NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF MEDICAL BIOLOGY

MOLECULAR MEDICINE PROGRAM

The Comparison of IL-11 gene expression analyzes in the use of cold atmospheric

Nitric Oxide (NO) gas alone and/or with NPH insulin cream in healing wounds with tissue loss in diabetic rats.

MASTER THESIS IN MOLECULAR MEDICINE

M.Sc. THESIS

BERKE IBRAHIM ERSOY

Nicosia

JULY , 2024

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Supervisor

Assoc. Prof. Dr. Mahmut Çerkez Ergören

Nicosia

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APPROVAL

We certify that we have read the thesis submitted by BERKE IBRAHIM ERSOY titled, " **The Comparison of IL-1 gene expression analyzes in the use of cold atmospheric Nitric Oxide (NO) gas alone and/or with NPH insulin cream in healing wounds with tissue loss in diabetic rats**' and that in our combined opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Educational Sciences.

Supervisor: Assoc. Prof. Dr. MAHMUT ÇERKEZ ERGOREN

Examining Committee	Name-Surname	Signature
Head of the Committee:	Dr. HAVVA ÇOBANOĞULLARI	.....
Committee Member*:		
Dr. ALI ÇURUKOĞLU		.....
Supervisor: Assoc. Prof. Dr. MAHMUT ÇERKEZ ERGOREN		.....

Approved by the Head of the Department

31/07/2024

Prof. Selma Yilmaz

Head of Department

Approved by the Institute of Graduate Studies

31/07/2024

Prof. Dr. Kemal Hüsnü Can Başer

Head of the Institute

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 original to this study

BERKE IBRAHIM ERSOY

31/07/2024

Acknowledgements

I want to start by saying thank you to my family for all of their help and encouragement when I was writing my thesis. I would like to thank all scientists for their expertise and technological opportunities that have enabled me to finish my thesis.

My sincere appreciation also goes out to **Assoc.Prof.Dr MAHMUT ÇERKEZ ERGÖREN** for their help all the requirement during the experaments either primer and samples and also encouragement during the entire thesis writing process.

I should also say that I could not have advanced scientifically without the knowledge they provided, and that finishing my thesis would have been extremely challenging without them. To sum up, I would want to express my gratitude to each one of my molecular medicine department lecturers for igniting my love of science and for sharing their expertise with me.

I must acknowledge my family, who have been my backbone, their trust, support, and patience in every challenge or joy in life have been the bedrock of my success Particularly my dear father Arhun Ersoy and my mother Deniz Ersoy and my brother Emre Ersoy, for his tireless support and significant contribution of my educational journey.

ABSTRACT

The Comparison of IL-11 gene expression analyzes in the use of cold atmospheric Nitric Oxide (NO) gas alone and/or with NPH insulin cream in healing wounds with tissue loss in diabetic rats.

M.Sc., Department of molecular medicine

JULY, 2024, 62 Pages

Diabetes mellitus (DM) is a widespread and growing global health concern, marked by persistent hyperglycemia due to impaired insulin production or action. According to the International Diabetes Federation, the prevalence of DM among individuals aged 20–79 reached 415 million in 2015, with projections indicating an increase to 615 million by 2040. This condition is linked with various severe complications, including microvascular and macrovascular diseases, which exacerbate health problems and pose significant risks to patients' well-being. One of the critical challenges faced by diabetic individuals is impaired wound healing, often leading to chronic wounds with tissue loss. Such wounds increase the risk of infections, amputations, and mortality, highlighting the urgent need for effective therapeutic strategies to promote wound repair and tissue regeneration.

Recent advances in biomedical research have introduced promising treatments for enhancing wound healing in diabetic patients. Among these, cold atmospheric nitric oxide (NO) gas and NPH insulin cream have emerged as potential therapeutic modalities. Cold atmospheric NO gas is known for its antimicrobial properties and its ability to modulate various cellular processes crucial for wound repair, while NPH insulin cream harnesses the regenerative effects of insulin to stimulate cellular proliferation and angiogenesis within the wound environment. Despite their individual efficacy, limited research has explored their combined impact on wound healing, particularly concerning the expression of key cytokines involved in the repair process.

Interleukin-11 (IL-11), a multifunctional cytokine, plays a pivotal role in wound healing by regulating inflammation, cell proliferation, and tissue remodeling. IL-11 promotes fibroblast proliferation, collagen synthesis, and angiogenesis, essential processes for effective tissue repair. This study seeks to fill the gap in knowledge by investigating the comparative effects of cold atmospheric NO gas alone and in combination with NPH insulin cream on IL-11 gene

expression in diabetic rats with tissue loss. By elucidating how these therapeutic modalities influence IL-11 expression, the study aims to provide insights into their mechanisms of action and potential synergistic effects.

The research will involve a detailed analysis of IL-11 gene expression in diabetic rats subjected to different treatment regimens, including cold atmospheric NO gas alone, NPH insulin cream alone, and a combination of both. Understanding the molecular mechanisms underlying these interventions will contribute to the optimization of wound healing strategies in diabetic patients, ultimately enhancing therapeutic approaches for managing chronic wounds associated with diabetes mellitus.

Keywords: *cold atmospheric plasma, diabetic wound healing, nitric oxide, diabetic rat, IL-11*

LIST OF ABBREVIATIONS

IL-11:Interleukin-11

NO:nitric oxide

NPH: Neutral Protamine Hagedorn

DNA: Deoxyribonucleic acid.

PCR:polymerase chain reaction.

RNA: Ribonucleic acid.

cDNA:complementary DNA.

RNS: Reactive nitrogen species

ROS:reactive oxygen species

Ct: Cycle Threshold

qPCR: Quantitative polymerase chain reaction

CAP: Cold atmospheric plasma

VEGFA:vascular endothelial growth factor A

VEGFR2: Vascular endothelial growth factor receptor — 2

JAK/STAT: Janus kinase (JAK)/signal transducer and activator of transcription

(TGFβ): Transforming growth factor-beta

TNF-α: Tumor necrosis factor alpha

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Chapter 1

Introduction

Persistent metabolic disease referred to as DM can be identified by continuous hyperglycemia. It might be caused by reduced manufacturing of insulin, resistance to insulin's peripheral impacts, or combined. In 2015, the number of people who were diagnosed with DM in the age bracket of 20-79 was roughly calculated by the International Diabetes Federation (IDF) to be 415 million. While this number is projected to grow to an extra 200 million by 2040, diabetes has been demonstrated to be a worldwide health concern.Those suffering from diabetes mellitus, prolonged high blood sugar levels may aggravate other disorders of metabolism and compromise numerous organs. This can result in fatal and crippling problems with health, the most widespread being microvascular, which includes nephropathy, neuropathy, and macrovascular causing higher likelihood of heart disorders with a fold of 2 to 4. (Rajeev Goyal,2023)(de Sousa-Uva,2016)

Based on its origin and clinical manifestation, diabetes mellitus can be separated into the following three primary categories: gestational diabetes (GDM), type 1 diabetes, and type 2 diabetes. Monogenic diabetes and secondary diabetes are two more, less prevalent forms of the disease. Diabetes mellitus, poses a significant challenge to wound healing processes. Among the myriad complications associated with diabetes, impaired wound healing often leads to chronic wounds characterized by tissue loss, increasing the risk of infections, amputations, and mortality. Addressing this critical issue necessitates exploring innovative therapeutic approaches that can effectively promote wound repair and tissue regeneration in diabetic individuals. (Rajeev Goyal,2023)(Sapra, 2023)

Recent advancements in biomedical research have highlighted the potential of cream of NPH insulin and cold atmospheric nitric oxide (NO) gas as promising modalities for enhancing healing of wounds in diabetic patients. Cold atmospheric NO gas, known for its antimicrobial properties and regulatory effects on various cellular processes involved in wound repair, has emerged as a novel therapeutic agent. Similarly, NPH insulin cream,

harnessing the regenerative potential of insulin, offers a targeted approach to stimulate cellular proliferation and angiogenesis within the wound microenvironment.

Central to the complex cascade of events underlying wound healing is the cytokine interleukin-11 (IL-11), which play pivotal roles in orchestrating the intricate interplay between inflammation, cell proliferation, and tissue remodeling. IL-11, a multifunctional cytokine, promotes fibroblast proliferation, collagen synthesis, and angiogenesis, thereby facilitating tissue repair.

While the individual roles of cream of NPH insulin and cold atmospheric NO gas in healing of wound have been explored to some extent, limited research has investigated their combined effects on the expression of IL-11 gene in diabetic wounds with tissue loss. Understanding how these therapeutic modalities modulate the expression of key cytokines involved in wound healing is essential for elucidating their mechanisms of action and optimizing treatment strategies.

Therefore, this master thesis aims to comprehensively investigate how the expression of the IL-11 gene is affected in diabetic rats who have wounds by cold atmospheric NO gas alone and in conjunction with NPH insulin cream. By elucidating the molecular processes that underpins the therapeutic efficacy of these interventions, this study seeks aid in the creation of focused strategies for enhancing wound healing in diabetic individuals.

Chapter 2

Diabetes mellitus presents a significant challenge in wound healing, often leading to chronic wounds with tissue loss. Cold atmospheric nitric oxide (NO) gas has emerged as a promising therapeutic modality due to its antimicrobial and wound healing properties. Additionally, NPH insulin cream has shown potential in promoting wound healing by enhancing cellular proliferation and angiogenesis. This literature review aims to compare the efficacy of utilizing cold atmospheric NO gas alone and in conjuction with NPH insulin cream in wounds that are healing with tissue loss in rats with diabetes, focusing on the expression analysis of interleukin-11 (IL-11)gene, a crucial cytokine involved in wound healing.

2.1Molecular biology of IL-11

A cytokine called interleukin-11 (IL-11) is important in maintaining tissue homeostasis and controlling immunological responses. Understanding their molecular biology is essential for elucidating their roles in health and disease.

