control ($P = 0.0171$, $P = 0.0013$, $P < 0.0001$, respectively). The cell proliferation was significantly increased in PVA/Gel/*P.emblica*-2 cryogel by $9.83\% \pm 5.31\%$, $5.25\% \pm 3.89\%$ and $17.50 \pm 6.68\%$, respectively, compared to control ($P = 0.0574$, $P = 0.2082$, $P = 0.0039$, respectively).

According to MTT assay, cell proliferation in PVA/Gel/*P.emblica*-0.5 cryogel was 15.22%, 24.44% and 62.09% higher at 24, 48 and 72 hours, respectively, compared to PVA/Gel ($P = 0.0003$, $P < 0.0001$, $P < 0.0001$). Moreover, the cell proliferation was increased in the PVA/Gel/*P.emblica*-1 cryogel compared to PVA/Gel by 17.83%, 34.60% and 70.37% ($P = 0.0014$, $P < 0.0001$, $P < 0.0001$). The cell proliferation was significantly increased in the

PVA/Gel/*P.emblica*-1.5 cryogel compared to PVA/Gel by 21.30%, 42.38% and 75.24% ($P=0.0005$, $P<0.0001$, $P<0.0001$). Also, the cell proliferation was 14.13%, 18.12% and 35.79% higher in the PVA/Gel/*P.emblica*-2 cryogel compared to PVA/Gel by ($P=0.0011$, $P=0.0002$, $P=0.0002$, respectively).

According to MTT assay, the cell proliferation in the PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5 and PVA/Gel/*P.emblica*-2 cryogels showed increases of 2.26%, 5.28% and -0.94% at 24 hour, respectively, compared to PVA/Gel/*P.emblica*-0.5 ($P=0.5198$, $P=0.0958$, $P=0.07148$). In addition, the cell proliferation significantly increased at 48 h and 72 h by 8.17%, 14.42%, -5.07% and 5.11%, 8.11%, -16.22% ($P=0.0016$, $P=0.0008$, $P=0.0187$ for 48th hour and $P=0.0252$, $P=0.0066$, $P=0.00297$ for 72nd h). Moreover, the cell proliferation was increased in the PVA/Gel/*P.emblica*-1.5 and *P.emblica*-2 cryogel compared to PVA/Gel/*P.emblica*-1 cryogel by 2.95% and -3.14% respectively at 24th hour ($P=0.5232$, $P=0.3486$). Also, the cell proliferation significantly increased at 48th and 72nd hours by 5.78%, -12.24% and 2.85%, -20.30% ($P=0.0479$, $P=0.0001$ for 48th hour and $P=0.0379$, $P=0.0010$ for 72nd hour, respectively). Also, the cell proliferation was decreased in the PVA/Gel/*P.emblica*-2 cryogel compared to PVA/Gel/*P.emblica*-1.5 by -5.91%, 17.04%, 22.51% respectively at 24, 48, 72 hours ($P=0.967$, $P=0.0005$, $P=0.0014$, respectively) (Figure 4.6B).

A



B

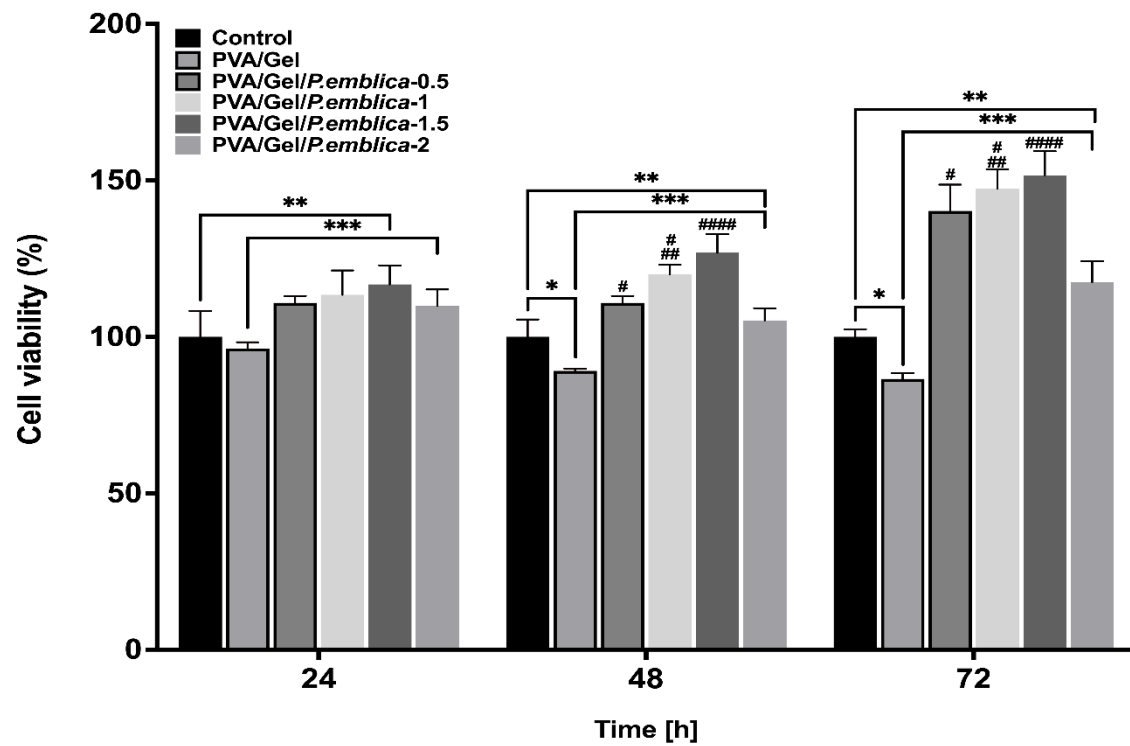


Figure 4.6. Determination of cytotoxic effect of PVA/Gel and PVA/Gel/*P.emblica* cryogels by MTT assay. The viability of HaCaT cell line on cryogels was assessed by MTT assay at 24, 48, and 72 hour. Cell cultured in polystyrene-culture plate was used as a control. **(A)** Quantitative evaluation of cell viability. The amount of MTT-formazan was determined by 570 nm absorbance as the wavelength. **(B)** 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, relative to the control cells. Three measurements were conducted from each well, and the data was reported as the mean $\pm$ standard deviation (SD). Tukey test and One-ANOVA were conducted for statistical analysis. * $P < 0.05$ is significantly different from 24 h. ** $P < 0.05$ is significantly different from 48 h. * $P < 0.05$ and ** $P < 0.05$ are significantly different from control. *** $P < 0.05$ is significantly different from PVA/Gel. # $P < 0.05$ is significantly different from PVA/Gel/*P.emblica*-2. ## $P < 0.05$ is significantly different from PVA/Gel/*P.emblica*-0.5. ### $P < 0.05$ is significantly different from other PVA/Gel/*P.emblica* cryogels. PVA/Gel: Poly (vinyl alcohol)/Gelatin cryogels; PVA/Gel/*P.emblica*: *P. emblica* loaded PVA/Gel cryogels; *P. emblica*: *Phyllanthus emblica*; MTT: (3-(4,5-dimethylthiazol-2) 2,5-diphenyltetrazolium bromide; SD: Standard error.

