NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF MEDICAL GENETICS

M.Sc. PROGRAM IN MEDICAL BIOLOGY AND GENETICS

Investigation of the *Vegf* and *Il1b* Gene Expression in Wound Healing in Rat Models

M.Sc. THESIS

Melis KALAYCI

Nicosia

February, 2023

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Nicosia
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Approval

We certify that we have read the thesis submitted by Melis Kalayci titled "Investigation of the *Vegf* and *Il1b* Gene Expression in Wound Healing in Rat Models" and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

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Declaration

I hereby declare that all information, documents, analysis, and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

09/02/2023

Melis KALAYCI

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Melis KALAYCI

Özet

Fare Modellerinde Yara İyileşmesine etki eden Vegf ve Il 1b Genlerinin Ekspresyon Seviyelerinin Araştırılması

Kalayci, Melis

Yüksek Lisans, Tıbbi Genetik Anabilim Dalı

Şubat, 2023, 60 Sayfa

Özet: Vücudun en büyüğü ve en önemli organlarından biri deridir. Vücudun en dış katmanını oluşturması nedeniyle, dış çevre ile en çok etkileşime girip, bariyer rolünü üstelenerek darbelere, hasarlara ve zararlı stres faktörlerine en çok maruz kalan organdır. Yara iyileştirme mekanizması, hasar onarımının yanı sıra cilt bütünlüğünün korunmasına ve yeniden oluşturulmasına da etki eder. Çok aşamalı, karmaşık bir biyolojik süreç olan yara iyileşmesi dinamik bir biyolojik süreçtir. Ne yazık ki, bu yaraların iyileşmesi veya önlenmesi için kullanılan evrensel bir tedavi yöntemi veya ilacı henüz yoktur. Etkili bir tedavi yöntemi belirleme ve hatta bir ilaç üretilebilmesi, yara iyileşme aşamalarının moleküler altyapısının anlaşılabilmesine bağlıdır. Keratinositler, sağlıklı cildin epidermisinde bulunan hücrelerdir. Herhangi bir yaranın varlığında, bu hücreler iyileşmek için yeniden oluşur ve farklılaşır ve yara bölgesine göç eder. Bu göç, yara iyileşmesinin temel mekanizmasıdır. Steroid tedavisi birçok yara modelinde denenmiş ve yara iyileşmesini etkilediği birçok kez kanıtlanmıştır. Yara iyileşmesinin temelinin immünojenik bir yanıta dayandığı düşünüldüğünde, steroid tedavisinin bağışıklık üzerindeki etkisinden dolayı birçok yara modeli çalışması için umut verici bir tedavi yöntemi olarak adlandırılmıştır. Bu çalışmanın amacı yara modeli oluşturulmuş sıçanlarda *Il1b* ve *Vegf* ekspresyon düzeylerinin araştırılmasıdır.

Gereç ve Yöntem: Manisa Celal Bayar Üniversitesi'nde sıçanlarda yara modelleri oluşturulup tedavi edilmiş ve dokular biyopsi yapıldıktan sonra parafin bloklara gömülmüştür. Yirmi dört erkek sıçan dört gruba ayrıştırılmış ve tedavi stratejilerinin

sayısına tabi tutulmuştur. Birinci grup, tedavi görmeyen kontrol grubu olarak belirlenmiştir. İkinci gruba farklılaşmış keratinositler, üçüncü gruba deksametazon, dördüncü gruba aynı anda deksametazon ve farklılaşmış keratinositler uygulanmıştır. İlgili dokulardan RNA izolasyonu ve cDNA sentezi yapılmıştır. Her örnekteki gen ekspresyon seviyesini değerlendirmek için gerçek zamanlı polimeraz zincir reaksiyonu (RT-PCR) kullanılmıştır.

Bulgular: Yara modelleri oluşturulmuş sıçan modelleri başarıyla oluşturulmuştur. Her gruba farklı tedavi yöntemleri uygulanmıştır. Her sıçan modelinden RNA ve cDNA örnekleri başarıyla elde edilmiştir. Sıçan modellerinden elde edilen örneklerde hem *Il1b* hem de *Vegf* genlerinin eksprese edildiği gösterilmiştir. Gen ekspresyon analizi sonuçları, ilaç tedavi uygulanan sıçan yara örnekleri ile kontrol grubu arasında gen ekspresyon seviyelerinde istatistiksel olarak anlamlı bir fark olmadığını göstermiştir.

Sonuç: *Il1b* ve *Vegf* gen seviyelerinin uygulanan farklılaşmış keratinosit ve/veya steroid tedavilerinden etkilenerek yara iyileşmesinde rol oynadığı bulunmuştur. Bu tedaviler altında *Il1b* ve *Vegf* gen seviyelerinin sıçan modellerinde araştırıldığı başka bir yayın literatürde bulunmamaktadır.

Anahtar Kelimeler: Yara iyileşmesi, gen ekspresyonu, sıçan modeli, *Il1b*, *Vegf*

Abstract

Investigation of the *Vegf* and *Il1b* Gene Expression in Wound Healing in Rat Models

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Background: The largest and one of the most important organs of the body is the skin. Since it is located outside the body, it is taking a role as a barrier by interacting the most with the outside environment, so in fact, the skin is the organ that is most exposed and open to impacts, damages, and harmful stress factors. The wound-healing mechanism acts to protect and re-establish skin integrity as well as repair the damage. A complex biological process with multiple phases, wound healing is a dynamic biological process. Unfortunately, there is no universal treatment method or medication that is used for the healing or prevention of these wounds. The ability to determine an effective treatment method and even to produce a drug depends on understanding the molecular infrastructure of the wound healing phases. Keratinocytes are cells found in the epidermis of healthy skin. In the presence of any wound, these cells re-form and differentiate for healing and migrate to the wound area. This migration is the basic mechanism of wound healing. Steroid therapy has been tried on many wound models and it has been proven many times that it affects wound healing. Considering that the basis of wound healing is based on an immunogenic response, it has been called a promising treatment method for many wound model studies due to the effect of steroid therapy on immunity. The aim of this study was to investigate the expression levels of *Il1b* and *Vegf* in rat wounded

skin rat models with steroid and/or differentiated keratinocytes and to understand the mechanism of how steroid and/or differentiated keratinocytes affect wound healing.

Materials and Methods: Rat wound models were established and treated in Manisa Celal Bayar University, and after tissues were biopsied, they were buried in paraffin blocks. Twenty-four male rats were divided into four groups and subjected to the number of treatment strategies. The first group was the control group, which received no therapy. Differentiated keratinocytes were administered to the second group, dexamethasone was administered to the third group, and dexamethasone and differentiated keratinocytes were administered simultaneously to the fourth group. RNA was then extracted and cDNA was synthesized. The real-time polymerase chain reaction (RT-PCR) was utilized to assess the level of gene expression in each sample.

Results: The rat models of wounded skin were successfully created. Different treatment modules were applied to each rat model. The RNA and cDNA samples were successfully obtained from each sample. Both *Il1b* and *Vegf* genes were shown to be expressed in samples obtained from the rat models. The gene expression analysis showed that there was no statistically significant difference in the gene expression levels between the drug-administered rat skin samples and the control group, respectively.

Conclusion: *Il1b* and *Vegf* are genes involved in wound healing whose expression levels have been found to be affected by the treatments used, such as steroid or keratinocyte. There are no previous studies in which these two treatments have been applied simultaneously and *Il1b* and *Vegf* gene expression leves were investigated.

Keywords: Wound healing, gene expression, rat model, *Il1b, Vegf*

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List of Abbreviations

AMP: Antimicrobial Peptides

BMP-4: Bone morphogenic protein-4

cDNA: complimentary DNA

CT: Cycle Threshold

DAB: Diaminobenzidine

DJE: Dermal-Epidermal Junction

DNA: Deoxiribonucleic Acid

DMEM: Dulbecco's Modified Eagle Medium

ECM: Extracellular Matrix

EDTA: Ethylenediaminetetraacetic acid

FFPE: Formalin-Fixed Paraffin-Embedded

FGF: Fibroblast Growth Factor

GF: Growth Factors

GM-CSF: Granulocyte-macrophage colony-stimulating factor

GT: Granulation Tissue

HIF-1: Hypoxia-Inducible Factor (HIF)-1

hsp90: heatshock protein

IGF: Insulin-like growth Factor

IL1B: Interleukin-1-Beta

KDR: Kinase Insert Domain Receptor

KGF: Keratinocyte Growth Factor

K/O: Knock Out

LIF: Leukemia inhibitory factor

MMPs: Matrix Metalloproteins

PBS: Phosphate Buffer Saline

PDFG: Platelet derived growth factor

RNA: Ribonucleic Acid

RNS: Reactive Nitrogen Species

PCR: Polymerase Chain Reaction

ROS: Reactive Oxygen Species

RT-PCR: Real-Time PCR

SEMPs: Sensing Enabling Metabolic Pathways

TGFβ: Tumor Growth Factor Beta

TNFa: Tumor Necrosis Factor Alpha

VEGF: Vascular Endothelial Growth Factor

WH: Wound Healing

CHAPTER I
Introduction

This chapter explains the structure and function of the skin, the wound healing process and the various elements of the treatment and side effects. Since these factors' parameters generate the bases of this research, this chapter also discusses the arrangement of the skin and the function of the *Il1b* and *Vegf* genes.

Structure and Function of the Skin

The biggest and most resistant organ in a mammal is the skin. Different layers of mammalian skin serve as functions for host defense and barrier integrity. The epidermis, the skin's outermost layer, is composed entirely of dead keratinocytes and has no blood vessels. This layer is named *Stratum Corneum.* Following this layer, there are *Stratum Lucideum*, *Stratum Granulosum*, *Stratum Spinosum*, and *Stratum Basale* layers. The last layer of the epidermis, *Stratum Basale*, is connected with the Dermal-Epidermal Junction (DEJ) where keratinocytes are also located as well as the base layer of the epidermis in the absence of a wound (in a healthy state) (Woodley et al., 2015). Each layer of the epidermis has unique morphologic and biochemical characteristics, specifically pointing to various functions in the skin barrier. After the DEJ layer, the dermis layer is presented, where fibroblasts, macrophages, collagens, hair follicles vessels, and nerves are located (Jiang et al., 2020). The dermis's predominant cell type is the fibroblast. The dermis also contains immune cells like mast cells, macrophages, and a limited number of lymphocytes which are important for immune response. The dermis is the thickest layer of skin, contributing flexibility and strength as well as helping with thermal regulation and sensory perception (Lopez-Ojeda et al., 2022).

Wound Formation and Wound Healing

Wounding can occur in all tissues and organs. So, repair phases are the same for all tissues. To maintain the coherence of the skin and surrounding tissue, wound healing is an requisite process. The healing of wound goes through four stages. These encompass the remodeling stage, the proliferative stage, the inflammatory stage, and the

hemostatic phase. Different molecules and cells take a role and affect these phases. As a result of these interactions, the type of wound and the healing process is determined. In particular, molecules and cells that affect or take a role in the inflammation phase can be decisive in whether the wound is acute or chronic. For a healthy recovery to take place, all molecules and cells must be in balance (Serra et al., 2021). But the main purpose of all cells and molecules is to induce tissue repair. A wound is considered fully healed when its anatomical structure, function, and appearance have all returned to normal within a reasonable amount of time (Robson et al., 2001). According to how long they take to heal, wounds can be classified into acute wounds, chronic wounds, and complicated wounds.