IL-11: Multifunctional cytokine interleukin 11 (IL-11) may be related to controlling the expansion and development of cells in the lymphoid and hematopoietic systems. A human cell line of fetal lung fibroblast is utilized to duplicate the human cDNA, and IL-11 function was first found in the media with the conditioning of a monkey cell line of bone marrow stromal. Adipogenesis, proliferation and differentiation of megakaryocyte progenitor cell, hepatocyte acute phase protein synthesis stimulation, growth of erythroid progenitor cell, maturation of B lymphocyte, and lymphohematopoietic stem cell proliferation are all influenced by the purified protein's multifunctional activity. IL-11 is distinct from other proteins at the molecular level because it lacks residues of cysteine and glycosylation sites that are asparagine-linked. (Steven Neben,1996)(Metcalfe et al.,2023)

The receptors foroncostatin M (OSM),leukemia inhibitory factor (LIF), IL-6, and ciliary neurotrophic factor (CNTF) are included in a receptor family which, has IL-11 receptors as a member. Following ligand binding, these receptors can all interact withgp130 which is a receptor for signal transduction. Preclinical models have shown promise in treating thrombocytopenia and, in certain situations, neutropenia with IL-11; trials are being

conducted to validate IL-11's clinical use in treating myelosuppression brought on by chemotherapy and bone marrow transplantation for cancer. (Steven Neben,1996)

Molecular Mechanisms and Biological Functions Multipurpose cytokine IL-11 has a range of biological activities across different tissues and kinds of cells. At the molecular level, a receptor complex, which is made up of the gp130 signal transducer and the IL-11 receptor alpha subunit (IL-11Rα) used for the signalling of IL-11 through the complex of receptors of IL-11. Upon ligand binding, IL-11 induces the stimulation of Janus kinase (JAK) and signal transducer and activator of transcription (STAT) signaling pathways(notably JAK2/STAT3), leading to the transcriptional regulation of target genes.IL6 communicates in a comparable manner, but it's important to note that IL6R plus IL11RA are activated on distinct kinds of cell, with stromal cells—such as fibroblasts, vascular smooth muscle cells (VSMCs), and adipocytes—expressing IL11RA at a higher level than immune cells. (Fung et al.2022)

IL-11 plays crucial roles in tissue homeostasis, hematopoiesis, and bone metabolism. In the bone marrow microenvironment, IL-11 promotes the differentiation and survival of hematopoietic progenitor cells, contributing to platelet production and thrombopoiesis. Additionally, IL-11 has anti-inflammatory properties and modulates immune responses by stimulating the regulatory T cell differentiation and suppressing the creation of cytokines that are pro-inflammatory. Furthermore, Furthermore, IL-11 has a part in both the renewal and repair of tissue. It stimulates the fibroblasts and epithelial cells expansion, enhances collagen synthesis, and promotes angiogenesis, thereby facilitating regeneration of tissues and healing of wounds. IL-11 signaling dysregulation has been suspected in numerous pathological conditions, counting inflammatory diseases, cancer, and fibrosis.(Fung et al.2022)(Hu et al.,1993)

IL-11 is a key regulator of immune responses and tissue homeostasis, exerting diverse biological functions in various physiological and pathological contexts. Elucidating the molecular mechanisms underlying IL-11 signaling pathway is essential for understanding Their significance in both health and illness and the creation of focused therapeutic interventions for immune-related disorders and inflammatory conditions. Morestudy is warranted to examine the therapeutic potential of modulating IL-11 signaling pathway in the therapy of autoimmune diseases, inflammatory disorders, and cancer. (Widjaja et al.,2021)

2.2.A. Type 2 Diabetes Mellitus

A disorder of metabolism called type 2 diabetes elevates levels of blood sugar. The severity of diabetes may differ tremendously: although some people regulate their medical condition efficiently others ultimately develop deeper medical problems as an outcome of their diabetes. (Sapra, 2023)

Diabetes is mainly separated into two types: childhood or adolescence usually is the time of onset for diabetes type 1. Either inadequate or no insulin creation is the cause of this illness due to an injury to the pancreas. (Sapra, 2023)

Although the pancreas manufactures insulin, ultimately it loses the capacity to receive and utilize it, this reason makes type 2 diabetes distinct from type 1. Because type 2 diabetes typically manifests later in life, it was formerly called as "adult-onset" diabetes. Contrary to type 1 diabetes, the frequency of type 2 diabetes is way more greater. 9 out of 10 diabetic people are impacted by type 2 diabetes. (Sapra, 2023)

Typically, type 2 diabetes strikes in the later part of life. Your blood sugar levels rise as a result of this illness. Sometimes, altering your diet and increasing your physical activity might already have a significant impact. Insulin injections or oral therapy are options if that proves insufficient. Diabetes may have long-term consequences, such as issues with the heart, kidneys, eyes, or feet. (Sapra, 2023)

Symptoms

Unmanaged type 2 diabetes results in consistently elevated blood sugar levels. Since the condition mayoccur gradually over several years without symptoms that are appearent the identification of type 2 diabetes can sometimes be unanticipated. Nevertheless over a protracted period of elevated blood sugar, these symptoms progressively manifest: (Overview: Type 2 diabetes, 2023)

- excessive thirst
- recurring urination
- Weary, weary, and without motivation
- difficulty focusing
- emesis

- lightheadedness
- issues with erection
- confusion, drowsiness, or
- diabetes coma

Causes

Adequate sugar or glucose from our diet is acquired by our organs and is guaranteed by our metabolism via equal distribution around the body. For this mechanism to be carried out efficiently, a hormone called insulin is essential. Its point of origin is the pancreas. The elevation of blood sugar levels occurs when insulin is secreted into the circulation. Muscle and liver cells take up glucose by the help of insulin. The glucose in the blood can not be effectively processed if this function of insulin is suspended. A rise in blood glucose levels results from this.Hyperglycemia is a term in medicine given to describe excessively elevated glucose concentrations. (Overview: Type 2 diabetes, 2023)

In The pancreas generates adequate insulin in individuals with type 2 diabetes, but it does not anymore influence the body's organs. This is known by medical professionals as "insulin resistance." For a time frame, insulin is created by the pancreas to deal with this. However, eventually, it becomes unable to sustain itself, which causes the levels of glucose to begin to climb. (Overview: Type 2 diabetes, 2023)

Effects

Individuals diagnosed with type 2 diabetes are more susceptible to the following issues of health:

- Heart attacks, strokes, and circulation issues in the legs and feet (peripheral arterial disease, or PAD) are examples of cardiovascular disorders. These kinds of problems are referred to as complications of macrovascular disease. The term "macrovascular" refers to their impact on the bigger blood arteries. Those with high blood pressure are more at risk of acquiring them. (Overview: Type 2 diabetes, 2023)

- Renal, nerve, and ocular damage are examples of microvascular issues resulting from diabetes. The reference of "Microvascular" means impacting the smallest blood vessels. Over time, this may cause your vision to deteriorate. Microvascular injury is more likely to result in health issues in persons who have type 2 diabetes early in life, such as around age 50. Individuals with diabetes who acquire it later in life are less likely to experience these issues. (Overview: Type 2 diabetes, 2023)

- In the case of diabetic foot, the foot's nerve degeneration is so severe that the discomfort is barely perceptible.. The blood flow to the legs and feet is also inadequate. Then, injuries such as blisters and bruises can quickly result in poorly healing wounds. A wound may lose part of its tissue if treatment is delayed.(Overview: Type 2 diabetes, 2023)

2.2.B.Pathophysiology of Diabetic Wounds

Elevated blood sugar levels and compromised insulin production are typification of diabetes mellitus, a complex disease of metabolism, leading to a myriad of complications, including diabetic wounds. Comprehending the pathophysiology of diabetic wounds is crucial for formulating effective treatmentapproaches to mitigate their impact on patient outcomes. We need to explore the multifactorial mechanisms underlying diabetic wound pathophysiology, focusing on the interplay between hyperglycemia, oxidative stress, impaired angiogenesis, inflammation, and impaired extracellular matrix (ECM) remodeling. (Yadav et al.,2024)

Hyperglycemia and Oxidative Stress

Hyperglycemia, a hallmark feature of diabetes, contributes to the development and progression of diabetic wounds through multiple pathways. The generation of advanced glycation end-products (AGEs) is allowed by elevated glucose levels, leading to endothelial dysfunction, impaired angiogenesis, and increased oxidative stress. Oxidative stress is defined as a conflict among antioxidant defense systems and the emergence of reactive oxygen species (ROS), exacerbates tissue damage and impairs wound healing processes in diabetic individuals. (Joel M Raja,2023)

Impaired Angiogenesis

In wound healing, a vital event is called angiogenesis which is the establishment of fresh vascularate from pre-existing blood vessels. In diabetic wounds, impaired angiogenesis results from endothelial malfunction, lowered vascular endothelial growth factor (VEGF) activation, and abnormal signaling pathways. Decreased microvascular density and altered angiogenic responses compromise blood flow delivery to the wound site, leading to ischemia and delayed wound healing. (Joel M Raja,2023)

Inflammation and Immune Dysfunction

Chronic inflammation is a hallmark feature of diabetic wounds, characterized by dysregulated immune responses and prolonged inflammatory signaling. Persistent hyperglycemia initiates the stimulation of pro-inflammatory pathways, enabling immune cells to be directed to the site of injury, including neutrophils, T cells, and macrophages. Dysfunctional immune cell phenotypes, impaired phagocytosis, and prolonged inflammatory cytokine production contribute to tissue damage, delayed healing, and increased susceptibility to infections in diabetic wounds. (Joel M Raja,2023)(Worsley et al.,2023)

Impaired Extracellular Matrix (ECM) Remodeling

The ECM provides structural support and regulates cellular behavior in the wound microenvironment. In diabetic wounds, alterations in ECM composition, remodeling dynamics, and mechanical properties impair tissue regeneration and repair processes. Reduced synthesis of collagen and other ECM components, increased matrix metalloproteinase (MMP) activity, and aberrant cross-linking of collagen fibers disrupts the integrity of the wound bed and impairs wound closure. (Huang, 2020)

The pathophysiology of diabetic wounds is multifaceted, involving complex interactions between hyperglycemia-induced metabolic disturbances, oxidative stress, impaired angiogenesis, chronic inflammation, and dysfunctional ECM remodeling. Targeting these underlying mechanisms through multifaceted therapeutic approaches, including wound debridement, topical agents, growth factors, and advanced wound care modalities, holds promise for improving outcomes in diabetic wound management. Future research endeavors should focus on elucidating the molecular pathways driving diabetic wound pathophysiology

and developing innovative strategies to promote wound healing and tissue regeneration in diabetic individuals (Joel M Raja,2023)(Yadav et al.,2024)

2.3.Mechanisms of Cold Atmospheric Nitric Oxide (NO) Gas

Hemostasis, inflammation, proliferation, and remodelling are the phases that make up normal wound healing. These phases start as soon as an injury happens and continue until full epithelialization.

In diabetic wounds, hyperglycemia inhibits organization of collagen and proliferation of cell, leading to the development of microangiopathy and neuropathy. Slowness in the healing of wounds and boosted vulnerability to infections caused by bacteria are the consequences of suppressing the proliferation of cells and the creation of collagen, which also reduces the growth factors needed for fibroblastic and angiogenic functions and hinders chemotaxis as well as phagocytotic performance.Since it is an endogenous controller of inflammation plus a non-resistant antibacterial substance nitric oxide (NO), an endogenous gasotransmitter essential to the healing of wounds, has gained popularity as a wound therapy. In normal wounds, nitric oxide synthase 2 is expressed at elevated levels by macrophages, keratinocytes, and fibroblasts; in wounds caused by diabetes, however, its expression is inhibited. The processes of wound healing are significantly influenced by the NOS (nitric oxide synthase) isoenzymes, namely NOS2 (nitric oxide synthase 2) plus NOS3 (nitric oxide synthase 3). Significant quantities of NO generated by NOS2 operate to eliminate bacteria that induce sudden infections, whereas smaller amounts of NO created via NOS3 control the inflammation necessary for the long-term healing of wounds. The lack of NOS2 has been demonstrated to affect cutaneous wound healing significantly. Diabetes-related wounds show reduced NOS2 and NOS3 expression by fibroblasts. Additionally, because fibroblasts cannot generate collagen, they are unable to construct the extracellular matrix (ECM), which reduces wound resistance. (Curukoglu, 2023)

Furthermore, throughout the recovery period of a wound, inadequate NO inhibits the mobility and migration of different cell types. Lately, NO regulation has been explored as a potential treatment for inadequate healing of wounds. By using both the processes of oxidative and nitrosative together, NO functions as both an endogenous controller of inflammation and a non-resisting antibacterial substance. The goal of therapeutic NO dosage, both exogenous plus endogenous, is to control remodeling of tissue and inflammation in long-

term wounds. Thus, among the present efforts to speed up the healing process of wounds is research on the administration of exogenous NO in wounds caused by diabetes as well as the implantation of long-term NO-supplying hydrogels made from chitosan and nanoparticles made of polymers in the wound bed. Promising outcomes and greater angiogenesis have been observed when NO-releasing hydrogels composed of chitosan are used to boost the effectiveness of mesenchymal stem cells that are produced by the placenta in the treatment of ischemic hind leg injuries in humans.(Curukoglu, 2023)

Cold atmospheric nitric oxide (NO) gas has garnered significant attention in biomedical research for its diverse physiological effects and therapeutic potential. Understanding the mechanisms underlying the actions of NO gas is essential for harnessing its benefits in various clinical settings. Cold atmospheric NO gas, has antimicrobial properties, regulation of cellular signaling pathways, modulation of inflammatory responses, and promotion of tissue repair and regeneration.