Trypan blue assay was performed to investigate the efficacy of PVA/Gel, PVA/Gel/*P.emblica*-0.5, PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5 and PVA/Gel/*P.emblica*-2 cryogels on keratinocytes viability at 24th, 48th and 72nd h (Figure 4.7.). In comparison with control, the cell number of PVA/Gel cryogel was declined by $7.23\% \pm 8.45\%$, $7.84\% \pm 3.81\%$, and $10.94\% \pm 3.47\%$, respectively ($P = 0.0913$, $P = 0.0471$, $P = 0.0641$ respectively). Moreover, the cell number in the PVA/Gel/*P.emblica*-0.5 cryogel was significantly increased by $11.45\% \pm 7.69\%$, $20.41\% \pm 2.3\%$, and $39.29 \pm 4.72\%$, respectively, compared to control ($P = 0.0177$, $P < 0.0001$, $P = 0.0003$, respectively). Also, the cell number in the PVA/Gel/*P.emblica*-1 cryogel was increased by $31.93\% \pm 10.36\%$, $43.71\% \pm 3.94\%$, and $40.85 \pm 2.31\%$, respectively, compared to control ($P = 0.0002$, $P < 0.0001$, $P = 0.0001$, respectively). The cell number in the PVA/Gel/*P.emblica*-1.5 cryogel was significantly increased by $48.19\% \pm 4.88\%$, $56.08\% \pm 4.10\%$ and $67.63 \pm 3.83\%$, respectively, compared to control ($P < 0.0001$ for all). The cell number in the PVA/Gel/*P.emblica*-2 cryogel was significantly increased by $1.41\% \pm 2.69\%$, $8.87\% \pm 2.07\%$ and $10.71 \pm 4.99\%$, respectively, compared to control ($P = 0.0585$, $P = 0.0013$, $P = 0.0393$, respectively).

According to trypan blue assay, the number of cells in the PVA/Gel/*P.emblica*-0.5 cryogel was increased by 20.13%, 30.65% and 56.39% at 24, 48 and 72 hours compared to PVA/Gel, respectively ($P = 0.0061$, $P < 0.0001$, $P < 0.0001$). The number of cells was 42.21%, 55.93% and 58.15% higher in PVA/Gel/*P.emblica*-1 cryogel compared to PVA/Gel ($P = 0.0004$, $P < 0.0001$, $P < 0.0001$). The cell number was significantly increased in the PVA/Gel/*P.emblica*-1.5 cryogel compared to PVA/Gel by 59.74%, 69.35% and 88.22% ($P < 0.0001$, for all). In addition, the number of cells was 9.31%, 18.12% and 24.31% higher in the PVA/Gel/*P.emblica*-2 cryogel compared to PVA/Gel ($P = 0.0766$, $P = 0.0007$, $P = 0.0005$, respectively).

According to trypan blue assay, the viable cell number was significantly increased in the other PVA/Gel/*P.emblica*-1, PVA/Gel/ *P.emblica*-1.5 and PVA/Gel/ *P.emblica*-2 cryogels compared to PVA/Gel/*P.emblica*-0.5 by 18.38%, 32.97% and -9.01%, respectively at 24 h ($P = 0.0139$, $P = 0.0002$, $P = 0.0379$). In addition, the cell number significantly increased at 48 and 72 hours by 19.35%, 29.62%, -9.59% and 1.12%, 20.35%, -20.51% ($P < 0.0001$, $P < 0.0001$, $P = 0.0006$ for 48 h and $P = 0.4492$, $P = 0.0003$, $P = 0.0008$ for 72 h). Furthermore, the cell number in the PVA/Gel/*P.emblica*-1.5 and PVA/Gel/*P.emblica*-2 cryogel showed significant increases of 12.33% and -23.14% compared to PVA/Gel/*P.emblica*-1 cryogels at 24 hour, respectively ($P = 0.0047$, $P = 0.0004$). Also, the cell number significantly increased at 48 and 72 hours by 8.61%, -24.25% and 19.02%, -21.39% ($P = 0.0024$, $P < 0.0001$ for 48 h and $P < 0.0001$, $P < 0.0001$ for 72 h). Also, the cell number was significantly decreased in the PVA/Gel/*P.emblica*-2 cryogel compared to PVA/Gel/*P.emblica*-1.5 by 31.57%, 30.25%, 33.95% at 24, 48, 72 hours, respectively ($P < 0.0001$ for all).

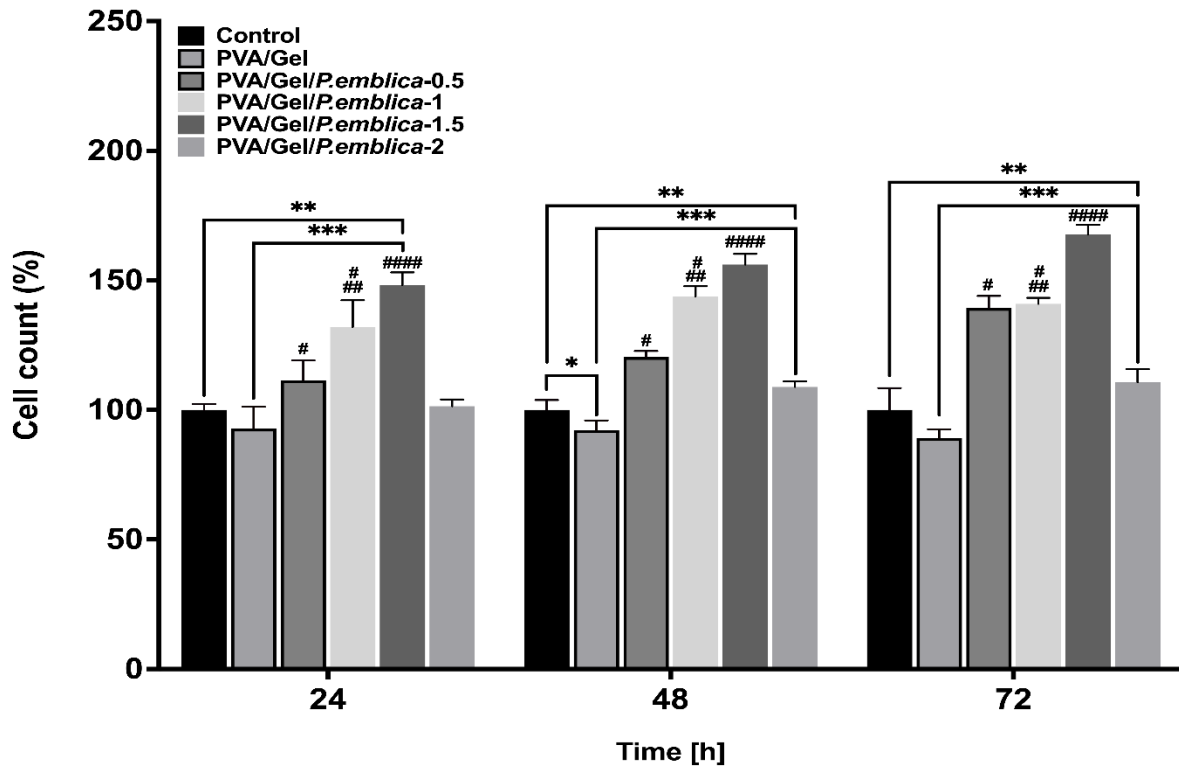


Figure 4.7. The efficacy of PVA/Gel and PVA/Gel/*P.emblica* cryogels on number of viable cells. The viability of HaCaT cell line on cryogels was assessed by trypan blue exclusion assay at 24, 48 and 72 hours. Cell cultured in polystyrene-culture plate was utilized as a control. Three measurements were conducted from each well, and the data was reported as the mean $\pm$ standard deviation (SD). Statistical analysis was performed using the Tukey and One-way ANOVA tests. * $P < 0.05$ and ** $P < 0.05$ are significantly different from control. *** $P < 0.05$ is significantly different from PVA/Gel. # $P < 0.05$ is significantly different from PVA/Gel/*P.emblica*-2. ## $P < 0.05$ is significantly different from PVA/Gel/*P.emblica*-0.5. ### $P < 0.05$ is significantly different from other PVA/Gel/*P.emblica* cryogels. PVA/Gel: Poly (vinyl alcohol)/Gelatin cryogels; PVA/Gel/*P.emblica*: PVA/Gel cryogels based loaded with *P. emblica*; *P. emblica*: *Phyllanthus emblica*; SD: Standard error.

