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Effect of Oasis-Ultra Matrix on the Healing Rate of Stage IV Pressure Wounds

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Effect of Oasis-Ultra Matrix on the Healing Rate of Stage IV Pressure Wounds

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By

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Abdelfatah Shaban .T. Abou Issa ENTITLED Effect of Oasis Ultra Matrix on the Healing Rate of Stage IV Pressure Wounds BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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ABSTRACT

Abou Issa, Abdelfatah. M.S., Department of Pharmacology and Toxicology, Wright State University, 2016. Effect of Oasis-ultra Matrix on the Healing Rate of Stage IV Pressure Wounds.

Introduction: (Oasis-ultra) is an extra cellular collagen rich matrix derived from porcine intestinal sub-mucosa. A prospective, multi-centered, randomized, single-blinded clinical trial was conducted to study the effects of Oasis-ultra combined with negative pressure wound therapy (NPWT) on the healing rate of stage IV pressure wounds versus NPWT alone.

Materials and Methods: Twelve subjects were involved in the study: six patients in the study group and six in the control group. NPWT was changed twice a week for all subjects, and Oasis-ultra was applied weekly. The wounds were measured weekly, and the healing rate was calculated for each subject for 12 weeks. The canisters were collected monthly for three months. For cytokine and growth factors analysis, 100 µl 1XPBS were added to the sample, and protein concentration was determined using the Bradford assay. A Bio-Rad BioPlex 96 well plate was set up with 50 µl of the sample and duplicated for cytokine analysis using Bio-Plex.

Results: In the study group, the healing rate calculated at 12 weeks was found to be ~87% when compared to the control group, which was ~55%. Analysis of different growth factors, normally present in stage IV pressure wounds, revealed higher concentrations in the oasis-ultra treated group when compared with the control group. Additionally, the other proinflammatory cytokines that accused of wound chronicity were down regulated as a result of treating the subjects in the study group with oasis-ultra.

Conclusion: Our study demonstrates that the use of Oasis-ultra accelerates the healing rate of stage IV pressure wounds when combined with NPWT. Also, in the Oasis-ultra treated group, the proinflammatory cytokines were successfully inhibited. At the same time, Oasis-

ultra promoted and upregulated the beneficial growth factors that had positive impact on the healing rate.

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Introduction:

Skin is the largest organ in the body. It works as a protective layer against toxins and microorganisms, and provides chemical protection against invasion by toxins and microorganisms. Also, skin plays an important role in thermoregulation and prevention of dehydration. (Choi, Uyama, Lee, & Sung, 2015) A wound can be defined as a breakdown in the protective function of the skin. Also, wounds can be defined as disruption in the epithelial lining of the skin or mucosa due to either physical or thermal injury (Dhivya, Padma, & Santhini, 2015; Lazarus et al., 1994).

A better understanding of wound healing is essential to help reducing patient morbidity and mortality related to abnormal or prolonged wound healing. Wound healing is a dynamic and complex process occurring in response to tissue injury, which is accomplished through four highly integrated, matriculated and overlapping phases: hemostasis or coagulation, inflammation, proliferation, and remodeling or maturation. (Dhivya et al., 2015; Guo & Dipietro, 2010; Rosique, Rosique, & Farina Junior, 2015) Wound healing phases are affected by active biological substances either directly or indirectly, like growth factors and cytokines. Also, other factors affecting wounds (Guo & Dipietro, 2010) which are classified into local factors and systemic factors. The local factors include, oxygen supply which is very critical for proliferation, angiogenesis and collagen production (Schreml et al., 2010; Zhong, 2011), infection, foreign body, venous sufficiency. The systemic factors include, age, gender, sex hormones, stress, ischemia,

diabetes, obesity, medications, like glucocorticoid, non-steroidal anti-inflammatory medications, chemotherapy, alcohol and smoking, nutrition, immune-compromised conditions, such as cancer, radiation therapy, AIDS (Dhivya et al., 2015; Guo & Dipietro, 2010).

In general, acute wounds heal within 2–4 weeks, (Schreml et al., 2010; Zhong, 2011) while chronic wounds show failure of healing within 12 weeks despite treatment, or delayed-healing which can extend up to 48 weeks without treatment (Guo & Dipietro, 2010). Nowadays, the concept of an ‘‘ideal dressing’’ should not only provide appropriate moisture and protective role to the wounds, nevertheless, should also directly stimulate cellular proliferation, differentiation and migration. Researchers for 3 previous decades, focused on cellular growth factors and opportunities of their use in the treatment of chronic wounds. (Stanirowski, Wnuk, Cendrowski, & Sawicki, 2015)

Chronic wounds:

Chronic wounds affect millions of Americans and cost the country annually around $50 billion (Brem, Kirsner, & Falanga, 2004; L. Shi & Ronfard, 2013). Also, around 35 million cutaneous wounds require major intervention every year (Shapira et al., 2015; Tonnesen, Feng, & Clark, 2000). In the U.S. alone, chronic wounds not only consume a great deal of healthcare resources all over the world, but they are really a big challenge to wound-care professionals as well. Different underlying pathologies make different types of chronic wounds difficult to heal. From the literature, many research articles mentioned the high prevalence of people who complain of chronic wounds. Here is some factors that are mentioned in previous research articles associated with chronic wounds like, age

(Gosain & DiPietro, 2004), smoking (Goldminz & Bennett, 1991), obesity and other comorbid diseases, such as diabetes, venous and arterial insufficiency (Gainza, Villullas, Pedraz, Hernandez, & Igartua, 2015; Menke, Ward, Witten, Bonchev, & Diegelmann, 2007). The most abundant inflammatory cells in chronic wounds are neutrophils which are accused of releasing large amounts of proteases. As a result, with more inflammation, wounds will remain in the inflammatory phase with failure to progress to proliferative phase. Therefore, the best way for better understanding of the differences between various types of chronic wounds, we should have a deep background understanding of wound phases and the molecular and cellular biology that happens in order to improve our treatment approaches, leading to better healing rates, and facilitating the development of new effective therapies, which leads to better clinical outcome (Frykberg & Banks, 2015). Chronic, non-healing wounds could occur as a complication of different disorders related to lack of oxygen and nutrients which are necessary to the wound cells proliferation and migration (Guo & Dipietro, 2010). As a result, chronicity makes wounds a potential source of infection and ends by necrosis of the surrounding tissues and makes healing hard to obtain (Stanirowski et al., 2015). Reassessment of underlying pathology and consideration of the need for collagen and advanced therapeutic agents should be introduced when wounds fail to achieve sufficient healing after 4 weeks of standard care (Frykberg & Banks, 2015). The care of chronic wounds has become very important, with providing advanced therapies, including, extracellular matrices (ECMs), growth factors, engineered skin, and negative pressure wound therapy (NPWT) as a standard of care, etc (Frykberg & Banks, 2015; Rice et al., 2014). Different other studies have shown increased levels of the pro-inflammatory cytokines like IL-1, IL-6, and MMPs in chronic wounds. Also, a lot of

studies have proved strong correlations between chronic wounds and these pro-inflammatory cytokines (Patel, Maheshwari, & Chandra, 2016).

Chronic wounds can be classified as vascular ulcers (venous and arterial ulcers), diabetic ulcers, and pressure ulcers (decubitus ulcers) (Nunan, Harding, & Martin, 2014). The most common characteristics shared by each of these chronic wounds are: prolonged or excessive inflammation (Schultz et al., 2003), persistent infections, formation of biofilms (bioburden), and the inability of dermal and epidermal cells to respond to reparative stimuli (Attinger et al., 2006; Demidova-Rice, Salomatina, Yaroslavsky, Herman, & Hamblin, 2007; Edmonds, 2012; Frykberg & Banks, 2015; A. Stojadinovic, Carlson, Schultz, Davis, & Elster, 2008; Woo, Ayello, & Sibbald, 2007).

Collagen types:

Collagen is the most abundant protein in the body. It constitutes around ¼ of total protein in the human body, which is mainly produced by fibroblasts and secreted into the ECM. Collagen is involved in all phases of the wound-healing cascade. It stimulates cellular migration and contributes to new tissue development (Fleck & Simman, 2011).

Collagen mainly presents in the bones, connective tissue, tendons, blood vessels, and skin. In the skin, collagen in conjunction with other proteins such as elastin, forms the basic flexible and pliable matrix that incorporates living dermal cells, blood vessels, sebaceous glands, and other components of the extracellular matrix (glycosaminoglycans, glycoproteins) (Fleck & Simman, 2011).

Type III collagen is first produced in adult wounds in a form of granulation tissue, followed largely by type I. However, in fetal and newborn tissue, type III collagen is still the dominant type of collagen. Collagen is synthesized in fibroblasts and secreted to support ECM in a triple helix form known as a procollagen. Procollagen has propeptides attached to the carboxy and amino terminals. At the ECM these monomers self-assemble into collagen fibrils. Vitamin C is an important factor which is essential for all wound healing phases (Moores, 2013). Ascorbic acid is also needed for the hydroxylation of proline and the formation of stable triple helices for collagen formation. Therefore, wound-care professionals should check the nutritional status of chronic wound patients who might have vitamin C deficiency as the cause of their wound healing delay (Moores, 2013). Research by Szarka & Lőrincz revealed the possibility of ascorbic acid involvement in oxidative protein folding and hydroxylation that is needed for collagen and ECM formation (Szarka & Lorincz, 2014).

Based on many scientific articles, collagen-based dressings could contribute toward wound healing by changing wound molecular biology levels and may trap more growth factors that help accelerate the healing process (Fleck & Simman, 2011). Actually, fibroblasts produce ECM in the form of collagen and that occurs by interaction between fibroblast and myofibroblast (Werner, Krieg, & Smola, 2007). Many studies reveal the clear interaction between fibroblasts and keratinocytes (Werner et al., 2007), the latter one will activate fibroblasts to produce different growth factors. These growth factors basically will help keratinocyte to proliferate.

Growth factors and cytokines literature review:

Table 1: Shows the important Growth Factors and Cytokines Involved in Wound Healing

Growth factor	Abbreviation	Source	Functions
Platelet-derived growth factor	PDGF	Platelets, keratinocytes, fibroblasts, endothelial cells, perivascular cells	Fibroblast proliferation, chemotaxis & collagen metabolism; angiogenesis
Transforming growth factor-β	TGF-β	Platelets, keratinocytes, fibroblasts, endothelial cells, macrophages	Fibroblast proliferation, chemotaxis & collagen metabolism; angiogenesis; immunomodulation
Transforming growth factor-α	TGF-α	Platelets, keratinocytes	Keratinocyte proliferation & migration
Epidermal growth factor	EGF	Platelets	Keratinocyte proliferation & migration
Interleukins	IL-1, IL-10	Leukocytes, keratinocytes	Fibroblast proliferation; promotes inflammation
Tumor necrosis factor-α	TNF-α	Leukocytes, keratinocytes	Promotes inflammation
Fibroblast growth factor	FGF	Keratinocytes, macrophages	Fibroblast and epithelial cell proliferation; matrix deposition, wound contraction; angiogenesis
Vascular endothelial growth factor	VEGF	Platelets, keratinocytes, macrophages, neutrophils	Angiogenesis; vascular permeability; macrophage chemotaxis

Since growth factors and cytokines are very important to wound healing, they have been extensively studied over the past decades. They contribute and regulate the activity of the cells in order to accomplish healing as a response to tissue injury (Molloy, Wang, & Murrell, 2003; Rosique et al., 2015). Complement cascade and various growth factors have been shown to have a role in the inflammation and have recently been shown to augment

wound healing. In our study, we investigate around 27 cytokines and growth factors that many of them have direct relation to wound healing; for example, interleukin- (IL-1a), (IL-1b), (IL-6), and tumor necrosis factor (TNF-alpha), which have been linked to regulate the function of neutrophils and recruit other inflammatory cells toward the wound location. During the inflammation phase of wound healing, TNF-alpha is a major cytokine secreted mainly by macrophages and neutrophils. It is elevated in early wound healing and could stay elevated in chronic wounds and contribute to the inflammation phase (Goel, Kumar, Singh, & Bhatia, 2010; Rosique et al., 2015; Streit, Beleznay, & Braathen, 2006).

Studies have shown that neutrophils are considered as the fastest eukaryotic cells because their speed has been measured through an *in vitro* study and reached 10 μm/min using two-dimensional (2-D) surfaces (Halilovic, Wu, Alexander, & Lin, 2015; Moghe, Nelson, & Tranquillo, 1995). Some of these pro-inflammatory cytokines accused for delaying wounds by degrading the extracellular matrix and growth factors that are necessary for wound healing and also by inhibiting cell proliferation and migration (Neuman, Nanau, Oruna, & Coto, 2011). R. Rosique. M. Rosique & Farina, (2015) mentioned that tumour necrosis factor-alpha antibody (Infliximab) can inhibit TNF-alpha activity and was effective in treating many chronic inflammatory diseases. In a case series done by Streit, Markus, Beleznay, & Braathen (2006) were they applied Infliximab topically to treat 14 patients with chronic venous ulcers for more than a 4-month duration (Streit et al., 2006). Infliximab was applied as a solution or as a gel formulation and was repeatedly applied to the ulcers for more than 4 months duration. After 8 weeks, the authors noted that five ulcers completely achieved healing, while the other four ulcers did achieve more than 75% of healing rate (Rosique et al., 2015; Streit et al., 2006). Therefore, further

studies should be conducted to evaluate the effect of topical Infliximab on chronic wound healing.

Another growth factor that we analyzed is Fibroblast growth factor (FGF), which has a crucial contribution in angiogenesis, cell growth, and tissue repair. FGF consists of 22 members, 18 of them are mammalian FGF, which are grouped into 6 subfamilies according to the differences in their sequence and the range of their biological functions (Beenken & Mohammadi, 2009; W. H. Lin et al., 2015). China was the first and the only country in the world for clinical application of FGF-1, FGF-2, and FGF-10 which have been approved by the SFDA for wound healing. Other therapeutic usages that are mentioned in the literature interfere with FGF2-induced angiogenesis, for example, Thalidomide. This medication has been tested and shows in phase II trials that its efficacy in treating cancer, including renal and prostate cancers, was notable (Beenken & Mohammadi, 2009; Eisen et al., 2000; Figg et al., 2001).

In our experiment, we tested basic fibroblast growth factor (bFGF), which is the same as FGF2. Suzuki et al., 2015, showed that bFGF does accelerate wound healing by enhancing granulation and epithelialization. bFGF is increased mainly in acute wounds (S. Barrientos, Stojadinovic, Golinko, Brem, & Tomic-Canic, 2008) and different research papers mentioned that bFGF is responsible for granulation tissue formation by stimulation of the growth of fibroblasts and endothelial cells. (Greenhalgh, Sprugel, Murray, & Ross, 1990; Powers, McLeskey, & Wellstein, 2000). bFGF was found to be involved in granulation tissues and new capillary formation (Masuoka, Morimoto, Sakamoto, Ogino, & Suzuki, 2015). Also, bFGF is considered as an angiogenic factor (Basilico & Moscatelli, 1992) that is needed for angiogenesis (Greenhalgh et al., 1990). FGF-2 works as a

fibroblast stimulus in an autocrine manner (Powers et al., 2000). Research studies that were done on diabetic mice db/db have revealed that treatment with human recombinant PDGF-BB, bFGF, or a combination of both increased the rate of cellular infiltration and capillary ingrowth into the wounds of db/db mice. In another study, scientists tested the fibroblast migration from human dermal skin and they found that it was significantly impaired in presence of a high glucose level; however, bFGF has increased the number of cells and enhanced the migration of human dermal fibroblasts in diabetic patients (H. Shi et al., 2015). Researchers also emphasized in many articles that diabetes itself has negative effects on the activity of fibroblasts, which mainly affect proliferation and migration function of fibroblasts (Hehenberger & Hansson, 1997; Hehenberger, Heilborn, Brismar, & Hansson, 1998). Hehenberger et al. (1998) have addressed in his research that fibroblast proliferation inhibition and growth factors resistance occur in presence of high glucose concentrations. Around 4 decades or more, researchers started a clinical trial using recombinant human basic fibroblast growth factor (RbFGF). Actually, in June 2001 Japan launched a product named "Fiblast Spray" and it was used to treat pressure ulcers (decubitus ulcers) and different types of skin ulcers.

One of the growth factors responsible for dermal fibrosis is transforming growth factor-beta1 (TGF-β1). TGF-β1 induced fibroblast cell proliferation and excessive accumulation of collagen type I (L. Lin, Wang, Liu, & Huang, 2015). In this paper, Wang and Huang (2015) run experiments by obtaining keloid tissue donating from a male, 22 years old. After that, they took pieces of keloids and implanted them into subcutaneous cavity on the 24 nude mice model of keloid (8-week old). Lin et al. (2015) have been revealed the results of this interesting *in vivo* experiment after 6 weeks from the injection

of BMP and activin membrane-bound inhibitor (BAMBI) which acts as a functional inhibitor for TGF-β receptor. They have shown that growth of the implanted keloids significantly diminished through suppressing TGF-β1 after injection of BAMBI. Therefore, we can infer from this experiment that TGF-β1 has an effective role on fibroblast cell proliferation and collagen type I production (L. Lin et al., 2015; C. Liu et al., 2014). Transforming growth factor beta1 (TGF-β1) is a cytokine that has the broadest spectrum of effects which have involved in a variety of processes like fibroblast proliferation and differentiation, cellular migration, extracellular matrix (ECM) production, and cell apoptosis (Finnson, McLean, Di Guglielmo, & Philip, 2013; Roux, Borbely, Sloniecka, Backman, & Danielson, 2015).

Mainly macrophages and platelets are the main cells contributing to release transforming growth factor beta (TGF-β) which has a major role as a potent chemoattractant and activator of fibroblasts that are involved in collagen synthesis. TGF-β is described in the literature as the best fibrogenic mediator in wound healing. In the study that was done by Wang et al. (2011) where they added exogenous metalloproteinase 2, (MMP-2) to the wound culture *in vitro* and they did close observation to the activity of ACL fibroblast toward the healing process. What they found is the TGF-β1 was significantly increased and as a result, it did stimulate ACL fibroblasts to produce more collagen that was needed for complete wound healing (Wang et al., 2011). Therefore, the main point that we infer from this experiment is the TGF-β1 has a crucial part of wound healing by advancing the fibroblast proliferation and migration (Wang et al., 2011). TGF-β family is composed of three main isoforms: TGF-β1, TGF-β2, and TGF-β3, which formed essentially by interrelated and interconnected dimeric polypeptide chains

(Poniatowski, Wojdasiewicz, Gasik, & Szukiewicz, 2015). TGF- β1, in acute skin injury is mainly secreted from different cell types, such as platelets, keratinocytes, fibroblasts, and macrophages (Faler, Macsata, Plummer, Mishra, & Sidawy, 2006). On the other hand, in chronic wounds, TGF-beta levels are low (Robson, 1997) which could be destructed due to various proteolytic enzymes, like elastase, which are produced mainly from neutrophil cells that could make these kind of growth factors very low and ineffective in wound healing (S. M. Chen, Ward, Olutoye, Diegelmann, & Kelman Cohen, 1997).

Interleukin-6 (IL-6) is a proinflammatory cytokine that increased in acute wound phase and probably mounted in chronic wounds as well. IL-6 also is produced by a variety of cell types; however, IL-6 receptors are expressed only in a few cells (O'Reilly, Ciechomska, Cant, Hugle, & van Laar, 2012). IL-6 plays an important role in the inflammation phase (O'Reilly et al., 2012). Therefore, good level of IL-6 signaling is needed to kick off the wound healing mechanism. In addition to chronic wounds, IL-6 is involved in other systemic diseases like chronic inflammatory diseases, such as inflammatory bowel diseases, diabetes, systemic sclerosis, and asthma (O'Reilly et al., 2012).

Recent studies suggest the possibility of treating all of these chronic autoimmune diseases just by blockage of IL-6 signaling (Jones, Scheller, & Rose-John, 2011). Researchers think that way is a very effective strategy and may be a promising therapy for such chronic inflammatory diseases and cancer (X. Liu, Jones, Choy, & Jones, 2016; Neurath & Finotto, 2011). One of the unique functions of IL-6 is acting as an organizer to the flow of chemokines that are responsible for leukocyte movements. IL-6 also plays an

important role in shifting from innate to adaptive immunity which ultimately leads to leukocyte differentiation and proliferation (Jones, 2005; Jones et al., 2011).

Sloniecka, Le Roux, Zhou, & Danielson, (2016) addressed the effect of substance-P (SP) stimulation over the proinflammatory IL-8. IL-8, after being activated, acts as a chemotactic to inflammatory cells, mainly neutrophils to migrate into wound location (Sloniecka, Le Roux, Zhou, & Danielson, 2016). Interestingly, IL-8 promotes keratinocyte migration and differentiation through SP activation. IL8 is produced by epithelial and fibroblast cells that are the main cells producing IL-8 which is responsible for the inflammation in the wound healing (Dobreva, Waeber, James, & Widmann, 2006; Matsushima & Oppenheim, 1989). Baggiolini et al. (1994) emphasised IL-8 aggregation especially after migration of monocytes to the site of injury and also the strong correlation between IL-8 and chronic inflammatory conditions in presence of neutrophils (Padrines, Wolf, Walz, & Baggiolini, 1994).

Previous research studies discussed the fact that especially during the phagocytosis process, neutrophils produce IL-8 (Bazzoni et al., 1991) after adding LPS to neutrophils; however, the exact role of neutrophil proteinases in activating IL-8 is still unclear.

IL-8 is secreted by neutrophils in two forms, IL-8(77) and IL-8(72). One contains 77 amino acids and the other one has 72 amino acids (Bazzoni et al., 1991). Here, Bazzoni, et al. (1991) explained in his research paper that IL-8 is converted into the active form only after neutrophils' protineases-releasing enzymes, like elastase, cathepsin-G and proteinase-3 during the inflammatory phase.