Acute Wounds

The healing process of an average of wound can take from 5 to 10 days to 30 days. The main causes of acute wounds are traumatic injuries and small cuts. In acute wounds, all phases of the wound healing mechanism must function correctly for a successful healing process (Lazarus et al., 1994).

Chronic Wounds

Chronic wounds cannot be repaired in a systematic or timely manner. These wounds cannot follow the usual stage of healing (Szycher & Lee, 1992). They are frequently mired in the stages of inflammation, which results in an increase in inflammatory cells including neutrophils, lymphocytes, and macrophages (Wicke et al., 2000). Therefore, the prominence of molecules being in balance during the WH process is great. The existence of a chronic wound is indicated by the prolonged inflammatory phase. Failure to complete wound healing and inability to proceed to the next phase may be due to many reasons, such as the presence of infection, tissue hypoxia, necrosis and excess level of cytokines (Vanwijck, 2001). A persistent state of inflammation in the wounds causes structural and functional disruption. These injuries recur frequently (Degreef, 1998). Sepsis or amputation of the wounded area could be the most severe effects of poor wound healing (Mieczkowski et al., 2022). The reason for chronic wounds may be burns, vasculitis, or pressure. All wounds have the risk and potential to

turn into chronic wounds but immunological status, age, stress level, and ethnicity have a great impact on this situation. Also, individuals who have darker skin prone to develop chronic wounds (Velnar et al., 2009).

Complicated Wounds

Condition that combines an infection with a tissue defect referred as complicated wounds. The cause of the tissue defect may be caused by a traumatic event or it can rise post-infection and in the presence of tumors or virulence (Degreef, 1998). Five symptoms of infection are observed in these wounds; redness, heat, pain, oedema, loss of functions. Even though timing and communications between the molecules of phases differ for the acute and chronic wounds, main phases persist same for repair (Harding, 2008).

Hemostatic Phase

This phase occurs right away after injury. This phase mainly has two roles. First aim of hemostatic phase is to control the blood loss to protect vascular system by activation of platelets. The second aim (long-term) is to supply a temporary matrix for foreign and encroaching cells. Chemokines, growth factors (GFs) and anti-inflammatory mediators such as *Il1, Tgfβ,* keratinocytes, and *Cxcl4* are secreted by platelets for the recruitment of neutrophils, macrophages and fibroblasts to the damaged/wounded area to prepare the site for further phases of tissue repair (Chen et al., 2020). Since the effect of platelets and the products they secrete on wound healing is known, they are being tried and even used as a treatment for wounds (Barton et al., 2013). T-cells, which are the immune cells found in tissues, simultaneously produce a variety of GFs, including *Fgf7, Fgf10, Igf10,* and *Kgfs*, to provoke the proliferation and differentiation of keratinocytes (Pesce et al., 2013). If these processes are not well organized (as in patients with diabetes), it may result in the wound not being able to heal. This condition also causes the formation of chronic wounds, which is much more difficult to heal (Iqbal et al., 2017).

With the formation of a wound, cut or blow, the vessels in the wound area are damaged and causes exposure of Sub-Endothelial Matrix Proteins (SEMPs) which are fibronectin

and collagens. Thus, a suitable infrastructure is created for scar formation. Apart from these, noxious substances produced by the wound and cause the formation of microvascular injuries. This situation also increases the amount of blood flowing to the wound area with the help of vasoactive amines, such as serotonin that platelets have (Broughton et al., 2006). This intense blood flow induces the neuronal reflex mechanism, causing the injured vessel to contract rapidly. The formation of edema also occurs with the sprayed blood, resulting in a transition to the inflammation phase (Richardson, 2004). At the same time, blood components and platelets reaching the wound area contact with exposed collagen and other extracellular matrix (ECM) molecules to form a temporary matrix structure (through the platelets and blood clot that are stuck in the wound area) and allowing cells to migrate. This temporary matrix will also be used during the inflammation phase (Lawrence, 1998).

Inflammation Phase

Eicosanoids and other metabolism products are released after injury to the cell membrane to initiate the inflammatory retaliation. The inflammation stage of WH is divided into two depending on the molecules involved in; early phase (neutrophils involved) and late phase (macrophages involved) (Ghosh et al., 2021).

Early Phase of Inflammation Phase

An early phase of the inflammation stage initiated during the late stage of coagulation (24-36 hours of injury). Due to the secreted molecules, changes occur in the mechanism that regulates the surface adhesion molecules of neutrophils. These changes make neutrophils "sticky" and lead to binding to endothelial cells and capillaries that surround wounds with weak attachment. This process is called margination. Attached neutrophils push and roll over the endothelium's surface with the aid of blood flow. At this stage, the rolling mechanism is regulated by selectin-dependent interactions, while the weak bond formed is strengthened by chemokines and integrins secreted by endothelial cells (Skover, 1991). Through strengthened bonds, neutrophils stop rolling and migrating, float out of the capillaries, and are placed between endothelial cells. This process is called diapedesis (Flanagan, 2013). Neutrophils reached the wound area by

platelets act as phagocytic agents and provide cleaning of residual microorganisms, bacteria, and necrotic/dead tissue in the wound area till the whole site becomes free of cell debris and opportunistic microorganisms. It has been reported in many articles that in the case of accumulation of tissue debris, accumulated debris can replace connective tissue, soft tissue, and parenchymal tissue over time, causing tissue fibrosis and organ loss (Miao et al., 2021). This process is done by secreting antimicrobial peptides (AMPs), reactive oxygen species (ROS), and various chemoattractant agents such as *Tnfα* (Velnar et al., 2009).

After completing their functions, neutrophils have to undergo apoptosis to remove from the wound area (Gonzalez et al., 2016). This allows the neutrophils to be removed from the tissue and the inflammation response to be strengthened before tissue damage is caused. The neutrophils destroyed by *Tgfβ,* are replaced by macrophages to continue phagocytosis. Since the life span of macrophages is longer than neutrophils, macrophages are attracted to the wound area (48-72h after injury) by a chemoattractant agent such as *Tgfβ,* platelet factor IV, and collagen (Ramasastry, 2005).

Late Phase of Inflammation Phase

It is well recognized that ROS and reactive nitrogen species (RNS) may raise *Il-6* levels, which then raise other exhilarating factors like granulocyte-macrophage colony-stimulating factor (GM-CSF). *GM-CSF* led secretion of *Vegf* in the macrophages that have called to the damaged area. Based on their differentiated phenotype, macrophages play a dual role in the late phase of the inflammatory phase. The establishment of inflammatory cytokines involved *Il-1, Il-6, Tnf,* and *Vegf* as well as the eradication of infections is facilitated by pro-inflammatory phenotype macrophages (M1) that are typically activated (Wilkinson & Hardman, 2020). As anti-inflammatory phenotype macrophages (M2), which are alternatively activated, stimulate the fibroblast found within the cells and prepare them for further stages by affecting keratinocytes. It is expected that switching M1 macrophages into M2 macrophages during a healthy wound-healing process and provide the conversion from the inflammation stage to the proliferation phase (Aitcheson et al., 2021). In the existence of a bacterial condition, pro-inflammatory cytokines such as *Tnfα* and *Il1b*, constantly extravasated to the wound site.

In this case, an irregularity occurs in the numbers of M1 and M2 macrophages due to competition. This deregulation, on the other hand, causes the inflammation phase to prolong and the wound to become chronic (Xiao et al., 2020).

The last cells that are associated with wound healing during the late inflammatory stage are lymphocytes by secretion of Il1b and immunoglobulin G (IgG) (Hunt et al., 2000). It is shown that collagenase modulation, which is crucial for the remodeling phase, is regulated by *Il1b* in a significant way (Sieggreen, 1987).

Proliferative Phase

On day three, the proliferative stage of wound healing begins and lasts for up to two weeks. Once hemostasis has been reached, an effective immunological response has been started, and further harm has stopped, the focus of the acute wound switches to tissue healing. This phase focus on newly synthesized ECM deposition instead of a temporary matrix that has been produced in previous phases. As a result, the main goal of this phase is by interacting with different cell types, creating new connective tissue (Schultz et al., 2011).

For the first three days after injury, fibroblasts and myofibroblasts in the circumjacent tissues are stimulated for proliferation. Platelets and inflammatory cells secrete *Pdfg* and *Tgfβ* which results in the activation of fibroblast. Activated fibroblasts are responsible for migration to the wound site and produce matrix proteins (fibronectin and collagen). Thus, fibroblasts and their accumulations are first seen in the damaged site (Goldman, 2004). Collagens generated by fibroblasts act as a scaffold for a new permanent matrix by differentiating into myofibroblasts (Ramasastry, 2005). Apart from matrix formation, myofibroblasts help regulation of wound contraction as well (Servold, 1991). Fibroblasts can affect soluble mediators which are produced by keratinocytes during interaction with fibroblasts. By regulating these soluble mediators, vitality, proliferation, and differentiation rate can be determined by cross-talk mechanisms. This mechanism aims to achieve adequate skin homeostasis (Jevtić et al., 2020). Keratinocyte starts to migrate at that stage. This phase can be seen under the microscope as a granulation tissue formation (Rodrigues et al., 2019).

Statement of the Problem

Wound formation is a complex mechanism. This mechanism is still not fully understood. The incomplete understanding of this mechanism limits the treatment methods that can be developed for wound healing. The development of an effective treatment method is based on a full understanding of the wound formation mechanism. Although the wound healing process differs from person to person, the basic stages are the same. In these basic stages, keratinocytes differentiate and migrate through the wound area and take part in wound closure. An inflammatory environment occurs due to the cytokines and immune cells secreted during the wound healing process. In cases where the inflammation is more than necessary, acute wounds turn into chronic wounds and the healing process is delayed. Therefore, drugs used for wound healing suppress inflammation. Steroids are known to suppress inflammation. Therefore, in this study, differentiated keratinocyte and/or steroid treatment was applied to rat models after wound models were established.

Purpose of Study

The aim of this study was to investigate the changes in the expression levels of *Vegf* and *Il1b* genes, which play a major role in wound healing pathways, under differentiated keratinocyte and/or steroid treatments applied.

Research Question/Hypothesis

It is known that *Vegf* and *IL1b* genes have important roles in wound healing. It is hypothesized that the expression levels of these genes change under the applied treatments in rat models of wounds.