A live/dead assay was performed to evaluate the cell viability of cells, where green fluorescence indicated viable cells and red fluorescence indicated dead cells. (Figure 4.8.). Cells were seeded on PVA/Gel, PVA/Gel/*P.emblica*-0.5, PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5 and PVA/Gel/*P.emblica*-2 cryogels for 24th, 48th and 72nd h. As seen in Figure 4.8A, 4.8B, 4.8C relatively similar numbers were determined in live and dead cells cultured in control and PVA/Gel/*P.emblica*-0.5, PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5 and

PVA/Gel/*P.emblica*-2 cryogels for all hours. The cells cultured on the PVA/Gel *P.emblica*-0.5, PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5 and PVA/Gel/*P.emblica*-2 cryogels exhibited a higher percentage of green fluorescence, which indicates viable cells compared to the PVA/Gel. The majority of cells cultured on the PVA/Gel/*P.emblica*-0.5, PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5 and PVA/Gel/*P.emblica*-2 cryogels emitted green fluorescence, which indicates a high level of cell viability and cell proliferation. As shown in Figures 5A, 5B and 5C, the addition of *P. emblica* extract to the PVA/Gel cryogels did not adversely affect cell viability relative to the live/dead assay. The percentage of viable (green) cells was higher in cultured in cryogels loaded with *P. emblica*. Unlike controls, cells cultivated especially in PVA/Gel/*P.emblica*-0.5, PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5 cryogels showed viable (green) cells with more rounded contours. In addition, compared to PVA/Gel cryogels, PVA/Gel/*P.emblica*-0.5, PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5 and PVA/Gel/*P.emblica*-2 cryogels have more viable cells.

According to live/dead assay, viable cells in the PVA/Gel cryogel decreased by $11.81\% \pm 1.38\%$, $12.86\% \pm 1.72\%$, and $25.77\% \pm 2.16\%$ at 24, 48, and 72 hours compared to control, respectively ($P= 0.0288$, $P= 0.0145$, $P= 0.0008$). The viable cell ratio in PVA/Gel/*P.emblica*-0.5 was significantly increased by $10.48\% \pm 3.97\%$, $14.86\% \pm 2.74\%$, $34.05\% \pm 1.37\%$ respectively cryogel compared to control ($P= 0.0004$, $P= 0.0008$, $P< 0.0001$). Also, the viable cell ratio in PVA/Gel/*P.emblica*-1 cryogel was significantly increased by $10.67\% \pm 1.03\%$, $17.07\% \pm 1.86\%$ and $52.76\% \pm 0.75\%$ respectively cryogel compared to control ($P= 0.0002$, $P= 0.0034$, $P= 0.0001$). Also, the viable cell ratio in PVA/Gel/*P.emblica*-1.5 cryogel was significantly increased by $9.33\% \pm 1.17\%$, $21.95\% \pm 1.79\%$, $66.87\% \pm 2.61\%$ 10.06 respectively cryogel compared to control ($P= 0.0060$, $P= 0.0004$, $P< 0.0001$). Also, the viable cell ratio in PVA/Gel/*P.emblica*-2 cryogel was significantly enhanced by $1.52\% \pm 1.37\%$, $5.99\% \pm 1.03\%$, $15.03\% \pm 1.63\%$, respectively cryogel compared to control ($P> 0.9999$, 0.0342 , $P> 0.9999$). Moreover, the viable cells in the PVA/Gel/*P.emblica*-0.5 cryogel increased significantly by 25.27%, 31.38% and 80.58%, respectively compared to PVA/Gel ($P= 0.0042$, $P= 0.0017$, $P< 0.0001$). Also, the viable cells in the PVA/Gel/*P.emblica*-1 cryogel increased by 25.49%, 34.35% and 105.79%, respectively compared to PVA/Gel ($P= 0.0026$, $P= 0.0005$, $P< 0.0001$). The viable cells in the PVA/Gel/*P.emblica*-1.5 cryogel increased by 23.97%, 39.95% and 129.79%, respectively compared to PVA/Gel ($P= 0.0069$, $P= 0.0003$, $P< 0.0001$). The

viable cells in the PVA/Gel/*P.emblica*-2 cryogel increased significantly by 15.12%, 21.63% and 54.96%, respectively compared with PVA/Gel ($P= 0.0361$, $P= 0.0036$, $P= 0.0002$).

According to live/dead assay, dead cells in the PVA/Gel cryogel increased by $82.67\% \pm 1.38\%$, $38.93\% \pm 1.72\%$, and $30.66\% \pm 2.16\%$ at 24, 48, and 72 hours, respectively, compared to control ($P= 0.0288$, $P= 0.0145$, $P= 0.0008$). Furthermore, the dead cell ratio in PVA/Gel/*P.emblica*-0.5 was decreased significantly by $73.33\% \pm 3.97\%$, $44.97\% \pm 2.74\%$, and $40.51\% \pm 1.37\%$, respectively cryogel compared to control ($P= 0.0004$, $P= 0.0008$, $P< 0.0001$). Also, the dead cell ratio in PVA/Gel/*P.emblica*-1 was significantly decreased by $74.67\% \pm 1.17\%$, $51.68\% \pm 1.86\%$, and $62.77\% \pm 0.75\%$, respectively compared to control ($P= 0.0002$, $P= 0.0034$, $P= 0.0001$). Moreover, the dead cell ratio in PVA/Gel/*P.emblica*-1.5 was significantly decreased by $65.33\% \pm 1.17\%$, $66.44\% \pm 1.79\%$, and $79.56\% \pm 2.61\%$, respectively cryogel compared to control ($P= 0.0060$, $P= 0.0004$, $P< 0.0001$). The dead cell ratio in PVA/Gel/*P.emblica*-2 was decreased by $10.67\% \pm 1.37\%$, $18.12\% \pm 1.03\%$, and $17.88\% \pm 1.63\%$, respectively cryogel compared to control ($P> 0.9999$, $P= 0.0342$, $P> 0.9999$). The dead cells in the PVA/Gel/*P.emblica*-0.5 cryogel decreased significantly by 85.40%, 60.39% and 54.47%, respectively compared to PVA/Gel ($P= 0.0042$, $P= 0.0017$, $P< 0.0001$). Also, the dead cells in the PVA/Gel/*P.emblica*-1 cryogel declined by 86.13%, 65.22% and 71.51%, respectively compared to PVA/Gel ($P= 0.0026$, $P= 0.0005$, $P< 0.0001$). Moreover, the dead cells in the PVA/Gel/*P.emblica*-1.5 cryogel decreased significantly by 81.02%, 75.85% and 84.36%, respectively compared to PVA/Gel ($P= 0.0069$, $P= 0.0003$, $P< 0.0001$). The dead cells in the PVA/Gel/*P.emblica*-2 cryogel declined significantly by 51.09%, 41.06% and 37.15%, respectively compared with PVA/Gel ($P= 0.0361$, $P= 0.0036$, $P= 0.0002$) (Figure 4.8D, 4.8E, 4.8F).