Many research papers mentioned the function of IL-8 as a chemotactic to leukocytes and fibroblasts, as well as its huge role contributing in wound healing process (Dobreva et al., 2006).

Here is another important growth factor called Platelet Derived Growth factor (PDGF) molecule, which consists of two polypeptide chains connected by an intermolecular disulfide bond (Hughes, Clunn, Refson, & Demoliou-Mason, 1996). There are five isoforms of PDGF: PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD (Gilbertson et al., 2001; Hughes et al., 1996; LaRochelle et al., 2001). PDGF is a hydrophilic glycoprotein, released by a wide range of cells including platelets, macrophages, and different other cells like endothelial and vascular smooth muscle cells (Hughes et al., 1996).

PDGF is considered as a weaker angiogenic growth factor compared to FGF and VEGF (S. Barrientos, Stojadinovic, Golinko, Brem, & Tomic-Canic, 2008); however, PDGF is considered as one of the earliest growth factors which acts as a chemotactic to different cell types responsible for migration and wound healing (Heldin & Westermark, 1999). In addition, PDGF works as a good stimulus to fibroblast cells to proliferate and lay collagen that is necessary to promote the ECM network as the end result (Clark, 1993; Heldin & Westermark, 1999).

In our research, we specifically analyzed PDGF-BB type. In the Unites States, the only randomized clinical trials that were successfully completed until now used PDGF-BB (S. Barrientos et al., 2008a). In a previous diabetic-mouse model-wound impairment study, where researchers are used to test some of the growth factors and how they could improve

wound healing, like PDGF-BB and basic fibroblast growth factor (Greenhalgh et al., 1990). PDGF has two different receptors: PDGFα receptor and PDGF-β receptor. Interestingly, the binding specificity of these receptors are different toward the two PDGF chains; for example, PDGF-A and PDGF-B chains bind to PDGFR-α specifically. While the PDGF-β receptor binds only to PDGF B-chain type. (Hughes et al., 1996; LaRochelle et al., 2001).

PDGF was approved for clinical use in 1997, and was named commercially as (Regranex) and the first time was used for treating diabetic foot ulcers. In many clinical usages of synthetic PDGF, they have shown increasing granulation tissue of cutaneous wounds. Steed et al. (2006) in their clinical trials have evaluated the efficacy of rhPDGF-BB. Application of rhPDGF-BB only one time daily is considered adequate and safe treating chronic wounds like diabetic foot ulcers (Steed, 2006).

VEGF-A is considered the most potent type of VEGF family, which consists of other member types: VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEG-F, and placental growth factor (PLGF). VEGF receptor (VEGFR) expression in the very early time of literature was thought only present in the endothelial cells, but researchers found out recently that VEGF receptors can be present and expressed by other cell types like macrophages and keratinocytes. That clearly indicates that VEGF as a growth factor may have other roles during wound healing process than was previously believed (Johnson & Wilgus, 2014). The literature reported that VEGF active levels were significantly low in people who have chronic wounds (S. Barrientos et al., 2008; Bodnar, 2015; Johnson & Wilgus, 2014).

Presence of excessive amounts of inflammatory cytokines/chemokines in the wound is regarded to be inhibitory to the neovascularization process. On the other hand, the ability of wounds to form a new vasculature will definitely improve wound healing (Bodnar, 2015).

Angiogenesis:

Angiogenesis is a process of new blood vessel growth from already existing vessels (Johnson & Wilgus, 2014). Failure of wounds to form a new vasculature will lead to inability of wounds to heal. Therefore, this process is considered as an essential step for successful wound healing. Providing oxygen and nutrients to injured tissues via new vessels formation (angiogenesis) is crucial for cell growth and ultimate wound healing (Johnson & Wilgus, 2014). Also, angiogenesis helps tissue to survive after ischemia, and it supports damaged tissues. Angiogenesis also can be defined as an amplification operation that happens at the level of already existing microvascular vessels in order to increase tissue growth and regeneration (Cuevas & Boudreau, 2009). Vascular endothelial growth factor (VEGF) is considered to be one of the strongest proangiogenic growth factors present in the skin. Also, the presence of VEGF in the wound can give us a very good indication about the future of the wound healing course (Johnson & Wilgus, 2014).

Neovascularization is a process of a new blood vessel formation (Bauer, Bauer, & Velazquez, 2005), which is essential in many physiological conditions including wound healing, and other pathological conditions, for example, diabetic retinopathy and cancer. Neovascularization consists of two processes: angiogenesis and vasculogenesis (Djonov, Baum, & Burri, 2003; Harry & Paleolog, 2003; Z. J. Liu et al., 2003; Patan, 2004).

The mechanism of angiogenesis occurs in two different ways: intussusception and sprouting (Flamme, Frolich, & Risau, 1997; Folkman, 1995; Molinas et al., 2003; Paku & Paweletz, 1991). Sprouting angiogenesis happens after proteolytic enzymes degrade the basement membrane of small blood vessels. After that, endothelial cells migrate and proliferate in the ECM. As a result, vasodilation and blood vessel permeability occur. This type of bud formation that mainly occurs internally and reconnects the lumen of small blood vessels together, is known as sprouting (Djonov et al., 2003). Caduff et al. (1986) were the first scientists who addressed the second form of angiogenesis called (non-sprouting or intussusception), which was first discovered after study had been applied in a rat's lung; they have noticed that pulmonary capillary regrew very quickly (Caduff, Fischer, & Burri, 1986).

Van Groningen et al. (1991) & Zhou et al. (1998) reported in their research that intussusception type of angiogenesis can o ccur in myocardium and skeletal muscle respectively (Djonov et al., 2003).

Pressure Ulcers (PUs):

Pressure ulcers are defined as breaks in the skin or underlying tissue which are caused by continuous pressure of the body weight against the skin, usually over a bony prominence. Pressure ulcers has other names: bedsores and decubitus ulcers (Dumville, Webster, Evans, & Land, 2015). PUs can arise from a combination of shearing and/or friction against a hard surface. Pressure ulcers are regarded as one of the most frequent causes of death among elderly and bed-bound patients. (J. M. Levine & Zulkowski, 2015; S. M. Levine, Sinno, Levine, & Saadeh, 2013; O. Stojadinovic et al., 2013).

Pressure ulcers can be devastating to patients and families. There are a couple of factors that can contribute to or cause pressure ulcers which can be divided into two main groups.

- Intrinsic factors, which are mainly within the body, like chronic disease, poor nutrition, low prealbumin and albumin, elderly, dehydration, bedridden or wheelchair bound.
- Extrinsic factors, which include external effects that can cause skin damage such as friction, shear, or moisture. According to Wound Ostomy and Continence Nurses Society, 2006-2011, injury to the skin or underlying tissue mostly affects certain areas, like sacrum, ischium, coccyx, heel, and trochanter. Therefore, changing patient position every 2 hour is very important for the prevention.

We need to know pressure ulcer stages as a key for understanding pressure ulcers clearly and managing them properly (Black et al., 2007). Pressure ulcers are staged into (Spear, 2013):

Stage I: A stage I pressure ulcer is an area of intact skin. It is a non-blanchable redness of a localized area over a bony prominence. The area might be painful, firm or soft compared to adjacent tissues.

Stage II: In this stage, the wound is a superficial, partial-thickness wound that looks like a shallow open ulcer with partial loss of dermis without slough. The wound bed looks pink and red.

Stage III: Stage III is a full thickness wound with tissue loss. The subcutaneous tissue may be visible. Nevertheless, bone, tendon or muscle are not exposed. There can be slough tissue at the wound bed. Also, undermining and tunneling might be present as well.

Stage IV: Full thickness tissue loss with palpable bone or tendon and exposed muscle. At some parts of the wound bed, slough or eschar may be present. Also, undermining and tunneling are often present in this stage.

Unstageable: It is a full thickness tissue loss. Actually, in this stage the base of the ulcer is covered by slough or eschar. It is difficult to get an accurate staging until necrotic tissue is removed.

Prevention measurements:

Management of Pressure ulcers are best prevented in the first instance by using risk assessment tools and ensuring pressure is relieved frequently, either by turning or using pressure-relieving mattress devices.

The Braden scale (H. L. Chen, Cao, Wang, & Huai, 2015), which is widely used as a tool risk assessment by the nurses and wound-care team, provides the information regarding the individual status and the suitable intervention necessary. Based on the clinical practice evidence and retrospective research study, there are three risk levels to assess pressure ulcers. A total score below 11 is considered high risk, scores of 12 to 16 is moderate risk, and the score of 17 or above is considered mild risk (H. L. Chen et al., 2015).

Pressure Ulcer management based on Braden score:

Patient with a Braden score of 17 or above (H. L. Chen et al., 2015): We should encourage patient to change his position if he can. Also, make sure to maximize patient's mobility and activity. We encourage the wound-care team to use pressure offloading surfaces. Braden scores of 12-16 which considered moderate risk: Use the previous methods plus routine reposition. Also, we should use bolsters or wedges to maintain position as necessary. Braden score of 11 or less, which is high risk: We recommend increasing patient's turning frequency. Wound-team should manage moisture, nutrition, and keep eyes on friction sites. In addition to that, we should add pressure offloading tools when the patient is in the bed or in the chair (H. L. Chen et al., 2015).

There is an important phrase to remember, called pressure redistribution that is now used instead of pressure reduction and pressure relief. Therefore, Braden scale can predict pressure ulcer by giving us the score risk level which is very effective in pressure ulcer prevention (H. L. Chen et al., 2015). Also, the gold standard for preventing pressure ulcers is by repositioning the bedridden patient at least every 2 hours; use pull sheet to pull the patient and turn him; perform passive range of motion; and elevate the head of the bed to an angle not exceeding 60º.

Negative-pressure wound therapy (NPWT) / vacuum-assisted closure (VAC):

Morykwas et al. (1997), applied NPWT first as a controlled suction through a foam to create an environment that promoted wound healing (Malmsjo, Huddleston, & Martin, 2014). After that, this therapy has successfully become popular and widely distributed to cover health care centers through the last 18 years. However, the exact

physiological properties of its effects are not yet fully understood (Kairinos, Solomons, & Hudson, 2010). NPWT is a very effective modality for treating chronic and difficult wounds (Argenta & Morykwas, 1997; Morykwas, Argenta, Shelton-Brown, & McGuirt, 1997). Dumville, Webster, Evans, and Land (2015) mentioned that NPWT is a treatment choice for decubitus ulcers.

NPWT is widely applied to different surgical wounds by creating negative pressure atmosphere that is needed to drain wound exudate in order to achieve wound healing (Bradbury, Walkley, Ivins, & Harding, 2015; Ubbink, Westerbos, Evans, Land, & Vermeulen, 2008). To summarize the main goal of using VAC therapy, is by removing as much as possible of moisture and drainage from the wound bed, and also other proinflammatory cytokines/chemokines that are involved in chronic wounds (Greene et al., 2006; Stechmiller, Kilpadi, Childress, & Schultz, 2006; Trengove et al., 1999).

NPWT Device structure:

NPWT is considered a new technology in wound care. NPWT provides a constant negative pressure to the wound bed. It consists of five main components: 1- A foam (sponge) with fenestrated pores which is placed in the wound bed. 2- A semi-permeable sheet to isolate the wound environment and provide a seal to the wound. Also, it allows the vacuum system to transmit subatmospheric pressures to the wound bed. 3- Connecting suction or drainage tube which acts as a connection suction between the sponge and the canister. 4- NPWT pump or suction device. 5- A canister which keeps exudates and wound drainage until full and replaced by a new one. The device permits the negative pressure to

be distributed all over the wound throughout the foam and improves exudate drainage (Bradbury et al., 2015; Malmsjo et al., 2014).

How does NPWT work?

NPWT is applied directly to the wound bed using an electrical battery, or mechanically powered pump. NPWT should be applied after the wounds are cleaned, debrided from any slough or nonviable tissue/eschar. The sterile foam should be cut to fit the wound size and placed in a wound bed. Also, we make sure that any areas of undermining or tunneling should also be filled with foam pieces. We recommend using skin sealant plus padding of periwound area to prevent skin irritation. The wound is covered with film drape to ensure an airtight seal. The tube clamps are opened, and the pump is turned on to allow wound exudate and drainage to travel into the collecting canister.

In our study, we adjusted the pressure to 125 mmHg; similarly, most studies have used 125 mmHg of pressure as well (Argenta & Morykwas, 1997; Argenta et al., 2006; Morykwas et al., 1997). Early research done by Morykwas et al., 1997 mentioned the blood flow improvement to the wound with setting NPWT at higher levels; however, recent research studies have supported this finding (Borgquist et al., 2011) while other studies rejected that (Kairinos et al., 2010). Many research studies have mentioned the risk of high negative pressure that result from the NPWT application, as potential cause of ischemia to the tissues and adjacent wound structures (Kairinos et al., 2010).

Studies that done on animals revealed that NPWT may hasten removal of any blood or serum collection in the wound bed through the lymphatic system (Kilpadi & Cunningham, 2011). The main things that NPWT provides to wounds as a standard of care are, elimination of wound exudate, minimizes bacterial colonization, and supports to the granulation tissue formation (Gupta et al., 2004).

NPWT can be clinically applied for a wide range of wounds; however, NPWT may have other mechanisms of action depends on the wound type and location. Therefore, in order to get a better idea of mechanism of action of NPWT, further studies are required (Malmsjo et al., 2014). Previous study which have mentioned the benefit of applying NPWT on stage III or Stage IV pressure ulcers, the result have shown great reduction in the hospitalization and urgent care admission with NPWT treated group compared to other type of wound treatment (Schwien, Gilbert, & Lang, 2005). Another research article supports the fact that NPWT is suitable technique for treating stage III and IV decubitus ulcers (Gupta et al., 2004).

From the literature, also VAC did help shrink the wound surface area which can be observed including poorly healing ulcers. Also, NPWT is commonly used to treat different type of pressure ulcers as well (Argenta et al., 2006), and it is regarded as a good option for treating pressure ulcers (Dumville et al., 2015).

Wounds indicated for NPWT:

1. Acute wounds

2. Chronic wounds (Dumville et al., 2015)

3. Traumatic wounds

4. Partial Thickness Burns.

5. Dehisced Wounds.

6. Diabetic Ulcers.

7. Pressure Ulcers (Schwien et al., 2005)

8. Flaps and Grafts (Dumville et al., 2015)

Contraindication of using NPWT:

1. Wounds with sloughing and necrotic tissues with eschar present.
2. Osteomyelitis untreated wounds.
3. Exposed blood vessels, nerves, or unexplored fistula.

Passive contraindications:

1. History of bleeding or taking oral anticoagulants
2. Wounds with exposed tendon or bone

To sum up, from the literature, we can say that the application of controlled subatmospheric pressure creates an environment that promotes wound healing.

Steps to apply NPWT successfully:

First, clean wound by irrigating wound with normal saline or solution. Second, clean and dry periwound area. Then, apply skin prep to periwound tissue. An appropriate foam should be selected. Make sure to cut foam away from wound bed, and try to match

the size of wound. After that, put the sponge into wound bed. The next step, drape to cover the foam dressing. Make a hole in drape for the suction tube pad placement. Fix the sterile canister into NPWT and connect suction tube to it. Make sure both clamps are open. Select NPWT pressure settings and initiate therapy.

Notion of wound dressing:

Winter (1962) demonstrates the concept of active dressing. He states clearly that moist dressings can accelerate re-epithelialization and wound healing compared to traditional dry dressings (Stanirowski et al., 2015; WINTER, 1962). They have mentioned in the literature that wet or moist wounds have shown great stimulation to reepithelization and decreasing to scar formation comparing to dry wounds (Junker, Kamel, Caterson, & Eriksson, 2013).

Many research articles have mentioned that dressings should be inexpensive, simple, and highly absorptive (Sood, Granick, & Tomaselli, 2014). Also, dressings should have antibacterial properties as well. However, absorptive and antibacterial properties should be selected according to the wound needs (Dhivya et al., 2015).

The idea of wound dressing is to apply non-harm, non-adhesive and non-allergenic types of dressing, and should absorb wound exudates and maintain wound moisture (Czaja, Krystynowicz, Bielecki, & Brown, 2006; Liang, Lu, Yang, Gao, & Chen, 2016). However, still to our time, there is no specific wound dressing that clinically have met the criteria of optimal wound dressings. Clinicians and wound-care teams should take into consideration that ideal dressings must be selected based on two things: risk of infection and inflammation

Blood glucose control and good nutrition supply are regarded as important factors that clinicians should adjust to achieve successful treatment (Sood et al., 2014). Clinicians and health-care providers nowadays are using a huge variety of wound dressings for different kinds of wounds. Basically, in the market, probably there are more than 3000 wound dressing products for managing different wounds (Dhivya et al., 2015; Sood et al., 2014).

Vitamins and minerals affect wound healing; for example, patients who take steroids, they get benefit of using vitamin A, which helps decrease wound inflammation and improve healing. Vitamin C is essential for the hydroxylation of lysine and proline in collagen synthesis and cross-linking. Zinc is a very important cofactor for protein synthesis and cellular proliferation. Also, zinc is a part of the MMPs' family of proteases (zinc-dependent endopeptidases) which have a big role in ECM degradation. Usually zinc deficiency occurs through wound drainage or gastrointestinal fluid loss like diarrhea (Lobmann et al., 2002). Essential fatty acids are also needed for all kind of cell synthesis. One of the other important nutrient factors is protein, which affects wound healing and its deficiency can retard fibroblast proliferation, ECM, collagen synthesis, and angiogenesis (Guo & Dipietro, 2010). Trace elements like copper which plays an important role as a cofactor enzyme is needed for cross-linking of collagen and makes collagen have more tensile strength. Therefore, in addition to an appropriate dressing to the wound, we should make sure that these vitamin and mineral supplements are given to patients, to avoid any complication as a result of inadequacy of intake, especially in malnourished patients.

Phases of wound healing:

Wound healing involves four integrated and overlapping phases which include, homeostasis, inflammation, proliferation, and remodeling (& MD, and Thomas A. Mustoe, MD, FACS, ; Gosain & DiPietro, 2004).

Homeostasis:

After the injury, blood-vessel constriction and platelet aggregation occur at the injury site. After that, a fibrin clot forms, which will minimize blood leak and secure the bleeding. Fibrin clot contains important molecules, for example, fibronectin, thrombospondin, and many growth factors. Some of the important growth factors secreted by the clot are: platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), fibroblast growth factor (FGF), epidermal growth factor (EGF), and insulin-like growth factor-1 (IGF-1) (Clark et al., 1982). Also, platelets contain a huge storage amount of growth factors like TGF-beta, PDGF, and VEGF which are necessary for promoting tissue healing and enhancing the blood vasculature to the wound area (Sanchez, Anitua, Orive, Mujika, & Andia, 2009). Furthermore, the clot acts as a glue which helps cells to bind to ECM proteins. Also, the clot works as a bridge for cells to migrate and cross over during wound healing (Werner & Grose, 2003).

Inflammatory phase:

Neutrophils and macrophages are the main inflammatory cells that start this phase. Also, they act as chemotactic agents to other cells that will enhance the inflammation (& MD, and Thomas A. Mustoe, MD, FACS, ; Dyugovskaya, Berger, Polyakov, Lavie, & Lavie, 2016).Neutrophils in this phase act as a cleaner of any cellular debris (& MD, and Thomas A. Mustoe, MD, FACS,) Also, neutrophils help in phagocytosis of bacteria and tissue debris in the wound area; however, neutrophils can cause more damage by releasing

proteases, which can extend the inflammatory phase and delay wound healing (Ghatak et al., 2015). The most advanced wound therapy is focusing on reducing the inflammatory phase to make wounds heal faster. In normal healing wounds, neutrophils will be engulfed by macrophages and terminated within the first 48hrs (Broughton, Janis, & Attinger, 2006; Campos, Groth, & Branco, 2008; Ghatak et al., 2015; Gosain & DiPietro, 2004). Scientists nowadays are using exogenous growth factors and cytokines for treating chronic wounds in order to shift this phase, inflammatory, to the proliferative phase (Menke et al., 2007). As we know failure of progression from inflammatory phase to the other phases will remain stuck at the same phase, vicious cycle (Mast & Schultz, 1996), and chronicity will definitely occur.

The Proliferative phase:

This phase can be extended from 4 to 12 days. Macrophages in this phase play an important role to decrease inflammation. In addition, macrophages are a very rich source of growth factors and cytokines which are needed for wound healing (Ghatak et al., 2015). Therefore, macrophages have big role for shifting from inflammatory to proliferative phase. During this phase also T- Lymphocyte plays a crucial role; however, its role is not fully understood. Some studies mentioned that T- helper cells have a positive role in wound healing, whereas T-suppressor cells have the opposite action on wound healing (Ghatak et al., 2015). Collagen is produced in this stage by fibroblasts which will enhance wound structure and ECM. Angiogenesis is an important factor that is required in order for tissue to get a new blood supply to bring nutrients and oxygen to the tissue and support fibroblast proliferation and allow keratinocytes to enhance epithelialization of the wound (Bodnar, 2015).

Maturation and Remodeling phase:

Usually this phase starts after 2 to 3 weeks and can continue up to 24 months. At this phase, matrix metalloproteinases (MMPs), which are proteolytic enzymes, act to degrade extracellular matrix (ECM) and break down the collagen, and the net result of that is a balance between collagen degradation and collagen synthesis. There are two main things that are important to consider in cases of newly formed wounds, with a newly deposited collagen, which include wound strength and mechanical integrity. Also, in this phase, angiogenesis, cell migration, differentiation, and at the end collagen production toward building ECM, are considered a part of the remodeling mechanism (Hodde, Badylak, Brightman, & Voytik-Harbin, 1996).

Biology of cytokines:

Cytokines are defined as small sized proteins produced by a variety of cells communicating with host cells via signaling to accomplish a specific function to the tissue and the immune system (Blackwell & Christman, 1996). Cytokines are considered the regulatory key that operates the communication between the innate and acquired immunity via sending signals between the immune system and tissues in cases of inflammation. Cytokines are considered as specialized cells or messengers that convey signals; for example, IL-2 promotes T cells proliferation and differentiation, GM-CSF and G-CSF affect macrophages, and different other cytokines: IL-1, IL-6, IL-8, and TNF-alpha are responsible for the acute phase inflammation (Ghosh, May, & Kopp, 1998).