CHAPTER II

Literature Review

Granulation Tissue (GT)

Wounds follow processes either for regeneration or tissue repair depending on the severity. That means wounds can be healed by primary intention or secondary intention. The primary intention is applicable in cases where there are small cuts, the wound edges are distinct and the wound edges close easily. In cases where there are very large and deep wounds, the wound edges cannot be easily approached. In these cases, secondary intention is applicable. The formation of GT is observed in wounds that heal with secondary intention. Granulation tissue acts as connective tissue for large and non-closing wound openings. The main components of GT are macrophages and myofibroblasts. Formation of GT is complex and requires lots of different growth factors, such as *Tgfβ*. Fibroblasts and keratinocytes secrete *Tgfβ* and induce GT formation with the help of myofibroblast differentiation. Initially, the ECM of GT consists of type III collagen. To create a firmer form, type I collagen replaces type III collagen when proliferation and migration take place. Contraction of GT results in wound margination and keratinocyte migration from wound edges. According to the knock-out experiments performed, in the absence of *Tnfα*, GT cannot be formed correctly or GT can be accumulated. This can result in unhealed or chronic wound formation or tissue fibrosis. *Tnfα* knock-out rat models showed that without *Tnfα* expression, GT contraction cannot be regulated that can be followed up by abnormalities in keratinocyte migration (Alhajj et al., 2022).

Remodeling Phase

The last stage of WH is the remodeling stage. This phase can take up to 2 years contingent on the severity of the wound. The main aim of this phase is the production of new epithelial and final scar tissue. The granulation tissue formed in the previous phase initiates this phase. The destruction-synthesis events take place continuously in this phase until the wound reaches a steady state (Baum & Arpey, 2005). In this phase, to strengthen the scar tissue, the amount of collagen is increased, the intracellular matrix

matures, the destruction of hyaluronic acid occurs, and fibronectins are degraded. For the tissues formed in this phase to be permanent, the collagens synthesized *via* matrix metalloproteins (MMPs) undergo destruction. MMPs is produced by *Tnfa* and *Il1b*. The increase in *Tnfa* and *Il1b* expression levels induces MMP production (Raziyeva et al., 2021). However, overproduction of MMPs due to overexpression of Il1b and *Tnfa* can cause inflammation and chronic wound formation. Therefore, as wound healing occurs, the number of MMP inhibitors increases and decreases activity. Following that, underlying connective tissue shrinks in size which brings wound edges/margins closer and led fibroblast-ECM interactions (Velnar et al., 2009). For the wound to be completely closed, keratinocyte migration must be completed (Woodley et al., 2015).

Keratinocyte Migration

Eight to twenty-four hours after the formation of the wound, keratin 6 and keratin 16 are expressed by the keratinocytes located in the suprabasal, which makes the keratinocytes more active. Through keratin 1 and 10 expressed from activated keratinocytes, keratinocyte migration is initiated for the initiation of wound closure/epithelialization (Usui et al., 2008). IL1B, which is a major pro-inflammatory cytokine, and *Tnfa* expression occurs from keratinocytes, which are activated due to stress and keratin expression caused by the formation of the wound. By the expression of *Il1b* and *Tnfa*, fibroblasts are induced and KGF is expressed for epidermal regeneration. This expression is regulated by *Ap*-1, *cJun*, and *JunB* transcription factors. The occurrence of any damage to the epithelial tissue causes disturbances in the functioning of these transcription factors, disrupting the wound-healing process. Growth factors such as *Vegf* secreted from platelets induced by the formation of scar tissue and immune response also contribute to the induction of Kgf and the realization of epidermal regeneration. Keratinocyte migration occurs by *Il1* secretion from keratinocytes and *GM-CSF* induced by ROSs (Werner & Smola, 2001).

In the absence of a wound, keratinocytes are located in the stratum basal. With the formation of a scar, keratinocytes activated, by the methods described previously, begin to migrate toward the skin surface i.e., the stratum corneum. Basal keratinocytes have proliferative properties. While migrating, when they reach each layer of the skin, they

lose these proliferative properties a little more and turn into dead cells without a nucleus. This change is called terminal differentiation. Dead keratinocytes form the outermost layer of the skin. Keratinocytes reprogrammed with wound formation do not lose their proliferative properties during migration. This reprogramming occurs when the damage caused to the vessels by the formation of a wound creates a change in the oxygen level and stresses the keratinocytes. Keratinocytes under stress become acute hypoxia. These keratinocytes secrete *Hif-1* and *hsp90*, causing migration and an increase in collagen, accelerating wound healing (Woodley et al., 2015).

Vascularization in Wound Healing

Collagen storage, re-formation, and vascular formation are very important in wound healing. *Vegf* serves as a chemotactic substance, a persuader of vascular permeability, and an endothelial cell mitogen. The effects of *Vegf* on several stages of the WH cascade, including angiogenesis, epithelization, and collagen demission, set it apart from other angiogenic growth factors that have been described (Brem et al., 2009). Following days of wound healing, macrophages and epidermal cells initiate *Vegf* expression. Activated *Vegf* induces chemotactic activities by binding to *Flt-1* and *Kdr* receptors. The binding of *Vegf* to the *Flt-1* receptor is responsible for organizing blood vessels. On the other hand, attachment to the *Kdr* receptor is responsible for the formation of new vessels. Therefore, the *Vegf-Kdr* interaction corresponds to the formation of new vessels in wound healing. It causes an increase in the expression levels of MMPs due to the induction of chemotactic activities. As a result of chemotactic activities, keratinocytes are induced and thickening of the skin is achieved. The hypoxic environment formed by the wound is reduced by the expression of *Vegf*. Following this, the capillaries sprout towards the wound area and allow new vessels to form. After the vascular formation is achieved *Vegf* expression is reduced by integrins and sprouting is stopped (Bao et al., 2009).

Treatment *via* Application of Dexamethasone

Steroid and derivative drugs are used in wound healing. Dexamethasone is also one of these drugs. The use of steroids in wound treatments can have contradictory

results. The common outcome of steroid treatment is that steroids should be used at the right time and at the right dose as a treatment. It has been proven that long-term steroid use does not contribute to wound healing, on the contrary, it raises blood sugar and can cause osteoporosis. The application of short-term (7 days) and minor doses of treatment reduces inflammation by reducing the grade of inflammatory cytokines. This condition prevents the formation of chronic wounds. In addition, steroid therapy (especially dexamethasone) accelerates wound healing by increasing vascularization and skeletal muscles which also increase wound contraction (Tu et al., 2020).

CHAPTER III
Materials and Methods

The supplies and procedures used in the research are highlighted in this chapter's content. The research aimed to assess the expression of *Il1β* and *Vegf* in rat models of wounded skin treated with differentiated keratinocytes and/or steroid and to provide a deeper knowledge of the mechanism underlying how these treatments affects wound healing.

Sample Preparation, Collection and Sample Size

Keratinocytes were differentiated from mouse embryonic stem cells in Manisa Celal Bayar University, Turkey. Mouse embryonic stem cell line (CGR8) and mouse embryonic fibroblasts (STO) were already available at the Department of Histology and Embryology, Faculty of Medicine, Manisa Celal Bayar University, Turkey. Rat wound models were established and treated with different treatment strategies in Manisa Celal Bayar University, Turkey. A total of 24 male rats were divided into 4 groups. These groups were; group 1: rats with surgical wound (no other treatment was performed), group 2: rats with surgical wound where differentiated keratinocyte was applied, group 3: rats with surgical wound where dexamethasone was applied, group 4: rats with surgical wound where a combination of differentiated keratinocyte and steroid was applied.

Biopsy samples were taken from the subjects from the wound site on the tenth and twenty first day after cell transplantation in accordance with the WH process. In total, biopsies were obtained from six subjects from each group, which is the lowest limit for data analysis in a statistical significance.

Sample Preparation

Cell culture part of the study was performed at Manisa Celal Bayar University under the supervision of Prof. Seda H. Vatansever. Briefly, the CGR8 mouse embryonic stem cell line was cultured with STO mouse embryonic fibroblasts in embryonic culture mediocre (15% fetal bovine serum-FBS, 1% L-glutamine, 1% penicillin/streptomycin,

0.1 mm non-essential amino acid, 10-6M β-mercaptoethanol, 1000 U/mL leukemia inhibitory factor (LIF), 4500 mg/L glucose and the DMEM-Dulbecco Modified Eagle's Medium containing sodium pyruvate). The expression of cytokeratin 8 and cytokeratin 14 was examined by flow cytometry and immunocytochemistry methods for the characterization of keratinocytes differentiated from CGR8. Dexamethasone was administered intramuscularly at a dose of 2 mg/kg per day for 1 week to the subjects belonging to Groups 3 and 4 before the wound model was created. Then, a wound model containing the epidermis and dermis with a size of 0.5x0.5 cm was created for the subjects in all groups using a sterile surgical set in the area cleaned of hair in the nape area. After the wound model was created, dexamethasone was continued to be administered to Group 3 and Group 4 at the specified dose every day for 3 weeks. Keratinocytes were transferred to the wound site of the subjects belonging to Group 2 and Group 4. After dressing was performed every two days, biopsy samples were taken from the wound site on days 10 and 21 after transfer. Taken biopsies were embedded in paraffin for further experiments.

Interpretation of the Formalin-Fixed Paraffin-Embedded (FFPE) Samples: RNA Isolation, cDNA Synthesis, and Real-Time PCR

The level of gene expression was investigated in the samples obtained from the biopsied wounded region of the rat models. A total of 24 FFPE samples acquired from male rats of wound skin model was divided into four groups; control (no treatment), differentiated keratinocyte treatment, steroid treatment and simultaneous differentiated keratinocyte and steroid treatment, respectively. This section of the study was carried out at NEU DESAM Research Institute laboratory, Nicosia, North Cyprus. RNA extraction was performed using the Norgen FFPE RNA Isolation Kit (Norgen, Canada) following the manufacturer protocol. The purity of the extracted RNA was estimated using NanoDrop® ND-1000 UV-Vis Spectrophotometer (Thermo-scientific, Pittsburg). All kits were used in accordance with the manufacturers' instructions without any changes to reverse transcribe RNA into cDNA. RNA concentration level of samples was about 20.5ng/µl. The ratio of absorbance at 260 and 280 nm (A260/280) following NanoDrop® ND-1000 UV-Vis Spectrophotometer analysis was used to evaluate the

purity and concentration of nucleic acids. A pure RNA has a 260/280 ratio of 2.0. It should be noted that this ratio differs from what was predicted indicates the existence of protein contamination or undesirable components. Accordingly, ratio of 260/280 and concentration of RNAs are given in results section.

Real-time PCR was conducted using the LightCycler® 480 SYBR Green I Master kit . Table 1 shows the list of primer sequences. The real-time PCR setup followed the conditions in Table 2. In the real-time PCR, a negative-control consisting just of the SYBR Green master-mix was also run to ensure that the reaction functioned properly and without contamination.

While optimizing the real-time PCR conditions used for the analysis of the expression level of the *Il1B* gene, annealing temperatures in the range of 60 to 64 C^{o} were tested in order to find the most optimal melting temperature. The primer concentration was tested in the range of 0.05 to 1µM, the amount of template (cDNA) used was increased up to 2 µl (20%) and $MgCl_2$ and glycerol were added when necessary. The annealing time between 30 seconds to 10 seconds were tested to identify the optimum conditions. Melting curve analysis and gel electrophoresis were performed in order to choose the optimum condition for the amplification and to make sure that the results obtained are not primer dimers, respectively.