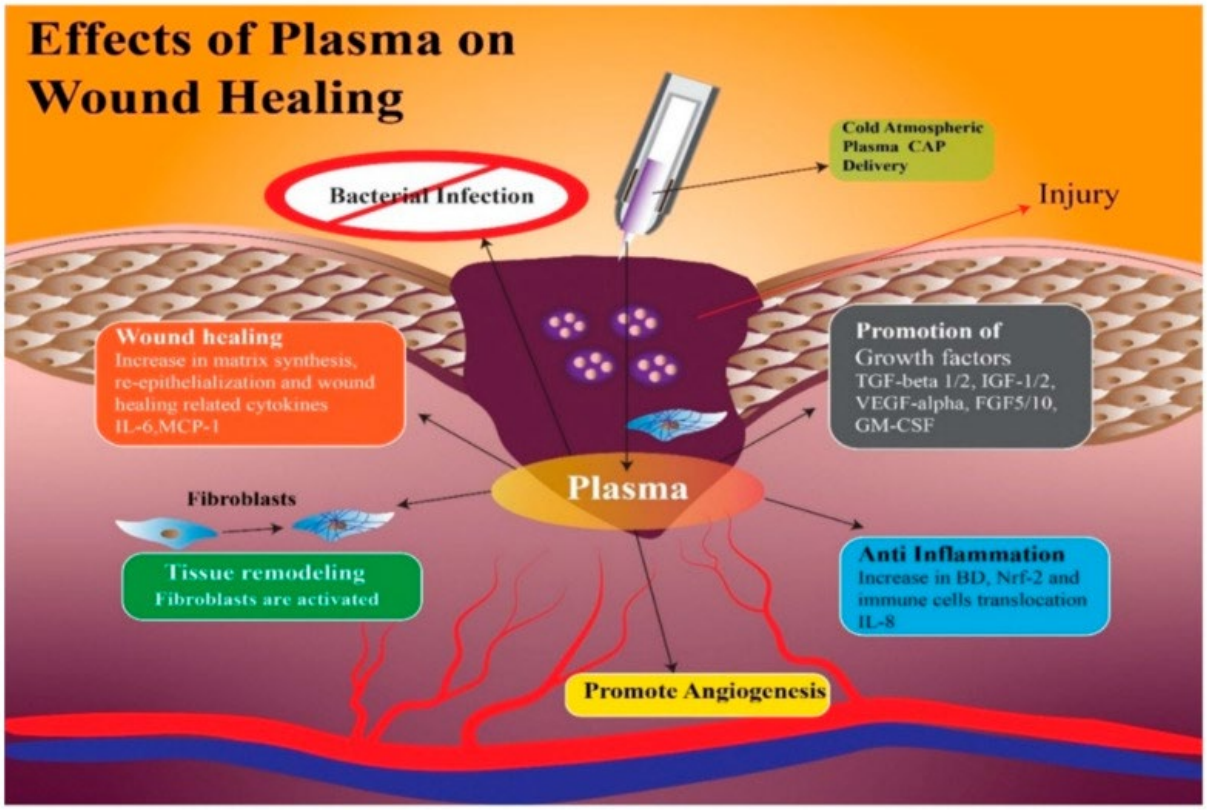


Figure1: shows how wound recovery is impacted by the plasma (Adapted from Tatiana Bolgeo et al.,2023)

Antimicrobial Properties

The strong antibacterial action of NO gas is exhibited against various kinds of pathogens, such as fungi, viruses and bacteria. Through the direct inhibition of microbial growth and biofilm formation, as well as the disruption of microbial membrane integrity and DNA/RNA synthesis, NO gas effectively suppresses microbial colonization and infection. Furthermore, NO gas induces microbial cell death via oxidative stress-mediated mechanisms, leading to the eradication of antibiotic-resistant strains and persistent infections. (Schairer et al.,2012)

Regulation of Cellular Signaling Pathways

NO gas exerts pleiotropic effects on cellular signaling pathways involved in diverse physiological processes. As a gaseous signaling molecule, NO diffuses freely across cell membranes and modulates the activity of various enzymes, ion channels, and transcription factors. By activating cyclic guanosine monophosphate (cGMP) signaling pathways, NO gas regulates smooth muscle relaxation, vasodilation, and platelet aggregation, thereby modulating cardiovascular function and blood flow regulation. Additionally, NO gas modulates neuronal signaling, emission of neurotransmitter, and synaptic plasticity in the central and peripheral nervous systems, contributing to its roles in neuroprotection and cognitive function.(Tuteja et al., 2004)

Modulation of Inflammatory Responses

NO gas has a majorduty in modulating inflammatory responses and immune cell activity. By inhibiting pro-inflammatory cytokine production, leukocyte adhesion, and endothelial activation, NO gas attenuates the inflammatory cascade and suppresses excessive immune responses. Furthermore, NO gas regulates macrophage polarization, promoting the transition from pro-inflammatory M1 to anti-inflammatory M2 phenotypes, which facilitates tissue repair and resolution of inflammation. Through its anti-inflammatory properties, NO gas mitigates tissue damage, prevents fibrosis, and promotes wound healing in various pathological conditions. (Tripathi et al., 2007)

Promotion of Tissue Repair and Regeneration

NO gas exerts proangiogenic, proproliferative, and proregenerative effects on tissues and organs. By stimulating endothelial cell proliferation, migration, and tube formation, NO gas enhances angiogenesis and neovascularization, thereby improving tissue perfusion and oxygenation. Additionally, NO gas promotes fibroblast proliferation, collagen synthesis, and extracellular matrix remodeling, facilitating tissue repair and wound healing. Furthermore, NO gas enhances stem cell proliferation, differentiation, and mobilization, contributing to tissue regeneration and organ repair in response to injury or disease. (Ding et al.,2023)

Numerous investigations conducted on animals have demonstrated the positive benefits of prolonged contact to gas plasma of therapeutically approved technologies and experimental prototypes of plasma in enhancing wound healing. In two investigations, punch biopsies are performed on 129 Sv/Ev mice on the dorsum were subjected to an older iteration of the widely accessible SteriPlas device by Arndt and colleagues. Interleukin (IL) 6, collagen type I,fibroblast growth factor 2,monocyte-chemoattractant protein 1, and wound closure were all increased, as well as macrophage and neutrophil infiltration. Numerous research endeavors examined the influence ofkINPen, which is an atmospheric pressure argon plasma jet, on recovery ofwound. Theoretically, these results correspond to comparable mechanisms found in wound recovery models involving the employment. (Strohal, 2022)

Breathnach and associates observed that rats with Punch wounds dealt with using gas plasma had faster healing of wound and re-epithelization in addition to increased acute inflammation and decreased fibrosis. Research conducted on injuries on ears from SKH1-hr mice confirmed and expanded upon these findings to include rapid healing, granulation, angiogenesis, keratin and collagen fiber formation, inflammation, re-epithelization, immigration of neutrophils and macrophages, and proliferative (p53) and antioxidant (Nrf2) responses. The latter study also showed that differences between quick (3 seconds) and extended (20 seconds) exposure intervals were found for multiple outcomes (re-epithelization;TGF-β,HMOX1, IL-1β, Nrf2,IL-6,SOD1,KGF, CAT,NQO1), and that H2O2 exposure did fail to completely recreate the outcomes acquired with gas plasma therapy. This implies that the overall amount quantity of RNS/ROS incorporated into the area of injury determines the degree to which differential redox regulation occurs.Interestingly, a year after treatment, an examination of the tissues showed no evidence of detrimental long-term effects

from the gas plasma therapy with respect to carcinogenesis or defects in skin structures. (Bekeschus et al., 2021)(Strohal, 2022)

However, early after gas plasma therapy (up to day 15), modifications in matrix remodeling for example, TIMPs and MMPs and focal adhesion control such as,integrin adhesion complexes and fibrillary adhesions, were noted in gas plasma-treated wounds. It has also been noted that singlet oxygen-inducing light conditions cause MMP1 and MMP5 to rise. Apart from the previously discussed safety and molecular characteristics, gas plasma therapy resulted in improved oxygenation of wound tissue in both superficial and deep layers. Additionally, it was associated with raised tissue hemoglobin and water indices. (Bekeschus et al., 2021)

It has been shown that administering cold atmospheric plasma (CAP) therapy to animals accelerates the healing of wounds. It's unclear, nevertheless, how this process is handled. This study looked at the processes that underpin CAP's enhanced effects on wound healing and the functions of related nitrogen species (RONS), andreactive oxygen,which are manufactured by plasma. Utilizing in vitro models that replicated several stages of angiogenesis, we were able to show that CAP increased the generation of nitric oxide (NO), facilitated cell movement, and improved assembly of endothelial cell into structures resembling vessels. These two characteristics characterize the proliferative stage of wound healing.(Duchesne et al., 2019)

We next demonstrated that CAP therapy was linked to greater angiogenesis, which is characterized by faster in vivo recovery of the wound and more division of cells employing a mouse model that has burn lesion of third-degreeHere, PDGFRβ and CD31, pro-angiogenic markers were considerably upregulated in mouse injuries, and CAP additionally boosted the endothelial NO synthase (eNOS) in vivo manufacture of, an enzyme that is used in endothelial cells for catalyzes of NO generation. In terms of mechanism, we demonstrated that CAP increased endogenous NO levels in endothelial cells by inducing eNOS phosphorylation and activation. Significant pro-angiogenic VEGFA/VEGFR2 signaling was further enhanced in vitro by enhanced NO production made possible by CAP. Future research addressing the usage of physical plasma and its therapeutic uses in many disease circumstances may be guided by the findings of this proof-of-concept study. (Duchesne et al., 2019)

2.4.Role of NPH Insulin Cream in Wound Healing

NPH (Neutral Protamine Hagedorn) insulin, a commonly used insulin formulation, has shown promise in promoting wound healing through its multifaceted effects on cellular proliferation, collagen synthesis, angiogenesis, and inflammation. Understanding the mechanisms underlying the role of NPH insulin cream in wound healing is essential for optimizing its therapeutic use in various clinical settings.