Cytokine signaling occurs in four different patterns: Endocrine, paracrine, autocrine, and juxtacrine depending on the body needs.

The main purpose of studying cytokine biology is to show how these cytokines act at the level of gene transcription and how these cytokines can suppress each other via signals and feedback mechanisms. Also, a lot of cytokine mechanisms and signal transductions are still not clearly understood; however, many ideas were applied in this field by creating models to make understanding of cytokines signal transmission better and make it easy for different quantitative measures (Schmitz, Weber, Roxlau, Gaestel, & Kracht, 2011).

Anti-cytokine, in a form of antibodies or immune-modulating treatment, is promising for treating different chronic inflammatory and autoimmune diseases.

MMPs and chronic wounds:

In normal wound healing, MMPs are very essential for degrading ECM, but in a very moderate precise manner. As the literature mentioned, excessive MMPs will definitely delay wound healing (Gibson & Schultz, 2013). Researchers pointed out to MMPs as the direct cause of destroying different growth factors, and cell surface receptors for these growth factors as well. As a result, cells will lack a communication with each other, with ECM, and will never show any response to normal growth and repair signals. In addition to that, with different affinity of growth factors' binding to extracellular matrix and the great chance that these growth factors get degraded by the MMPs. All of that can negatively impact on the concentration of growth factors present in the wound bed. As a result, wounds will be very challenging to heal on time (Greenhalgh et al., 1990). Researchers found out that high percentage of MMPs can dehisce acute wounds (Mast & Schultz, 1996; Trengove et al., 1999). Increasing MMPs can occur by various different mechanisms though MMPs are mandatory for normal wound cure and tissue revival

(Lobmann et al., 2002). Another important thing in order to achieve wound healing, equilibrium should occur between collagen deposition and other ECM degradation products like MMPs and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs) (Kahari & Saarialho-Kere, 1997; Madlener, 1998; Ravanti & Kahari, 2000).

The MMPs are produced by different cell types, like macrophages, endothelial cells, and fibroblasts. MMPs family is composed of around 20 members that have been distinguished so far (Ravanti & Kahari, 2000). MMPs are considered as zinc-dependent motives that are strongly expressed in chronic wounds and are involved in ECM degradation. There are important inhibitors to the MMPs known as TIMPs. Lobmann et al., 2002 in their study implied that concentrations of TIMP-2 protein were approximately threefold lower in the diabetic ulcers than in the acute wounds.

Another study that was done on chronic pressure ulcers where the collected fluid samples from these ulcers were analyzed and have shown notable increase in the amount of pro-inflammatory cytokines and active proteases such as MMPs; whereas they showed far less concentration of growth factors, less activity, and reduced TIMPs as well (Mast & Schultz, 1996).

Major sources of TGF-β include platelets, leukocytes, bone cells and placental tissue. TGF-β1 can be both inhibitory and stimulatory and is a potent chemoattractant for monocytes, macrophages, lymphocytes, neutrophils, fibroblasts, and keratinocytes (Peplow & Chatterjee, 2013). Also, TGF-β1 stimulates the release of different cytokines (e.g. IL-l, IL-6, TNF-Alpha, and bFGF) from these cells (Lafyatis et al., 1990).

Many previous studies that had been conducted *in vitro* assays demonstrated the fact that TGF-B3 has similar activities to TGF-B1 and TGF- B2; however, it is not exactly the same (Lafyatis et al., 1990). Interestingly, many research articles have shown that TGFβ1 and its receptors are highly expressed in acute wounds, while in chronic wounds its expression is markedly reduced. (Pastar et al., 2010). TGF-β1 is a master regulator of fibroblasts mitogenesis, cell growth, and apoptosis. TGF-β3 has shown enhanced scarless healing in the fetus and minimal scarring in adults while TGF-β1 may be responsible for fibrosis in adult wounds (Lichtman, Otero-Vinas, & Falanga, 2015).

Research studies have mentioned the strong suppression effect of TGF-beta on T cells (Wahl, Allen, Wong, Dougherty, & Ellingsworth, 1990), and also TGF-beta is responsible for decreased activation and proliferation of other cytokines; for example, TGF-Beta downregulates IL-1 receptors (Wahl et al., 1988) at the level of different cells.

Biochemical and physiological characteristics of Oasis-Ultra:

OASIS-ultra; Cook Biotech, Inc., West Lafayette, IN; exclusively marketed by Smith and Nephew, Inc., Fort Worth, TX) is an extracellular collagen-rich matrix derived from porcine small intestinal submucosa (SIS) (AbouIssa, Mari, & Simman, 2015). Oasis-ultra is known by this name because it consists of a biocompatible, acellular, nonimmunogenic, and biodegradable tri-layer of porcine small intestine submucosa (SIS) with thickness of ~0.30 mm (Cazzell, Lange, Dickerson, & Slade, 2015).

The composition of Oasis-ultra is mostly collagen, around 90%. The majority of it is type I, and less amount of collagen type III, IV, V, & VI (Badylak et al., 1995), and other ECM proteins, such as glycoproteins and glycosaminoglycan which are crucial for wide

range of wound functions. Oasis-ultra acts as a native tissue scaffold; not only it provides support to the structure, but also provides other vital functions to the wounds; for example, it has a very important contribution in stimulation of cell proliferation and migration, as well as minimizing the inflammation that results from proinflammatory cytokines (AbouIssa et al., 2015). Ahn, et al. (2007) mentioned in their study using SIS as a scaffold treatment for human bone marrow stem cells, SIS contains glycosaminoglycans, chondroitin sulfates, fibronectins, hyaluronic acids, heparins, and heparin sulfates. Also, it has other types of growth factors like basic fibroblast growth factor (bFGF), transforming growth factor (TGF-), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and insulin-like growth factor-1 (IGF-1) (Huang, Liu, Huang, & Liu, 2014); however, in our present study, we found out when we analyzed the Oasis-ultra (the three layer-SIS) using the Bio-Plex system, it just has bFGF and GM-CSF; but the wet form of SIS might have more growth factors retained.

Also, Oasis-ultra is characterized by easy absorbability and handling, which makes it suitable as a xenogenic tissue replacement that closely mimics the natural ECM. SIS showed great tissue remodeling when it was applied in different animal models, such as vascular, dermatological, urological, and orthopedic wound injuries (Hodde et al., 1996). There are many factors that could affect Oasis-ultra remodeling behavior when it was applied *in vivo*, such as local tissue situation, the rate of ECM degradation, forces at the level of wound, and the ability of cells to deposit new collagen toward the ECM (Badylak, Freytes, & Gilbert, 2009).

Usage of Oasis-Ultra:

Since Oasis-ultra is composed of rich collagen-biomaterials that mimic ECM, with low antigenicity; that makes Oasis-ultra a good collagen-based matrix to heal different types of wounds (AbouIssa et al., 2015). For example, Oasis-ultra was successfully applied in different kinds of wounds, such as, vascular ulcers, diabetic ulcers, vasculitis at achilles tendon, stage IV pressure ulcer, post-traumatic wound, and post-marjolin's ulcer excision (AbouIssa et al., 2015), and partial-thickness and full-thickness wounds. Therefore, Oasis-ultra successfully healed different types of wounds by up-regulating the important growth factors necessary and restitution of the old ECM to make wound healing fast and ultimate. The ability of Oasis-ultra as collagen rich matrix, to deliver certain growth factors to the wound cells, makes it a wonderful and promising type of wound-dressing treatment (Huang et al., 2014).

Hypothesis and Specific Aims:

Hypothesis:

Treating chronic wounds with Oasis-ultra may alter the molecular environment of the wound by disrupting the prolonged inflammatory phase and allowing normal progression of the healing cycle.

Specific aims:

1- To test the hypothesis that Oasis-ultra treatment would improve the healing rate of chronic wounds when compared with the NPWT only.

2- To test the hypothesis that Oasis-ultra treatment would decrease proinflammatory cytokines and increase the growth factors needed to achieve wound healing.

Materials and Methods:

The study was a prospective, multi-centred, randomized, single-blinded clinical trial that approved by the Ethics Committee of the Copernicus. Written informed consent was obtained from all patients. We discussed and answered patients' concerns before obtaining the consent. Twelve patients enrolled into the study: six were placed in a control group, receiving only negative pressure wound therapy (NPWT) and the rest of the patients were selected as a study group receiving both treatment: Oasis-ultra and NPWT. Both devices are cleared for use by the U.S. Food and Drug Administration (FDA). However, the use of both devices together is considered investigational in this study. Subjects will be in the study for approximately 3 months. The canister that contained the wound exudate was taken every month to the lab. Wound exudates were extracted from the canisters and aliquoted to 1.5 ml eppendorph tubes along with the addition of a protease inhibitor and stored at -80°C until use. At wound center, during the weekly examinations, we did measurements and took photos of the wound. The inclusion and exclusion criteria were:

Inclusion criteria:

1. Adults aged 18-89 who exhibit stage III or IV trunk pressure wounds with no signs of infection.
2. HbA1c < 8 (if a patient is diabetic)
3. Adequate nutrition including albumin ≥ 2.0 and prealbumin ≥ 12.5.

Exclusion criteria include:

1. Subjects who have wounds that cannot have a NPWT device properly applied due to location (too close to anus), or due to other causes, such as diarrhea and peri-wound skin issues.
2. Patients with infected wounds.
3. Patients with HbA1c >8.
4. Malnourished patients.
5. Immunodeficient or immunocompromised patients.
6. Patients who have a religious or ethical aversion to porcine products or any allergy.
7. Patients who are at risk of bleeding.
8. Patients who are DNR/DNI.
9. Lab results for albumin and prealbumin up to 30 days prior to enrollment and HbA1c results up to 100 days prior to enrollment were further examined for determination of their eligibility.

For three months, wounds were measured weekly until the end of the study in terms of wound closure and the healing rate. Dressings were changed twice a week or more as necessary at the physician's discretion. Drainage canisters were collected monthly for the following three months. The skin surrounding the wound was cleaned and prepped well before we applied the Oasis-ultra in the study group. Then, we put Adaptec or alginate for protecting oasis-ultra before we put the sponge. After that, the vacuum assisted closure foam dressing was trimmed to match the size of the wound and placed directly over the Oasis-ultra and protective layer. In control subjects, we put the foam directly over the wound bed. The skin adhesive prep was applied to the skin around the wound

approximately 3-5cm away from the wound edge and allowed to dry. Next, the appropriate size of adhesive drape was applied. Then, the suction tube was applied to a small opening made in a dressing area and was sealed tightly. The tube then was connected to a vacuum machine, which had a canister to collect the wound exudate. The machine was set to pressure 125 mm Hg. NPWT was changed 3 times a week in a control group, while in a study group, it was recommended to be changed 2 times a week. During the three-month period of the study, the canisters were collected 3 times. If the seal failed before the follow-up time, the wound-care team changed the dressing and reapplied the NPWT as needed to keep the wound sealed.

Growth Factors and Cytokine Analysis:

For cytokine analysis using the Bio-PLex system, wound exudate was extracted from the canisters and aliquoted to 1.5 ml eppendorph tubes; 100µl of protease inhibitor were added and stored at -80°C until use. For cytokine analysis, 100µl 1XPBS were added to the sample, and protein concentration was determined using the Bradford assay. A (Bio-Rad) BioPlex 96 well plate was set up with 50 ml of the sample and duplicated for cytokine analysis using Bio-Plex (Bio-Rad) Human Cytokine 27-Plex Group 1 assay kits. To each well, buffer, magnetic beads were added and washed on the magnetic plate washer. The plate was run on the Bio-Plex 200 system at a low PMT setting followed by high PMT according to Bio-Rad instructions, and the data were grouped using the BioPlex software package. The data was then analyzed using SPSS software and GraphPad Prism5.

TGF-Beta Analysis: TGF-β1, TGF-β2, & TGF-β3

For TGF-Beta family analysis, the Bio-PLex system protocol was repeated: wound fluid was extracted from the canisters and aliquoted to 1.5 ml eppendorph tubes, 100µl protease inhibitors were added and stored at -80°C until use. For TGF-betas analysis, 100µl 1XPBS were added to the samples, and protein concentration was determined using the Bradford assay. A (Bio-Rad) BioPlex 96 well plate was set up with 50 µl of the sample and duplicated for cytokine analysis using Bio-Plex (Bio-Rad) Human TGF-β1, TGF-β2, and TGF-β3 Plex assay kits. To each well, buffer, magnetic beads were added and washed on the magnetic plate washer. The plate was run on the Bio-Plex 200 system at a low PMT setting followed by high PMT according to Bio-Rad instructions, and the data were grouped using the BioPlex software package. The data were analyzed using SPSS software and GraphPad Prism5.

Table 2: Demographic features of patients participating in this study		
Total number/ 12	**6**	**6**
Variables	**Control**	**Oasis-ultra + NPWT**
Male	2	4
Female	4	2
Age	62.5 year	63.5 year
Race	white	white
Alcohol	1 patient drinks around 4 cup/day	N/A
Caffeine	5 out of 6 drink average of 1.8 cup of coffee/day	5 out of 6 drink average of 2.2 cup of coffee/day
Smoking	N/A	N/A
Weight	75.74kg	86.02kg
BMI	27.85 kg/m^2	28.69 kg/m^2
Albumin	3.05mg/dl	2.58mg/dl
Prealbumin	22.21mg/dl	17.51mg/dl
HbA1C	7.0 mg%	7.02 mg%
Wound Location	5 candidates had wound at sacrum and only one is in the Lt. Ischium	Sacrum
Wound duration prior to enrolment	25.6 weeks	19.6 weeks

Statistical Analysis

Results are expressed as means ± SEM. Differences between 2 groups were performed by multiple comparisons and were analyzed by 1 or 2-way ANOVA. For all tests, a $P<0.05$ was considered significant. All comparisons were performed using the statistical package SPSS 16.0 for Window and Prism5.

Results:

Patient demographics and baseline features presented in table 1. The total number of patients who have been screened during the time frame of 8/5/2014-10/9/2015 were 63 subjects and 21 were enrolled; 12 patients completed the study successfully, and 8 patients were excluded from the study.

The healing rate was analyzed over a period of time with weekly measurements taken from 0 to 12 weeks (Table 3). The ANOVA for difference in groups was not statistically significant, but it is close to be considered significant; therefore, further study ought to be done with a larger sample size. Using these data, a boxplot that shows the healing rates for the two groups was done (Figure 6).

Table 3: Average of the closure% in the study determined over a 12 week period.

Dependent Variable: Healing rate			
Groups	**Mean**	**Std. Deviation**	**N**
Control	55.29	38.91919	6
Oasis	86.59	9.63933	6
Total	70.94	31.59039	12

Oasis vs Control: F (df,df) **3.657 (1,10);**p-value **0.085;** Est. effect size **0.268;** Observed power **0.409**

Table 4: Comparison of the healing rate between the two groups at different times.				
Groups	**0-4 weeks**	4-8weeks	8-12weeks	**N**
Control	9.4cm³/week	4.43 cm³/week	0.3 cm³/week	6
Oasis	12.8 cm³/week	14.3 cm³/week	5.4 cm³/week	6
Healing rate in wounds treated with Oasis-ultra increases in the 4-8 week window compared to NPWT alone. In addition, the healing rate slows down but continues from 8-12 weeks in Oasis-ultra treated groups compared to the control group where the healing rate almost stopped at 8 weeks.				

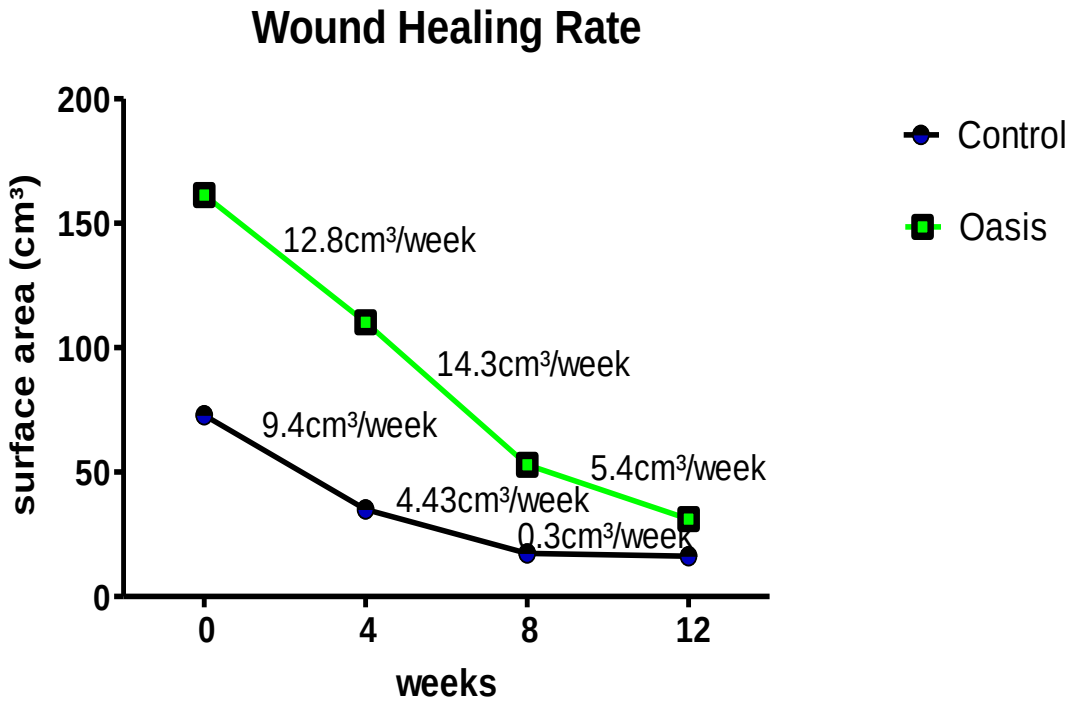

Figure 1. Healing rate in wounds treated with OASIS-ULTRA increases in the 4-8 week window compared to NPWT alone even though the size of the wound is 2X larger in the Oasis-ultra treated group. Also, the healing rate slows down but continues from 8-12 weeks in OASIS-ULTRA treated groups compared to the group treated with NPWT alone where the healing stopped at 8 weeks.

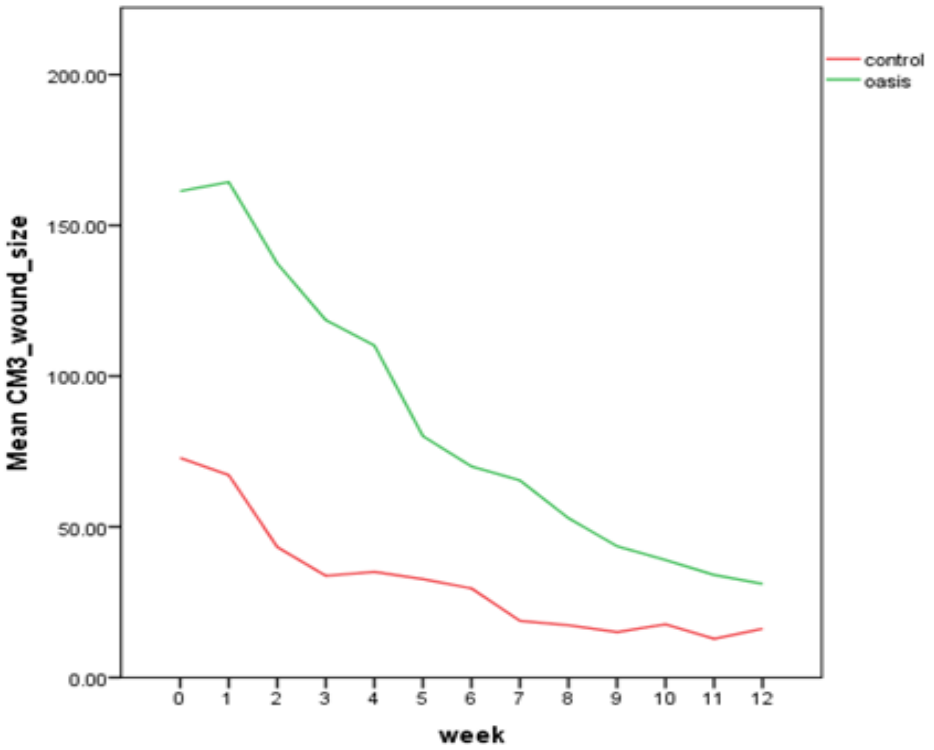

Figure 2. The oasis group had a lower variability, meaning that the healing was going on for those six patients at a steadier rate. For the control group, the variability nearly covered the entire scale (0% to 100%) meaning that the healing could progress at nearly any rate. Even though, the average wound size of the study group was 161.36, and of the control group was 72.83, the healing rate of the Oasis treated group was significant compared to the control group.