The CT values obtained from the qRT-PCR (Insta Q96^{Tm} Plus Real Time PCR Detection System, HiMedia Laboratories PVT.Ltd., Mumbai, India) were used to evaluate the level of gene expression between each group. Housekeeping gene was used for normalization. The level of gene expression in the four groups were examined and evaluated using the ANOVA analysis.

The gene expression levels of *Il1b* and *Vegf* were studied in four groups of rat models of damaged skin tissue using different treatment techniques. The first group was the control group, which included just rat models with injured skin with no therapy. The second group included rat models of damaged skin treated with differentiated keratinocytes, whereas the third group included rat models of wounded skin treated with steroids. The last group contained rat models of damaged skin treated with a mixture of steroids and differentiated keratinocytes. The optimum PCR master mixture condition performed for the experiments are shown in Table 3.

Statistical Analysis

The ΔΔCt values of samples were statistically analyzed using GraphPad prism v8.4.2. One-way ANOVA statistical analysis was used to evaluate the experimental data.

Table 1

List of Primers Sequences and Their Melting Temperature.

Genes	Forward Primer	Reverse Primer	Tm
Il1b	TCAGGCAGGCAGTATCACTC	AGCTCATATGGGTCCGACAG	63 C^0
Vegf	CCGGTTTAAATCCTGGAGCG	GAGAGGTCTGGTTCCCGAAA	64 C^0
Gapdh	CATCACTGCCACCCAGAAGACTG	ATGCCAGTGAGCTTCCCGTTCAG	62 C^0

Table 2

Optimized Real-Time PCR Conditions

		Temperature C⁰	**Time**	**Cycles**
PCR Steps	Initial Denaturation	95^0 C	10 min	1
	Denaturation	95^0 C	10 sec	40
	Annealing	63^0 C *Il1b* 64^0 C *Vegf*	10-20 sec	
	Elongation	72^0 C	30 sec	
Melting Stage	High resolution melting curve	95^0 C	15 sec	0.3^0 C decrease in 20 sec
		60^0 C	1 min	
		95^0 C	15 sec	

Table 3

Table of PCR Master Mixture Conditions

Components	***Il1b***	***Vegf***
Tm	63Cº	64Cº
Annealing Time	30sec	10sec
Forward Primer (final concentration)	0.1µM	0.1µM
Reverse Primer (final concentration)	0.1µM	0.1µM
SYBR Green	5µl	5µl
Glycerol (%50)	2µl	1µl
$MgCl_2$	15%	12.5%
dH_2O	0.35µl	1.35µl
Template	1.25µl	2µl

CHAPTER IV
Results

Il1b Expression Analysis of Samples

A pure RNA has a 260/280 ratio of 2.0. It should be noted that any deviation from this predicted ratio indicates the existence of protein contamination or other undesirable components Accordingly, 260/280 ratios for samples used in this study and RNA concentration measurements are given in Table.4. Table 5 shows the CT values obtained from Real-Time PCR experiments. Results of melting curve analysis for, *Vegf* and *Il1b* are given in Figure 1.

One-way ANOVA analysis was used to assess the levels of gene expression in samples of wounded and treated skin obtained from male rats. Figure 2 illustrates the investigation results. According to the statistical analysis, the ΔΔCT value for the group 2, 3 and 4 in the instance of the *Il1b* gene is shown in Table 6. Statistical findings suggest that *IL1b* expression did not differ with differentiated keratinocyte and/or steroid influence ($p>0.05$). The results show that a minor elevation in *Il1b* expression when the keratinocyte treatment was applied to the rat models with wounded skin (groups 2 and 4, respectively). As a result, the application of differentiated keratinocyte therapy appears to have an influence on the gene expression levels. Expression levels of the *Il1b* gene were found to be rather lower in the third group with steroid therapy compared to the control group with no treatment ($p> 0.9999$). When compared to the control group, the fourth group with differentiated keratinocytes and steroid therapy had slightly higher levels of *Il1b* gene expression ($p= 0.9799$).

Table 4

Nanodrop Results

Sample ID	Concentration (ng/µl)	260/280
1	9.3	1.80
2	10.2	1.78
3	8.7	1.82
4	8.4	1.83
5	7.3	1.81
6	9.1	1.81
7	7.7	1.82
8	10.8	1.79
9	12.1	1.83
10	8.4	1.84
11	9.6	1.87
30	7.3	1.77
12	9	1.83
13	8.2	1.83
14	11.3	1.81
15	10.1	1.80
16	9.9	1.79
17	7.2	1.77
18	7.9	1.84
19	10.3	1.80
20	9.7	1.82
21	10.5	1.83
22	12	1.84
23	11.8	1.80

Table 5

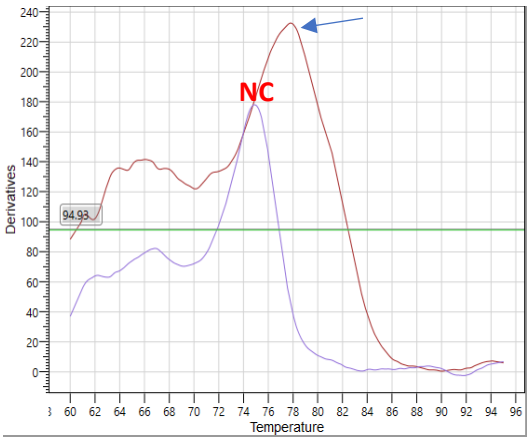
Mean Ct values of RT-PCR results for Il1b and Vegf genes.

Groups	Sample ID	Gapdh	Vegf	Il1b
Ct Values of Group 1	1	25.6	32.6	28.7
	2	22.9	31.5	-
	3	19.6	33.8	30.0
	4	19.2	32.3	27.0
	5	20.1	34.7	-
	6	19.7	32.5	28.7
Ct Values of Group 2	7	22.1	27.6	25.3
	8	22.7	33.3	25.7
	9	22	32.7	28.28
	10	22	34.9	28.26
	11	18.9	31.5	24.7
	30	25.8	32.0	25.2
Ct Values of Group 3	12	22.5	29.7	24.2
	13	19.2	32.9	26.0
	14	19.9	34.1	27.2
	15	17.9	34.5	25.8
	16	19.8	33.4	26.2
	17	22.5	30.9	29.04
Ct Values of Group 4	18	24.6	32.5	25.2
	19	18.9	31.1	28.66
	20	18.4	30.3	24.9
	21	18.2	36.1	-
	22	19.3	28.7	23.1
	23	19.7	28.9	27.73

Figure 1

The melting curve images of Vegf (A) and Il1b (B). The curve of the negative control is represented with NC. The curve of the sample is represented with an arrow.

A



B

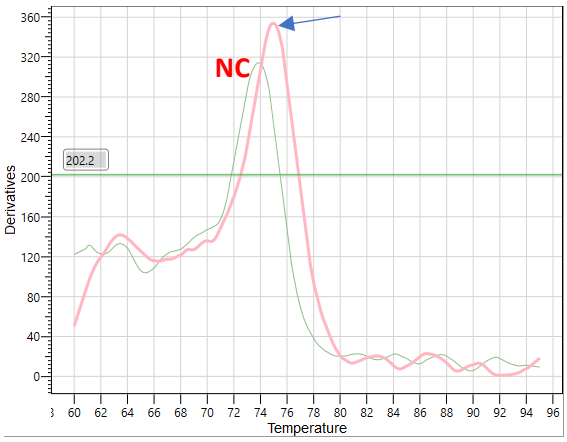


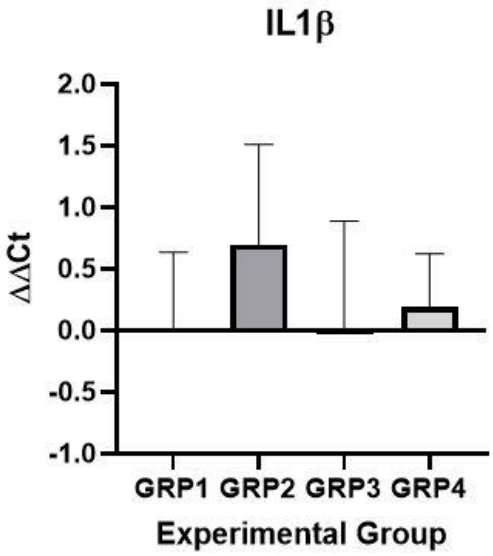
Table 6

Table summarizing the statistical analysis in the expression level of Il1b between different groups

Groups (G)	p-value (ANOVA)	p-value (t-test)
G1 vs G2	p= 0.4671	p=0.1866
G1 vs G3	p> 0.9999	p=0.9719
G1 vs G4	p= 0.9799	p=0.6113

Figure 2

The statistical analysis of the ΔΔCT values of Il1b in each one of the rat groups.



***Vegf* Expression Analysis of Samples**

The level of gene expression for the *Vegf* gene was also determined using one-way ANOVA. The graph of ΔΔCT values for the experimental groups' is shown in Figure 3. The results were statistically insignificant between the control and treatment groups, respectively (Table 7). Additionally, there were no statistically significant differences between the groups. The expression levels of the *Vegf* gene were found to be somewhat lower in the third group with steroid therapy compared to the control group with no treatment (p=0.7883). On the other hand, fourth group had lower lever of *Vegf* gene expression level compared to control group (p= 0.9980).

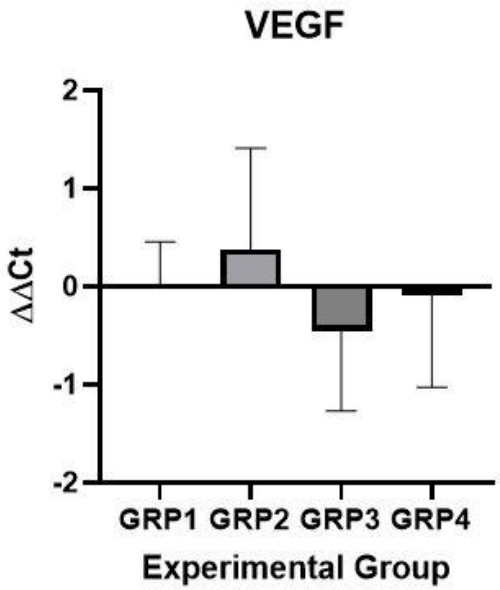
Table 7

Table summarizing the statistical analysis in the expression level of VEGF between different groups

Groups (G)	p-value (ANOVA)	p-value (t-test)
G1 vs G2	p= 0.8595	p= 0.4248
G1 vs G3	p=0.7883	p= 0.2643
G1 vs G4	p= 0.9980	p= 0.8457

Figure 3.

A graph chart representing the statistical analysis data following ANOVA in four groups for Vegf gene expression



CHAPTER V
Discussion

Wound healing is a complex process with different genes and pathways involved. Proinflammatory cytokines, such as *Il1* and *Tnfa*, and *Vegf*, play a role in different phases wound healing process (Barrientos et al., 2008). The need of fast and effective treatment strategies is always important in the wound healing process. Thus, the purpose of this study was to examine the effects of differentiated keratinocyte and/or steroid therapy on genes involved in wound healing.