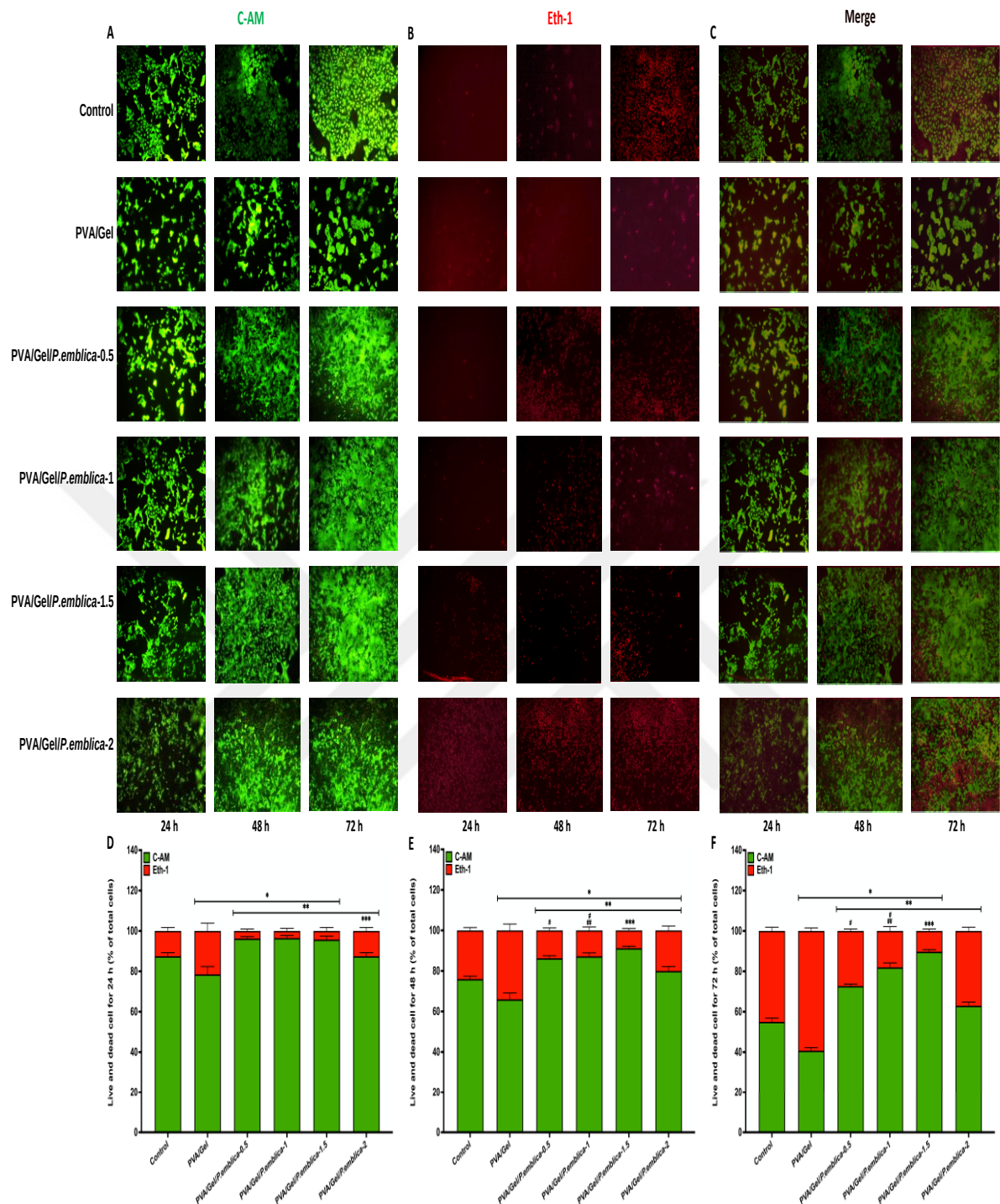


Figure 4.8. Determination of cell viability of PVA/Gel and PVA/Gel/*P.emblica* cryogels by live/dead staining. Live-dead assay was performed at 24, 48 and 72 hours. Cell cultured in polystyrene-culture plate was utilized as a control. Fluorescent microscope images showed cells on PVA/Gel, PVA/Gel/*P.emblica*-0.5, PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5 and PVA/Gel/*P.emblica*-2 cryogels stained with **(A)** C-AM (green fluorescent) and **(B)** Eth-1 (red

fluorescent) and (C) both C-AM and Eth-1 to obtain merged images (Scale bar: 100 μ m). The quantitative data for live and dead cells was obtained using the live/dead assay at (D) 24 h, (E) 48 h, (F) 72 h. Cryogels were visualized and analyzed using a microscope and ImageJ software. The data are expressed as the total percentage of live and dead cells, accompanied by the mean $\pm$ standard deviation (SD). Statistical analysis was conducted using the Tukey test and One-way ANOVA. * $P < 0.05$ is significantly different from control. ** $P < 0.05$ is significantly different from PVA/Gel. *** $P < 0.05$ is significantly different from other PVA/Gel/*P. emblica* cryogels. # $P < 0.05$ is significantly different from PVA/Gel/*P. emblica*-2. ## $P < 0.05$ is significantly different from PVA/Gel/*P. emblica*-0.5. C-AM: Calcein Acetomethoxy; Eth-1: Ethidium homodimer-1; PVA/Gel: Poly (vinyl alcohol)/Gelatin cryogels; PVA/Gel/*P. emblica*: *P. emblica* loaded PVA/Gel cryogels; *P. emblica*: *Phyllanthus emblica*; SD: Standard error.

Cell scaffolds must be mechanically stable, biocompatible, biodegradable, but also porous with good interconnectivity for cell viability, proliferation, migration and other metabolic needs (Shakya et al., 2014). PVA is a synthetic polymer widely used to produce macroporous, spongy matrices in tissue engineering applications due to its biocompatibility, non-carcinogenic and non-toxic properties (Muppalaneni and Omidian, 2013). Gel is frequently preferred in biomaterial synthesis as an attractive natural polymer with its chemical structure, biocompatibility, and biodegradability properties that increase cell adhesion (Farris et al., 2010; He et al., 2021). However, studies have revealed that biocompatible PVA and Gel-based scaffolds maintain the functionality of cells metabolically thanks to their strong, elastic and porous 3D structure, at the same time they support cell viability, adhesion, proliferation, mobility and allow the absorption of the necessary substrate for cell nutrition. For these reasons, it has been reported to be a promising platform in tissue engineering (He et al., 2021; Charron et al., 2020; Cheaburu-Yilmaz et al., 2019; Canatar et al., 2023). Pterostilbene-loaded PVA/Gel cryogels have been reported as a potential dressing material in wound therapy, promoting cell viability and proliferation (Canatar et al., 2023). However, it has been reported that PVA/Gel cryogel promotes the viability and proliferation of endothelial cells and is particularly suitable for vascular tissue engineering applications (Vrana et al., 2010). In addition, it was determined that there was no cytotoxic effect for human skin cells cultured in PVA/Gelatin cryogel and the scaffold exhibited good biocompatibility (Choi et al., 2013). The antioxidant, antidiabetic, anti-inflammatory, antiaging, wound healing, cryoprotective and hepatoprotective activities of *P. emblica* extracts used in traditional medicine were studied (Bhandari and Kamdod, 2012;

Kulkarni and Ghurghure, 2018; Khan et al., 2019). In in vitro studies, it has been reported that *P. emblica* extract promotes wound healing by supporting endothelial cells, fibroblast, and keratinocyte vitality, proliferation, migration, and angiogenesis through its high bioactive component content (Chularojmontri et al., 2013; Talekar et al., 2017; Ahmad et al., 2021). In addition, it has been shown in an in vivo animal model that *P. emblica* treatment increases tissue regeneration by reducing oxidative stress in the wound area, inducing collagen expression and angiogenesis (Klimis-Zacas and Rodriguez-Mateos, 2022; Liao et al., 2023). In a different study, the potential of using the spray solution prepared with polyvinylpyrrolidone solution containing silver nanoparticles and *P. emblica* extract as an antibacterial, antioxidant and biocompatible wound dressing for fibroblasts and keratinocytes was demonstrated (Suvandee et al., 2022). In addition, green tea, ginger, and *P. emblica* extracts loaded hydrogel have been reported to treat acne and accelerate wound healing in humans (Lin et al., 2021). However, to the best of our knowledge, the effect of *P. emblica* binding to cryogel on cytotoxicity and its potential in tissue regeneration as a wound dressing are not yet known. Evaluation of cell viability and proliferation in the microenvironment as an indicator of biocompatibility in synthesized scaffold structures is very important in tissue engineering applications (Lin et al., 2021; Abd El-Aziz et al., 2021). Therefore, in our study, for the first time in the literature, cell viability, and proliferation were evaluated after different incubation times with MTT and trypan blue exclusion assay in cells cultured in PVA/Gel cryogels to which different concentrations of *P. emblica* were added. In addition, live/dead assay was applied to visualize live and dead cells in culture medium to understand the effect of scaffolds on cell behavior (Abd El-Aziz et al., 2021; Canatar et al., 2023).