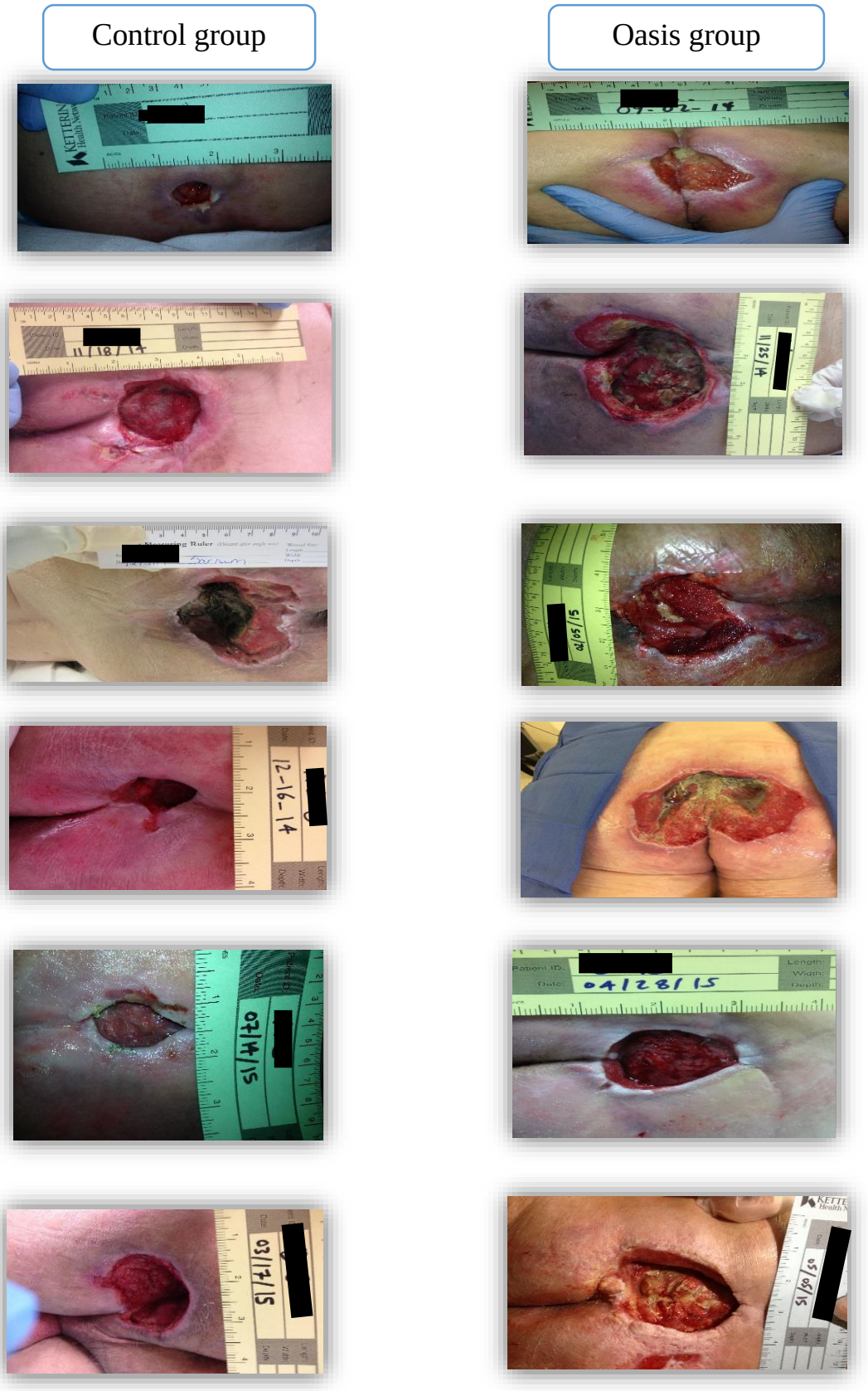

Figure 3. The wound size of the control group vs. the oasis-treated group during week 0.

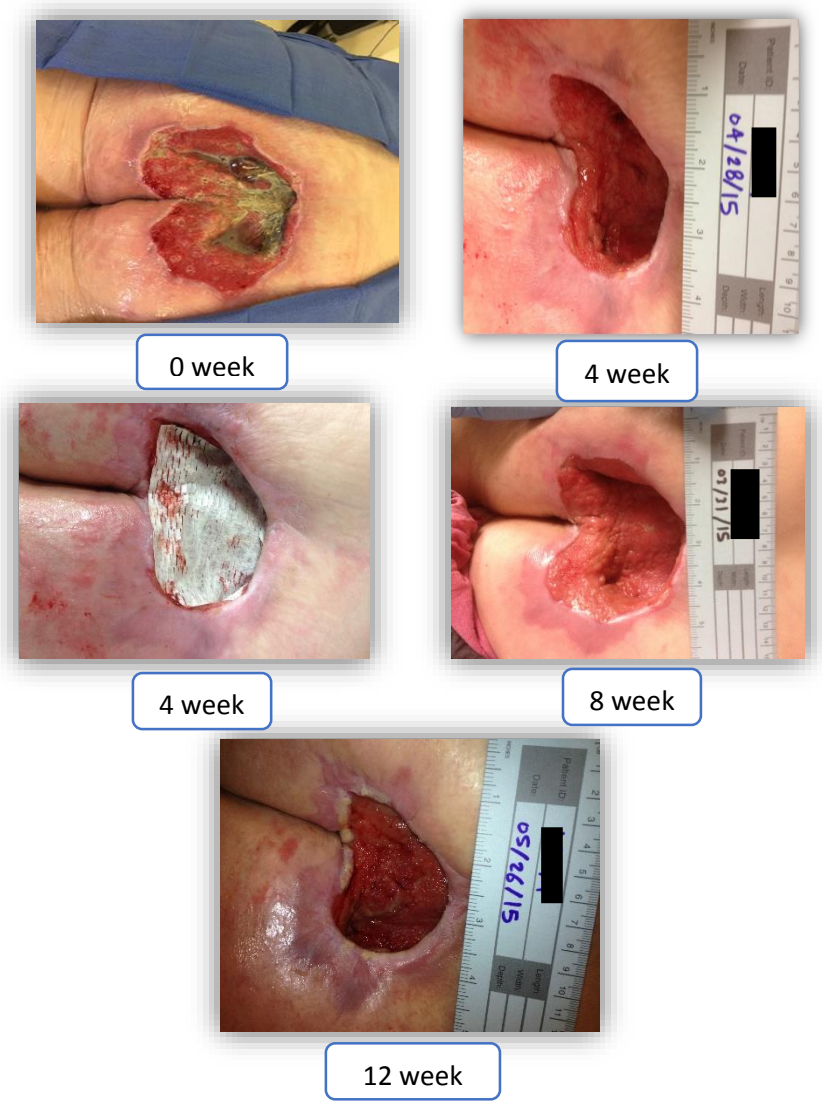

Figure 4. Stage IV pressure wounds randomized as a study candidate received Oasis-ultra plus NPWT

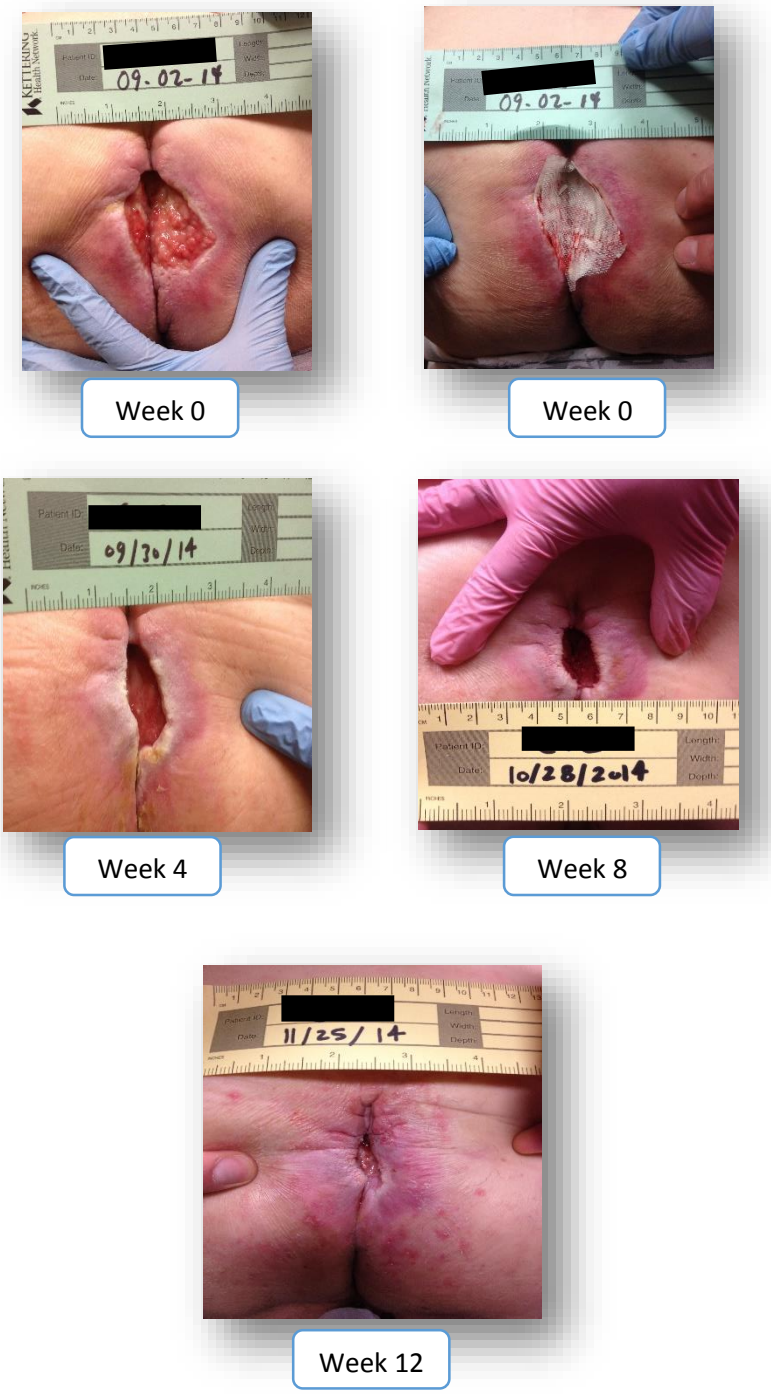

Figure 5. Shows stage IV pressure ulcer which completely healed after 11 applications of Oasis-ultra plus NPWT.

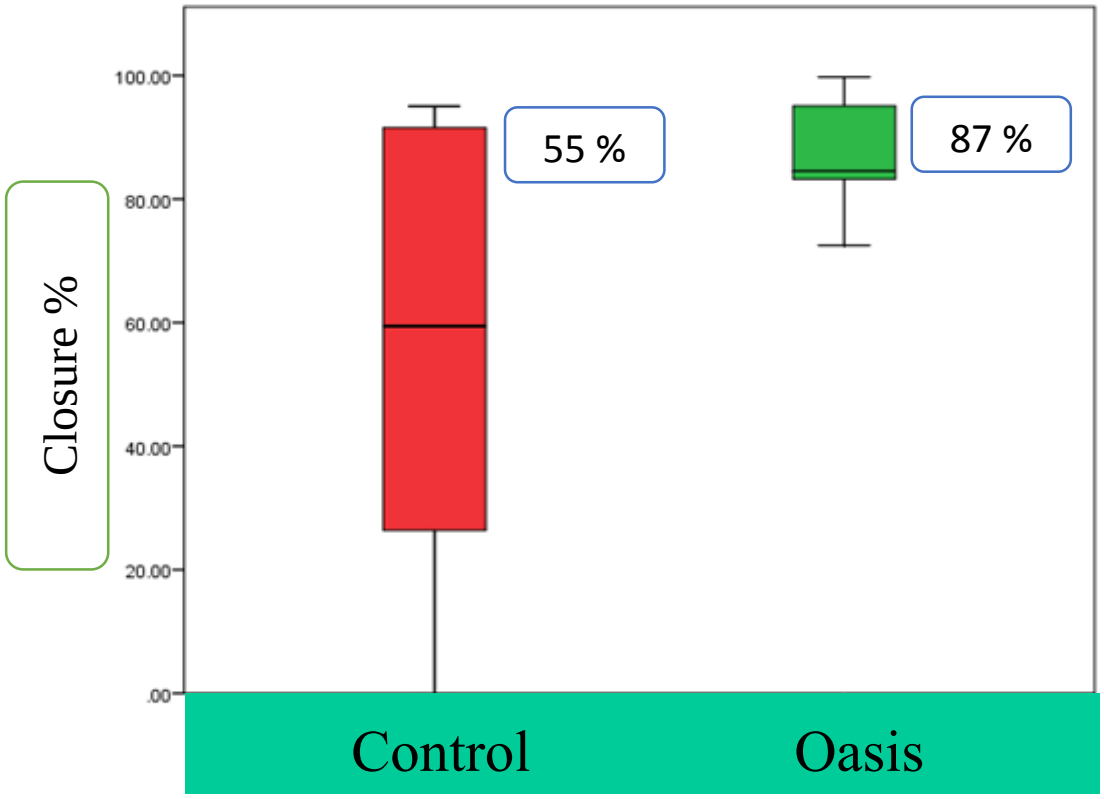

Figure 6. This box plot is showing the closure % achieved by the two groups; it is clear that the median healing rate for the oasis group is much higher than the median healing rate for the control group, and the variability for the two groups is significantly different.

According to previous reports, several key cytokines were found to have been changed in wound healing. We chose to use the Bio-Plex (Bio-Rad) system for analyzing cytokine levels from the wound exudates (lavage). There were 27 different cytokines analyzed using this assay plus the TGF- βs (Table 5).

Table 5: Cytokines analyzed in the Human Cytokine 27-Plex Group 1 Assay.			
FGF basic	IL-2	IL-10	MIP-1α
Eotaxin	IL-4	IL-12	MIP-1β
G-CSF	IL-5	IL-13	PDGF-BB
GM-CSF	IL-6	IL-15	RANTES
IFN-γ	IL-7	IL-17	TNF-α
IL-1β	IL-8	IP-10	VEGF
IL-1ra	IL-9	MCP-1 (MCAF)	
TGF-β1	TGF-β2	TGF-β3	

In the previous reports, the cytokines reported in the following tables and graphs were indicated to have changed during wound healing. We are showing their results, whether they were statistically significant or not, i.e.: IL-1Ra, IL-1b, IL-6, IL-8, MCP-1, MIP-1α, VEGF, bFGF, PDGF-BB, and TGF-β1. All cytokine levels were normalized to total protein levels that were determined by Bio-Rad (Bradford) protein assay.

ANOVA results IL-1Ra:

When IL-1Ra was analyzed, there was a statistically significant difference between the control and experimental groups; the *p*-value= 0.05. The significant difference was observed between control and experimental groups during weeks 4 and 8.

Table 6: IL-1Ra was not significantly changed during wound healing during the 12 week period.						
Week	**Control**			**Oasis**		
	n	**Mean**	**St. dev.**	**n**	**Mean**	**St. dev.**
4	6	474.77	410.46	6	2010.93	1421.14
8	6	951.48	1642.07	6	3506.19	2755.59
12	6	448.95	712.88	6	2021.27	3081.51
F (df,df) **4.607 (1,10);** *p*-value= **0.05;** Est. effect size **0.315;** Observed power **0.492**						

ANOVA results IL-1b:

The ANOVA for the effect over time is statistically significant. The ANOVA for the interaction between weeks and the experimental group is not statistically significant.

Finally, the results of the test between the control and oasis groups were analyzed.

The results are shown in the table 7:

Table 7: IL-1b, there were no statistically significant difference between the control and the experimental groups.						
Week	**Control**			**Oasis**		
	n	**Mean**	**st. dev.**	**n**	**Mean**	**st. dev.**
4	6	1998.68	1773.82	6	2170.42	1143.33
8	6	1753.06	1588.87	6	2762.03	1078.33
12	6	1196.67	970.40	6	1725.57	1315.54
F (df,df) **0.639 (1,10);** *p*-value= **0.443;** Est. effect size **0.060;** Observed power **0.112**						

ANOVA results VEGF:

For a two-way and repeated measures analysis, ANOVA was used. The results are presented in the following table: Neither the ANOVA for measurements at 4, 8, and 12 weeks nor the analysis of the interaction between the control and the experimental groups are statistically significant.

Table 8: VEGF, there were no statistically significant difference between the control and the experimental groups.						
Week	**Control**			**Oasis**		
	n	**Mean**	**st. dev.**	**n**	**Mean**	**st. dev.**
4	6	315.13	568.39	6	107.31	145.65
8	6	77.96	146.75	6	171.24	265.35
12	6	73.29	62.22	6	65.03	83.56
F (df,df) **0.172 (1,10);** *p*-value= **0.687;** Est. effect size **0.017;** Observed power **0.066**						

ANOVA results FGF basic:

For a two-way and repeated measures analysis, ANOVA was used. The results are presented in the following table. Neither the ANOVA for measurements at 4, 8, and 12 weeks nor the analysis of the interaction between the control and the experimental groups are statistically significant (the *p*-value was 0.73).

Table 9: bFGF, there were no statistically significant difference between the control and the study group.						
Week	**Control**			**Oasis**		
	n	**Mean**	**st. dev.**	**n**	**Mean**	**st. dev.**
4	6	46.92	35.33	6	23.71	38.54
8	6	27.42	31.92	6	99.36	167.46
12	6	25.59	33.53	6	9.35	14.98
F (df,df) **0.124 (1,10);** *p*-value=**0.732;** Est. effect size **0.012;** Observed power **0.062**						

ANOVA results PDGF-bb:

For a two-way and repeated measures analysis, ANOVA was used. The results are presented in the table 10: Neither the ANOVA for weeks nor the one for the interaction between weeks and experimental group are statistically significant.

There is no statistically significant difference between the control and the experimental groups. While performing the Kruskal-Wallis tests, separate tests were done for the groups during the periods of 4 weeks, 8 weeks, and 12 weeks. The results did not show significant differences between the control and the experimental groups on week 4 or 8, but there was a significant difference during 12 weeks.

Table 10: PDGF-bb, there were no significant differences between control and experimental groups at 4 or 8 weeks, but there was a significant difference at 12 weeks.

Week	**Control**			**Oasis**		
	n	**Mean**	**st. dev.**	**n**	**Mean**	**st. dev.**
4	6	24.20	28.04	6	31.56	54.36
8	6	11.32	9.15	6	28.52	52.22
12	6	41.93	52.69	6	2.30	2.93
F (df,df) **0.146 (1,10);** *p*-value=**0.710;** Est. effect size **0.014;** Observed power **0.064**						

ANOVA results IL-6:

The ANOVA for difference in weeks is not statistically significant, even though the means vary greatly. Again, this is likely due to the immense variance of both groups.

Table 11: IL-6, there was no statistically significant difference between the control and experimental groups.

Week	**Control**			**Oasis**		
	n	**Mean**	**st. dev.**	**n**	**Mean**	**st. dev.**
4	6	5036.11	8227.51	6	104.31	145.37
8	6	148.711	232.89	6	1732.62	2979.43
12	6	2796.21	6430.85	6	20.34	20.81
F (df,df) **1.653 (1,10);** *p*-value=**0.228;** Est. effect size **0.142;** Observed power **0.214**						

ANOVA results IL-8:

For a two-way and repeated measures analysis, ANOVA was used. While the ANOVA for weeks was not significant, the one for the interaction between weeks and experimental group was statistically significant at week 8. There were no significant differences between control and experimental groups at 4 or 12 weeks, but the difference at 8 weeks is statistically significant, *p*-value = 0.02.

The results are presented in the table 12:

Table 12: IL-8, There were no significant differences between control and experimental groups at 4 or 12 weeks, but the difference at 8 weeks is statistically significant, *p*-value = 0.02.

Week	**Control**			**Oasis**		
	n	**Mean**	**st. dev.**	**n**	**Mean**	**st. dev.**
4	6	2749.24	2958.83	6	3216.87	2837.87
8	6	1193.69	1915.48	6	5148.94	5957.32
12	6	2050.58	1713.33	6	3665.58	5597.86
F (df,df) **0.896 (1,9)**; p-value=**0.024;** Est. effect size **0.091;** Observed power **0.136**						

ANOVA results of TGF-Beta 1:

For a two-way and repeated measures analysis, ANOVA was used. One way ANOVA shows significant difference on week 4 and 8 were healing mostly achieved at this time. The results are presented in the table 13.

Table 13: TGF-β1.						
Week	**Control**			**Oasis**		
	n	**Mean**	**st. dev.**	**n**	**Mean**	**st. dev.**
4	6	0.40	0.98	6	33.38	70.29
8	6	0.57	1.41	6	26.29	64.41
12	6	8.92	16.77	6	20.15	23.16
F (df,df) **2.937 (1,10);** *p*-value=**0.117;** Est. effect size **0.227;** Observed power **0.341**						

ANOVA results MIP-1a:

There were no significant differences between control and experimental groups at 4 weeks, but the difference at 8 weeks is statistically significant, and the difference at 12 weeks is worthy of further study.

Table 14: MIP-1a.						
Week	**Control**			**Oasis**		
	n	**Mean**	**st. dev.**	**n**	**Mean**	**st. dev.**
4	6	95.89	175.23	6	32.04	45.41
8	6	5.28	3.78	6	17.35	10.88
12	6	82.77	178.60	6	4.41	1.80
F (df,df) **1.893 (1,10);** *p*-value=**0.199**; Est. effect size **0.159**; Observed power **0.238**						

ANOVA results MCP-1:

There were no significant differences between control and experimental groups at 4, 8, or 12 weeks.

Table 15: MCP-1 **(**MCAF). Neither the ANOVA for weeks nor the one for the interaction between weeks and experimental group are statistically significant.

Week	**Control**			**Oasis**		
	n	**Mean**	**st. dev.**	**n**	**Mean**	**st. dev.**
4	6	185.90	232.17	6	94.59	188.74
8	6	20.79	36.38	6	60.16	117.11
12	6	141.70	207.20	6	1.17	1.86
F (df,df) **1.176 (1,10)**; *p*-value= **0.304**; Est. effect size **0.105**; Observed power **0.166**						

Here are tables for the most relevant growth factors and cytokines involved in wound healing, taken at 4, 8, and 12 weeks. (Table 16) is for the control group and (table 17) is for the Oasis treated group.

Table 16: The control group for the most important growth factors and cytokines involved in wound healing analyzed at 4, 8, and 12 weeks.