Keratinocytes play a crucial part in the healing process by quickly covering cutaneous and mucosal injury sites to re-establish an epithelial shield with the surrounding environment (Wang & Graves, 2020). In order for the wound to heal fully and properly, keratinocytes must be able to move through the fibrin and newly formed ECM of the wound. Furthermore, they need to detach from the underlying basal lamina, a process made possible by MMPs. Moreover, keratinocyte migration on type 1 collagen is supported by MMPs, which is abundantly secreted from the edges of wounds (Pilcher et al., 1999). MMP synthesis is induced by *Il1b* and *Tnfa,* that are released during the healing of wounds (Ravanti & Kähäri, 2000). In this study, differentiated keratinocytes from mouse embryonic stem cells and steroids were applied in wound rat models for the first time. The effect of these treatment strategies on the level of *Il1b* and *Vegf* gene expression patterns were tested. It is important to note that the results show a minor elevation in *Il1b* expression when the keratinocyte treatment was applied to the animal models with wounded skin (groups 2 and 4). This observation is consistent with findings of Xiao and colleagues (2020) which concluded that *Il1b* was expressed by keratinocytes during the wound healing process and promotes wound closure (Xiao et al., 2020). Increases in group 2 and group 4 compared to group 1, can be an indicative that steroid administration is the reason why group 4 has a lower level of *Il1b* expression compared to group 2. This situation is supported by the data obtained from Group 3.

Various different cell types involved in wound repair can express *Vegf*. For instance, keratinocytes and macrophages, which both play crucial roles in the healing of wounds, produce *Vegfs* and are capable of reacting immediately to *Vegf*. The previous

experiments demonstrated that *Vegf* encourages angiogenesis and can directly influence the activity of a number of nonendothelial cell types found in skin (Johnson & Wilgus, 2014). Although statistically inconsequential, the result of this study demonstrates a minor elevation in *Vegf* expression when a keratinocyte treatment was applied to the wound. This finding is consistent with a previously published study by Frank and colleagues (Frank et al., 1995).

Numerous early stages of an inflammatory response are inhibited by glucocorticoids (Coutinho & Chapman, 2011). Therefore, it is known that steroid administration delays wound healing (Kato et al., 2017). Despite this, steroids and their derivatives are often used in wound healing. The reason of this is to ensure that the autoimmune response to the wound is kept in balance. Dysregulated autoimmune response to wounds are associated with attacking of innate immune cells to the response formed against the wound and cause delayed healing of the wound. This condition can result in organ dysfunction (Sun et al., 2020). Accordingly, the slight decrease in *Il1b* and *Vegf* expression levels is consistent with the previously published results, as wound healing will be delayed in steroid applied to group 3 (Cho et al., 2014). However, the findings are yet again statistically insignificant.

Previous research has shown that corticosteroids can reduce edema or prevent the development of new blood vessels by inhibiting *Vegf* expression (Nauck et al., 1998). *Vegf*, which plays a role in the regulation of keratinocyte migration, delays wound healing by causing abnormal keratinocyte migration in case of any decrease in its expression (Streit et al., 2000). *Vegf* also regulates collagen accumulation in wounds. Collagen is the component necessary for wound healing and for ensuring tissue integrity after wound healing (Bao et al., 2009). In light of all of this information, the differentiated keratinocyte and steroids applied in Group 4 might lead to the assumption that keratinocyte application may be insufficient to suppress corticosteroid side-effects on collagen production, thus leading to reduced *Vegf* expression. Although, it is imperative to remember that these experiments should be repeated since these findings were statistically insignificant.

CHAPTER VI
Conclusion and Limitations

This study evaluated the gene expression related to wound healing. A total of 24 male rats were divided into four groups and given differentiated keratinocytes and/or steroid treatments. Keratinocytes are known to differentiate and migrate during the healing process of wounds. There are four main stages of wound healing. Hemostasis, inflammation, proliferation, and remodeling stages are among them. The prolongation of the inflammation phase due to various reasons has been associated with the transformation of the wound from acute to chronic form. Chronic wounds are much more difficult to heal and treat.

It has been shown in experiments that steroids can prevent inflammation by suppressing the immune response produced by the body and therefore shorten the inflammation phase. However, on the contrary, it has also been shown that skin thinning is observed in cases where high doses of steroid therapy are applied, and the wound may worsen due to over-suppressed immunity. Because of this, when used appropriately and in the proper dosage, steroid treatment might be beneficial. Due to all these findings, differentiated keratinocyte and/or steroid administration was preferred as a treatment method in this experiment. The experiment anticipated a significant result for the evaluation of gene expression.

The constraints of this study were the number of samples is small and that the collected samples were buried in paraffin blocks. If taken into account, these limitations could improve the experiment's accuracy. Larger sample sizes will initially yield more reliable and accurate results because using more samples will lead to a more thorough analysis, but using more rats is unacceptable due to ethical concerns. The treatment that the tissues collected when they are embedded in paraffin blocks may reduce the expression levels of genes. It was considered appropriate to bury the samples in paraffin blocks for transfer and storage. It is believed that the results may be significant if they were studied from fresh tissues taken by biopsy.

In conclusion, no research has been done on wounded rat models to ascertain the role of differentiated keratinocytes and/or steroid treatment in the genes connected to the wound

healing (*Il1b and Vegf*). According to the study's limitations and the findings evaluated, even if the hypothesis based on this data appears to be consistent with the literature, there are no substantial differences between the rat groups that received the therapy when compared with the control group using the ANOVA test.

References

Aitcheson, S. M., Frentiu, F. D., Hurn, S. E., Edwards, K., & Murray, R. Z. (2021). Skin Wound Healing: Normal Macrophage Function and Macrophage Dysfunction in Diabetic Wounds. *Molecules*, *26*(16), 4917–4928. https://doi.org/10.3390/MOLECULES26164917

Alhajj, M., Bansal, P., & Goyal, A. (2022). Physiology, Granulation Tissue. *StatPearls*. https://www.ncbi.nlm.nih.gov/books/NBK554402/

Bao, P., Kodra, A., Tomic-Canic, M., Golinko, M. S., Ehrlich, H. P., & Brem, H. (2009). The Role of Vascular Endothelial Growth Factor in Wound Healing. *Journal of Surgical Research*, *153*(2), 347–358. https://doi.org/10.1016/J.JSS.2008.04.023

Barrientos, S., Stojadinovic, O., Golinko, M. S., Brem, H., & Tomic-Canic, M. (2008). PERSPECTIVE ARTICLE: Growth factors and cytokines in wound healing. *Wound Repair and Regeneration*, *16*(5), 585–601. https://doi.org/10.1111/J.1524-475X.2008.00410.X

Barton, A., Wende, K., Bundscherer, L., Hasse, S., Schmidt, A., Bekeschus, S., Weltmann, K. D., Lindequist, U., & Masur, K. (2013). Nonthermal Plasma Increases Expression of Wound Healing Related Genes in a Keratinocyte Cell Line. *Plasma Medicine*, *3*(1–2), 125–136. https://doi.org/10.1615/PLASMAMED.2014008540

Baum, C. L., & Arpey, C. J. (2005). Normal Cutaneous Wound Healing: Clinical Correlation with Cellular and Molecular Events. *Dermatologic Surgery*, *31*(6), 674–686. https://doi.org/10.1111/J.1524-4725.2005.31612

Brem, H., Kodra, A., Golinko, M. S., Entero, H., Stojadinovic, O., Wang, V. M., Sheahan, C. M., Weinberg, A. D., Woo, S. L. C., Ehrlich, H. P., & Tomic-Canic, M. (2009). Mechanism of Sustained Release of Vascular Endothelial Growth Factor in Accelerating Experimental Diabetic Healing. *Journal of Investigative Dermatology*, *129*(9), 2275–2287. https://doi.org/10.1038/JID.2009.26

Broughton, G., Janis, J. E., & Attinger, C. E. (2006). The basic science of wound healing. *Plastic and Reconstructive Surgery*, *117*(7 SUPPL.). https://doi.org/10.1097/01.PRS.0000225430.42531.C2

Chen, Y., Zhong, H., Zhao, Y., Luo, X., & Gao, W. (2020). Role of platelet biomarkers in inflammatory response. *Biomarker Research*, *8*(1), 1–7. https://doi.org/10.1186/S40364-020-00207-2/FIGURES/1

Cho, J. S., Kang, J. H., Park, I. H., & Lee, H. M. (2014). Steroids inhibit vascular endothelial growth factor expression via TLR4/Akt/NF-κB pathway in chronic rhinosinusitis with nasal polyp. *Experimental Biology and Medicine*, *239*(8), 913–921. https://doi.org/10.1177/1535370214537742

Coutinho, A. E., & Chapman, K. E. (2011). The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. *Molecular and Cellular Endocrinology*, *335*(1), 2–13. https://doi.org/10.1016/J.MCE.2010.04.005

Degreef, H. J. (1998). HOW TO HEAL A WOUND FAST. *Dermatologic Clinics*, *16*(2), 365–375. https://doi.org/10.1016/S0733-8635(05)70019-X

Flanagan, M. (2013). The physiology of wound healing. *Journal of Wound Care*, *9*(6), 299–300. https://doi.org/10.12968/JOWC.2000.9.6.25994

Frank, S., Hubner, G., Breier, G., Longaker, M. T., Greenhalgh, D. G., & Werner, S. (1995). Regulation of Vascular Endothelial Growth Factor Expression in Cultured Keratinocytes.: IMPLICATIONS FOR NORMAL AND IMPAIRED WOUND HEALING. *Journal of Biological Chemistry*, *270*(21), 12607–12613. https://doi.org/10.1074/JBC.270.21.12607

Ghosh, B., Mukhopadhyay, M., & Bhattacharya, D. (2021). Biopolymer-based nanofilms for the treatment of burn wounds. *Biopolymer-Based Nano Films: Applications in Food Packaging and Wound Healing*, 311–336. https://doi.org/10.1016/B978-0-12-823381-8.00005-3

Goldman, R. (2004). Growth Factors and Chronic Wound Healing: Past, Present, and Future. In *ADVANCES IN SKIN & WOUND CARE* • (Vol. 17, Issue 1).

Gonzalez, A. C. D. O., Andrade, Z. D. A., Costa, T. F., & Medrado, A. R. A. P. (2016). Wound healing - A literature review. *Anais Brasileiros de Dermatologia*, *91*(5), 614–620. https://doi.org/10.1590/ABD1806-4841.20164741

Harding, K. G. (2008). Wound Infection in Clinical Practice: An International Consensus. *International Wound Journal*, *5*(Suppl 3), iii. https://doi.org/10.1111/J.1742-481X.2008.00488.X

Hunt, T. K., Hopf, H., & Hussain, Z. (2000). Physiology of wound healing. *Advances in Skin & Wound Care*, *13*(6), 6–11. https://www.proquest.com/openview/27e35e3198a7463126b102c68ef7bade/1?cbl=46070&pq-origsite=gscholar&parentSessionId=yUFmvEbhfohdfKd5A7LAptbaVOtbSofe45a4ewXmhGM%3D

Iqbal, A., Jan, A., Wajid, M. A., & Tariq, S. (2017). Management of Chronic Non-healing Wounds by Hirudotherapy. *World Journal of Plastic Surgery*, *6*(1), 9. /pmc/articles/PMC5339604/

Jevtić, M., Löwa, A., Nováčková, A., Kováčik, A., Kaessmeyer, S., Erdmann, G., Vávrová, K., & Hedtrich, S. (2020). Impact of intercellular crosstalk between epidermal keratinocytes and dermal fibroblasts on skin homeostasis.

Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, *1867*(8), 118722. https://doi.org/10.1016/J.BBAMCR.2020.118722

Jiang, Y., Tsoi, L. C., Billi, A. C., Ward, N. L., Harms, P. W., Zeng, C., Maverakis, E., Michelle Kahlenberg, J., & Gudjonsson, J. E. (2020). Cytokinocytes: the diverse contribution of keratinocytes to immune responses in skin. *JCI Insight*, *5*(20). https://doi.org/10.1172/JCI.INSIGHT.142067

Johnson, K. E., & Wilgus, T. A. (2014). Vascular Endothelial Growth Factor and Angiogenesis in the Regulation of Cutaneous Wound Repair. *Https://Home.Liebertpub.Com/Wound*, *3*(10), 647–661. https://doi.org/10.1089/WOUND.2013.0517

Kato, Y., Iwata, T., Washio, K., Yoshida, T., Kuroda, H., Morikawa, S., Hamada, M., Ikura, K., Kaibuchi, N., Yamato, M., Okano, T., & Uchigata, Y. (2017). Creation and Transplantation of an Adipose-derived Stem Cell (ASC) Sheet in a Diabetic Wound-healing Model. *JoVE (Journal of Visualized Experiments)*, *2017*(126), e54539. https://doi.org/10.3791/54539

Lawrence, W. T. (1998). Physiology of the Acute Wound. *Clinics in Plastic Surgery*, *25*(3), 321–340. https://doi.org/10.1016/S0094-1298(20)32467-6

Lazarus, G. S., Cooper, D. M., Knighton, D. R., Margolis, D. J., Percoraro, R. E., Rodeheaver, G., & Robson, M. C. (1994). Definitions and guidelines for assessment of wounds and evaluation of healing. *Wound Repair and Regeneration*, *2*(3), 165–170. https://doi.org/10.1046/J.1524-475X.1994.20305.X

Lopez-Ojeda, W., Pandey, A., Alhajj, M., & Oakley, A. M. (2022). Anatomy, Skin (Integument). *StatPearls*. https://www.ncbi.nlm.nih.gov/books/NBK441980/

Miao, H., Wu, X. Q., Zhang, D. D., Wang, Y. N., Guo, Y., Li, P., Xiong, Q., & Zhao, Y. Y. (2021). Deciphering the cellular mechanisms underlying fibrosis-associated diseases and therapeutic avenues. *Pharmacological Research*, *163*, 105316. https://doi.org/10.1016/J.PHRS.2020.105316

Mieczkowski, M., Mrozikiewicz-Rakowska, B., Kowara, M., Kleibert, M., & Czupryniak, L. (2022). The Problem of Wound Healing in Diabetes—From Molecular Pathways to the Design of an Animal Model. *International Journal of Molecular Sciences 2022, Vol. 23, Page 7930*, *23*(14), 7930. https://doi.org/10.3390/IJMS23147930

Nauck, M., Karakiulakis, G., Perruchoud, A. P., Papakonstantinou, E., & Roth, M. (1998). Corticosteroids inhibit the expression of the vascular endothelial growth factor gene in human vascular smooth muscle cells. *European Journal of Pharmacology*, *341*(2–3), 309–315. https://doi.org/10.1016/S0014-2999(97)01464-7

Pesce, M., Patruno, A., Speranza, L., & Reale, M. (2013). Extremely low frequency electromagnetic field and wound healing: implication of cytokines as biological mediators. *European Cytokine Network*, *24*(1), 1–10. https://doi.org/10.1684/ECN.2013.0332

Pilcher, B. K., Wang, M., Qin, X. J., Parks, W. C., Senior, R. M., & Welgus, H. G. (1999). Role of Matrix Metalloproteinases and Their Inhibition in Cutaneous Wound Healing and Allergic Contact Hypersensitivity. *Annals of the New York Academy of Sciences*, *878*(1), 12–24. https://doi.org/10.1111/J.1749-6632.1999.TB07671.X

Ramasastry, S. S. (2005). Acute Wounds. *Clinics in Plastic Surgery*, *32*(2), 195–208. https://doi.org/10.1016/J.CPS.2004.12.001

Ravanti, L., & Kähäri, V. M. (2000). Matrix metalloproteinases in wound repair (review). *International Journal of Molecular Medicine*, *6*(4), 391–407. https://doi.org/10.3892/IJMM.6.4.391/HTML

Raziyeva, K., Kim, Y., Zharkinbekov, Z., Kassymbek, K., Jimi, S., & Saparov, A. (2021). Immunology of Acute and Chronic Wound Healing. *Biomolecules 2021, Vol. 11, Page 700*, *11*(5), 700. https://doi.org/10.3390/BIOM11050700

Richardson, M. (2004). Acute wounds: an overview of the physiological healing process. *Nursing Times*, *100*(4), 50–53. https://europepmc.org/article/med/14974265

Robson, M. C., Steed, D. L., & Franz, M. G. (2001). Wound healing: Biologic features and approaches to maximize healing trajectories. *Current Problems in Surgery*, *38*(2), A1-140. https://doi.org/10.1067/MSG.2001.111167

Rodrigues, M., Kosaric, N., Bonham, C. A., & Gurtner, G. C. (2019). Wound healing: A cellular perspective. *Physiological Reviews*, *99*(1), 665–706. https://doi.org/10.1152/PHYSREV.00067.2017/ASSET/IMAGES/LARGE/Z9J0041828900006.JPEG

Schultz, G. S., Chin, G. A., Moldawer, L., & Diegelmann, R. F. (2011). Principles of Wound Healing. *Diabetic Foot Problems*, 395–402. https://doi.org/10.1142/9789812791535_0028

Serra, F., Aielli, L., Costantini, E., Serra, F., Aielli, L., & Costantini, E. (2021). The role of miRNAs in the inflammatory phase of skin wound healing. *AIMS Allergy and Immunology 2021 4:264*, *5*(4), 264–278. https://doi.org/10.3934/ALLERGY.2021020

Servold, S. A. (1991). Growth factor impact on wound healing. *Clinics in Podiatric Medicine and Surgery*, *8*(4), 937–953. https://europepmc.org/article/med/1933739

Sieggreen, M. Y. (1987). Healing of Physical Wounds. *Nursing Clinics of North America*, *22*(2), 439–447. https://doi.org/10.1016/S0029-6465(22)01291-9

Skover, G. R. (1991). Cellular and biochemical dynamics of wound repair. Wound environment in collagen regeneration. *Clinics in Podiatric Medicine and Surgery*, *8*(4), 723–756.

Streit, M., Velasco, P., Riccardi, L., Spencer, L., Brown, L. F., Janes, L., Lange-Asschenfeldt, B., Yano, K., Hawighorst, T., Iruela-Arispe, L., & Detmar, M. (2000). Thrombospondin-1 suppresses wound healing and granulation tissue formation in the skin of transgenic mice. *The EMBO Journal*, *19*(13), 3272–3282. https://doi.org/10.1093/EMBOJ/19.13.3272

Sun, J., Zhang, J., Wang, X., Ji, F., Ronco, C., Tian, J., & Yin, Y. (2020). Gut-liver crosstalk in sepsis-induced liver injury. *Critical Care*, *24*(1), 1–8. https://doi.org/10.1186/S13054-020-03327-1/FIGURES/3

Szycher, M., & Lee, S. J. (1992). Modern Wound Dressings: A Systematic Approach to Wound Healing. *Journal of Biomaterials Applications*, *7*(2), 142–213. https://doi.org/10.1177/088532829200700204

Tu, H., Zhang, D., Barksdale, A. N., Wadman, M. C., Muelleman, R. L., & Li, Y. L. (2020). Dexamethasone Improves Wound Healing by Decreased Inflammation and Increased Vasculogenesis in Mouse Skin Frostbite Model. *Wilderness & Environmental Medicine*, *31*(4), 407–417. https://doi.org/10.1016/J.WEM.2020.07.003

Usui, M. L., Mansbridge, J. N., Carter, W. G., Fujita, M., & Olerud, J. E. (2008). Keratinocyte migration, proliferation, and differentiation in chronic ulcers from patients with diabetes and normal wounds. *Journal of Histochemistry and Cytochemistry*, *56*(7), 687–696. https://doi.org/10.1369/JHC.2008.951194/ASSET/IMAGES/LARGE/10.1369_JHC.2008.951194-FIG5.JPEG

Vanwijck, R. (2001). [Surgical biology of wound healing]. *Bulletin et Memoires de L'academie Royale de Medecine de Belgique*, *156*(3–4), 175–184; discussion 185. https://europepmc.org/article/med/11789398

Velnar, T., Bailey, T., & Smrkolj, V. (2009). The Wound Healing Process: an Overview of the Cellular and Molecular Mechanisms. *The Journal of International Medical Research*, *37*(5), 1528.

Wang, Y., & Graves, D. T. (2020). Keratinocyte function in normal and diabetic wounds and modulation by FoxO1. *Journal of Diabetes Research*, *2020*. https://doi.org/10.1155/2020/3714704

Werner, S., & Smola, H. (2001). Paracrine regulation of keratinocyte proliferation and differentiation. *Trends in Cell Biology*, *11*(4), 143–146. https://doi.org/10.1016/S0962-8924(01)01955-9