In our study, the MTT test showed that cells were viable in PVA/Gel and PVA/Gel/*P.emblica* cryogels. Furthermore, a highly increased cellular metabolic activity was observed over time in cryogels with different concentrations of *P.emblica*. However, in the control, although the cell viability increased until the 2nd day of the cell culture, the cell viability decreased. This may be due to the rapid confluence of cells in the flat culture system compared to the 3D culture system, as the doubling time of the HaCaT cell is about 26.4-48 h (Pavez Lorie et al., 2020; Pessina et al., 2001). Cells in monolayer culture with limited substrate area proliferate rapidly until they are confluent, but when the limit is reached, cell viability decreases due to contact inhibition (Stoker and Rubin, 1967). In agreement with the characterization study data, the 3D macroporous structure, high swelling rate, pore sizes, the large surface area of the

PVA/Gel/*P.emblica* cryogels were sufficient to facilitate the diffusion of macromolecules necessary for the metabolic activity of the cells and the removal of wastes. In this way, unlike the 2D culture medium, cell viability and proliferation continuously increased in PVA/Gel/*P.emblica* cryogels until the end of the 3rd day of culture, so that the cells filled the pores of the matrix (Guo et al., 2021). Also, the increase in cell viability and proliferation was very limited in PVA/Gel cryogels compared to containing *P. emblica* cryogels. It has been reported in the literature that high antioxidant activity originating from bioactive secondary compounds reduces intracellular oxidative stress and increases cell viability (Lemos et al., 2017; Talekar et al., 2017; Chularojmontri et al., 2013). It is known that *P. emblica* has strong antioxidant activity mainly due to the presence of phenolics, flavonoids and various gallic acid derivatives (Khan et al., 2019; Liu et al., 2008; Ahmad et al., 2021). This high bioactive capacity of *P. emblica* may have promoted the viability and proliferation of cells cultured in cryogels. Similarly, in agreement with the MTT assay data, the trypan blue exclusion test confirmed that PVA/Gel/*P. emblica* cryogels provide a biosafe environment that promotes cell viability and increase in number. More importantly, the live/dead assay results confirmed other results from direct cytotoxicity studies by providing visible evidence that the *P. emblica* extract, together with the polymers used in the manufacture of cryogels, promotes cell viability by a functionally significant difference. These results demonstrate the high potential of the macroporous scaffold synthesized with natural *P. emblica* extract as a suitable dressing material with its good biocompatibility.

4.2.3. Investigation of cell morphology, adhesion, viability, cell-cell and cell-matrix interactions on PVA/Gel and PVA/Gel/*P.emblica* cryogels via phase contrast microscopy, giemsa staining, immunofluorescence staining and SEM

Cells secrete ECM proteins in response to chemical-mechanical signals through their receptors and exhibit a characteristic morphology (Boudreau and Jones, 1999). Cell-cell and cell-cryogel interaction can be investigated by using microscopic imaging of features such as focal adhesion of the cell, cytoskeleton with fluorophores, and nuclear distribution. (Entcheva et al., 2004). Microscopic examination, giemsa, F-Actin/DAPI and Calcein-AM/Eth-1 staining was performed to assess the proliferation and morphology of cells cultured on control, PVA/Gel, PVA/Gel/*P.emblica*-0.5, PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5 and PVA/Gel/*P.emblica*-2 cryogels at 72nd h (Ma et al., 2022; Canatar et al., 2023) (Figure 4.9.).

Phase contrast microscopy and giemsa staining showed that the cells adhered to the surface of the cryogels and penetrated the pores. Moreover, especially in the PVA/Gel/*P.emblica* cryogels, cells proliferated extensively, forming large-sized contiguous colonies/spheroids. In addition, cells in PVA/Gel/*P.emblica* cryogels were dose-dependently affected by *P. emblica* in terms of proliferation and distribution. In contrast, cells in PVA/Gel grew in scattered colonies and in fewer numbers. Unlike control cells, which grew in a monolayer in polystyrene, adherence and infiltration of cells into the cryogel were observed and their 3D structure was clear (Figure 4.9A, 4.9B). Cells show different structural conformations from typical images of cells adhering to 2D surfaces when in contact with 3D scaffolds. (Eyckmans and Chen, 2017). Cells in PVA/Gel and PVA/Gel/*P.emblica* cryogels retained a tightly arranged cubic/spherical morphology. F-Actin/DAPI staining showed peripheral accumulation of strongly organized F-actin following cell contours (Figure 4.9C) (Boukamp et al., 1988). In this thesis, it was observed that loading *P. emblica* on cryogel effectively enhanced cell number and adhesion in spherical morphology. However, morphological studies should be confirmed molecularly and biochemically for known morphological differentiation markers such as involucrin, cytokeratin, keratin and vimentin (Borowiec et al., 2013; Breitkreutz et al., 1993). According to C-AM/Eth-1 staining, the compounds used in the synthesis of PVA/Gel and PVA/Gel/*P.emblica* cryogels did not affect cell viability. It was observed as live (green) and dead (red) cells with cubic boundaries inside the scaffold (Figure 4.9D). Our microscopic imaging data confirmed the cell viability and proliferation data, demonstrating good compatibility of PVA/Gel/*P.emblica* cryogels to keep a high number of cells viable in healthy morphology. Our data showed that PVA/Gel/*P.emblica* cryogels are suitable for use in skin tissue engineering applications and can be developed with advanced studies.