Analyte	Week	N	Mean	S.E.M	St. dev.	Median
MIP-1b	4	6	409.33	189.31	463.72	309.87
	8	6	43.01	17.98	44.06	23.94
	12	6	239.65	161.22	394.91	91.50
IL-6	4	6	5036.11	3358.86	8227.51	1347.48
	8	6	148.71	95.07	232.89	23.47
	12	6	2796.21	2625.38	6430.85	10.06
IFN-g	4	6	52.70	18.59	45.55	39.21
	8	6	40.24	20.42	50.02	20.17
	12	6	30.72	8.15	19.96	35.19
IL-1ra	4	6	474.77	167.57	410.46	481.78
	8	6	951.48	670.37	1642.07	164.75
	12	6	448.95	291.03	712.88	236.02
GM-CSF	4	6	66.09	17.21	42.17	75.57
	8	6	56.30	16.61	40.70	65.36
	12	6	53.89	10.44	25.59	53.32
TNF-a	4	6	112.44	67.05	164.25	23.77
	8	6	47.52	27.55	67.50	15.09
	12	6	52.28	22.66	55.52	42.66
IL-1b	4	6	1998.68	724.16	1773.82	1883.11
	8	6	1753.08	648.65	1588.87	1336.13
	12	6	1196.67	396.16	970.40	1094.13
FGF basic	4	6	46.92	14.42	35.33	34.85
	8	6	27.42	13.03	31.92	18.50
	12	6	25.59	13.69	33.53	15.03
VEGF	4	6	315.13	232.04	568.39	102.49
	8	6	77.96	59.91	146.75	23.50
	12	6	73.29	25.40	62.22	85.97
PDGF-bb	4	6	24.20	11.44	28.04	10.41
	8	6	11.32	3.73	9.15	11.94
	12	6	41.93	21.51	52.69	17.95
MCP-1	4	6	185.90	94.78	232.17	88.66
	8	6	20.79	14.85	36.38	1.97
	12	6	141.70	84.59	207.20	42.47
IL-8	4	6	2749.24	1207.93	2958.83	1491.15
	8	6	1193.69	781.99	1915.48	492.80
	12	6	2050.58	699.46	1713.33	1125.22
MIP-1a	4	6	95.89	71.53	175.23	11.27
	8	6	5.28	1.54	3.78	4.78
	12	6	82.77	72.91	178.60	9.79
G-CSF	4	4	1112.36	617.37	1234.74	770.79
	8	4	123.79	73.46	146.92	67.15
	12	4	950.54	894.53	1789.06	75.42

Table 17: The study group for the most important growth factors and cytokines involved in wound healing analyzed at 4, 8, and 12 weeks.

Analyte	Week	N	Mean	S.E.M	St. dev.	Median
MIP-1b	4	6	338.41	262.75	643.61	89.51
	8	6	139.55	65.64	160.79	65.22
	12	6	64.19	24.92	61.05	68.65
IL-6	4	6	104.31	59.34	145.37	32.83
	8	6	1732.62	1216.35	2979.43	351.14
	12	6	20.34	8.49	20.81	18.77
IFN-g	4	6	21.82	7.93	19.43	11.92
	8	6	43.25	19.62	48.08	32.56
	12	6	15.55	5.69	13.94	10.51
IL-1ra	4	6	2010.93	580.18	1421.14	1973.56
	8	6	3506.19	1124.96	2755.59	3026.28
	12	6	2021.27	1258.02	3081.51	981.87
GM-CSF	4	6	56.11	10.39	25.46	56.38
	8	6	54.99	11.74	28.75	47.41
	12	6	81.21	11.60	28.42	88.83
TNF-a	4	6	33.88	15.28	37.43	18.88
	8	6	43.16	25.82	63.25	11.18
	12	6	17.93	8.37	20.52	12.41
IL-1b	4	6	2170.24	466.76	1143.33	2653.84
	8	6	2762.03	440.22	1078.33	3052.77
	12	6	1725.57	537.06	1315.54	1820.23
FGF basic	4	6	23.71	15.73	38.54	0.00
	8	6	99.36	68.36	167.46	18.35
	12	6	9.35	6.11	14.98	0.00
VEGF	4	6	107.31	59.46	145.56	46.34
	8	6	171.24	108.32	265.35	0.00
	12	6	65.03	34.11	83.56	25.31
PDGF-bb	4	6	31.56	22.19	54.36	5.15
	8	6	28.52	21.32	52.22	8.14
	12	6	2.30	1.19	2.93	1.57
MCP-1	4	6	94.59	77.01	188.74	10.08
	8	6	60.16	47.81	117.11	0.00
	12	6	1.17	0.76	1.86	0.00
IL-8	4	5	3216.87	1269.13	2837.87	2606.94
	8	6	4803.05	2202.63	5395.32	2491.14
	12	6	3054.54	2133.39	5225.73	295.06
MIP-1a	4	6	32.04	18.53	45.41	10.55
	8	6	17.35	4.44	10.88	16.07
	12	6	4.41	0.73	1.80	4.90
G-CSF	4	2	1997.42	1940.59	2744.40	1997.42
	8	2	4258.21	1217.95	1722.44	4258.21
	12	2	534.02	511.23	722.98	534.02

Here are tables for TGF-beta 1, TGF-beta2, and TGF beta 3, taken at 4, 8, and 12 weeks. (Table 18) is for the control group and (table 19) is for the Oasis treated group.

Table 18: Showing TGF-beta 1,2 ,&3 in the Control group

TGF-β	Week	N	Mean	S.E. Mean	St. dev.	Median
TGF-β 1	4	6	0.40	0.40	0.98	0.0
	8	6	0.57	0.57	1.41	0.0
	12	6	8.92	6.84	16.77	0.0
TGF-β 2	4	6	0.00	0.00	0.00	0.0
	8	6	0.00	0.00	0.00	0.0
	12	6	1.97	1.97	4.83	0.0
TGF-β 3	4	6	8.04	2.98	7.30	6.17
	8	6	6.93	3.01	7.37	4.95
	12	6	5.55	1.75	4.30	5.85

Table 19: Showing TGF-beta 1,2,&3 in the study group

TGF-β	Week	N	Mean	S.E. Mean	St. dev.	Median
TGF-β 1	4	6	33.38	28.69	70.29	0.0
	8	6	26.29	26.28	64.41	0.0
	12	6	20.15	9.45	23.15	14.88
TGF-β 2	4	6	8.13	7.85	19.23	0.0
	8	6	0.77	0.77	1.9	0.0
	12	6	8.12	5.48	13.44	1.74
TGF-β 3	4	6	5.69	2.99	2.99	2.52
	8	6	4.38	2.56	2.56	2.45
	12	6	12.83	8.08	8.08	5.45

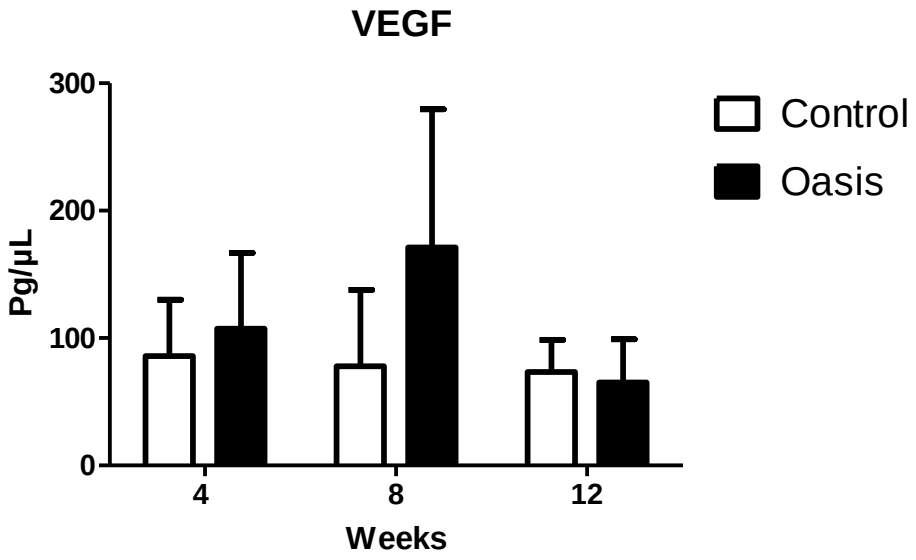

Figure 7. In the oasis treated group, VEGF was steadily increasing by week 8 where the healing rate mostly achieved at this time; however, there were no significant differences between control and experimental groups at any of the three time points.

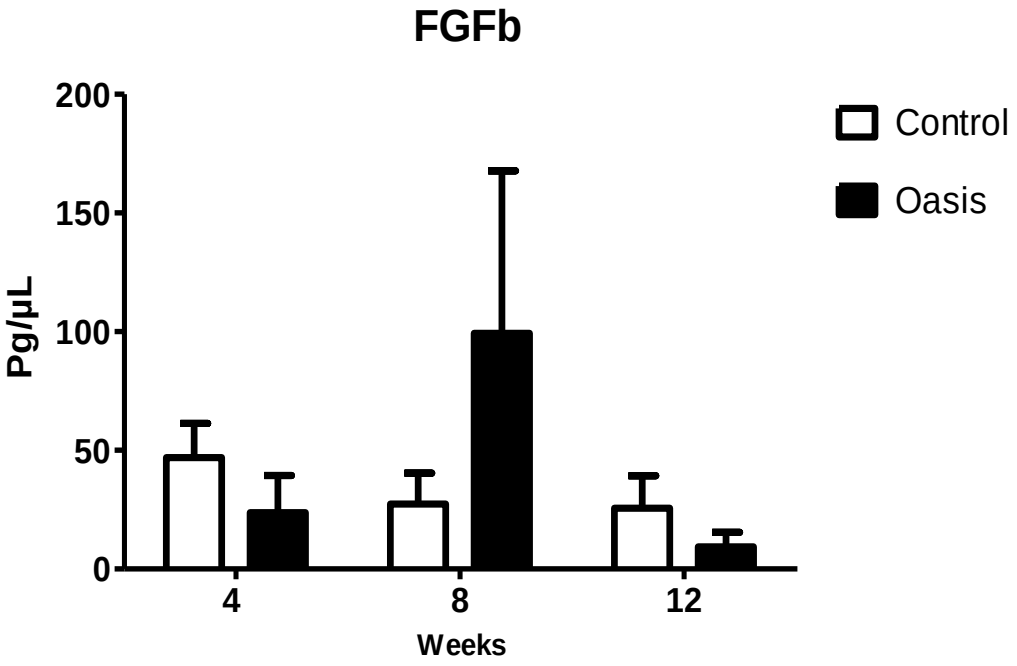

Figure 8. The mean of bFGF in the oasis treated group tripled by week 8 during fibroblast proliferative phase of wound healing. In the control group, the healing process was hindered by the constant decrease of the bFGF throughout the research period.

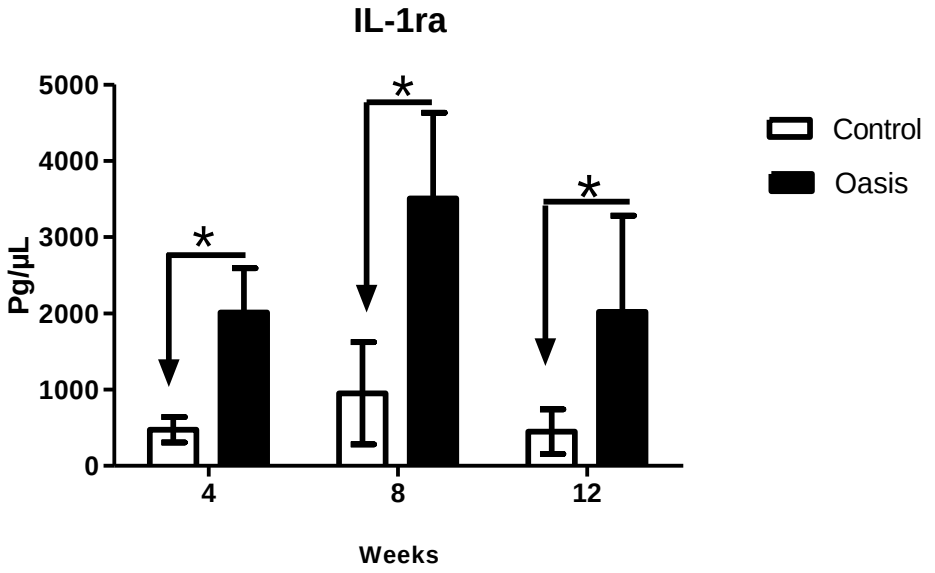

Figure 9. Showing the IL-1ra was significantly increased in the study group in the week 4, 8, and 12, which is strongly correlated with the progression of the healing rate that achieved by Oasis-ultra.

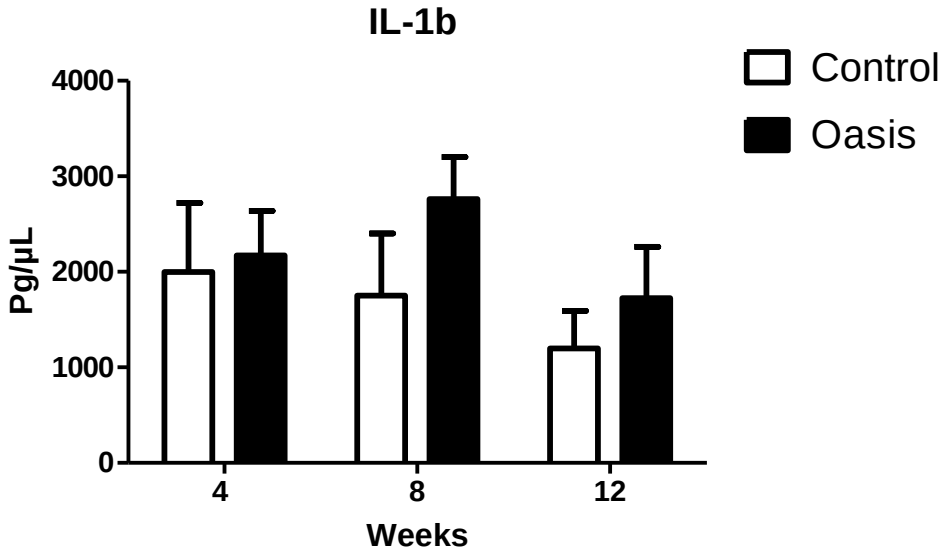

Figure 10. We found that IL-1b was highly expressed in both groups; however, there were no significant difference between the two groups.

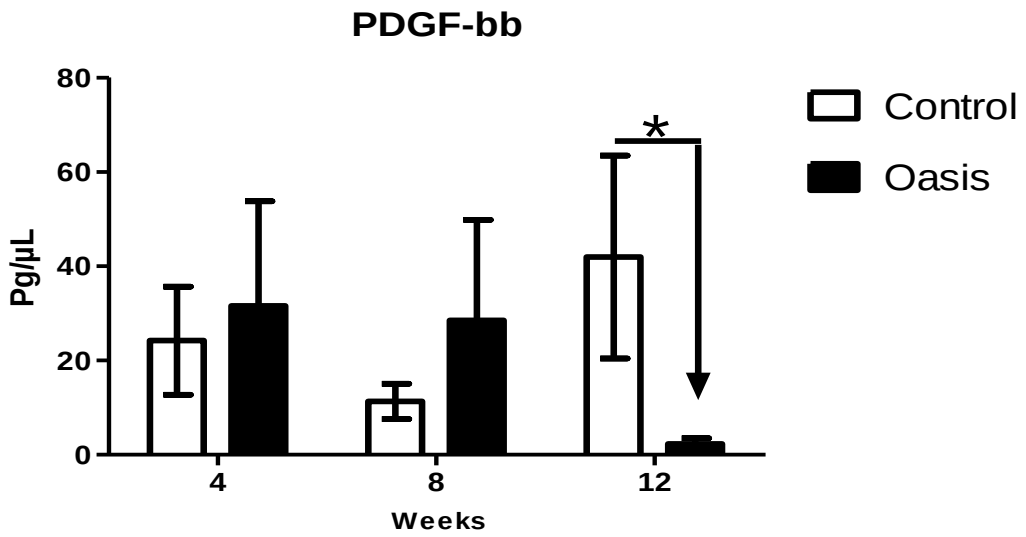

Figure 11. Showing an increase of PDGF-bb in the oasis treated group compared to the control group in the period between 4 and 8 week followed by a decrease during week 12, by time wound healed.

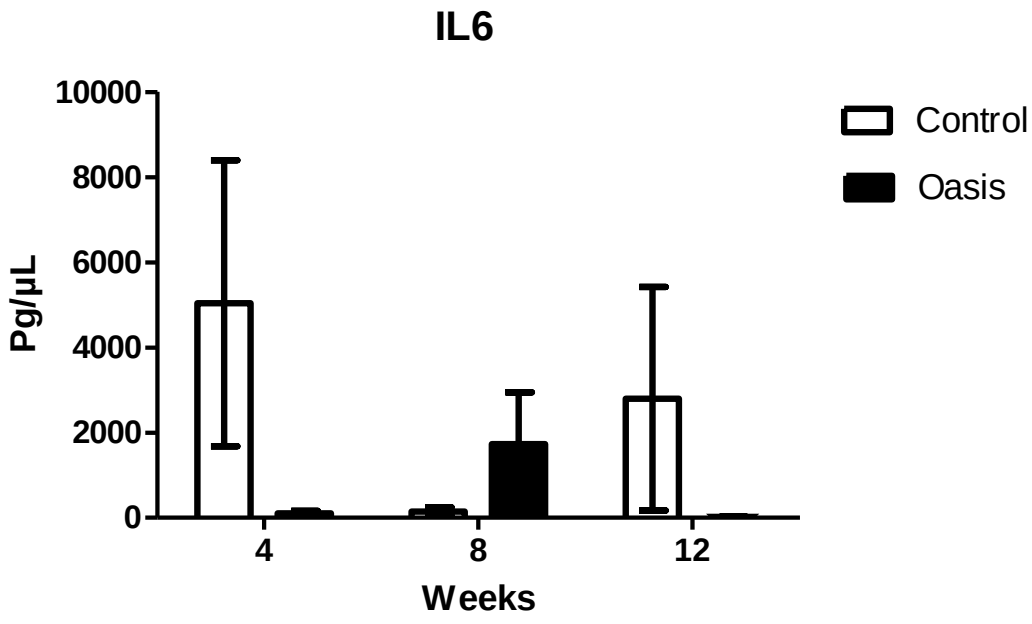

Figure 12. IL6, there are no significant difference between the oasis treated group and the control group at any time point. Even though the means vary greatly, this is likely due to the immense variance of both groups.

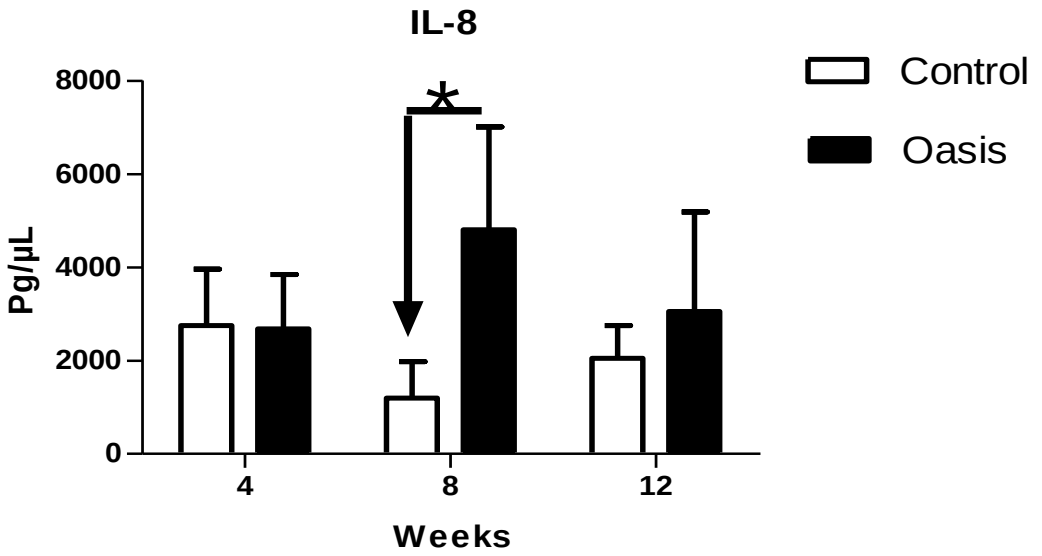

Figure 13. During the first measurement, on week 4, the levels of IL-8 are very close in the oasis treated and the control groups. During week 8, IL-8 was increased in the study group compared to the control group. On week 12 there were no significant difference between the two groups.

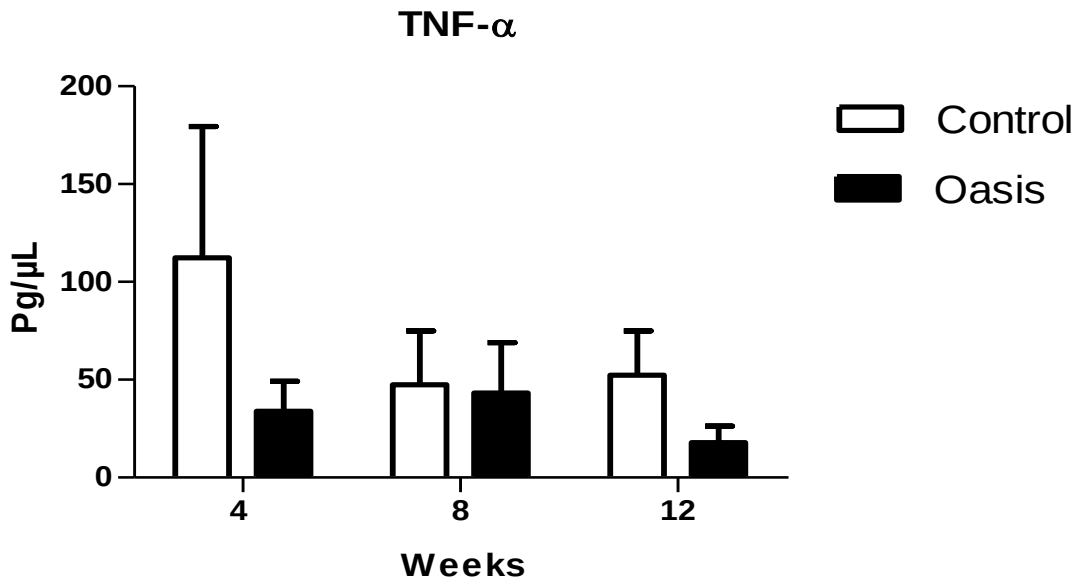

Figure 14. Showing that TNF-α was increased in the control group between weeks 4 and 12, while it was almost similar between the two groups on week 8. We see that oasis decrease the level of TNF-α specifically on weeks 4 and 12.