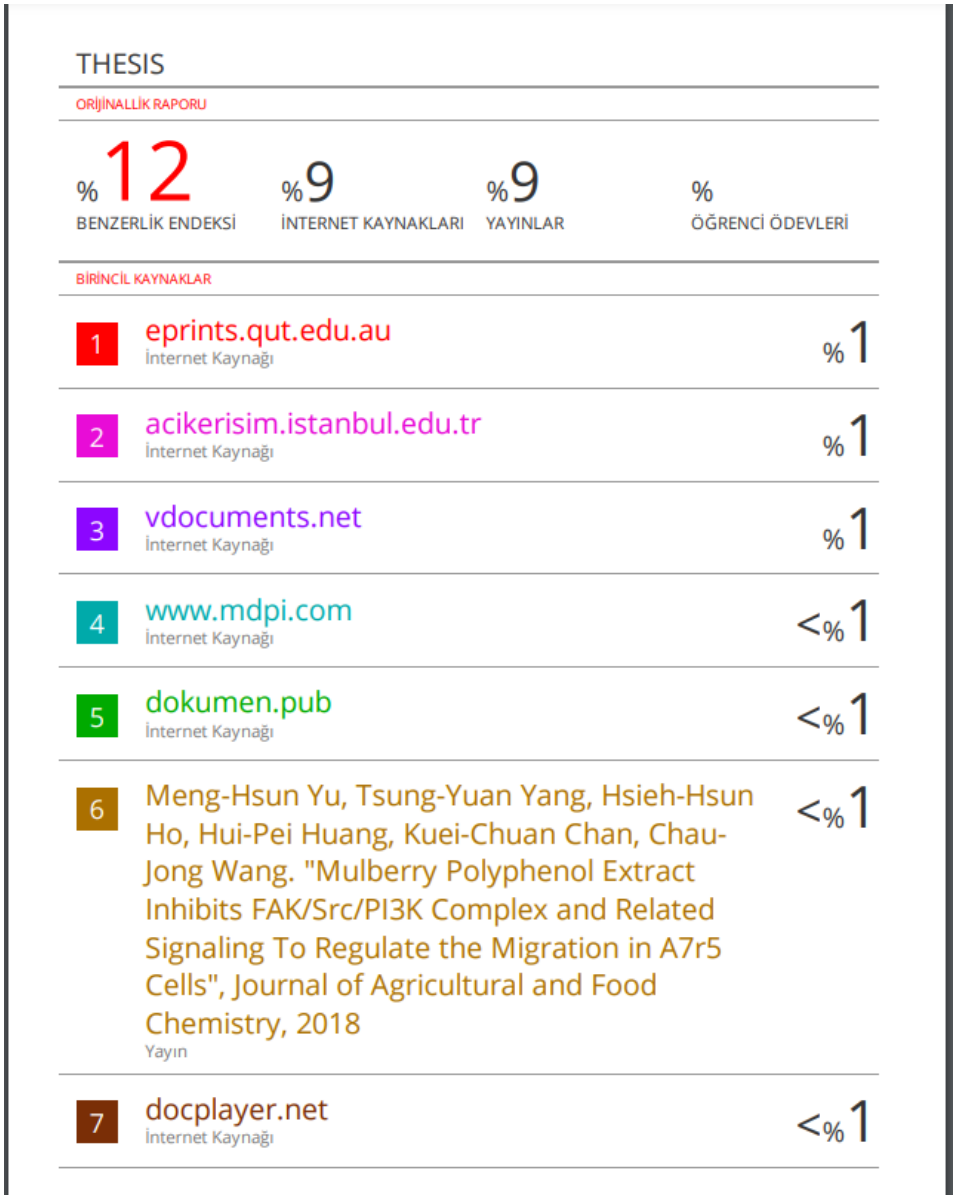
Wicke, C., Halliday, B., Allen, D., Roche, N. S., Scheuenestuhl, H., Spencer, M. M., Roberts, A. B., & Hunt, T. K. (2000). Effects of Steroids and Retinoids on Wound Healing. *Archives of Surgery*, *135*(11), 1265–1270. https://doi.org/10.1001/ARCHSURG.135.11.1265

Wilkinson, H. N., & Hardman, M. J. (2020). Wound healing: cellular mechanisms and pathological outcomes: Cellular Mechanisms of Wound Repair. *Open Biology*, *10*(9). https://doi.org/10.1098/RSOB.200223/

Woodley, D. T., Wysong, A., DeClerck, B., Chen, M., & Li, W. (2015). Keratinocyte Migration and a Hypothetical New Role for Extracellular Heat Shock Protein 90 Alpha in Orchestrating Skin Wound Healing. *Advances in Wound Care*, *4*(4), 203. https://doi.org/10.1089/WOUND.2014.0566

Xiao, T., Yan, Z., Xiao, S., & Xia, Y. (2020). Proinflammatory cytokines regulate epidermal stem cells in wound epithelialization. *Stem Cell Research and Therapy*, *11*(1), 1–9. https://doi.org/10.1186/S13287-020-01755-Y/TABLES/1

Appendix
Turnitin Report



THESIS

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5	dokumen.pub İnternet Kaynağı	<%1
6	Meng-Hsun Yu, Tsung-Yuan Yang, Hsieh-Hsun Ho, Hui-Pei Huang, Kuei-Chuan Chan, Chau-Jong Wang. "Mulberry Polyphenol Extract Inhibits FAK/Src/PI3K Complex and Related Signaling To Regulate the Migration in A7r5 Cells", Journal of Agricultural and Food Chemistry, 2018 Yayın	<%1
7	docplayer.net İnternet Kaynağı	<%1

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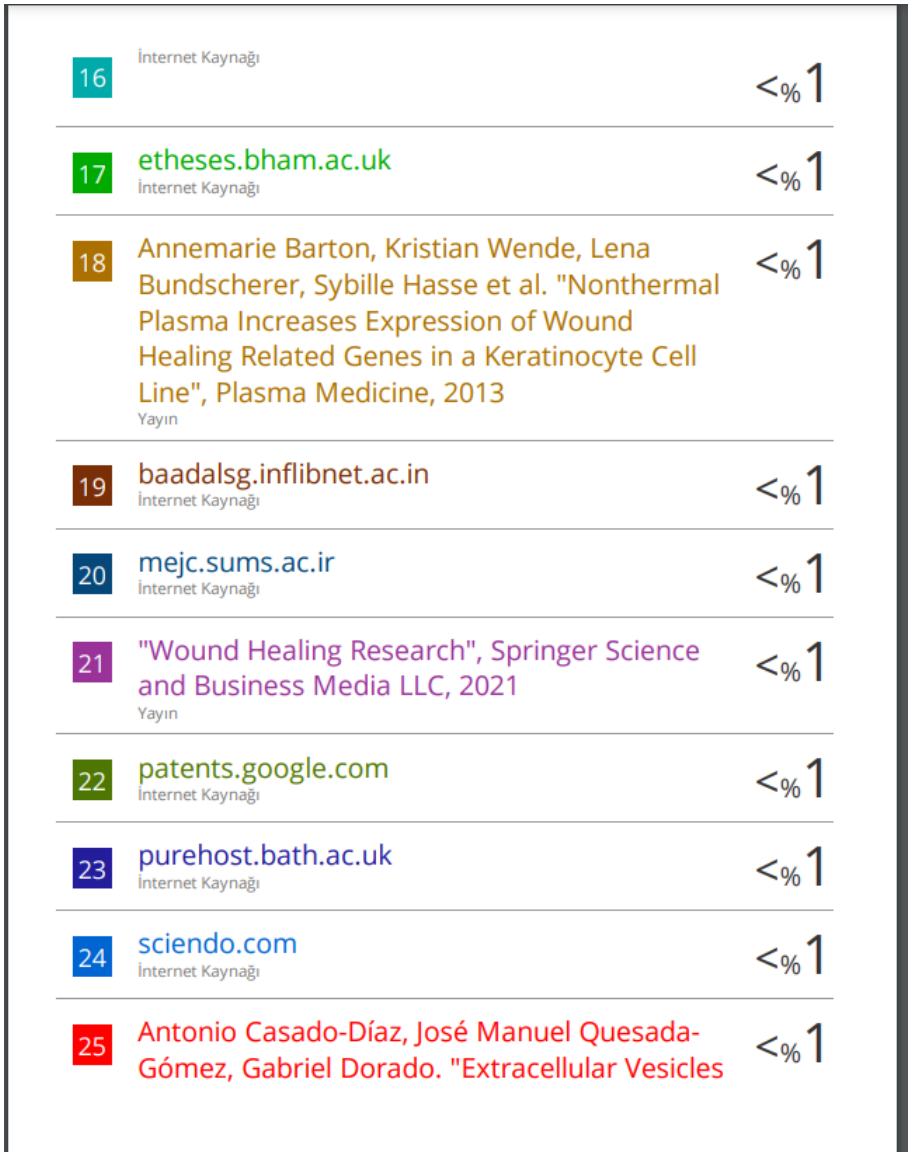
9	d-nb.info İnternet Kaynağı	<%1
10	www.hindawi.com İnternet Kaynağı	<%1
11	Hongfei Yu, Jinliang Peng, Yuhong Xu, Jiang Chang, Haiyan Li. "Bioglass Activated Skin Tissue Engineering Constructs for Wound Healing", ACS Applied Materials & Interfaces, 2016 Yayın	<%1
12	iv.iiarjournals.org İnternet Kaynağı	<%1
13	www.ncbi.nlm.nih.gov İnternet Kaynağı	<%1
14	H. Seda Vatansever, Elgin Turkoz Uluer, Hasan Aydede, M. Kemal Ozbilgin. "Analysis of transferred keratinocyte-like cells derived from mouse embryonic stem cells on experimental surgical skin wounds of mouse", Acta Histochemica, 2013 Yayın	<%1
15	discovery.ucl.ac.uk İnternet Kaynağı	<%1
	www.science.gov	



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18	Annemarie Barton, Kristian Wende, Lena Bundscherer, Sybille Hasse et al. "Nonthermal Plasma Increases Expression of Wound Healing Related Genes in a Keratinocyte Cell Line", Plasma Medicine, 2013 Yayın	<%1
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24	sciendo.com İnternet Kaynağı	<%1
25	Antonio Casado-Díaz, José Manuel Quesada-Gómez, Gabriel Dorado. "Extracellular Vesicles	<%1

	Derived From Mesenchymal Stem Cells (MSC) in Regenerative Medicine: Applications in Skin Wound Healing", Frontiers in Bioengineering and Biotechnology, 2020 Yayın	
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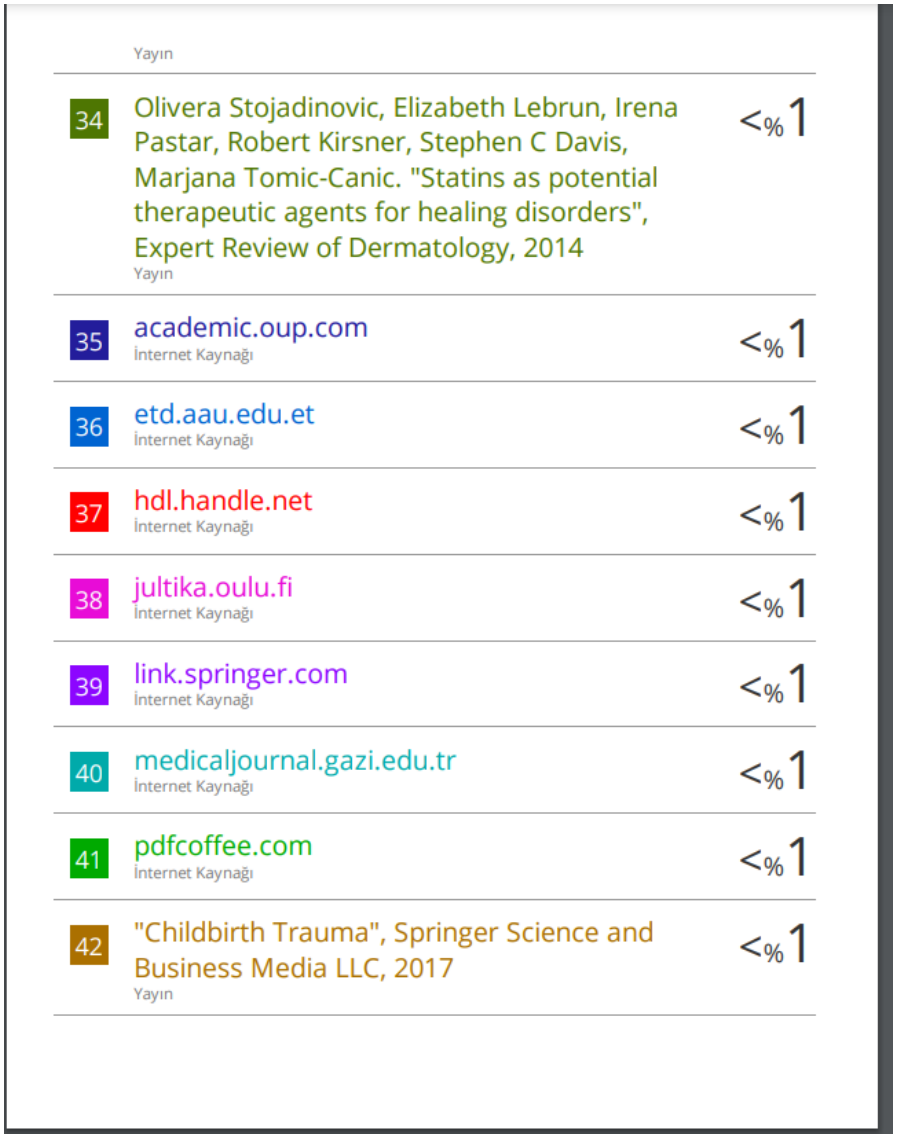
30	repositorium.uni-muenster.de İnternet Kaynağı	<%1
31	rupress.org İnternet Kaynağı	<%1
32	www.researchgate.net İnternet Kaynağı	<%1
33	Huiyin Tu, Dongze Zhang, Michael C. Wadman, Yu-Long Li. "Dexamethasone ameliorates recovery process of neuromuscular junctions after tourniquet-induced ischemia-reperfusion injuries in mouse hindlimb", European Journal of Pharmacology, 2020	<%1