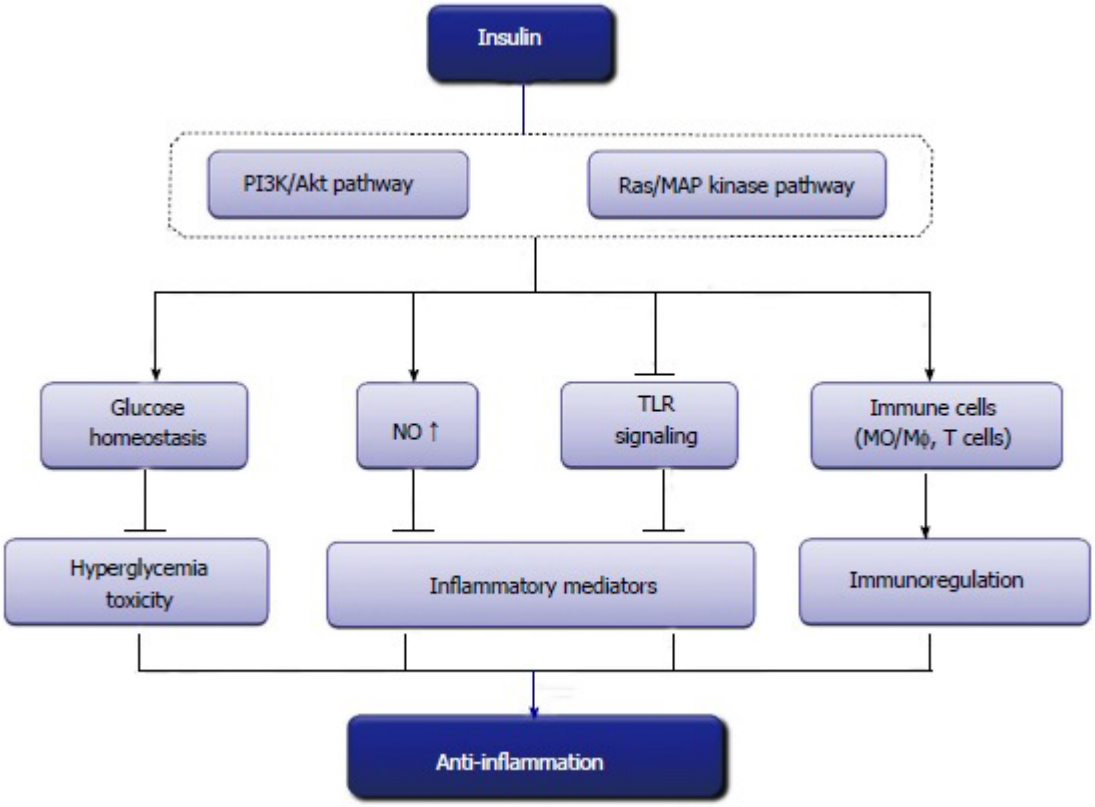


Figure 2:TLR: Toll-like receptor, the impacts of insulin on suppression of inflammation (Adapted from Qiang Sun et al.,2014)

Enhancement of Cellular Proliferation

NPH insulin exerts mitogenic effects on endothelial cells, keratinocytes, and fibroblasts—all of which are cell types implicated in the healing of wounds. By activating insulin receptor signaling pathways NPH insulin stimulates the manufacture of DNA, progression of the cycle of cells, and proliferation of cells, thereby accelerating the emergence of tissues with granulation and epithelialization at the wound site. Furthermore, NPH insulin promotes the recruitment and differentiation of progenitor cells, such as mesenchymal stem

cells, to the wound microenvironment, augmenting repair of tissue and renewal processes. (Ozaydin et al.,2018)

Stimulation of Collagen Production and Remodeling of Extracellular Matrix

Production of collagen and remodeling of extracellular matrix (ECM) are essential steps in wound healing, facilitating tissue repair and scar formation. NPH insulin enhances collagen production by fibroblasts and myofibroblasts, promoting the deposition of mature collagen fibers and the organization of ECM components within the wound bed. Additionally, NPH insulin modulates the function of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs), regulating ECM turnover and preserving tissue integrity whilewound healing remodeling stage.(Corrêa, 2020)

Angiogenesis and NeovascularizationPromotion

Angiogenesis is the process by which pre-existing vasculature divides into new blood vessels. It is essential for delivering nutrients as well as oxygen to the area of the wound and promoting tissue healing. NPH insulin stimulates endothelial expansion of cells, migration, and development of tube, enhancing angiogenesis and neovascularization in ischemic tissues. By allowing endothelial nitric oxide (NO) synthase and allowing the emission of vascular endothelial growth factor (VEGF) and other proangiogenic factors, NPH insulin enhances microvascular perfusion and capillary density, accelerating wound closure and healing. (Liu,2008)

Modulation of Inflammatory Responses

NPH insulin exhibits anti-inflammatory properties, attenuating the inflammatory cascade and promoting a change in stance from pro- to anti-inflammatory cytokine profiles within the wound microenvironment. By suppressing the production of cytokines that allows inflammation, such asinterleukin-1 beta (IL-1β) plus tumor necrosis factor-alpha (TNF-α), and boosting the secretion of anti-inflammatory cytokines, such as interleukin-10 (IL-10), NPH insulin mitigates tissue damage, reduces inflammation, and promotes a favorable environment for wound healing.(Apolinário et al.,2023)

Some Studies' methods of action provide evidence for insulin's roles in accelerating wound healing. According to Liu et al., topical insulin administered toinjuries of skin excision facilitates re-epithelialization and fosters "maturation" of the recovering tissue. The insulin receptor is necessary for these effects, and the PI3K-Akt-Rac1 signaling pathways have a crucial part in stimulating keratinocyte migration and integrin-α3 and LN332 production, which are essential for both in vitro and in vivomigration of cells. Additionally, their results demonstrated that insulin acts via activating receptors for the insulin-like growth factor (IGF) 1. Transforming growth factor-beta in dermal fibroblasts is stimuated by IGF-1, which promotes injuryrecovery, as demonstrated by Ghahary et al.According to Rezvani et al., IGF and insulin have the chemically similar structure, particularly IGF-1, a growth factor. The findings state that longer neutrophil infiltration times and a higher neutrophil count are present in diabetic wounds. As a result, wounded individuals with diabetes experience longer healing periods and a higher rate of infected wounds. (Liu et al., 2021)

Chen et al. postulated that topical insulin might regulate the inflammation that occurs in the injured region by inhibiting the invasion of neutrophils into injury site chemokine macrophage inflammatory protein-2 (MIP-2) production. Additionally, they discovered that insulin improved neutrophil activities, suggesting that insulin regulates the inflammatory response to wounds throughout the healing process.Negrini et al. believed that Chen et al.'s perspective was conflicting since they believed that insulin-induced increased function of mouse neutrophils was responsible for the better healing and that early neutrophil infiltration is a crucial initial stage in the healing process. They also demonstrated how insulin, in the early stages of wound healing, promotes faster healing by modifying the inflammatory response to the wound, particularly the quantity and activity of fibroblasts, heterophils, and macrophages.(Liu et al., 2021)

Neovascularization is essential for wound healing to be successful. Research on insulin's topical effects shows that it first irritates local angiogenesis, which creates a favorable environment for wound healing.Topical insulin increases the development of blood vessels and progresses fibrosis, as proven by Martínez-Jiménez et al. According to Li et al., insulin promotes blood vessel development during the healing process by increasing the production of α-smooth muscle actin (α-SMA) and platelet-derived growth factor-B (PDGFR-β), as well as causing an increase in the angiopoietin-1 (Ang-1) activity. These findings may be connected to the mechanisms underlying insulin-induced healing of wounds.According to

reports, Ang-1's primary role in the maturation of vessels may only be indirect because of its ability to prevent apoptosis in plastic stage endothelial cells. ÖZAYDIN et al. discovered that the topical utilization of NPH insulin, applied as an ointment, contributes positively to the healing of complicated wounds involving tissue damage, granulation tissue development, and epithelization because open wounds heal through all stages of the healing process more quickly.(Liu et al., 2021)

Even though several studies have demonstrated that using insulin topically helps accelerate wound healing, further research is still required to address the following concerns: First, he kind of insulin (medium-, long-, or short-acting) and levels of insulin used in clinical trials should be determined through randomized controlled trials; second, the proper number of samples ought to be projected; and an evaluation on all crucial components about randomness, hiding, and blindness, as well as subgroup analysis, is required for patients with injuries of various etiologies. Thirdly, the outcomes—for examplespeed of healing of wound, wound recovery duration, site of wound, percentage of granulation tissue, microvascular density, and so forth—that contribute to assessing the efficacy and effectiveness of local insulin should be documented in as much information as feasible.Fourth, when local insulin is being applied, it's also important to keep an eye on the experiment's safety and record the participants' blood glucose levels. In animal research, several animal models were employed, the size and location of the wounds made varied, and in certain cases, the sample size was rather small.(Liu et al., 2021)

Growth factors represent significant scientific advancements with enormous potential to alter the way wound healing appears. Unfortunately, growth factors are highly costly and challenging to keep for long periods of time, which places a significant financial strain on patients with wounds, particularly those who have diabetes. But insulin is cheap and simple to get. It has been reported that incorporating insulin into hydrogels, liposomal chitosan gels, creams, crystals, or ointments can stabilize its instability and uncontrollably release it. This improves the stickiness of the insulin to the wound surface and increases compliance from patients, particularly in cases of painful cuts. Insulin used topically does not result in hypoglycemia or any other negative side effects.In addition to being safe, affordable, and effective, it accelerates the healing process and decreases the need for medication, length of therapy, and hospital stays. This makes it an excellent therapeutic option for both acute and chronic wounds, particularly diabetic foot ulcers. (Liu et al., 2021)

2.5. Interactions Between NO Gas and NPH Insulin Cream

The combination of cold atmospheric nitric oxide (NO) gas and NPH (Neutral Protamine Hagedorn) insulin cream holds promise as a synergistic therapeutic approach for enhancing wound healing. Understanding the interactions between NO gas and NPH insulin cream is essential for optimizing their combined efficacy in promoting tissue repair and regeneration. The interactions between NO gas and NPH insulin cream, may have potential synergistic effects on angiogenesis, collagen synthesis, inflammatory modulation, and overall wound healing outcomes.

Angiogenic Synergy

Both NO gas and NPH insulin cream exert proangiogenic effects on endothelial cells, promoting the development of new vascularates and neovascularization in ischemic tissues. By triggering endothelial nitric oxide synthase (eNOS) and manufacturing nitric oxide, a powerful vasodilator, and agent of angiogenesis, NO gas enhances endothelial cell growth, migration, and creation of tubes. Similarly, NPH insulin stimulates endothelial proliferation of cell and angiogenesis by triggering insulin receptor signaling pathways and promoting the emission of vascular endothelial growth factor (VEGF), a vital angiogenesis controller. The combination of NO gas and NPH insulin cream may synergistically enhance angiogenesis and microvascular perfusion in the wound microenvironment, facilitating oxygen and nutrient delivery to the injured tissues and accelerating wound healing processes. (Liu,2008),(Ding.,2023)

Collagen Synthesis and Extracellular Matrix Remodeling

NO gas and NPH insulin cream both modulate collagen synthesis and extracellular matrix (ECM) remodeling, essential processes for tissue repair and scar formation. NO gas enhances fibroblast proliferation and collagen synthesis by activating cyclic guanosine monophosphate (cGMP) signaling pathways and promoting the expression of collagen genes. Similarly, NPH insulin stimulates collagen production and ECM remodeling by activating insulin receptor signaling pathways and modulating the expression of matrix metalloproteinases (MMPs) and tissue blockers of metalloproteinases (TIMPs). The combined effects of NO gas and NPH insulin cream may synergistically enhance collagen deposition,

cross-linking, and maturation within the wound bed, improving tissue integrity and tensile strength during wound healing. (Corrêa, 2020),(Ding.,2023)

Inflammatory Modulation

NO gas and NPH insulin cream both exhibit anti-inflammatory properties, suppressing pro-inflammatory cytokine production and attenuating the inflammatory cascade in the wound microenvironment. NO gas promotes the production of cytokines that reduce inflammation, like interleukin-10 (IL-10), while inhibiting the expression of mediators that allow inflammation like interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α), by immune cells. Similarly, NPH insulin attenuates inflammation by modulating immune cell function, suppressing leukocyte activation, and promoting a shift from pro-inflammatory to anti-inflammatory cytokine profiles. The synergistic effects of NO gas and NPH insulin cream may mitigate tissue damage, reduce inflammation, and create a favorable environment for wound healing by modulating the inflammatory response in a coordinated manner. (Apolinário et al.,2023),(Zhang,2009)

The interactions between cold atmospheric nitric oxide (NO) gas and NPH (Neutral Protamine Hagedorn) insulin cream represent a promising therapeutic strategy for enhancing wound healing through synergistic effects on angiogenesis, collagen synthesis, and inflammatory modulation. Additionally, clinical trials are a must to evaluate the safety, efficiency as well as affordability of combined NO gas and NPH insulin cream therapy in comparison to standard wound care modalities, paving the way for its translation into clinical practice.