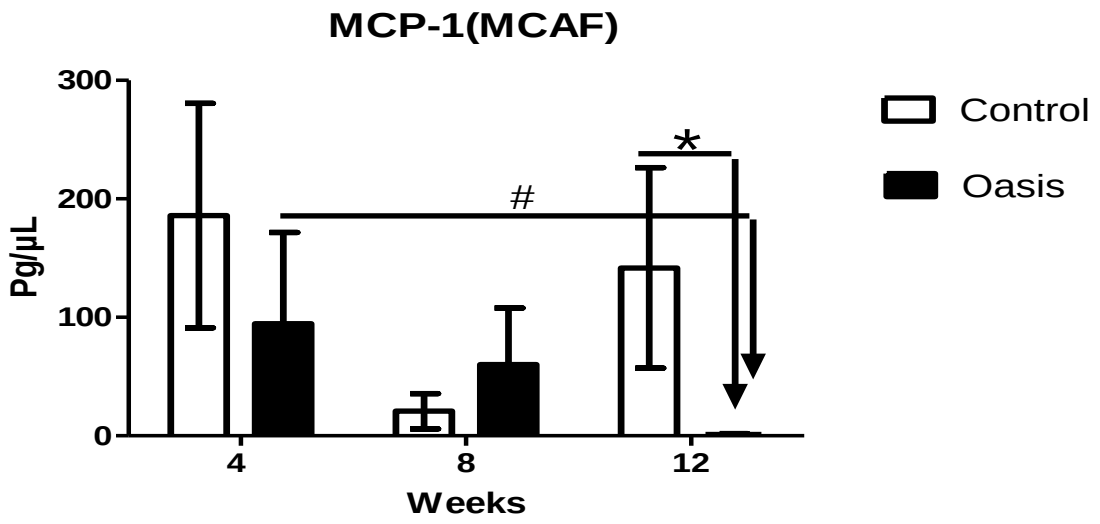

Figure 15. Showing the inhibitory effect of Oasis on MCP-1: MCP-1 was steadily decreasing between time points 4, 8, and 12 with a significant difference between weeks 4 and 12 in the same study group. However, the control group had increased levels of MCP-1 on 4 and 12 weeks, the important stages in wound healing thus interfering with the recovery process.

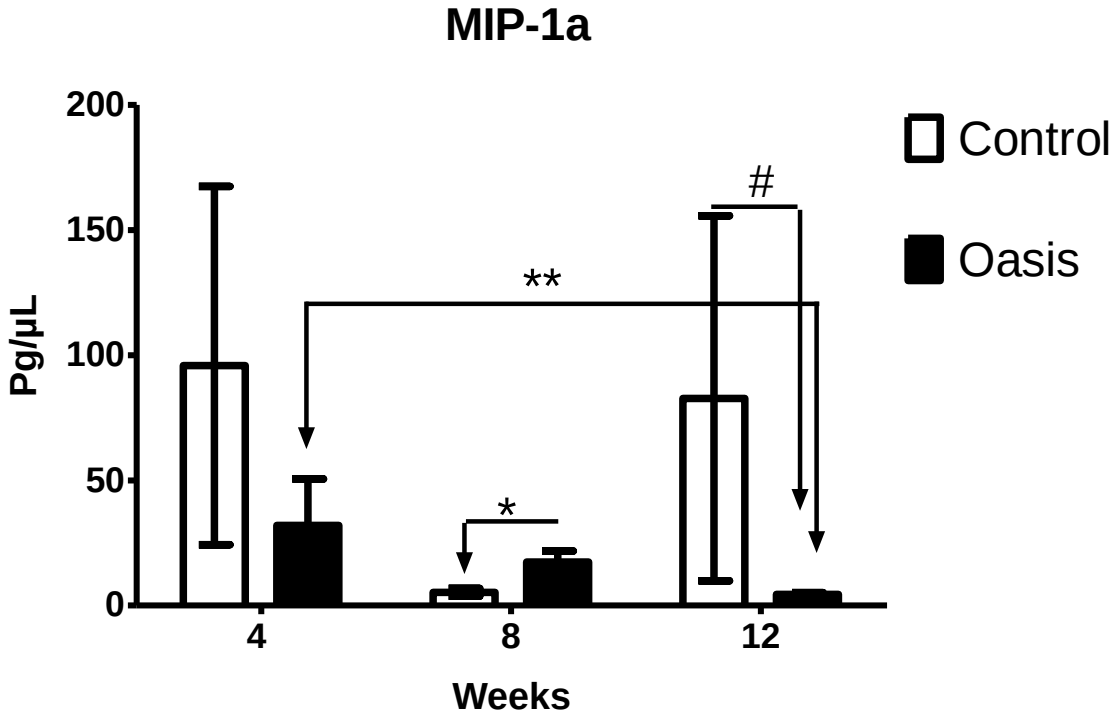

Figure 16. MIP-1 that is responsible for chronic inflammation and delay in wound healing was significantly increased in the control group compared to the study group during weeks 4 and 12 while it was steadily decreasing in the study group thus facilitating the healing process.

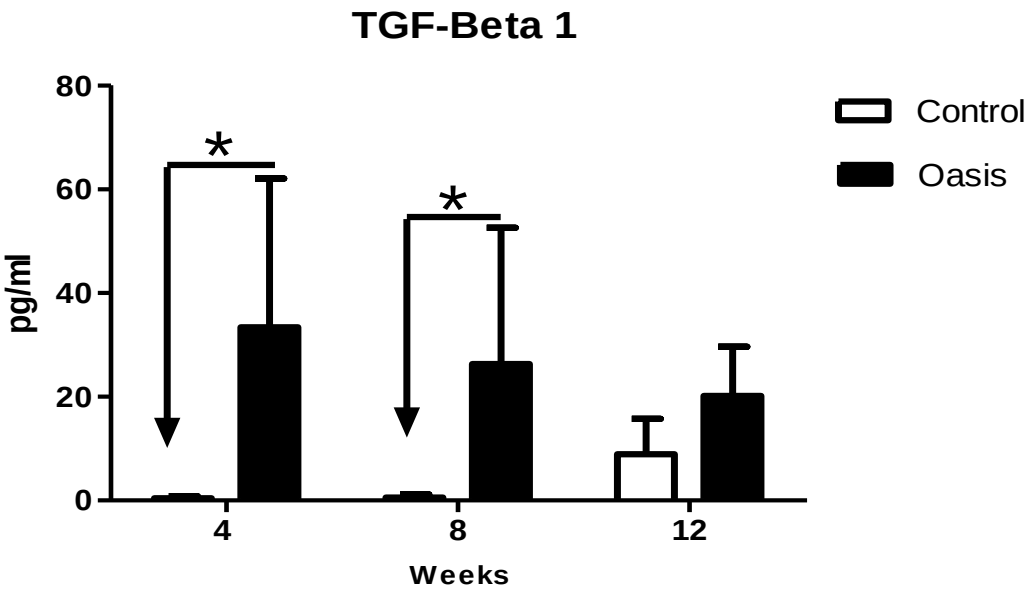

Figure 17. Showing the significant difference in TGF-β1 levels during weeks 4 and week 8 compared to the control group. Higher levels of TGF-β1 are required for wound healing. On the other hand, TGF- β1 was very minimal expressed in the control group during all time points: 4, 8, and 12.

Discussion and Conclusion:

Wound healing is a sophisticated process that requires coordinating work of several different cell types: leukocytes, neutrophils, keratinocytes, fibroblasts, epithelial and connective tissue cells, inflammatory cells, coagulation factors, growth factors, and cytokines (Guo & Dipietro, 2010). Each stage of tissue repair involves a highly orchestrated sequence of events to make the healing process successful (Ghatak et al., 2015). Thus, wound healing requires precision that is regulated and accomplished by a complex signaling network of various growth factors, cytokines, and chemokines involved in a complex integration of signals that coordinate cellular processes (S. Barrientos et al., 2008b). The aim of this study was to evaluate and assess the key growth factors involved in wound healing.

In our study, 27 growth factors and cytokines were analyzed using the Bio-Plex system, and a number of these cytokines were known to be relevant to wound healing process, for example, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and transforming growth factor beta-1 (TGF-β1). Each of these growth factors played a crucial role in the angiogenic process during wound healing. Moreover, FGF and VEGF are essential factors in the generation of endothelium. Numerous clinical trials that used collagen-based matrices for treatment of chronic wounds showed great results in accelerating the healing rate of non-healing wounds (Robson, 1997).

In our present study, we observed that prolonged inflammatory phase in a special type of chronic wounds, decubitus ulcers or pressure ulcers can alter the important steps of

wound healing downstream, such as collagen synthesis and angiogenesis. The cytokines found in high concentrations in chronic wounds (Kasiewicz & Whitehead, 2015) and involved in the inflammatory phase of wound healing are: IL-1, IL-6, IL-8, TNF-α, MIP-1, and MCP-1 (Singer & Clark, 1999). In our research study, we found that IL-1b was highly expressed in both groups; however, it was expressed higher in the study group compared to the control group. Vegesna et al. (2005) explained the role of IL-1b to be enhancing tensile strength of certain defective wounds. We could reach the assumption that such fluctuation in IL-1b concentration was a natural sequence of events needed to achieve healing in this type of wounds.

High levels of MCP-1, MIP-1a, and TNF-α have a negative impact on the healing process thus explaining the rationale of chronicity of pressure ulcers, when these cytokines are present, as one of the leading causes of inflammation. Pro-inflammatory cytokines, such as tumor necrosis factor (TNF-α) and interleukin 1b (IL-1b) are the first cells that show early response during the inflammatory phase (Blackwell & Christman, 1996). Also, TNF-□ is mainly secreted from macrophages and neutrophils during the inflammatory phase of wound healing (Goel et al., 2010). TNF-α is produced by various cells during the inflammatory phase thus causing activation of different types of MMPs, and at the same time suppression of ECM synthesis (Mauviel, Qiu Chen, Dong, Evans, & Uitto, 1993).

Neutrophils are considered to be potent phagocytic cells playing important roles for keeping wounds clean from any debris. Nevertheless, in the absence of pathogens, neutrophils are not important in healing wounds (Simpson & Ross, 1972). In their research paper (1972), Simpson and Ross emphasized that mice with neutropenia, treated with neutrophils antibodies, showed no significant differences affecting wound closure speed

compared to the controls. Conversely, most studies have shown that macrophages are the most predominant cells in the early phase of wound healing that have a significant role in the healing process (Yates, Hebda, & Wells, 2012). Werner and Grose (2003) mentioned in their research that macrophages' function was not only restricted on phagocytosis, but they were also a rich source of different growth factors playing a crucial role in the wound healing process: transforming growth factor alpha (TGF-α), epidermal growth factor (EGF), transforming growth factor beta (TGF-β) platelet-derived growth factor (PDGF) (Penn, Grobbelaar, & Rolfe, 2012). Active macrophages have two phenotypes: M1 and M2. M1 phenotype is responsible for releasing some of the proinflammatory cytokines, such as IL-1, IL-6, and TNF-alpha (Koh & DiPietro, 2011); however, M2 phenotype is regarded as anti-inflammatory and, at the same time, a good source for different growth factors' production that are needed for proliferation and wound healing (Martin, Walton, & Harper, 2009).

In our study, we noticed that certain growth factors and cytokines were linked to the wound healing process. Some of the important cytokines were: IL-1b, IL-1Ra, IL-6, IL-8, TNF-alpha, PDGF-bb, bFGF, VEGF, and TGF-β1. Researchers have shed light on TGF-beta when applied exogenously and how amazing its effect in treating acute wounds was (Mustoe et al., 1987; Shah, Foreman, & Ferguson, 1995). Also, Pastar et al. (2010) mentioned in their research that animal wound models showed great healing rates after recombinant TGF-β application (Pastar et al., 2010). In our study, we intended to investigate TGF-β1, the growth factor that plays an essential role for cell proliferation and migration. Although, TGF- β1 total concentration for the three time points, 4, 8, and 12 weeks was low, it shows a great elevation in the study group (Oasis-ultra plus NPWT).

TGF-β1 was significantly increased in the study group on week 4 and week 8 compared to the control group; however, overall there is no significant difference between both groups In the literature, TGF-β1 plays an important role of regulation and stimulation of the extracellular matrix (ECM) by stimulating fibroblasts to produce collagen. Also, TGF-β1 protects ECM from degradation by inhibiting different proteases (Lafyatis et al., 1990). TGF-β particularly at the level of epithelial tissues, acts as suppressor to different cytokines' activity (Siegel & Massague, 2003). In another highlight study, researchers used quantitative PCR specifically to test TGF-β receptors obtained from human non-healing venous ulcers (Pastar et al., 2010). They reached the assumption that chronic venous ulcers lack TGF-β receptor type II. This result is consistent with Robson (1997) who mentioned in his research the decreased levels of TGF-beta in chronic wounds (Robson, 1997).

TGF-β1 elicits diverse cellular responses depending on cell type, state of differentiation, and culture conditions (Barnard, Lyons, & Moses, 1990). In advanced studies knockout TGF-β1 mice were used. The mice were born without any morphological defect, but after 2-3 weeks of treatment, they displayed a generalized inflammatory syndrome, a form of mononuclear cells infiltration to different vital organs, such as heart, lung, also tissue necrosis, that became lethal (Shull et al., 1992). Also, chronic non-healing wounds are very strongly associated with reduction in TGF-β signaling (Penn et al., 2012). Another study found the clear reduction in macrophages, fibronectin, and collagen I & III deposition in cutaneous rat wounds after adding exogenous neutralizing antibody to TGF-β1 compared to control wounds (Shah et al., 1995). This finding indicates the important role of TGF-β1 especially during the early wound healing phases.

To determine the role of the TNF receptor p55 in cutaneous wound repair, Mori et al., (2002) emphasized the role of TNF-alpha receptors in cutaneous wound healing. He found that TNF-alpha-receptor-knockout mice were showing great wound healing by improving angiogenesis and collagen production compared to the wild type (Mori, Kondo, Ohshima, Ishida, & Mukaida, 2002). Also, among the TNF-receptor-deficient mice group, the expression of TGF-β1, connective tissue growth factor (CTGF) and VEGF genes were high (Mori et al., 2002), which explains the increased healing rate through increasing the proliferation of fibroblasts and increasing the new blood vessels formation via VEGF.

In our research experiments, the study group (Oasis-ultra plus NPWT) did achieve marked reduction in the level of TNF-α at different time points, 4, 8, and 12 weeks. Although the reduction was not significant, the clinical healing rate was markedly improved in the study group as correlated with the reduction in TNF-α and compared to the control group. Moreover, the TGF-β1 expression level increased in the study group due to the decrease in the inhibitory effect of TNF-α; at the same time, the level of the TGF-β1 was low in the control group due to high levels of TNF- α and other proinflammatory cytokines. Therefore, our results were consistent with the findings described by Mori et al. (2002) in their research. In a number of previous studies, chronic wounds, similar to those in our target patients, were arrested in the inflammatory phase. Therefore, our focus was on decreasing the pro-inflammatory cytokines and MMPs concentration (Salazar, Ennis, & Koh, 2015). While increasing the growth factors that are important for angiogenesis and fibroblast proliferation and differentiation. That was achieved by applying Oasis-ultra to stage IV pressure wounds. We need to facilitate the transition of the wound healing process from the inflammatory phase to the next phase in order to achieve the full healing.

The patients' wounds were measured weekly, length x width x depth (cm^3). The closure % calculations were performed using the following formula: % of healing rate = [(Area on Day 0 - Open Area on Final Day)/Area on Day 0] X 100. Adding Oasis-ultra to the study group improved the healing rate which reached 87% compared to the control group with the healing rate of 55%. The median healing rate of the Oasis-ultra treated group was much higher than the median healing rate of the control group, and the rates between the groups varied significantly. The Oasis-ultra treated group had a lower variability, meaning that the healing rate was going on for those six patients at a steadier rate compared to the control group. Therefore, based on these results, Oasis-ultra was found to be associated with a more rapid improvement and a higher likelihood of achieving the complete ulcer closure than the ulcers treated with only NPWT.

Although the inflammatory phase is crucial in the wound healing mechanism, the proinflammatory cytokines that are produced during this phase will definitely damage wound tissues leading to ischemia and necrosis, typical events occurring in pressure ulcers (decubitus ulcers) (Stechmiller et al., 2006).

IL-1ra is a member of the interleukin-1 cytokine family, and it is a natural inhibitor of the pro-inflammatory effect of IL-1-β. This finding is consistent with Karstoft and Pedersen (2016), who emphasized in their diabetic research the ability of exercise to increase the levels of IL-6. The increase of IL-6 lead to diminished TNF-α inflammatory effect and at the same time stimulated IL-1ra, which is considered to have an anti-inflammatory effect on IL-1b. IL-1ra is attached to IL-1 receptors; however, no intracellular signaling can happen (Goto et al., 2015). Previous clinical studies have shown increases in IL-1a and IL-1b in chronic inflammatory conditions, for example, periodontitis and gingival tissue from

patients, compared with healthy subjects (Ishihara et al., 1997; Rawlinson, Dalati, Rahman, Walsh, & Fairclough, 2000). In a study that was conducted *in vivo* on a rabbit model of osteoarthritis (OA), the scientists applied gene therapy and co-expression of interleukin-1 receptor antagonist (IL-1Ra) and transforming growth factor-β1 (TGF-β1) to change the course of disease. Fluid from the joint was analyzed using ELISA; IL-1ra and TGF-β1 in the single and double transfection groups showed extraordinary increase. At the same time, levels of IL-1b and tumor necrosis factor alpha (TNF-α) were measured and showed a drastic decrease in the study group compared to the control group (Zhang, Zhong, Yu, & Liu, 2015). We conclude from this experiment that IL-1ra and TGF-β1 have improved healing of cartilage degeneration and helped the repairing process. From our result, we noticed high expression of IL-1ra among the Oasis-ultra treated group when compared to the group treated by NPWT only. Also, during the study that was done on IL-1ra-knockout mice, the scientists found this group of mice were more likely to contract infection due to the unopposed effect of IL-1a, which further lead to chronic inflammation, such as Rheumatoid arthritis (Hirsch, Irikura, Paul, & Hirsh, 1996; Horai et al., 2000; Nicklin, Hughes, Barton, Ure, & Duff, 2000). In our research, IL-1Ra was significantly increased in the study group during week 4, week 8, and 12, which meant good prognosis toward the healing. Also, as we discussed previously that IL-1ra acts as anti-inflammatory cytokine, which is significantly upregulated by Oasis-ultra treated group compared to the control group.

Interleukin-6 (IL-6) plays an important role in the wound healing process. Moreover, it promotes the expression of other cytokines necessary for initiating the wound healing process, such as, IL-1alpha, IL-1beta, etc. In the *in vitro* study using corneal

epithelial cells of rabbits Nakamura and Nishida (1999) mentioned that IL-6 as pro-inflammatory cytokine promoted cell migration of the rabbit corneal epithelial cells and wound closure. In another research study that was conducted using IL-6 depleted mice versus the wild type, it was observed that wounds healed faster in the control group compared to IL-6 depleted mice, which led us to the conclusion that IL-6 played a role in wound healing (Z. Q. Lin et al., 2005). In our present study, IL-6, is considered as a proinflammatory cytokines. Even though the means vary greatly between the oasis-ultra treated group and the control group, there were no any significant difference between the two groups at any time point. At the same time, the mean concentration of IL-6 was low on week 4 in the study group followed by its increase on week 8, which is necessary in order to stimulate fibroblast and keratinocytes proliferation and migration. Later on, IL-6 decreased again thus leading to the maturation phase that does not need any proinflammatory cytokines.

The other cytokines that were down regulated by adding oasis-ultra included MIP-1a, MIP-1b, and MCP-1 (MCAF). Many research studies have mentioned that in case of burn wounds, there was a significant increase in the skin proinflammatory cytokines, such as interleukin 1b, interleukin 6, tumor necrosis factor-α, and the macrophage inflammatory protein MIP-1b (Rani, Zhang, & Schwacha, 2014). Rani, Zhang, and Schwacha (2014) in their research study, used special γδ T-cell deficient (δTCR−/−) mice that were exposed to the third degree burn by using (25% TBSA), and the study revealed that TNF-α, MIP-1a, and MIP-1b levels doubled or tripled in treated mice compared to the wild type. Similarly, in our research, these proinflammatory cytokines were highly expressed during stages IV pressure ulcers in the control group; however, in the study group, where oasis-ultra was

applied, TNF-α, MIP-1a, and MIP-1b cytokines responsible for delaying healing in these type of wounds, were inhibited. For example, MIP-1a and MCP-1 were steadily decreased in the period between week 4 to week 12 in the study group; however, MIP-1a and MCP-1 were highly increased during week 4. Even though, these proinflammatory cytokines decreased on week 8, which could have been caused by the factors like debridement either mechanical by NPWT following the removal of the sponge that adhered to the wound bed or using the scalper and scissors, these factors highly increased thus contributing directly or indirectly to the chronicity of these types of wounds.

Based on the literature and our findings, we reached the assumption that in the Oasis-ultra treated group, the proinflammatory cytokines were successfully inhibited. The mediators associated with the wound healing (growth factors and cytokines) can be further used as a reliable biomarker giving prognosis of the wound healing progress observed in the clinical settings (Patel et al., 2016).

In conclusion, based on our research results and the data presented, Oasis-ultra combined with NPWT provides good outcomes for treating stage IV pressure wounds. Additionally, supplementing Oasis-ultra with NPWT in the study group showed a successful inhibition of the proinflammatory cytokines while promoting and upregulating the beneficial growth factors that have a good contribution toward the healing rate.