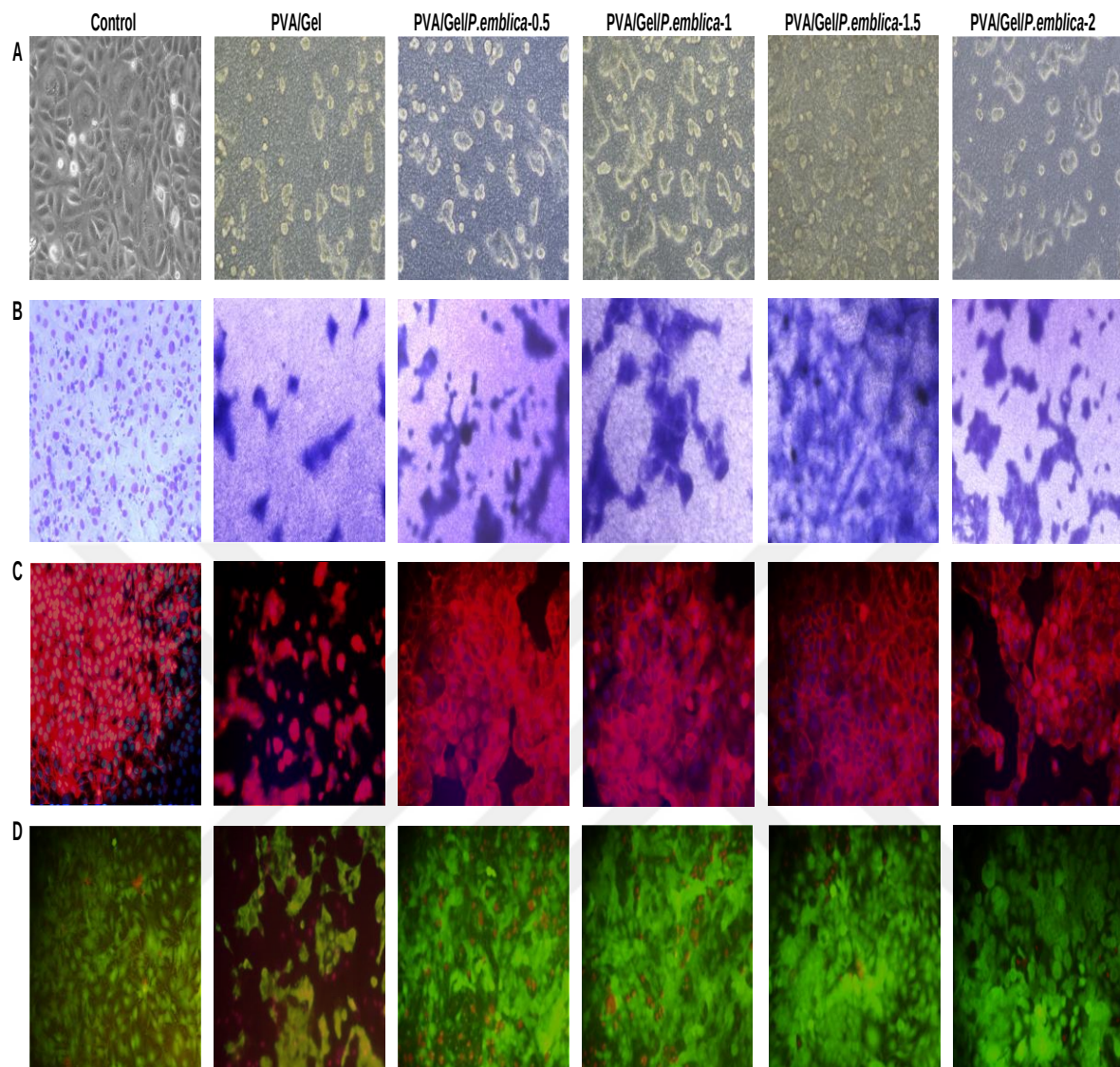


Figure 4.9. The effect of PVA/Gel and PVA/Gel/*P.emblica* cryogels on cells morphology, adhesion, viability cell-cell and cell-matrix interactions. Cell morphology was analysed at 72 hour. The cells cultivated in polystyrene-culture plate were utilized as a control. Representative **(A)** phase contrast microscopy, **(B)** giemsa staining (pink-purple), **(C)** immunofluorescence of F-Actin (red)/ DAPI (blue) staining merge and **(D)** C-AM (green)/Eth-1 (red) staining images of seeded cells on control, PVA/Gel, PVA/Gel/*P.emblica*-0.5, PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5 and PVA/Gel/*P.emblica*-2 cryogels (all magnifications X20, scale bar: 100 μ m). DAPI: 4',6-diamidino-2-phenylindole; C-AM: Calcein Acetomethoxy; Eth-1: Ethidium homodimer-1; PVA/Gel: Poly (vinyl alcohol)/Gelatin cryogels; PVA/Gel/*P.emblica*: *P. emblica* loaded PVA/Gel cryogels; *P. emblica*: *Phyllanthus emblica*.

In a skin injury, migration and proliferation of keratinocytes are triggered first due to the inflammatory response, then they interact with the ECM and their adhesion changes (Stojadinovic et al., 2005). Activation of keratinocyte migration is closely related to intercellular transmission and signal transduction regulated by Connexins, (Cx) proteins, which form an intercellular space junction (Langlois et al., 2007). In particular, gap junction protein Cx43 is involved in inducing signaling pathways including growth factors and ECM deposition that are key in wound closure (Faniku et al., 2018). Cx43 distribution in cells cultured on control, PVA/Gel, PVA/Gel/*P.emblica*-0.5, PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5 and PVA/Gel/*P.emblica*-2 cryogels was visualized under the microscope at 72nd h by immunofluorescence staining (Figure 4.10.). Cx43 emits intense and bright fluorescent light in direct proportion to the increase in cell-cell interaction (Entcheva et al., 2004). An overall increase in gap junction density was observed for cells cultured on PVA/Gel/*P.emblica* cryogels compared to control and PVA/Gel (Figure 4.10A, 4.10B, 4.10C). According to the quantitative analysis of immunofluorescent staining, Cx43 antibody fluorescence intensity in PVA/Gel/*P.emblica*-0.5, PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5 and PVA/Gel/*P.emblica*-2 cryogels was increased by 38.41% $\pm$ 8.02%, 131.03% $\pm$ 7.80%, 186.87% $\pm$ 10.62%, 311.93% $\pm$ 14.42% and 63.21% $\pm$ 8.35%, respectively, compared to control ($P < 0.0001$ for all). Moreover, gap junction density increased by 275.12%, 365.77%, 568.84% and 165.51% in PVA/Gel/*P.emblica*-0.5, PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5 and PVA/Gel/*P.emblica*-2 compared to PVA/Gel cryogel ($P < 0.0001$ for all). In addition, the fluorescence intensity of Cx43 increased by 56.09%, 69.64% and 39.70% in PVA/Gel/*P.emblica*-1.5 compared to other PVA/Gel/*P.emblica* cryogels, respectively (Figure 4.10D) ($P < 0.0001$ for all). This data generally indicates enhanced cell-to-cell interaction in cryogels loaded with *P. emblica* ($P = 0.0007$ for *P.emblica*-0.5/*P.emblica*-1; $P < 0.0001$ for *P.emblica*-0.5/*P.emblica*-2 and $P < 0.0001$ for *P.emblica*-1/*P.emblica*-2).