2.6. Gene Expression Analysis of IL-11

IL-11 Gene Expression Analysis

A complicated system of post-transcriptional and transcriptional processes controls the expression of the IL-11 gene upon exposure to several distinct triggers., including cytokines, growth factors, and environmental cues. Gene expression profiling studies have identified tissue-specific expression patterns of IL-11 in diverse cell types and organs, including bone marrow, liver, heart, and gastrointestinal tract. Dysregulated IL-11 expression has been implicated in various pathological conditions, including inflammation, fibrosis, cancer, and cardiovascular diseases. Gene expression analysis of IL-11 isoforms and splice variants

further elucidates the molecular diversity and functional heterogeneity of IL-11 signaling pathways. (Fung et al.2022)

Functional Implications and Clinical Relevance

Gene expression analysis of IL-11 offers valuable insights into their functional roles in immune regulation, inflammatory responses, and tissue repair processes. IL-11 gene expression is associated with tissue remodeling, angiogenesis, and wound healing. Dysregulated IL-11 gene expression profile serve as potential biomarkers for disease diagnosis, prognosis, and therapeutic response prediction in various pathological conditions. Targeting IL-11 signaling pathway holds promise for developing novel therapeutic interventions for immune-related disorders, inflammatory diseases, and cancer. (Fung et al.2022)

2.7. Interleukin 11 in Wound Healing

Anti-inflammatory Effects

IL-11 has potent anti-inflammatory properties, which are vital during the earlieststeps of healing of wound. Although inflammation is a normal reaction to pathogens and tissue damage, unnecessary or protracted inflammation can impede the healing process. IL-11 helps to regulate inflammation. IL-11 can influence the polarization of macrophages towards an anti-inflammatory M2 phenotype. M2 macrophages promote tissue repair and resolution of inflammation by secreting growth factors and cytokines that stimulate wound closure and tissue remodeling. IL-11 inhibits the manufacturing of cytokines that allow inflammation such as IL-1β, TNF-α, and IL-6. By reducing the levels of these cytokines, IL-11 helps to dampen the inflammatory reaction and facilitate a bettermanaged healing process.Excessive inflammation during wound healing can lead to fibrosis and scar formation. IL-11 helps regulate the balance between tissue repair and scar formation by promoting a controlled inflammatory response and supporting tissue regeneration without excessive collagen deposition. This anti-fibrotic effect of IL-11 is crucial for ensuring functional tissue repair and minimizing scar tissue formation. (Fung et al.2022) (Metinko,2004)

Promotion of Cell Proliferation and Differentiation

IL-11 promotes the proliferation and differentiation of various cell types involved in wound healing.

Fibroblasts are key cells that produce collagen and other extracellular matrix components essential for wound closure. IL-11 stimulates expansion of fibroblast and collagen synthesis, contributing to the development of granulation tissue and wound contraction. IL-11 enhances the multiplication and movement of endothelial cells, promoting angiogenesis—the development of new vascularates. Adequate supply blood is vital for distributing nutrients oxygen to the wound site, supporting repair of tissue and renewal. The process of angiogenesis, or the growth of fresh vessels of blood, is essential for delivering oxygen as well as nutrients to the site of the wound and supporting tissue repair. IL-11 has a duty in promoting angiogenesis by stimulating endothelial cell expansion and movement. This process is mediated in part through the increased expression of vascular endothelial growth factor (VEGF), a vitalplayer in angiogenesis. By enhancing blood vessel formation, IL-11 helps create a conducive environment for cell proliferation and differentiation during wound healing. Bone marrow stromal cells (BMSCs) are multipotent stem cells that can differentiate into various cell types, including chondrocytes (cartilage-forming cells) and osteoblasts (bone-forming cells). IL-11 has been shown to influence the differentiation of BMSCs towards osteogenic and chondrogenic lineages. This effect is relevant in situations where wound healing involves bone or cartilage repair, such as in fractures or joint injuries. (Cialdai, 2022) (Hu et al.,1993)

Matrix Metalloproteinase (MMP) Regulation

IL-11 regulates the activity of matrix metalloproteinases (MMPs), enzymes that play a role in tissue remodeling and wound closure:

IL-11 can inhibit certain MMPs, such as MMP-1 and MMP-3, which are involved in breaking down and remodeling the extracellular matrix during wound healing. This regulation ensures that MMP activity is balanced to support effective tissue repair without excessive degradation. (Alicja Krejner, 2016)

Effects on Keratinocytes and Epithelial Cells

IL-11 influences the behavior of keratinocytes and epithelial cells, which are essential for re-epithelialization—the process by which the epidermis closes over the wound:

IL-11 promotes keratinocyte migration across the wound bed and enhances their differentiation into mature epithelial cells. This process is critical for restoring the protective barrier function of the skin.

Clinical Implications and Research:

Research into the therapeutic potential of IL-11 in wound healing is ongoing. Studies have explored its application in various wound models, including chronic wounds and surgical incisions, to assess its ability to accelerate healing and improve outcomes. The understanding of IL-11's precise mechanisms in wound repair continues to evolve, with potential implications for developing targeted therapies to enhance healing in clinical settings.

2.8 Pro-Infammatory properties of IL-11

A variety of pro-fibrotic proteins are up-regulated by IL11 in cultured human fibroblasts without causing alterations in their transcript quantity. Although the exact nature of this fascinating phenomena is yet unknown, it has been postulated that IL11 communicates via pathways beyond transcript abundance regulation and JAK/STAT. Stuart A. Cooket al. said that 'IL11-promoted MEK/ERK expression has been found to be especially significant for VSMC mesenchymal transition (VMT) and fibroblast mesenchymal transition (FMT), also known as phenotypic switching, in both our and other research'. A recognized non-canonical signaling pathway located downstream of gp130, MEK/ERK is expressed by IL11 in vitro in two phases: a sustained phase that occurs afterwards and can be inhibited by elevated DUSP function. As anticipated, JAK/STAT3 is activated by IL11 in human fibroblasts and epithelial cells, such as hepatocytes and renal tubular epithelial cells (TECs). (Cook, 2023)

In comparison to the impacts of IL6 or OSM, the induction of JAK/STAT in cultured fibroblasts and TECs is instantaneous, temporary, and of a smaller amplitude than that of IL11-triggered ERK expression, which is biphasic and persistent.The increased levels of several pro-inflammatory factors in fibroblasts is caused by IL11-dependent JAK/STAT3 tivity. These factors includeIL1RL1,CXCL3/5/8,ICAM1, CCL20,SERPINB2, TNFRSF18,

and IL33. The most current in vitro and in vivo results clearly indicate that IL11 has pro-inflammatory effects, despite the fact that previous research and even most recent reviews considered it to be anti-inflammatory. Although Akt has been linked to IL11 signaling, IL11-stimulated fibroblasts only seldom activate it. JNK activation in response to IL11 stimulation of some cell types (hepatocytes, for example) has been seen by us and others; nevertheless, this is most likely a secondary or indirect event. Rapid, enhanced expression of IL33, CCL20, and IL8 facilitated by STAT3 as well as decreased levels of TEC-specific genes such aquaporin 6 (AQP6) and renal tubular urea transporter (SLC14A2) are stimulated by IL11 in TECs.(Cook, 2023)

Although MEK/ERK activation is recognized to be a pro-fibrotic pathway, it remained unclear to us how IL11-stimulated ERK activation resulted in the translation of fibrogenic proteins. Fibrosis is mostly caused by AMPK inhibition and mTOR activation; earlier research has demonstrated that ERK/P90RSK inhibits LKB1/AMPK. We then demonstrated that IL11 activates P90RSK and ERK, which in turn phosphorylates LKB1 at serine 325 and 428, respectively, inducing LKB1 inactivation and blocking mTORC1 and AMPK. This IL11/ERK/LKB1/AMPK/mTOR pathway's discovery highlights a crucial signaling module, which allows fibrosis,that includes well-known and significant signaling elements which allows fibrosis. It is noteworthy to note that further genetic studies must be conducted to asctertain the functional significance of LKB1 phosphorylation at serine 428 and 325 in this pathway.(Cook, 2023)

A second and equally significant axis of effect for IL11-induced ERK/P90RSK activity is the simultaneous phosphorylation and termination of GSK3β, an alternate substrate. In this case, GSK3β is inactivated as a result of the concurrent phosphorylation of Thr43 and Ser9 by ERK and p90RSK. As a consequence, SNAI1 is freed from GSK3β-facilitatedblocking, which leads to SNAI1-facilitated decrease the activity of E-cadherin, the standard epithelial indicator. Consequently, the SNAI1:E-cadherin switch, which is crucial for the epithelial mesenchymal transition (EMT) and, in a similar manner, fibroblast FMT, is regulated by the IL11/ERK/P90RSK axis through the inactivation of GSK3β.(Cook, 2023)

Within the subject of IL6 biology, trans-signalling postulates that soluble IL6R signals are produced by IL6 when complexed with gp130. The utilization of a fusion protein known as HyperIL6, which has varying pathogenic or cytoprotective capabilities, is necessary for IL6

trans-signalling to occur. Utilizing a decoy protein (sgp130Fc), which is a soluble fragment of gp130 connected to an antibody Fc domain, is also necessary. Although sgp130Fc is specifically designed to block trans-signallingIL6, it also blockstrans-signalling of CNTF, OSM, and LIF cis-signalling, and theoretically, it may also block IL6 and IL11 cis-signalling.We adopt the stance that the available informationindicatescis-signalling of IL11 as the predominant type of IL11 activity and feel that additional research is necessary on trans-signalling in light of these fluctuations and the contradictory findings about the presence or lack of IL11 trans-signalling.(Cook, 2023)

To sum everything up, IL11 communicates with a variety of autocrine and paracrine stromal and epithelial cells to stimulate JAK/STAT3, which causes a brief spike in pro-inflammatory gene expression in vitro, and more persistently to stimulate ERK/P90RSK, which in turn hinder LKB1, turn on mTOR, and hamper GSK3β, which activates the SNAI1:E-Cadherin switch. All things considered, an inflammation and gene expression mesenchymal program is driven by these signaling events in many cell types.(Cook, 2023)

2.9 Factors That Affecting Wound Healing

The recovery of wounds can be hampered by a variety of circumstances. Overall, the two primary groups influencing recovery are systemic and local variables.Systemic variables affect an individual's general health or disease and may impair their capacity to recover, whereas local factors have a greater influence on the wound's features. There are several interconnected components, and systemic influences on the healing of wounds can be localized.