References

Mustoe, T. A., Pierce, G. F., Thomason, A., Gramates, P., Sporn, M. B., & Deuel, T. F. (1987). Accelerated healing of incisional wounds in rats induced by transforming growth factor-beta. *Science (New York, N.Y.), 237*(4820), 1333-1336.

AbouIssa, A., Mari, W., & Simman, R. (2015). Clinical usage of an extracellular, collagen-rich matrix: A case series. *Wounds: A Compendium of Clinical Research and Practice, 27*(11), 313-318.

Argenta, L. C., & Morykwas, M. J. (1997). Vacuum-assisted closure: A new method for wound control and treatment: Clinical experience. *Annals of Plastic Surgery, 38*(6), 563-76; discussion 577.

Argenta, L. C., Morykwas, M. J., Marks, M. W., DeFranzo, A. J., Molnar, J. A., & David, L. R. (2006). Vacuum-assisted closure: State of clinic art. *Plastic and Reconstructive Surgery, 117*(7 Suppl), 127S-142S. doi:10.1097/01.prs.0000222551.10793.51

Attinger, C. E., Janis, J. E., Steinberg, J., Schwartz, J., Al-Attar, A., & Couch, K. (2006). Clinical approach to wounds: Debridement and wound bed preparation including the use of dressings and wound-healing adjuvants. *Plastic and Reconstructive Surgery, 117*(7 Suppl), 72S-109S. doi:10.1097/01.prs.0000225470.42514.8f

Badylak, S. F., Freytes, D. O., & Gilbert, T. W. (2009). Extracellular matrix as a biological scaffold material: Structure and function. *Acta Biomaterialia, 5*(1), 1-13. doi:10.1016/j.actbio.2008.09.013

Badylak, S. F., Tullius, R., Kokini, K., Shelbourne, K. D., Klootwyk, T., Voytik, S. L., .Simmons, C. (1995). The use of xenogeneic small intestinal submucosa as a biomaterial for achilles tendon repair in a dog model. *Journal of Biomedical Materials Research, 29*(8), 977-985. doi:10.1002/jbm.820290809

Barnard, J. A., Lyons, R. M., & Moses, H. L. (1990). The cell biology of transforming growth factor beta. *Biochimica Et Biophysica Acta, 1032*(1), 79-87. Doi:0304419X(90)90013Q

Barrientos, S., Stojadinovic, O., Golinko, M. S., Brem, H., & Tomic-Canic, M. (2008a). Growth factors and cytokines in wound healing. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society, 16*(5), 585-601. doi:10.1111/j.1524-475X.2008.00410.x

Barrientos, S., Stojadinovic, O., Golinko, M. S., Brem, H., & Tomic-Canic, M. (2008b). Growth factors and cytokines in wound healing. *Wound Repair and Regeneration, 16*(5), 585-601. doi:10.1111/wrr.2008.16.issue-5

Basilico, C., & Moscatelli, D. (1992). The FGF family of growth factors and oncogenes. *Advances in Cancer Research, 59*, 115-165.

Bauer, S. M., Bauer, R. J., & Velazquez, O. C. (2005). Angiogenesis, vasculogenesis, and induction of healing in chronic wounds. *Vascular and Endovascular Surgery, 39*(4), 293-306.

Bazzoni, F., Cassatella, M. A., Rossi, F., Ceska, M., Dewald, B., & Baggiolini, M. (1991). Phagocytosing neutrophils produce and release high amounts of the neutrophil-activating peptide 1/interleukin 8. *The Journal of Experimental Medicine, 173*(3), 771-774.

Beenken, A., & Mohammadi, M. (2009). The FGF family: Biology, pathophysiology and therapy. *Nature Reviews.Drug Discovery, 8*(3), 235-253. doi:10.1038/nrd2792

Black, J., Baharestani, M., Cuddigan, J., Dorner, B., Edsberg, L., Langemo, D., National Pressure Ulcer Advisory Panel. (2007). National pressure ulcer advisory panel's updated pressure ulcer staging system. *Dermatology Nursing / Dermatology Nurses' Association, 19*(4), 343-9; quiz 350.

Blackwell, T. S., & Christman, J. W. (1996). Sepsis and cytokines: Current status. *British Journal of Anaesthesia, 77*(1), 110-117.

Bodnar, R. J. (2015). Chemokine regulation of angiogenesis during wound healing. *Advances in Wound Care, 4*(11), 641-650. doi:10.1089/wound.2014.0594

Borgquist, O., Anesater, E., Hedstrom, E., Lee, C. K., Ingemansson, R., & Malmsjo, M. (2011). Measurements of wound edge microvascular blood flow during negative pressure wound therapy using thermodiffusion and transcutaneous and invasive

laser doppler velocimetry. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society, 19*(6), 727-733. doi:10.1111/j.1524-475X.2011.00741.x

Bradbury, S., Walkley, N., Ivins, N., & Harding, K. (2015). Clinical evaluation of a novel topical negative pressure device in promoting healing in chronic wounds. *Advances in Wound Care, 4*(6), 346-357. doi:10.1089/wound.2014.0596

Brem, H., Kirsner, R. S., & Falanga, V. (2004). Protocol for the successful treatment of venous ulcers. *American Journal of Surgery, 188*(1A Suppl), 1-8. doi:10.1016/S0002-9610(03)00284-8

Broughton, G., 2nd, Janis, J. E., & Attinger, C. E. (2006). The basic science of wound healing. *Plastic and Reconstructive Surgery, 117*(7 Suppl), 12S-34S. doi:10.1097/01.prs.0000225430.42531.c2

Caduff, J. H., Fischer, L. C., & Burri, P. H. (1986). Scanning electron microscope study of the developing microvasculature in the postnatal rat lung. *The Anatomical Record, 216*(2), 154-164. doi:10.1002/ar.1092160207

Campos, A. C., Groth, A. K., & Branco, A. B. (2008). Assessment and nutritional aspects of wound healing. *Current Opinion in Clinical Nutrition and Metabolic Care, 11*(3), 281-288. doi:10.1097/MCO.0b013e3282fbd35a

Cazzell, S. M., Lange, D. L., Dickerson, J. E.,Jr, & Slade, H. B. (2015). The management of diabetic foot ulcers with porcine small intestine submucosa tri-layer matrix: A

randomized controlled trial. *Advances in Wound Care, 4*(12), 711-718. doi:10.1089/wound.2015.0645

Chen, H. L., Cao, Y. J., Wang, J., & Huai, B. S. (2015). A retrospective analysis of pressure ulcer incidence and modified braden scale score risk classifications. *Ostomy/wound Management, 61*(9), 26-30.

Chen, S. M., Ward, S. I., Olutoye, O. O., Diegelmann, R. F., & Kelman Cohen, I. (1997). Ability of chronic wound fluids to degrade peptide growth factors is associated with increased levels of elastase activity and diminished levels of proteinase inhibitors. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society, 5*(1), 23-32. doi:WRRwrr_050108

Choi, J. C., Uyama, H., Lee, C. H., & Sung, M. H. (2015). Promotion effects of ultra-high molecular weight poly-gamma-glutamic acid on wound healing. *Journal of Microbiology and Biotechnology,* doi:10.4014/jmb.1412.12083

Clark, R. A. (1993). Regulation of fibroplasia in cutaneous wound repair. *The American Journal of the Medical Sciences, 306*(1), 42-48.

Clark, R. A., Lanigan, J. M., DellaPelle, P., Manseau, E., Dvorak, H. F., & Colvin, R. B. (1982). Fibronectin and fibrin provide a provisional matrix for epidermal cell migration during wound reepithelialization. *The Journal of Investigative Dermatology, 79*(5), 264-269.

Cuevas, I., & Boudreau, N. (2009). Managing tumor angiogenesis: Lessons from VEGF-resistant tumors and wounds. *Advances in Cancer Research, 103*, 25-42. doi:10.1016/S0065-230X(09)03002-4

Czaja, W., Krystynowicz, A., Bielecki, S., & Brown, R. M.,Jr. (2006). Microbial cellulose--the natural power to heal wounds. *Biomaterials, 27*(2), 145-151. doi:S0142-9612(05)00704-0

Demidova-Rice, T. N., Salomatina, E. V., Yaroslavsky, A. N., Herman, I. M., & Hamblin, M. R. (2007). Low-level light stimulates excisional wound healing in mice. *Lasers in Surgery and Medicine, 39*(9), 706-715. doi:10.1002/lsm.20549

Dhivya, S., Padma, V. V., & Santhini, E. (2015). Wound dressings - a review. *Biomedicine, 5*(4), 22-015-0022-9. Epub 2015 Nov 28. doi:10.7603/s40681-015-0022-9

Djonov, V., Baum, O., & Burri, P. H. (2003). Vascular remodeling by intussusceptive angiogenesis. *Cell and Tissue Research, 314*(1), 107-117. doi:10.1007/s00441-003-0784-3

Dobreva, I., Waeber, G., James, R. W., & Widmann, C. (2006). Interleukin-8 secretion by fibroblasts induced by low density lipoproteins is p38 MAPK-dependent and leads to cell spreading and wound closure. *The Journal of Biological Chemistry, 281*(1), 199-205. doi:M508857200

Dumville, J. C., Webster, J., Evans, D., & Land, L. (2015). Negative pressure wound therapy for treating pressure ulcers. *The Cochrane Database of Systematic Reviews, 5*, CD011334. doi:10.1002/14651858.CD011334.pub2

Dyugovskaya, L., Berger, S., Polyakov, A., Lavie, P., & Lavie, L. (2016). Intermittent hypoxia affects the spontaneous differentiation in vitro of human neutrophils into long-lived giant phagocytes. *Oxidative Medicine and Cellular Longevity, 2016*, 9636937. doi:10.1155/2016/9636937

Edmonds, M. (2012). Body of knowledge around the diabetic foot and limb salvage. *The Journal of Cardiovascular Surgery, 53*(5), 605-616. doi:R37127314

Eisen, T., Boshoff, C., Mak, I., Sapunar, F., Vaughan, M. M., Pyle, L., Gore, M. E. (2000). Continuous low dose thalidomide: A phase II study in advanced melanoma, renal cell, ovarian and breast cancer. *British Journal of Cancer, 82*(4), 812-817. doi:S0007092099910042

Faler, B. J., Macsata, R. A., Plummer, D., Mishra, L., & Sidawy, A. N. (2006). Transforming growth factor-beta and wound healing. *Perspectives in Vascular Surgery and Endovascular Therapy, 18*(1), 55-62.

Figg, W. D., Dahut, W., Duray, P., Hamilton, M., Tompkins, A., Steinberg, S. M., Reed, E. (2001). A randomized phase II trial of thalidomide, an angiogenesis inhibitor, in patients with androgen-independent prostate cancer. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research, 7*(7), 1888-1893.

Finnson, K. W., McLean, S., Di Guglielmo, G. M., & Philip, A. (2013). Dynamics of transforming growth factor beta signaling in wound healing and scarring. *Advances in Wound Care, 2*(5), 195-214. doi:10.1089/wound.2013.0429

Flamme, I., Frolich, T., & Risau, W. (1997). Molecular mechanisms of vasculogenesis and embryonic angiogenesis. *Journal of Cellular Physiology, 173*(2), 206-210. doi:10.1002/(SICI)1097-4652(199711)173:2<206::AID-JCP22>3.0.CO;2-C

Fleck, C. A., & Simman, R. (2011). Modern collagen wound dressings: Function and purpose. *The Journal of the American College of Certified Wound Specialists, 2*(3), 50-54. doi:10.1016/j.jcws.2010.12.003

Folkman, J. (1995). Seminars in medicine of the beth israel hospital, boston. Clinical applications of research on angiogenesis. *The New England Journal of Medicine, 333*(26), 1757-1763. doi:10.1056/NEJM199512283332608

Frykberg, R. G., & Banks, J. (2015). Challenges in the treatment of chronic wounds. *Advances in Wound Care, 4*(9), 560-582. doi:10.1089/wound.2015.0635

Gainza, G., Villullas, S., Pedraz, J. L., Hernandez, R. M., & Igartua, M. (2015). Advances in drug delivery systems (DDSs) to release growth factors for wound healing and skin regeneration. *Nanomedicine: Nanotechnology, Biology, and Medicine, 11*(6), 1551-1573. doi:10.1016/j.nano.2015.03.002

Ghatak, S., Maytin, E. V., Mack, J. A., Hascall, V. C., Atanelishvili, I., Moreno Rodriguez, R., . . . Misra, S. (2015). Roles of proteoglycans and glycosaminoglycans in wound

healing and fibrosis. *International Journal of Cell Biology, 2015*, 834893. doi:10.1155/2015/834893

Ghosh, S., May, M. J., & Kopp, E. B. (1998). NF-kappa B and rel proteins: Evolutionarily conserved mediators of immune responses. *Annual Review of Immunology, 16*, 225-260. doi:10.1146/annurev.immunol.16.1.225

Gibson, D. J., & Schultz, G. S. (2013). Molecular wound assessments: Matrix metalloproteinases. *Advances in Wound Care, 2*(1), 18-23. doi:10.1089/wound.2011.0359

Gilbertson, D. G., Duff, M. E., West, J. W., Kelly, J. D., Sheppard, P. O., Hofstrand, P. D., . . . Hart, C. E. (2001). Platelet-derived growth factor C (PDGF-C), a novel growth factor that binds to PDGF alpha and beta receptor. *The Journal of Biological Chemistry, 276*(29), 27406-27414. doi:10.1074/jbc.M101056200

Goel, A., Kumar, S., Singh, D. K., & Bhatia, A. K. (2010). Wound healing potential of ocimum sanctum linn. With induction of tumor necrosis factor-alpha. *Indian Journal of Experimental Biology, 48*(4), 402-406.

Goldminz, D., & Bennett, R. G. (1991). Cigarette smoking and flap and full-thickness graft necrosis. *Archives of Dermatology, 127*(7), 1012-1015.

Gosain, A., & DiPietro, L. A. (2004). Aging and wound healing. *World Journal of Surgery, 28*(3), 321-326. doi:10.1007/s00268-003-7397-6

Goto, H., Ishihara, Y., Kikuchi, T., Izawa, A., Ozeki, N., Okabe, E., Mitani, A. (2015). Interleukin-1 receptor antagonist has a novel function in the regulation of matrix metalloproteinase-13 expression. *PloS One, 10*(10), e0140942. doi:10.1371/journal.pone.0140942

Greene, A. K., Puder, M., Roy, R., Arsenault, D., Kwei, S., Moses, M. A., & Orgill, D. P. (2006). Microdeformational wound therapy: Effects on angiogenesis and matrix metalloproteinases in chronic wounds of 3 debilitated patients. *Annals of Plastic Surgery, 56*(4), 418-422. doi:10.1097/01.sap.0000202831.43294.02

Greenhalgh, D. G., Sprugel, K. H., Murray, M. J., & Ross, R. (1990). PDGF and FGF stimulate wound healing in the genetically diabetic mouse. *The American Journal of Pathology, 136*(6), 1235-1246.

Guo, S., & Dipietro, L. A. (2010). Factors affecting wound healing. *Journal of Dental Research, 89*(3), 219-229. doi:10.1177/0022034509359125

Gupta, S., Baharestani, M., Baranoski, S., de Leon, J., Engel, S. J., Mendez-Eastman, S., Pompeo, M. Q. (2004). Guidelines for managing pressure ulcers with negative pressure wound therapy. *Advances in Skin & Wound Care, 17 Suppl 2*, 1-16. doi:00129334-200411002-00001

Halilovic, I., Wu, J., Alexander, M., & Lin, F. (2015). Neutrophil migration under spatially-varying chemoattractant gradient profiles. *Biomedical Microdevices, 17*(3), 9963-015-9963-8. doi:10.1007/s10544-015-9963-8

Harry, L. E., & Paleolog, E. M. (2003). From the cradle to the clinic: VEGF in developmental, physiological, and pathological angiogenesis. *Birth Defects Research.Part C, Embryo Today: Reviews, 69*(4), 363-374. doi:10.1002/bdrc.10024

Hehenberger, K., & Hansson, A. (1997). High glucose-induced growth factor resistance in human fibroblasts can be reversed by antioxidants and protein kinase C-inhibitors. *Cell Biochemistry and Function, 15*(3), 197-201. doi:10.1002/(SICI)1099-0844(199709)15:3<197::AID-CBF740>3.0.CO;2-7

Hehenberger, K., Heilborn, J. D., Brismar, K., & Hansson, A. (1998). Inhibited proliferation of fibroblasts derived from chronic diabetic wounds and normal dermal fibroblasts treated with high glucose is associated with increased formation of l-lactate. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society, 6*(2), 135-141.

Heldin, C. H., & Westermark, B. (1999). Mechanism of action and in vivo role of platelet-derived growth factor. *Physiological Reviews, 79*(4), 1283-1316.

Hirsch, E., Irikura, V. M., Paul, S. M., & Hirsh, D. (1996). Functions of interleukin 1 receptor antagonist in gene knockout and overproducing mice. *Proceedings of the National Academy of Sciences of the United States of America, 93*(20), 11008-11013.

Hodde, J. P., Badylak, S. F., Brightman, A. O., & Voytik-Harbin, S. L. (1996). Glycosaminoglycan content of small intestinal submucosa: A bioscaffold for tissue replacement. *Tissue Engineering, 2*(3), 209-217. doi:10.1089/ten.1996.2.209

Horai, R., Saijo, S., Tanioka, H., Nakae, S., Sudo, K., Okahara, A., Iwakura, Y. (2000). Development of chronic inflammatory arthropathy resembling rheumatoid arthritis in interleukin 1 receptor antagonist-deficient mice. *The Journal of Experimental Medicine, 191*(2), 313-320.

Huang, C. C., Liu, C. Y., Huang, C. Y., & Liu, H. W. (2014). Carbodimide cross-linked and biodegradation-controllable small intestinal submucosa sheets. *Bio-Medical Materials and Engineering, 24*(6), 1959-1967. doi:10.3233/BME-141005

Hughes, A. D., Clunn, G. F., Refson, J., & Demoliou-Mason, C. (1996). Platelet-derived growth factor (PDGF): Actions and mechanisms in vascular smooth muscle. *General Pharmacology, 27*(7), 1079-1089. doi:S0306362396000602

Ishihara, Y., Nishihara, T., Kuroyanagi, T., Shirozu, N., Yamagishi, E., Ohguchi, M., Noguchi, T. (1997). Gingival crevicular interleukin-1 and interleukin-1 receptor antagonist levels in periodontally healthy and diseased sites. *Journal of Periodontal Research, 32*(6), 524-529.

Johnson, K. E., & Wilgus, T. A. (2014). Vascular endothelial growth factor and angiogenesis in the regulation of cutaneous wound repair. *Advances in Wound Care, 3*(10), 647-661. doi:10.1089/wound.2013.0517

Jones, S. A. (2005). Directing transition from innate to acquired immunity: Defining a role for IL-6. *Journal of Immunology (Baltimore, Md.: 1950), 175*(6), 3463-3468. doi:175/6/3463

Jones, S. A., Scheller, J., & Rose-John, S. (2011). Therapeutic strategies for the clinical blockade of IL-6/gp130 signaling. *The Journal of Clinical Investigation, 121*(9), 3375-3383. doi:10.1172/JCI57158

Junker, J. P., Kamel, R. A., Caterson, E. J., & Eriksson, E. (2013). Clinical impact upon wound healing and inflammation in moist, wet, and dry environments. *Advances in Wound Care, 2*(7), 348-356. doi:10.1089/wound.2012.0412

Kahari, V. M., & Saarialho-Kere, U. (1997). Matrix metalloproteinases in skin. *Experimental Dermatology, 6*(5), 199-213.

Kairinos, N., Solomons, M., & Hudson, D. A. (2010). The paradox of negative pressure wound therapy--in vitro studies. *Journal of Plastic, Reconstructive & Aesthetic Surgery: JPRAS, 63*(1), 174-179. doi:10.1016/j.bjps.2008.08.037

Kasiewicz, L. N., & Whitehead, K. A. (2015). Silencing TNFalpha with lipidoid nanoparticles downregulates both TNFalpha and MCP-1 in an in vitro co-culture model of diabetic foot ulcers. *Acta Biomaterialia,* doi: S1742-7061(15)30261-0

Kilpadi, D. V., & Cunningham, M. R. (2011). Evaluation of closed incision management with negative pressure wound therapy (CIM): Hematoma/seroma and involvement of the lymphatic system. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society, 19*(5), 588-596. doi:10.1111/j.1524-475X.2011.00714.x

Koh, T. J., & DiPietro, L. A. (2011). Inflammation and wound healing: The role of the macrophage. *Expert Reviews in Molecular Medicine, 13*, e23. doi:10.1017/S1462399411001943

Lafyatis, R., Lechleider, R., Kim, S. J., Jakowlew, S., Roberts, A. B., & Sporn, M. B. (1990). Structural and functional characterization of the transforming growth factor beta 3 promoter. A cAMP-responsive element regulates basal and induced transcription. *The Journal of Biological Chemistry, 265*(31), 19128-19136.

LaRochelle, W. J., Jeffers, M., McDonald, W. F., Chillakuru, R. A., Giese, N. A., Lokker, N. A., Lichenstein, H. S. (2001). PDGF-D, a new protease-activated growth factor. *Nature Cell Biology, 3*(5), 517-521. doi:10.1038/35074593

Lazarus, G. S., Cooper, D. M., Knighton, D. R., Margolis, D. J., Pecoraro, R. E., Rodeheaver, G., & Robson, M. C. (1994). Definitions and guidelines for assessment of wounds and evaluation of healing. *Archives of Dermatology, 130*(4), 489-493.