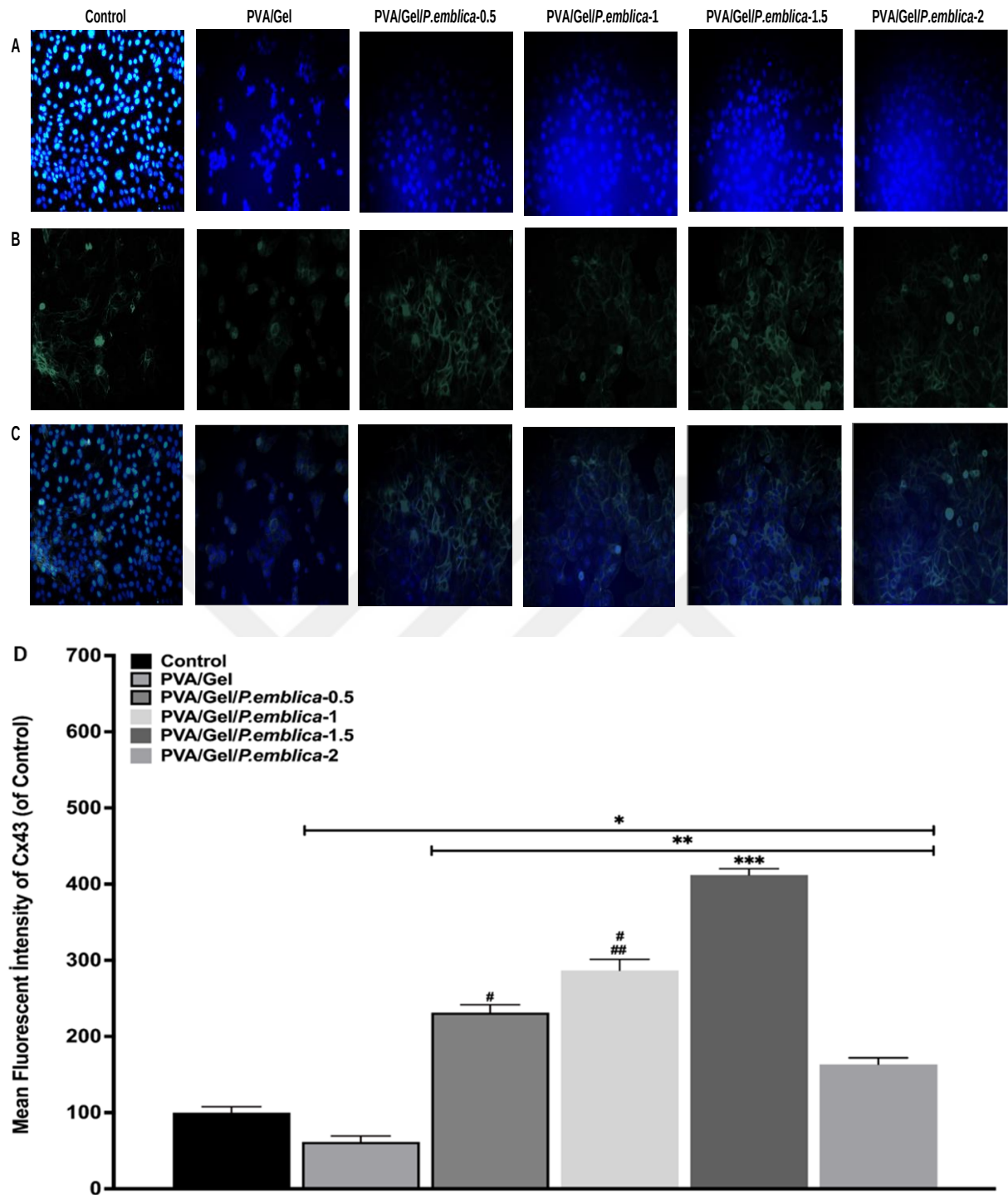


Figure 4.10. The effect of PVA/Gel and PVA/Gel/*P.emblica* cryogels on Cx43 distribution in cells by immunofluorescence staining. Cx43 staining was performed 72nd h after incubation. The cells cultivated in polystyrene-culture plate were utilized as a control. Representative **(A)** DAPI (blue), **(B)** Cx43 (green) and **(C)** immunofluorescence of Cx43 (green)/ DAPI (blue) staining merge images of seeded cells on control, PVA/Gel, PVA/Gel/*P.emblica*-0.5,

PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5 and PVA/Gel/*P.emblica*-2 cryogels (all magnifications X20, scale bar: 100 μ m). **(D)** Quantitative data of relative intensity of anti-Cx43 immunofluorescent staining. Six individual regions that randomly selected were imaged on each sample and the relative of fluorescent dye was measured using ImageJ software. Data were defined as relative variation of fluorescent signal intensities and mean $\pm$ SD (n= 3). One-way ANOVA test was used for statistical analysis. *P< 0.001 differ significantly from control. **P< 0.001 is significantly different from the PVA/Gel. ***P< 0.001 is significantly different from the PVA/Gel/*P.emblica*-1.5. DAPI: 4',6-diamidino-2-phenylindole; Cx43: Connexin 43; PVA/Gel: Poly (vinyl alcohol)/Gelatin cryogels; PVA/Gel/*P.emblica*: *P. emblica* loaded PVA/Gel cryogel; *P. emblica*: *Phyllanthus emblica*.

The purpose of biomaterial production is to form good cellular adhesion areas for controlling cell migration, proliferation, differentiation and ECM Synthesis (Langer and Tirrell, 2004). Cells were visualized 72 h after incubation to evaluate the effect of control, PVA/Gel, PVA/Gel/*P.emblica* cryogels on cell proliferation, morphology, localization and cell-matrix interaction by SEM. SEM can visualize cell attachment, spread, and morphology within the porous structure of the cryogel, revealing important information about the distribution of cells and their interaction with the surrounding matrix (Iandolo et al., 2019). Cells with indistinct borders located in the pores of the scaffold and inside the polymeric walls grew on the cryogels and the presence of ECM was observed. There was also strong contact between cells and formed colonies/spheroids. It was observed that cells also encompass the interpore spaces, as evidenced by the spherical morphology covering the surface (Boukamp et al., 1988). Notably, in the PVA/Gel/*P.emblica* cryogel compared to PVA/Gel, cells settled over the entire surface of the scaffold, forming diffuse, proliferating and contiguous colonies/spheroids with ECM formation. Cell number and density were affected by the *P. emblica* concentration in the cryogels (Figure 4.11.). *P. emblica* contains bioactive secondary metabolites such as gallic acid, ascorbic acid, quercetin, punigluconin, apigenin 7-glucoside, kaempferol and so on (Ahmad et al., 2021). *P. emblica* loaded cryogels possess bioactive components with multiple hydroxyl functional groups that promote cell attachment as well as cell proliferation. Macroporosity in biomaterials allows nutrient supply and metabolic waste diffusion for cell-cell network formation (Deb et al., 2018). The ECM is vital for cell adhesion, receiving-transmitting physical external stimuli, and has a significant impact on cellular behavior (Boudreau and Jones, 1999). SEM data revealed that the macroporous structure of PVA/Gel/*P.emblica* cryogels promotes

cell adhesion and growth to simulate epithelialization by synthesizing proinflammatory and growth factors from keratinocytes for wound healing (Werner et al., 2007). In vitro results showed that the PVA/Gel/*P.emblica* cryogel, which provides versatile support for cell-material interaction with its 3D structure, has the potential to pave the way for the design of more efficient next generation tissue engineering materials and implants.

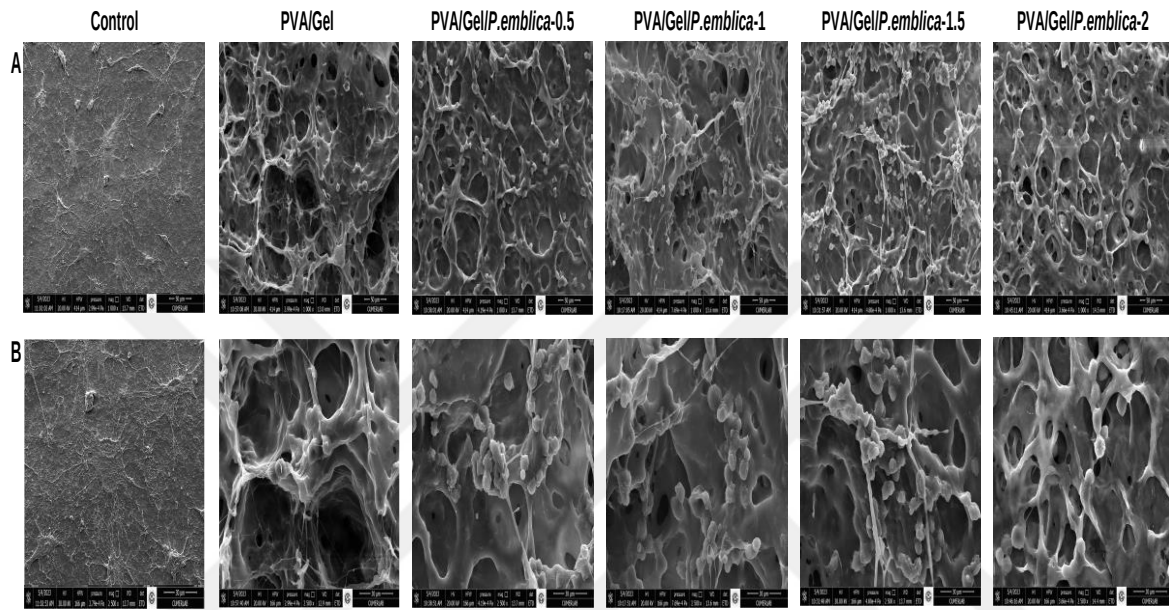


Figure 4.11. SEM images of PVA/Gel and PVA/Gel/*P.emblica* cryogels with cells. The cells cultivated in polystyrene-culture plate were utilized as a control. SEM images of seeded cells on control, PVA/Gel, PVA/Gel/*P.emblica*-0.5, PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5 and PVA/Gel/*P.emblica*-2 cryogels (**A**) at 1000X magnification, scale bar: 50 µm and (**B**) at magnification 2500X, scale bar: 30 µm. SEM: Scanning electron microscopy; PVA/Gel: Poly (vinyl alcohol)/Gelatin cryogels; PVA/Gel/*P.emblica*: *P. emblica* loaded PVA/Gel cryogels; *P. emblica*: *Phyllanthus emblica*.