Local Factors

Wound Type

Acute vs. Chronic: Acute wounds (such as surgical incisions, traumatic wounds) generally heal faster and have a predictable healing trajectory compared to chronic wounds (like diabetic ulcers, pressure ulcers), which may have underlying issues affecting healing, such as poor circulation or infection. (‘’Factors Affecting Wound Healing in Chronic Wounds’’, 2016)

Wound Size and Depth

Larger wounds generally take longer to heal because they involve more tissue damage and require more extensive cellular proliferation and tissue remodeling. Deeper wounds may involve damage to underlying structures (muscles, tendons, bones), which can complicate healing. (‘’Factors Affecting Wound Healing in Chronic Wounds’’, 2016)

Presence of Infection

Infection at the wound site is one of the most significant local factors that can delay healing. Bacterial colonization or infection increases inflammation, prolongs the inflammatory phase of healing, and can lead to tissue necrosis. Effective management with antimicrobial treatments is crucial for promoting healing. (‘’Factors Affecting Wound Healing in Chronic Wounds’’, 2016)

Blood Supply (Perfusion)

For the wound to commence recovering, an appropriate flow of blood is crucial for supplying the nutrition, oxygen, and immune cells needed. Complications such as venous insufficiency and disorder of peripheral arterial can cause inadequate circulation, which can hinder recovery and lead to chronic wounds. (‘’8 Factors That Affect Wound Healing’’, 2021)

Tissue Oxygenation

Oxygen is critical for cellular metabolism, collagen synthesis, and immune function. Hypoxia (low oxygen levels) at the wound site can impair healing processes and contribute to chronic wound formation. (‘’Factors Affecting Wound Healing in Chronic Wounds’’, 2016)

Presence of Foreign Bodies or Debris

Retained foreign bodies, necrotic tissue (eschar), or excessive wound exudate can create a barrier to healing. These factors can interfere with the granulation tissue development and delay epithelialization. (‘’Factors Affecting Wound Healing in Chronic Wounds’’, 2016)

Wound Bed Preparation

Proper preparation of the wound bed through debridement (removal of necrotic or non-viable tissue), wound cleansing, and management of wound exudate is crucial for creating an optimal environment for healing. A clean and moist wound bed supports cellular activity and facilitates tissue repair. (‘’8 Factors That Affect Wound Healing’’, 2021)

Mechanical Factors

Pressure, friction, and shear forces can disrupt wound healing, particularly in patients with reduced mobility or in areas prone to pressure ulcers (e.g., sacrum, heels). Proper wound care techniques, including offloading and use of appropriate dressings, are essential to mitigate mechanical stress on the wound. (‘’8 Factors That Affect Wound Healing’’, 2021)

Temperature and Moisture Balance

Maintaining an appropriate temperature and moisture level at the wound site is important for promoting cellular activity and preventing tissue dehydration or maceration. Excessive moisture (e.g., from wound exudate) or dryness can negatively impact healing. (‘’7 Factors that Affect Wound Healing’’, 2017)

Surgical Technique

For wounds resulting from surgical procedures, the technique used during surgery can influence healing outcomes. Factors such as tissue handling, closure method (e.g., sutures, staples), and post-operative care practices can affect wound healing speed and quality. (‘’Factors Affecting Wound Healing in Chronic Wounds’’, 2016)

Systemic Factors

Systemic factors refer to conditions or characteristics of the whole body that can influence the process of wound healing. These factors have a vitalduty in determining the overall speed, quality, and success of recovery. Here are the key systemic factors that affect wound healing:

Nutritional Status

Adequate nutrition is crucial for supporting cellular metabolism, production of collagen, and immune function, all of which are essential for healing of wounds. Deficiencies in proteins, minerals (e.g., zinc) and vitamins (e.g., vitamin C, vitamin A) can impede thehealing of wound and lead to delayed recovery. Malnutrition, commonly seen in elderly patients or those with chronic illnesses, significantly affects healing outcomes. (‘’Factors Affecting Wound Healing in Chronic Wounds’’, 2016)

Systemic Diseases

Chronic diseases such as diabetes mellitus, peripheral vascular disease, autoimmune disorders (e.g., lupus), chronic kidney disease, and cardiovascular diseases can impact wound healing. These conditions may affect circulation, immune function, and the body's capaability to react to injury, leading to delayed wound healing and increased risk of complications. (‘’8 Factors That Affect Wound Healing’’, 2021)

Age

Age-related changes in cellular function, collagen synthesis, and immune response can affect wound healing. Elderly individuals may have slower healing rates due to reduced cell proliferation, decreased collagen deposition, and impaired immune function compared to younger individuals.(‘’7 Factors that Affect Wound Healing’’, 2017)

Hormonal Factors

Hormonal imbalances, such as those seen in thyroid disorders, cortisol excess (e.g., Cushing's syndrome), or hormonal changes during pregnancy, can influence wound healing.

Immune reaction modulation, collagen synthesis, and tissue remodeling processesare influenced by hormones. (‘’8 Factors That Affect Wound Healing’’, 2021)

Medications

Certain medications can affect wound healing by various mechanisms. For example, corticosteroids suppress immune responses and collagen synthesis, immunosuppressants (e.g., used post-transplant) reduce immune function, and anticoagulants may increase the risk of bleeding complications at the wound site. (‘’8 Factors That Affect Wound Healing’’, 2021)

Smoking and Substance Abuse

Smoking tobacco and substance abuse (e.g., alcoholism) can impair wound healing by reducing tissue oxygenation, compromising immune function, and interfering with collagen synthesis. These factors contribute to delayed wound closure and increased risk of wound complications. (‘’Factors Affecting Wound Healing in Chronic Wounds’’, 2016)

Psychological Factors

Psychological stress, anxiety, and depression can impact wound healing through hormonal changes (e.g., increased cortisol levels), immune suppression, and altered inflammatory responses. Emotional well-being and mental health support are important considerations in optimizing wound healing outcomes. (‘’Factors Affecting Wound Healing in Chronic Wounds’’, 2016)

Immunodeficiency States

Conditions that compromise immune function, such as HIV/AIDS or immunosuppression due to chemotherapy or autoimmune diseases, increase the risk of infections and delay wound healing. Adequate immune function is essential for effective wound repair and defense against pathogens. (‘’8 Factors That Affect Wound Healing’’, 2021)

Vascular and Circulatory Conditions

Disorders affecting vascular health, such as peripheral arterial disease (PAD), venous insufficiency, or hypertension, can impair blood flow to the area of the wound. Distributing

the oxygen, nutrients, and immune cells crucial for healing requires adequate perfusion. (‘’Factors Affecting Wound Healing in Chronic Wounds’’, 2016)

Obesity

Obesity can impact wound healing due to metabolic changes, impaired circulation, and increased mechanical stress on wounds (e.g., pressure ulcers). Managing weight and addressing associated comorbidities are important for promoting healing in obese patients.(‘’7 Factors that Affect Wound Healing’’, 2017)

Chapter 3

Methodology

Material and method

The results, which included a comparison of the IL-11 gene expression analysis using cold atmospheric Nitric Oxide gas alone and with cream of NPH insulin inhealing of wound with tissue damageinjury in diabetic rats, were related using a genetics information set that was gathered. Before taking part in the experiment, a letter of research was completed and approved by the relevant Ethics Committee (approval number: YDU 2024/169.). Prior to taking part in this research, the subjects gave their informed permission.

Data Collection

The experiment was completed. Patients who had diabetes sent 46 samples to Near East University Hospital (NEUH). Blood samples were collected from 46 patients diagnosed with diabetes mellitus

Material Kits

cDNA Synthesis

The cDNA synthesis was carried out using Syber Green cDNA synthesis, which consists of several essential components. An essential component was the enzyme mix solution, which was made up of reverse transcriptase and RNase inhibitors.

REACTION SETUP and preparation of cDNA

Thaw all the components and carefully mix. When getting ready for a reaction, be cool. It is highly recommended to do a reverse transcriptase-free control reaction to check for potential contamination of DNA. (Optional information) RNA can be denatured for five minutes at 72°C using a reaction buffer. The mix is span down and immediately put on ice. Improved transcription of long mRNAs or GC-rich RNA can be obtained by using this

technique. For a negative control, 1 ul of nuclease-free dH20 was substituted for the enzyme mixture. Then they were combined in a sterile tube free of RNase:

Table 1.Protocolof the cDNA Kit Preparation

Components	**Volume**
Reaction	4µL
enzyme mix	1µL
Total RNA	10µL
Nuclease Free dh_2o	10µL
Total Volume	10µL

Table 2. Conditions for Polymerase Chain Reaction for cDNA Synthesis is demonstrated by this table

Step	**Temperature**	**Time**
cDNA synthesis	42°C	60 minutes
Inactivation of the kit	80°C	10 minutes

Following the diluting of the reaction by 200 ul of nuclease-free dH20, keep it at -20°C. Do not continuously freeze and thaw. For the PCR applications that come next, the diluted cDNA reaction ought to account for 10% of the overall reaction volume (e.g. 5 ul in a 50 ul reaction).

Components and Component in the 2X SYBR Green qPCR Mix

The SYBR Green qPCR Mix is utilized because it offers an easy-to-use and efficient means of tracking the real-time advancement of the PCR. More SYBR Green attaches to the copied DNA as the PCR continues, boosting the fluorescence signal proportionately. This makes it possible for the user to determine how much original DNA is in each sample. Since I have 46 samples and only require two negative, the entire amount will be 48 times.

2X SYBR Green qPCR Mix

The 2X SYBR Green qPCR Mix is not only user-friendly but also designed to maximize effectiveness and efficacy in Real-Time PCR (qPCR). Contained in the package is the Taq DNA Polymerase enzyme, that was created through the process of molecular evolution. For this reason, it's a unique blend created exclusively for qPCR using SYBR Green I. The HibriGen 2X SYBR Green qPCR Mix (High Rox Plus), which does not include primers, template DNA, or water, is the perfect premix of ingredients for improved precision and specificity when conducting real-time Polymerase Chain Reaction (PCR) using SYBR Green I dye. Double-chain DNA and SYBR Green I dye combine to provide a luminous indicator that displays the amount of double-chain DNA generated during PCR. During qPCR, this product is utilized in the ABI real-time equipment to detect and amplify DNA. A high Rox standard dye is used for the normalization procedure, with an ending concentration of 500 nM..

Table 3.Protocolof the 2X SYBR Green qPCR Mix Preparation

Component 1x	Component 1x
SYBR 10	460 µL
Forward 0.8	36,8 µL
Reverse 0.8	36,8 µL
H20	248
cDNA 3	17+3 cdna

PCR Machine

A preliminary polymerase chain reaction (PCR) test was conducted, strictly following the manufacturer's recommended primer concentration of 10 micromolar, using the supplied

primers and positive as well as negative controls. These focused on the primers' specificity for the target sequence and their capacity to amplify the desired DNA fragments. The desired sequence, or template DNA, was included into the samples under the positive control to serve as the standard for effective multiplication.However, because of a lack of template DNA in the negative control, an examination may reveal contamination or unintentional multiplication. The annealing temperature, cycle counts, and other adjustments performed before the start of the PCR process guarantee that the targeted DNA sequences are amplified in a dependable and repeatable manner. In this manner, it might ascertain whether or not there was an issue with the primers' efficiency.