	Yayın	
34	Olivera Stojadinovic, Elizabeth Lebrun, Irena Pastar, Robert Kirsner, Stephen C Davis, Marjana Tomic-Canic. "Statins as potential therapeutic agents for healing disorders", Expert Review of Dermatology, 2014 Yayın	<%1
35	academic.oup.com İnternet Kaynağı	<%1
36	etd.aau.edu.et İnternet Kaynağı	<%1
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40	medicaljournal.gazi.edu.tr İnternet Kaynağı	<%1
41	pdfcoffee.com İnternet Kaynağı	<%1
42	"Childbirth Trauma", Springer Science and Business Media LLC, 2017 Yayın	<%1

43	"Epidermal Cells", Springer Science and Business Media LLC, 2020 Yayın	<%1
44	JinPing Fu, Yue Zhang, Jing Chu, Xiao Wang, WenXia Yan, Qiong Zhang, HanPing Liu. "Reduced Graphene Oxide Incorporated Acellular Dermal Composite Scaffold Enables Efficient Local Delivery of Mesenchymal Stem Cells for Accelerating Diabetic Wound Healing", ACS Biomaterials Science & Engineering, 2019 Yayın	<%1
45	Nasser Ebrahimpour, Mehrnaz Mehrabani, Maryam Iranpour, Zeinab Kordestani et al. "The efficacy of a traditional medicine preparation on second-degree burn wounds in rats", Journal of Ethnopharmacology, 2020 Yayın	<%1
46	Vanessa Araújo Varela, Letícia Borges da Silva Heinen, Luciana Cavalheiro Marti, Victória Bulcão Caraciolo et al. "In vitro differentiation of myeloid suppressor cells (MDSC-like) from an immature myelomonocytic precursor THP-1", Journal of Immunological Methods, 2023 Yayın	<%1
47	ediss.uni-goettingen.de İnternet Kaynağı	<%1

48	epub.ub.uni-greifswald.de İnternet Kaynağı	<%1
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50	ro.uow.edu.au İnternet Kaynağı	<%1
51	theses.ncl.ac.uk İnternet Kaynağı	<%1
52	www.benthamopen.com İnternet Kaynağı	<%1
53	www.frontiersin.org İnternet Kaynağı	<%1
54	"Wound Healing", Wiley, 2018 Yayın	<%1
55	Bharat Bhushan, Huiwen Liu. "Chapter 18 Self-Assembled Monolayers for Controlling Adhesion, Friction and Wear", Springer Science and Business Media LLC, 2005 Yayın	<%1
56	S Kanda. "In vitro differentiation of hepatocyte-like cells from embryonic stem cells promoted by gene transfer of hepatocyte nuclear factor 3 β", Hepatology Research, 2003 Yayın	<%1

57	discovery.dundee.ac.uk İnternet Kaynağı	<%1
58	"Chronic Wounds, Wound Dressings and Wound Healing", Springer Science and Business Media LLC, 2021 Yayın	<%1
59	A. N. Otterço, P. Brassolatti, A. L. M. Andrade, L. R. S. Avó, P. S. Bossini, N. A. E. Parizotto. "Effect of photobiomodulation (670nm) associated with vitamin A on the inflammatory phase of wound healing", Lasers in Medical Science, 2018 Yayın	<%1
60	Carolina Manosalva, Pablo Alarcón, Karina González, Jorge Soto et al. "Free Fatty Acid Receptor 1 Signaling Contributes to Migration, MMP-9 Activity, and Expression of IL-8 Induced by Linoleic Acid in HaCaT Cells", Frontiers in Pharmacology, 2020 Yayın	<%1
61	Irena Pastar, Olivera Stojadinovic, Natalie C. Yin, Horacio Ramirez et al. "Epithelialization in Wound Healing: A Comprehensive Review", Advances in Wound Care, 2014 Yayın	<%1
62	Navid Hosseini Mansoub, Mehmet Gürdal, Elif Karadadaş, Hilal Kabadayi, Seda Vatansever,	<%1

Gulinnaz Ercan. "The role of PRP and adipose tissue-derived keratinocytes on burn wound healing in diabetic rats", BioImpacts, 2017
Yayın

63 Tuna Onal, Yildiz Uyar, H. Seda Vatansever. "Evaluation of Oocyte Maturation in Experimental PCOS Model", Research Square Platform LLC, 2022 <%1
Yayın

64 Xin Zhou, Yanling Guo, Kun Yang, Peng Liu, Jun Wang. "The signaling pathways of traditional Chinese medicine in promoting diabetic wound healing", Journal of Ethnopharmacology, 2021 <%1
Yayın

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ETHICAL APPROVAL

T.C.
Manisa Celal Bayar Üniversitesi
Hayvan Deneyleri Yerel Etik Kurulu

KARAR TARİH / NO	16/ 03 / 2021 / 77.637.435
ARAŞTIRMANIN ADI	Uzun Süreli Steroid Bankın Altındaki Farelerde Oluşturulan Yara Modelinde Fare Embriyonik Kök Hücrelerinden Farklılaştırılan Keratinositlerin Yara İyileşmesi Üzerine Olan Etkisi
SORUMLU ARAŞTIRMACI	Prof. Dr. Hasan Aydede - Genel Cerrahi Anabilim Dalı
ARAŞTIRMA EKİBİ	Araş.Gör. Bayda Gençer,- Prof.Dr. H.Seda Vatansever,- Araş. Gör. Damla AKOĞULLARI
ARAŞTIRMANIN NİTELİĞİ	UZMANLIK TEZİ ☒ YÜKSEK LİSANS–DOKTORA TEZİ ☐ AKADEMİK AMAÇLI ☐ Eğitim ☐
DEĞERLENDİRİLEN BELGELER	11 / 12 / 2020 / Tarih ve 40422 sayılı; araştırma dosyası
KARAR BİLGİLERİ	Araştırma dosyası incelenmiş, bilimsel ve etik açıdan **UYGUN** olduğuna oy birliği ile karar verilmiştir.

Unvanı/Adı/Soyadı		Araştırma İle İlgisi Olan Üye	Toplantıya Katılmayan Üye	Unvanı /Adı /Soyadı		Araştırma İle İlgisi Olan Üye	Toplantıya Katılmayan Üye
Prof. Dr. Ercüment ÖLMEZ		☐	☐	Prof. Dr. Gökhan TEMELTAŞ		☐	☐
Prof. Dr. İsmet TOPÇU Anestezi ve Reanimasyon AD		☐	☐	Prof. Dr. Özge YILMAZ Çocuk Hastalıkları AD.	—	☐	☒
Prof. Dr. Ertuğrul TATLISUMAK Anatomi AD		☐	☐	Dr. Öğr. Üyesi Fulya OCAK – Veteriner Hekim		☐	☐
Prof. Dr. Kıvanç GÜNHAN (DEHAM MD)	—	☐	☒	Saime ÖZKARA Sivil Toplum Üyesi	—	☐	☒
Dr. Öğr. Üyesi Selim ALTAN Tıp Tarihi ve Etik AD		☐	☐	Levent ÖDEMİŞ Sivil Üye		☐	☐

Etik Kurulumuzun kararı yukarda belirtilmiştir. Araştırma Başvuru Formunun Taahhütname kısmında belirtilmiş olan hususların dikkate alınarak istenilen bilgilerin Etik Kurulumuza zamanında iletilmesi konusunda bilgilerinizi ve gereğini rica ederim.

Ercüment ÖLMEZ
Başkan

CURRICULUM VITAE

1. PERSONAL INFORMATIONS

NAME, SURNAME: DATE OF BIRTH AND PLACE:	**Melis KALAYCI** 26.02.1998/İSTANBUL
POSITION: Student/Research Assistant ADRESS: Near East University Faculty of Medicine / KKTC PHONE: 0-533-829-82-99 E-MAIL: melis.kalayci@neu.edu.tr	

2. EDUCTAION

YEAR	DEGREE	ÜNİVERSİTE	FACULTY
2016-2020	BSc	Near East University	Molecular Biology and Genetics
2020-2022	MSc	Near East University	Medical Biology and Genetics

3. ACADEMIC EXPERIENCE

YEAR	TITLE	DEPARTMENT	UNIVERSITY
2018	Intern	Department of Medical Genetics, Molecular and Cytogenetic Lab.	Near East University
Jul 2019-Sept 2019	Intern	Department of Molecular Medicine, Aziz Sancar Institute of Experimental Medicine	Istanbul University-DETAM
Jan 2020- Feb 2020	Intern	Neuro-oncology, R.B.Darnell Lab.	The Rockefeller University
Sept 2020-March 2022	Full Time	Department of Medical Genetics, SARS-CoV-2 Lab	Near East University

Dec 2020-..	Full Time Research Assistant	DESAM-Kit Production and Genome Analysis Lab.	Near East University

4. RESEARCH INTERESTS

RESEARCH INTERESTS	KEY WORDS
Medical Genetics	Molecular Genetics, Epigenetics, Molecular Cytogenetics, Cancer Genetics, Infertility

5. PUBLICATION IN LAST 5 YEARS

1. Ergoren, M. C., Komurcu, K., Tuncel, G., Akan, G., Ozverel, C. S., Dalkan, C., **Kalayci, M**., & Sanlıdag, T. (2022). Impact of SARS-CoV-2 Delta and Omicron variants on viral burden and cycle threshold in BNT162b2-vaccinated 12-18 years group. *Brazilian journal of microbiology : [publication of the Brazilian Society for Microbiology]*, *53*(4), 1937–1940. https://doi.org/10.1007/s42770-022-00820-3