4.2.4. Cell adhesion and infiltration on PVA/Gel and PVA/Gel/*P.emblica* cryogels by DAPI and F-Actin staining

In tissue engineering applications, the suitability of the biomaterial is important for cells to attach, infiltrate, multiply, grow and differentiate (Shakya vd., 2014; Kathuria vd., 2009). Cell nuclei and cytoplasm were stained with DAPI and phalloidin to test cell adhesion and infiltration matrix cultured on PVA/Gel, PVA/Gel/*P.emblica*-0.5, PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5 and PVA/Gel/*P.emblica*-2 cryogels at 72 h (Afanasiev et al., 2020)

(Figure 4.12.). Thanks to the porous structure of the cryogels, homogeneous cells that adhered to the matrices were visualized under a fluorescent microscope by staining with DAPI and F-Actin. A strong fluorescence was observed showing a highly populated, matrix-penetrating cells evenly dispersed within the pores of the PVA/Gel/*P.emblica* cryogels compared to the control and PVA/Gel. The interconnected pores forming the structure of the cryogel allowed the cells to settle. In contrast, an opaque and weakly fluorescence was observed in PVA/Gel, indicating a limited number of diffuse, clustered, and few cells on the surface. 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 PVA/Gel cryogel (Vrana et al., 2010; Choi et al., 2013; Canatar et al., 2023). It is known that *P. emblica* is rich in phytochemicals, has high free radical scavenging and antioxidant activity, as well as wound healing ability by inducing cell attachment, proliferation and migration in many cell types (Datta et al., 2011; Talekar et al., 2017; Ahmad et al., 2021). The release of biomolecules in the *P. emblica* extract into the matrix was not specifically investigated in this study. However, considering the literature, it is thought that the molecules in the *P. emblica* remain active even after *P. emblica* added into PVA/Gel cryogel, thus ultimately increasing the bioactivity of the material and promoting cell attachment and penetration (Liu et al., 2008; Ahmad et al., 2021; Hixon et al., 2017).

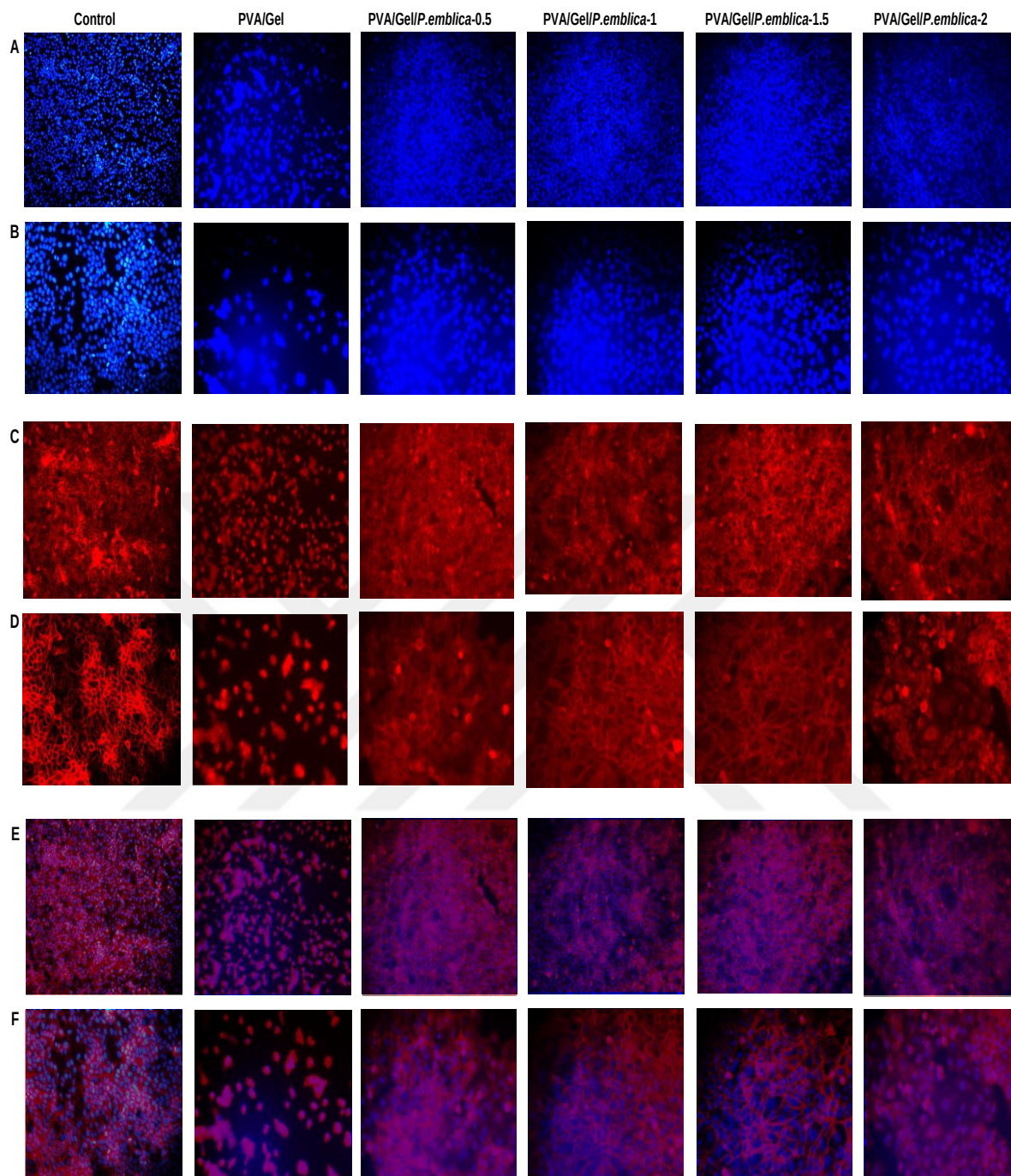


Figure 4.12. The effect of PVA/Gel and PVA/Gel/*P.emblica* cryogels on interaction of cells matrix via DAPI and F-actin staining. The cells cultivated in polystyrene-culture plate were utilized as a control. Representative **(A)**, **(B)** DAPI and **(C)**, **(D)** F-Actin and **(E)** **(F)** DAPI/F-Actin staining merge images of cells in control, PVA/Gel, PVA/Gel/*P.emblica*-0.5, PVA/Gel/*P.emblica*-1, PVA/Gel/*P.emblica*-1.5 and PVA/Gel/*P.emblica*-2 cryogels. Top row images at 10X magnification, scale bar: 150 μm and bottom row images at magnification 20X, scale bar: 100 μm . DAPI: 4',6-diamidino-2-phenylindole; PVA/Gel: Poly (vinyl

alcohol)/Gelatin cryogels; PVA/Gel/*P.emblica*: *P. emblica* loaded PVA/Gel cryogels; *P. emblica*: *Phyllanthus emblica*.



5. CONCLUSION

In this thesis, *P. emblica*, a natural compound known to have antioxidant, anti-inflammatory and antimicrobial properties, was synthesized by adding into PVA/Gel cryogels. *P. emblica* has a strong effect on accelerating wound healing, therefore PVA/Gel/*P.emblica* cryogels offered better cell viability and proliferation rate than PVA/Gel cryogels. Macroporous PVA-Gel/*P.emblica* cryogel was found to be non-cytotoxic and biocompatible. The PVA/Gel/*P.emblica*-1.5 cryogel showed promising results with the highest swelling rate, cell viability and cell infiltration. Overall, the incorporation of *P. emblica* extract into PVA/Gel cryogels enhanced their properties, making them promising candidates for wound dressing applications in tissue engineering and this biomaterial can developed with advanced studies. Further studies and evaluations can be needed to confirm and optimize the PVA/Gel/*P.emblica* cryogels for specific wound dressing applications and to understand the release profile of bioactive compounds from *P. emblica* within the cryogel.

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