Table 4. Contents of the qPCR mix

1x	46x
2x Syber green 10 µL	460 µL
µL Forward Primer F 0.8	36,8 µL
Reverse Primer F 0.8	36,8 µL
H20	248 µL
cDNA	3 µL

Gradient PCR

Temperature gradient PCR was used in this investigation to examine the whole range of annealing temperatures. The temperature gradient was calibrated to have values between 59°C and 62°C, which were then utilized as different annealing temperature settings to determine which setting was optimal for primers. This makes determining the ideal temperature for target sequence amplification easier and more successful. This will guarantee that, within the confines of the experiment, the annealing is done at the chosen temperature in order to maximize the PCR's efficacy.

Table 5. Specifies PCR Cycling Conditions: 40x Cycles

Stage	Temperature	time
Initial denaturation	95°C	1 minute
Denaturation	95°C	15 seconds
Annealing	59 °C	15seconds
Elongation	72 °C	45 seconds
Termination	72 °C	

Data Analysis Study

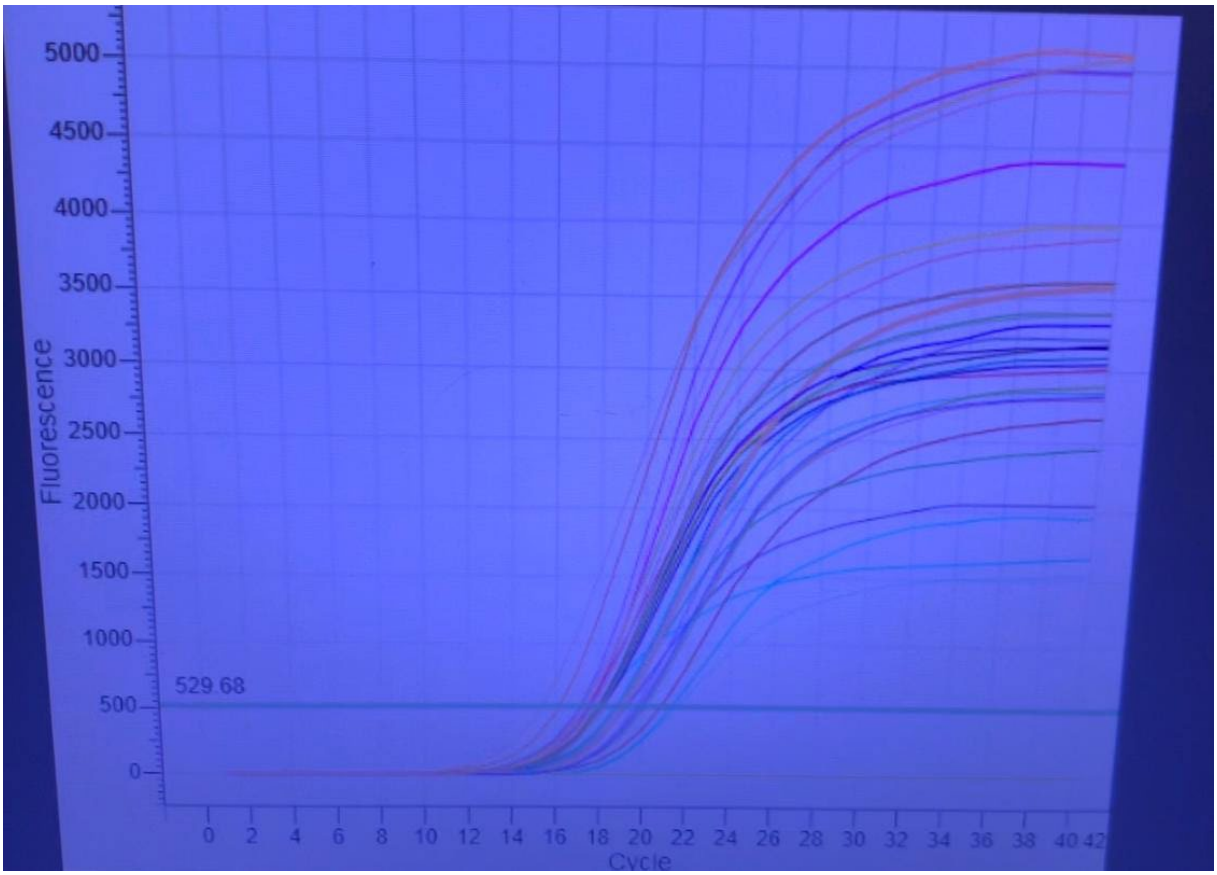
The gene expression datas are demonstrated as Cycle Threshold(Ct), which means the number of cycle where the logarithmic plot of PCR cross. GraphPad Prism 10.2.3 version is utilized to perform the statistical analysis. Fold change(2^-ΔΔCT) method is used in order to make comparison of IL-11 between the four groups ΔCT= Ct of IL-12 gene - Ct of ACTB (housekeeping gene). One way ANOVA analysis is performed in order to make comparison between the four groups.

Chapter 4

Results

The IL-11 Gene expression Analysis in Wound Tissue:

Figure 3: Ct PCR for 46 samples duplicated and negative control



DC, NO, I1-6, INO are our 4 groups that are composed of the 46 samples. Also our control reference, the housekeeping genes, are distributed in to same 4 groups. Correlation analysis will be done by using these four different groups. In these groups, the IL-11 is our target gene. The Housekeeping is ACTB(beta actin gene) in these groups. The recognition of effects will be done in a correct way by the aid of the study of applied therapies in each group.

We perform the analysis to gene expression by the analysis of (2^-ΔΔCT) used GraphPad Prism for the comparison of four groups.

Statistical Analysis

Table 6. Brown- Forsythe test results

Brown-Forsythe test					
F (DFn, DFd)	2,433 (3, 42)				
P value	0,0783				
P value summary	ns				
Are SDs significantly different (P < 0.05)?	No				

P Value Analysis

P value: 0.0783 indicates that the differences among the group means are not statistically significant (P > 0.05).

P value summary: ns (not significant)

Confirms that there is no significant difference among the means of the groups.

No Significant Difference Among Means. The ANOVA results (P value = 0.0783) indicate that there are no significant differences among the means of the four groups, which is also visually supported by the graph showing overlapping error bars for NOs, INOs, and I1-6.

Bartlett's Test

Table 7. Results for Bartlett's test

Bartlett's test					
Bartlett's statistic (corrected)	151,3				
P value	<0,0001				
P value summary	****				
Are SDs significantly different (P < 0.05)?	Yes				

To evaluate the homogeneity of variances among the different treatment groups, Bartlett's test was conducted. The test yielded a Bartlett's statistic (corrected) of 151.3 with a p-value of <0.0001, indicating that the variances across the groups are significantly different ($p < 0.05$)

Bartlett's statistic (corrected): 151.3

P value: <0.0001

Indicates a highly significant difference in variances among the groups.

P value summary: ****

This typically denotes a P value less than 0.0001, indicating a very high level of significance.

Are SDs significantly different (P < 0.05)?: Yes

Confirms significant differences in standard deviations among the groups.

The significant differences in variances (Bartlett's test) are evident, with DC showing much higher variability compared to NOs, INOs, and I1-6.

ANOVA Table

Table Analyzed	Data 1				
Data sets analyzed	A-D				
ANOVA summary					
F	2,407				
P value	0,0806				
P value summary	ns				
Significant diff. among means (P < 0.05)?	No				
R squared	0,1467				
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	921,8	3	307,3	F (3, 42) = 2,407	P=0,0806
Residual (within columns)	5361	42	127,6		
Total	6283	45			
Data summary					
Number of treatments (columns)	4				
Number of values (total)	46				

Table 8. Results from one-way ANOVA

A one-way ANOVA was performed to assess the differences in IL11 expression across the three conditions: nitric oxide atmosphere, insulin cream, and the combination of nitric oxide environment and insulin cream, as well as a control group. The one-way ANOVA revealed no statistically significant difference in IL11 expression among the four groups ($F(3,42) = 2.407$, $p = 0.0806$). Although the p-value is relatively close to the conventional threshold of 0.05, it does not provide sufficient evidence to reject the null hypothesis of no difference in IL11 expression across the groups.

Treatment (between columns)

SS: 921.8

DF: 3

MS (Mean Square): 307.3

F (DFn, DFd): 2.407 (3, 42)

P value: 0.0806

These values show the breakdown of the ANOVA calculation, indicating that the treatment effect is not statistically significant.

Residual (within columns)

SS: 5361

DF: 42

MS (Mean Square): 127.6

These values represent the within-group variance.

Figure 4: IL-11 mRNA exression on each group

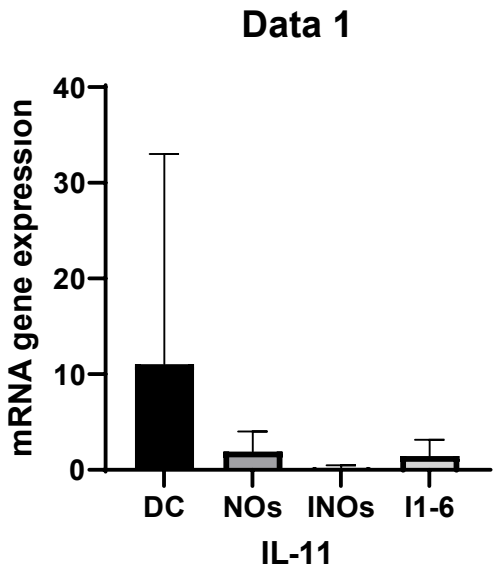


Figure 4 explains that x-axis shows the different variables measured: DC, NOs, INOs, and I1-6. Y-Axis demonstrates the measured values for mRNA gene expression, ranging from 0 to 40.

DC (black) represents the Control group which have the highest mean value with significant variability and highest mRNA expression. NOs (white) shows the samples that are treated only with nitric oxide. They have a lower mean value with smaller variability and low mRNA expression. I1-6 (white) illusturates the samples treated only with insulin which have a low mean value with some variability. Almost similar to NO group but lower. INOs (white) represents the samples treated with both insulin and nitric oxide. They are characterized by very low mean value with minimal variability and the lowest mRNA expression between the groups.

There is consistency with Prior Findings. The findings from the statistical analysis align with the graph(figure 2), where DC shows the highest mean and variability, while the other variables (NOs, INOs, I1-6) exhibit lower means and minimal variability.

To conclude, between these experimental conditions and IL-11 expression, there is no statistically significant difference. Thus we accept the effects of IL-11 expression have similarity between these conditions.

Chapter 5

Discussion

Hemostasis, skin proliferation, inflammation, and remodeling are the four main stages of the intricate procedure for wound healing. Generally, wounds fall into two categories: chronic wounds and acute wounds. Acute injuries can be burns, scalds, abrasions, or incisions made after surgery; persistent wounds, on the other hand, heal more slowly, often spending an excessive amount of time in the inflammatory phase and occasionally becoming worse due to age, systemic conditions, or repeated trauma. Examples of these wounds involve pressure sores, arterial, venous, and diabetic ulcers. Using its antiseptic properties, CAP may accelerate the skin cells growth and movement by either allowing or blocking receptors of integrin on the surface of the cell, or it may have a pro-angiogenic impact. (Tatiana Bolgeo et al.,2023)

Additionally, it seems to function by inducing the release of nitric oxide (NO), that facilitates movement of endothelial cell and the formation of vessel-like formations that aid in the neo-vascularization of wounds. The CAP treatment may be modified corresponding to the various steps of healing of wound; research has shown that helium plasma is more successful in healing wounds, while argon plasma is better at inducing coagulation. According to Amini et al., CAP therapy alters continuing levels of Growth-promoting factors and inflammatory cytokines like IL-8, IL-1, TGF-β, INF-γ, and TNF-α, which promotes recovery by starting the step of proliferation earlier.Furthermore, the production ofnitrogen species (RNS) and reactive oxygen species (ROS) by CAP can enhance the production of factors that allow angiogenesis, hence facilitating the recovery of wounds.(Tatiana Bolgeo et al.,2023)(Boeckmann et al.,2020)

Both the receptor and IL-11 are broadly generated. In addition to stimulating B-cell development, IL-11 also activates megakaryocytes, myeloid progenitors, erythroid precursors, and stem cells as well. It additionally impacts non-hematopoietic cells, such as those found in the liver and bones. Cytokines that induce inflammation (TNF, IL-1), as well as TGF-β, promote IL-11. (O'Shea, 2013)

IL-11 is induced by proinflammatory cytokines which are TNF, IL-1, and these cytokines are supressed by CAP therapy. Therefore, it is logical that in our research IL-11 expression was low because the cytokines that induce IL-11 were supressed in the presence of CAP nitric oxide.

TNF-α is the most potent pro-inflammatory cytokine in terms of inducing the generation of additional cytokines, including various expression molecules plus IL-6. For the first time, Qiang Sun et al. (2014)showed that insulin treatment in vitro reduced ischemia/reperfusion (I/R)-induced TNF-α creation in cardiomyocytes by the Akt-eNOS-NO signaling pathway. They also directly showed that insulin blocks TNF-α regionally and globally in myocardial I/R rats.Furthermore, in the setting of normoglycemia in rat and pig model, insulin has been shown to reduce TNF-α, IL-6 production, and increase the cascades that supresses inflammation, therefore mitigating the endotoxin-induced systemic inflammatory response. Each of these results clearly suggests that insulin functions as a drug that reduces inflammation by suppressing immune mediators and cytokines that are pro-inflammatory.(Qiang Sun et al.,2014)

TLR4-deficient animals showed a more than 50% reduction in the MI area, as initially shown by Oyama et al. This decline was linked to a lessened level of myocardial inflammation, as shown by a decrease in lipid peroxides and neutrophil invasion. Eritoran, a particular TLR4 antagonist, suppressed the manufacturing ofIL-6, MCP-1, TNF-α and,IL-1β resulting in a 40% decrease in MI. Furthermore, it has been discovered that TRL4 influences KC and MCP-1 expression, which in turn determines the invasion of neutrophils following global MI. TRL signaling inhibition is advantageous in I/R-based models of animals and is linked to a reduced MI size. The mRNA activities of TLR1, -2, -4, -7, and -9 in MNCs have been shown to be strongly repressed by infusions of insulin (2 U/h) in individuals with type 2 diabetes (T2D) in two hours. This fast inhibition may be achieved by the reduction of PU.1 interaction and followinginduction of TLRs. Insulin thereby mitigates TLR-mediated inflammatory damage by suppressing the transcriptional activation of many TLRs.(Qiang Sun et al.,2014)

Because NPH insulin supresses the production of TNF-α, IL-6 and increase cascade that supresses inflammation, in out experiment it is sensible to assume that NPH insulin causes the low expression of IL11. Moreover, deficiency of TLR-4 caused the suppression ofIL-6, IL-1β, TNF-α and it is shown that insulin causes repression of TLR-4 so ultimately this causes a decline in the production of IL11 by TNF-α.

By controlling oxidative stress and inflammatory reactions, topical insulin promotes wound healing. Insulin therapy reduces reactive oxygen species, this may have detrimental impacts on DNA, proteins, and lipids in rats with injuries caused by burns. Furthermore, topical insulin promotes the early recruitment of neutrophils and reduces inflammation in

injuries by elevating IL-10 quantities and the quantity of M2 macrophages, which helps to remove dead tissue. By controlling MCP-1 production at wound sites, insulin promotes the chemotaxis and phagocytosis of macrophages along with the release of inflammatory mediators in vitro.(Wang, 2020).

Furthermore, improvement of keratinocyte, promotion of fibroblastic response and acceleration of re-epithelialization are done by treatment of topical insulin on skin injuries. the PI3K-Akt-Rac1 network facilitates the insulin receptor-dependent but EGFR-dependent keratinocyte movement and maturation that are supported by insulin. Elevated hydroxyproline values show topical insulin therapy enhances the accumulation of collagen and development on damaged skin. (Wang, 2020)

Insulin has an angiogenic impact on injuries as well as controlling re-epithelialization and processes of inflammation in damaged areas. In recovering tissues, the growth of new blood vessels is promoted by insulin. Moreover, insulin administered subcutaneously promotes the movement of microvascular endothelial cells and the development of endothelial tubes. This biological action is linked to the signaling pathway PI3K-Akt-SREBP1. Furthermore, there is mounting proof showing that topical insulin induces angiogenesis and thickens blood vessels in diabetic wounds. This likely occurs through the recovery of compromised insulin signaling, including the pathways of PI3K/Akt and MAPK/ERK, as well as through upregulating the production of VEGF and angiopoietin-1. (Wang, 2020)

A number of interleukins decrease both innate and acquired immunity, which helps to regulate inflammation. A few that control the induction of T cells and growth are IL-10, IL-37, and IL-38. These agents do this by attaching to inhibiting receptors such as IL-18Ra (IL-R5) and IL-1R6. B cells and macrophages generate IL-38, which suppresses TNF, IL-17, IL-22, IL-1, and IL-6.IL-10 has the most anti-inflammatory effects of the various anti-inflammatory cytokines, inhibiting activated macrophages' production of cytokines associated with inflammation including TNF-α, IL-1, as well as IL-6. Furthermore, IL-10 has the ability to block cytokine receptors that allow inflammation and stimulate endogenous anti-cytokines. As a result, it has the ability to modulate the synthesis of cytokines that allow inflammation and activity on several different levels. Rapid IL-10 protein treatment has been shown in a number of studies involving animals, including peripheral neuritis, excitotoxic

damage of the spinal cord, and peripheral damaged nerves, to block the progression of spinally-mediated pain facilitation.(Zhang, 2009)

It has been demonstrated that IL-6 is required for the brain's response to nerve damage. Regenerative benefits were diminished when antibodies of anti-IL-6R were employed in vivo to disable IL-6R. Moreover, IL-6 controls the production of neuropeptides in neurons and is implicated in triggering the proliferation of microglial cells plus astrocytic cells. Proof suggests that after peripheral damage to the nerve, IL-6 plays a role in the occurrence of behavior for neuropathic pain. Sciatic cryoneurolysis, for instance, raises IL-6 immunoreactivity in the spinal cord by periodically thawing or freezing a sciatic nerve segment. Furthermore, in intact and nerve-damaged rats, administration of intrathecal IL-6 causes tactile allodynia and heat hyperalgesia, correspondingly. (Zhang, 2009)

Chapter 6

Conclusion

We were able to reach the endpoint of our research. We produced different expression levels for IL-11 by using NO gas therapy alone, NPH insulin therapy alone and finally we used them together. We hypothesized that synergistic effects of NPH insulin and cold atmospheric NO can enhance the wound healing compared to NO gas therapy alone. We can see that it is possible. First reason is that while NO gas therapy have a low IL-11 expression, insulin therapy alone resulted an even lower IL-11 expression than that. Secondly, IL-11 expression is at its lowest when NO gas therapy and insulin therapy used together. Finally,The most current in vitro and in vivo findings clearly demonstrate that IL11 has pro-inflammatory impacts, despite the fact that previous research and even recent reviews considered it to have an anti-inflammatory. To sum up, even if the IL-11 has anti-inflammatory effects, having a very low expression of IL-11 would prevent the activation of its pro-inflammatory effect and considering the anti-iflammatory effects of NPH insulin and NO gas therapy, because of these reasons we can expect an enhanced wound healing in the diabetic rats.

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YAKIN DOĞU ÜNİVERSİTESİ
HAYVAN DENEYLERİ YEREL ETİK KURULU
ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

Toplantı Tarihi **17/01/2024**
Toplantı No **: 2024/169**
Proje Başvuru No **: 169**

Yakın Doğu Üniversitesi, Veteriner Hekimliği Fakültesi'nden, sorumlu araştırmacı Dr. Ali Çürükoğlu tarafından hazırlanan 'Diabetik ratlada doku kayıplı yaraların iyileşmesinde soğuk atmosferik Nitrik Oksit (NO) gazının tek başına ve/veya NPH insulin krem ile kullanımında *IL-1, IL-2, IL-6, IL-8 , IL-11, IL-12, Il,22 ,CD4 ,CD8* gen ekspresyon analizlerinin karşılaştırılması başlıklı araştırma önerisi kurulumuz tarafından uygun bulunmuştur.

1. Prof. Dr. Emine KOÇ (BAŞKAN
2. Prof. Dr. Tamer YILMAZ (ÜYE)
3. Prof. Dr. Nurettin ABACIOĞLU (ÜYE)
4. Prof. Dr. Dilek ARSOY (ÜYE)
5. Prof. Dr. Aysel KÜKNER (ÜYE)
6. Prof. Dr. Vedat SAĞMANLIGİL (ÜYE)
7. Prof . Dr. Ahmet Özer ŞEHİRLİ (ÜYE)
8. Vet. Hek. Ahmet SERHATOĞLU (ÜYE)
9. Vet. Hek. Meliha TEMİZEL (ÜYE)
10. Avukat Ömür Güneş ÖZTÜRK (ÜYE)


turnitin
Sayfa 2 of 49 - Bütünlük Genel Bakış
Gönderi Kimliği trn:oid:::1:2989875623
9% Genel Benzerlik
Her veri tabanı için çakışan kaynaklar da dâhil tüm eşleşmelerin kombine toplamı
Eşleşme Grupları
80 Atıf ya da Alıntı Yapılmamış 8%
Ne metin içi atıf ne de tırnak işareti içeren eşleşmeler
12 Eksik Alıntılar 1%
Kaynak materyale hâlâ çok benzeyen eşleşmeler
0 Eksik Atıf 0%
Tırnak işaretleri olan ancak metin içi atıfları olmayan eşleşmeler
0 Atıf Yapılan ve Alıntılanan 0%
Metin içi atıf içeren ama tırnak işareti içermeyen eşleşmeler
Ön Sıradaki Kaynaklar
5% İnternet kaynakları
5% Yayınlar
4% Gönderilen çalışmalar (Öğrenci Makaleleri)
Bütünlük Bayrakları
İnceleme için 1 Bütünlük Bayrağı
Değiştirilen Karakterler
13 sayfada 84 şüpheli karakter
Harfler başka bir alfabeden benzer karakterlerle değiştirilir
Sistemimizin algoritmaları bir belgede, onu normal bir gönderiden ayırabilecek her türlü tutarsızlığı derinlemesine inceler. Tuhaf bir şey fark edersek incelemeniz için bayrak ekleriz.
Bir Bayrak mutlaka bir sorun olduğunu göstermez. Ancak daha fazla inceleme için dikkatinizi vermenizi öneririz.