Levine, J. M., & Zulkowski, K. M. (2015). Secondary analysis of office of inspector general's pressure ulcer data: Incidence, avoidability, and level of harm. *Advances in Skin & Wound Care, 28*(9), 420-8; quiz 429-30. doi:10.1097/01.ASW.0000470070.23694.f3

Levine, S. M., Sinno, S., Levine, J. P., & Saadeh, P. B. (2013). Current thoughts for the prevention and treatment of pressure ulcers: Using the evidence to determine fact or fiction. *Annals of Surgery, 257*(4), 603-608. doi:10.1097/SLA.0b013e318285516a

Liang, D., Lu, Z., Yang, H., Gao, J., & Chen, R. (2016). A novel asymmetric wettable AgNPs/Chitosan wound dressing: In vitro and in vivo evaluation. *ACS Applied Materials & Interfaces,* doi:10.1021/acsami.5b11160

Lichtman, M. K., Otero-Vinas, M., & Falanga, V. (2015). Transforming growth factors beta (TGF-beta) isoforms in wound healing and fibrosis. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society,* doi:10.1111/wrr.12398

Lin, L., Wang, Y., Liu, W., & Huang, Y. (2015). BAMBI inhibits skin fibrosis in keloid through suppressing TGF-beta1-induced hypernomic fibroblast cell proliferation and excessive accumulation of collagen I. *International Journal of Clinical and Experimental Medicine, 8*(8), 13227-13234.

Lin, W. H., Xiang, L. J., Shi, H. X., Zhang, J., Jiang, L. P., Cai, P. T., Xiao, J. (2015). Fibroblast growth factors stimulate hair growth through beta-catenin and shh expression in C57BL/6 mice. *BioMed Research International, 2015*, 730139. doi:10.1155/2015/730139

Lin, Z. Q., Dong, Y. Z., Zhang, X. D., Wang, T., Sun, K. L., & Niu, W. Y. (2005). Effect of interleukin-6 on gene expression of certain cytokines during wound healing process of mouse skin. *Yi Chuan Xue Bao = Acta Genetica Sinica, 32*(1), 46-51.

Liu, C., Chen, X., Yang, L., Kisseleva, T., Brenner, D. A., & Seki, E. (2014). Transcriptional repression of the transforming growth factor beta (TGF-beta) pseudoreceptor BMP and activin membrane-bound inhibitor (BAMBI) by nuclear factor kappaB (NF-

kappaB) p50 enhances TGF-beta signaling in hepatic stellate cells. *The Journal of Biological Chemistry, 289*(10), 7082-7091. doi:10.1074/jbc.M113.543769

Liu, X., Jones, G. W., Choy, E. H., & Jones, S. A. (2016). The biology behind interleukin-6 targeted interventions. *Current Opinion in Rheumatology,* doi:10.1097/BOR.0000000000000255

Liu, Z. J., Snyder, R., Soma, A., Shirakawa, T., Ziober, B. L., Fairman, R. M., Velazquez, O. C. (2003). VEGF-A and alphaVbeta3 integrin synergistically rescue angiogenesis via N-ras and PI3-K signaling in human microvascular endothelial cells. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology, 17*(13), 1931-1933. doi:10.1096/fj.02-1171fje

Lobmann, R., Ambrosch, A., Schultz, G., Waldmann, K., Schiweck, S., & Lehnert, H. (2002). Expression of matrix-metalloproteinases and their inhibitors in the wounds of diabetic and non-diabetic patients. *Diabetologia, 45*(7), 1011-1016. doi:10.1007/s00125-002-0868-8

Madlener, M. (1998). Differential expression of matrix metalloproteinases and their physiological inhibitors in acute murine skin wounds. *Archives of Dermatological Research, 290 Suppl*, S24-9.

Malmsjo, M., Huddleston, E., & Martin, R. (2014). Biological effects of a disposable, canisterless negative pressure wound therapy system. *Eplasty, 14*, e15.

Martin, W. J., Walton, M., & Harper, J. (2009). Resident macrophages initiating and driving inflammation in a monosodium urate monohydrate crystal-induced murine peritoneal model of acute gout. *Arthritis and Rheumatism, 60*(1), 281-289. doi:10.1002/art.24185

Mast, B. A., & Schultz, G. S. (1996). Interactions of cytokines, growth factors, and proteases in acute and chronic wounds. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society, 4*(4), 411-420. doi:WRRwrr040404

Masuoka, H., Morimoto, N., Sakamoto, M., Ogino, S., & Suzuki, S. (2015). Exploration of the wound healing effect of topical administration of nicotine in combination with collagen scaffold in a rabbit model. *Journal of Artificial Organs: The Official Journal of the Japanese Society for Artificial Organs,* doi: 10.1007/s10047-015-0873-6

Matsushima, K., & Oppenheim, J. J. (1989). Interleukin 8 and MCAF: Novel inflammatory cytokines inducible by IL 1 and TNF. *Cytokine, 1*(1), 2-13.

Mauviel, A., Qiu Chen, Y., Dong, W., Evans, C. H., & Uitto, J. (1993). Transcriptional interactions of transforming growth-factor-beta with pro-inflammatory cytokines. *Current Biology: CB, 3*(12), 822-831. doi:0960-9822(93)90216-B

Menke, N. B., Ward, K. R., Witten, T. M., Bonchev, D. G., & Diegelmann, R. F. (2007). Impaired wound healing. *Clinics in Dermatology, 25*(1), 19-25. doi:S0738-081X(06)00182-9

Moghe, P. V., Nelson, R. D., & Tranquillo, R. T. (1995). Cytokine-stimulated chemotaxis of human neutrophils in a 3-D conjoined fibrin gel assay. *Journal of Immunological Methods, 180*(2), 193-211. doi:002217599400314M

Molinas, C. R., Campo, R., Dewerchin, M., Eriksson, U., Carmeliet, P., & Koninckx, P. R. (2003). Role of vascular endothelial growth factor and placental growth factor in basal adhesion formation and in carbon dioxide pneumoperitoneum-enhanced adhesion formation after laparoscopic surgery in transgenic mice. *Fertility and Sterility, 80 Suppl 2*, 803-811. doi:S0015028203007684

Molloy, T., Wang, Y., & Murrell, G. (2003). The roles of growth factors in tendon and ligament healing. *Sports Medicine (Auckland, N.Z.), 33*(5), 381-394. doi:3354

Moores, J. (2013). Vitamin C: A wound healing perspective. *British Journal of Community Nursing, Suppl*, S6, S8-11.

Mori, R., Kondo, T., Ohshima, T., Ishida, Y., & Mukaida, N. (2002). Accelerated wound healing in tumor necrosis factor receptor p55-deficient mice with reduced leukocyte infiltration. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology, 16*(9), 963-974. doi:10.1096/fj.01-0776com

Morykwas, M. J., Argenta, L. C., Shelton-Brown, E. I., & McGuirt, W. (1997). Vacuum-assisted closure: A new method for wound control and treatment: Animal studies and basic foundation. *Annals of Plastic Surgery, 38*(6), 553-562.

Mustoe, T. A., Pierce, G. F., Thomason, A., Gramates, P., Sporn, M. B., & Deuel, T. F. (1987). Accelerated healing of incisional wounds in rats induced by transforming growth factor-beta. *Science (New York, N.Y.), 237*(4820), 1333-1336.

Neuman, M. G., Nanau, R. M., Oruna, L., & Coto, G. (2011). In vitro anti-inflammatory effects of hyaluronic acid in ethanol-induced damage in skin cells. *Journal of Pharmacy & Pharmaceutical Sciences: A Publication of the Canadian Society for Pharmaceutical Sciences, Societe Canadienne Des Sciences Pharmaceutiques, 14*(3), 425-437.

Neurath, M. F., & Finotto, S. (2011). IL-6 signaling in autoimmunity, chronic inflammation and inflammation-associated cancer. *Cytokine & Growth Factor Reviews, 22*(2), 83-89. doi:10.1016/j.cytogfr.2011.02.003

Nicklin, M. J., Hughes, D. E., Barton, J. L., Ure, J. M., & Duff, G. W. (2000). Arterial inflammation in mice lacking the interleukin 1 receptor antagonist gene. *The Journal of Experimental Medicine, 191*(2), 303-312.

Nunan, R., Harding, K. G., & Martin, P. (2014). Clinical challenges of chronic wounds: Searching for an optimal animal model to recapitulate their complexity. *Disease Models & Mechanisms, 7*(11), 1205-1213. doi:10.1242/dmm.016782

O'Reilly, S., Ciechomska, M., Cant, R., Hugle, T., & van Laar, J. M. (2012). Interleukin-6, its role in fibrosing conditions. *Cytokine & Growth Factor Reviews, 23*(3), 99-107. doi:10.1016/j.cytogfr.2012.04.003

Padrines, M., Wolf, M., Walz, A., & Baggiolini, M. (1994). Interleukin-8 processing by neutrophil elastase, cathepsin G and proteinase-3. *FEBS Letters, 352*(2), 231-235. doi:0014-5793(94)00952-X

Paku, S., & Paweletz, N. (1991). First steps of tumor-related angiogenesis. *Laboratory Investigation; a Journal of Technical Methods and Pathology, 65*(3), 334-346.

Pastar, I., Stojadinovic, O., Krzyzanowska, A., Barrientos, S., Stuelten, C., Zimmerman, K., Tomic-Canic, M. (2010). Attenuation of the transforming growth factor beta-signaling pathway in chronic venous ulcers. *Molecular Medicine (Cambridge, Mass.), 16*(3-4), 92-101. doi:10.2119/molmed.2009.00149

Patan, S. (2004). Vasculogenesis and angiogenesis. *Cancer Treatment and Research, 117*, 3-32.

Patel, S., Maheshwari, A., & Chandra, A. (2016). Biomarkers for wound healing and their evaluation. *Journal of Wound Care, 25*(1), 46-55. doi:10.12968/jowc.2016.25.1.46

Penn, J. W., Grobbelaar, A. O., & Rolfe, K. J. (2012). The role of the TGF-beta family in wound healing, burns and scarring: A review. *International Journal of Burns and Trauma, 2*(1), 18-28.

Peplow, P. V., & Chatterjee, M. P. (2013). A review of the influence of growth factors and cytokines in in vitro human keratinocyte migration. *Cytokine, 62*(1), 1-21. doi:10.1016/j.cyto.2013.02.015

Poniatowski, L. A., Wojdasiewicz, P., Gasik, R., & Szukiewicz, D. (2015). Transforming growth factor beta family: Insight into the role of growth factors in regulation of fracture healing biology and potential clinical applications. *Mediators of Inflammation, 2015*, 137823. doi:10.1155/2015/137823

Powers, C. J., McLeskey, S. W., & Wellstein, A. (2000). Fibroblast growth factors, their receptors and signaling. *Endocrine-Related Cancer, 7*(3), 165-197.

Rani, M., Zhang, Q., & Schwacha, M. G. (2014). Gamma delta T cells regulate wound myeloid cell activity after burn. *Shock (Augusta, Ga.), 42*(2), 133-141. doi:10.1097/SHK.0000000000000176

Ravanti, L., & Kahari, V. M. (2000). Matrix metalloproteinases in wound repair (review). *International Journal of Molecular Medicine, 6*(4), 391-407.

Rawlinson, A., Dalati, M. H., Rahman, S., Walsh, T. F., & Fairclough, A. L. (2000). Interleukin-1 and IL-1 receptor antagonist in gingival crevicular fluid. *Journal of Clinical Periodontology, 27*(10), 738-743.

Rice, J. B., Desai, U., Cummings, A. K., Birnbaum, H. G., Skornicki, M., & Parsons, N. B. (2014). Burden of diabetic foot ulcers for medicare and private insurers. *Diabetes Care, 37*(3), 651-658. doi:10.2337/dc13-2176

Robson, M. C. (1997). The role of growth factors in the healing of chronic wounds. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society, 5*(1), 12-17. doi:WRRwrr_050106

Rosique, R. G., Rosique, M. J., & Farina Junior, J. A. (2015). Curbing inflammation in skin wound healing: A review. *International Journal of Inflammation, 2015*, 10.1155/2015/316235. doi:316235

Roux, S. L., Borbely, G., Sloniecka, M., Backman, L. J., & Danielson, P. (2015). Transforming growth factor beta 1 modulates the functional expression of the neurokinin-1 receptor in human keratocytes. *Current Eye Research,* 1-9. doi:10.3109/02713683.2015.1088954

Salazar, J. J., Ennis, W. J., & Koh, T. J. (2015). Diabetes medications: Impact on inflammation and wound healing. *Journal of Diabetes and its Complications,* doi: S1056-8727(15)00506-1

Sanchez, M., Anitua, E., Orive, G., Mujika, I., & Andia, I. (2009). Platelet-rich therapies in the treatment of orthopaedic sport injuries. *Sports Medicine (Auckland, N.Z.), 39*(5), 345-354. doi:10.2165/00007256-200939050-00002

Schmitz, M. L., Weber, A., Roxlau, T., Gaestel, M., & Kracht, M. (2011). Signal integration, crosstalk mechanisms and networks in the function of inflammatory cytokines. *Biochimica Et Biophysica Acta, 1813*(12), 2165-2175. doi:10.1016/j.bbamcr.2011.06.019

Schreml, S., Szeimies, R. M., Prantl, L., Karrer, S., Landthaler, M., & Babilas, P. (2010). Oxygen in acute and chronic wound healing. *The British Journal of Dermatology, 163*(2), 257-268. doi:10.1111/j.1365-2133.2010.09804.x

Schultz, G. S., Sibbald, R. G., Falanga, V., Ayello, E. A., Dowsett, C., Harding, K., . . . Vanscheidt, W. (2003). Wound bed preparation: A systematic approach to wound management. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society, 11 Suppl 1*, S1-28. doi:1129

Schwien, T., Gilbert, J., & Lang, C. (2005). Pressure ulcer prevalence and the role of negative pressure wound therapy in home health quality outcomes. *Ostomy/wound Management, 51*(9), 47-60.

Shah, M., Foreman, D. M., & Ferguson, M. W. (1995). Neutralisation of TGF-beta 1 and TGF-beta 2 or exogenous addition of TGF-beta 3 to cutaneous rat wounds reduces scarring. *Journal of Cell Science, 108 (Pt 3)*(Pt 3), 985-1002.

Shapira, S., Ben-Amotz, O., Sher, O., Kazanov, D., Mashiah, J., Kraus, S., Arber, N. (2015). Delayed wound healing in heat stable antigen (HSA/CD24)-deficient mice. *Plos One, 10*(10), 1-15. doi:10.1371/journal.pone.0139787

Shi, H., Cheng, Y., Ye, J., Cai, P., Zhang, J., Li, R., Xiao, J. (2015). bFGF promotes the migration of human dermal fibroblasts under diabetic conditions through reactive oxygen species production via the PI3K/Akt-Rac1- JNK pathways. *International Journal of Biological Sciences, 11*(7), 845-859. doi:10.7150/ijbs.11921

Shi, L., & Ronfard, V. (2013). Biochemical and biomechanical characterization of porcine small intestinal submucosa (SIS): A mini review. *International Journal of Burns and Trauma, 3*(4), 173-179.

Shull, M. M., Ormsby, I., Kier, A. B., Pawlowski, S., Diebold, R. J., Yin, M., Calvin, D. (1992). Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature, 359*(6397), 693-699. doi:10.1038/359693a0

Siegel, P. M., & Massague, J. (2003). Cytostatic and apoptotic actions of TGF-beta in homeostasis and cancer. *Nature Reviews.Cancer, 3*(11), 807-821. doi:10.1038/nrc1208

Simpson, D. M., & Ross, R. (1972). The neutrophilic leukocyte in wound repair a study with antineutrophil serum. *The Journal of Clinical Investigation, 51*(8), 2009-2023. doi:10.1172/JCI107007

Singer, A. J., & Clark, R. A. (1999). Cutaneous wound healing. *The New England Journal of Medicine, 341*(10), 738-746. doi:10.1056/NEJM199909023411006

Sloniecka, M., Le Roux, S., Zhou, Q., & Danielson, P. (2016). Substance P enhances keratocyte migration and neutrophil recruitment through interleukin-8. *Molecular Pharmacology, 89*(2), 215-225. doi:10.1124/mol.115.101014

Sood, A., Granick, M. S., & Tomaselli, N. L. (2014). Wound dressings and comparative effectiveness data. *Advances in Wound Care, 3*(8), 511-529. doi:10.1089/wound.2012.0401

Spear, M. (2013). Pressure ulcer staging-revisited. *Plastic Surgical Nursing: Official Journal of the American Society of Plastic and Reconstructive Surgical Nurses, 33*(4), 192-194. doi:10.1097/PSN.0000000000000015

Stanirowski, P. J., Wnuk, A., Cendrowski, K., & Sawicki, W. (2015). Growth factors, silver dressings and negative pressure wound therapy in the management of hard-to-heal postoperative wounds in obstetrics and gynecology: A review. *Archives of Gynecology and Obstetrics, 292*(4), 757-775. doi:10.1007/s00404-015-3709-y

Stechmiller, J. K., Kilpadi, D. V., Childress, B., & Schultz, G. S. (2006). Effect of vacuum-assisted closure therapy on the expression of cytokines and proteases in wound fluid of adults with pressure ulcers. *Wound Repair and Regeneration : Official Publication of the Wound Healing Society [and] the European Tissue Repair Society, 14*(3), 371-374. doi:WRR134

Steed, D. L. (2006). Clinical evaluation of recombinant human platelet-derived growth factor for the treatment of lower extremity ulcers. *Plastic and Reconstructive Surgery, 117*(7 Suppl), 143S-149S; discussion 150S-151S. doi:10.1097/01.prs.0000222526.21512.4c

Stojadinovic, A., Carlson, J. W., Schultz, G. S., Davis, T. A., & Elster, E. A. (2008). Topical advances in wound care. *Gynecologic Oncology, 111*(2 Suppl), S70-80. doi:10.1016/j.ygyno.2008.07.042

Stojadinovic, O., Minkiewicz, J., Sawaya, A., Bourne, J. W., Torzilli, P., de Rivero Vaccari, J. P., Tomic-Canic, M. (2013). Deep tissue injury in development of pressure ulcers: A decrease of inflammasome activation and changes in human skin morphology in response to aging and mechanical load. *PloS One, 8*(8), e69223. doi:10.1371/journal.pone.0069223

Streit, M., Beleznay, Z., & Braathen, L. R. (2006). Topical application of the tumour necrosis factor-alpha antibody infliximab improves healing of chronic wounds. *International Wound Journal, 3*(3), 171-179. doi:IWJ233

Szarka, A., & Lorincz, T. (2014). The role of ascorbate in protein folding. *Protoplasma, 251*(3), 489-497. doi:10.1007/s00709-013-0560-5

Tonnesen, M. G., Feng, X., & Clark, R. A. (2000). Angiogenesis in wound healing. *The Journal of Investigative Dermatology.Symposium Proceedings / the Society for Investigative Dermatology, Inc.[and] European Society for Dermatological Research, 5*(1), 40-46. doi:10.1046/j.1087-0024.2000.00014.x

Trengove, N. J., Stacey, M. C., MacAuley, S., Bennett, N., Gibson, J., Burslem, F., Schultz, G. (1999). Analysis of the acute and chronic wound environments: The role of proteases and their inhibitors. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society, 7*(6), 442-452. doi:wrr442

Ubbink, D. T., Westerbos, S. J., Evans, D., Land, L., & Vermeulen, H. (2008). Topical negative pressure for treating chronic wounds. *The Cochrane Database of Systematic Reviews, (3):CD001898. doi*(3), CD001898. doi:10.1002/14651858.CD001898.pub2

Wahl, S. M., Allen, J. B., Wong, H. L., Dougherty, S. F., & Ellingsworth, L. R. (1990). Antagonistic and agonistic effects of transforming growth factor-beta and IL-1 in rheumatoid synovium. *Journal of Immunology (Baltimore, Md.: 1950), 145*(8), 2514-2519.

Wahl, S. M., Hunt, D. A., Wong, H. L., Dougherty, S., McCartney-Francis, N., Wahl, L. M., Roberts, A. B. (1988). Transforming growth factor-beta is a potent immunosuppressive agent that inhibits IL-1-dependent lymphocyte proliferation. *Journal of Immunology (Baltimore, Md.: 1950), 140*(9), 3026-3032.

Wang, Y., Tang, Z., Xue, R., Singh, G. K., Lv, Y., Shi, K., Yang, L. (2011). TGF-beta1 promoted MMP-2 mediated wound healing of anterior cruciate ligament fibroblasts through NF-kappaB. *Connective Tissue Research, 52*(3), 218-225. doi:10.3109/03008207.2010.516849

Werner, S., & Grose, R. (2003). Regulation of wound healing by growth factors and cytokines. *Physiological Reviews, 83*(3), 835-870. doi:10.1152/physrev.00031.2002

Werner, S., Krieg, T., & Smola, H. (2007). Keratinocyte-fibroblast interactions in wound healing. *The Journal of Investigative Dermatology, 127*(5), 998-1008. doi:5700786

WINTER, G. D. (1962). Formation of the scab and the rate of epithelization of superficial wounds in the skin of the young domestic pig. *Nature, 193*, 293-294.

Woo, K., Ayello, E. A., & Sibbald, R. G. (2007). The edge effect: Current therapeutic options to advance the wound edge. *Advances in Skin & Wound Care, 20*(2), 99-117; quiz 118-9. doi:00129334-200702000-00009

Yates, C. C., Hebda, P., & Wells, A. (2012). Skin wound healing and scarring: Fetal wounds and regenerative restitution. *Birth Defects Research.Part C, Embryo Today: Reviews, 96*(4), 325-333. doi:10.1002/bdrc.21024

Zhang, P., Zhong, Z. H., Yu, H. T., & Liu, B. (2015). Exogenous expression of IL-1Ra and TGF-beta1 promotes in vivo repair in experimental rabbit osteoarthritis. *Scandinavian Journal of Rheumatology, 44*(5), 404-411. doi:10.3109/03009742.2015.1009942

Zhong, W. (2011). 18 - Textiles for medical filters. In V. T. Bartels (Ed.), *Handbook of medical textiles* (pp. 419-433) Woodhead Publishing. doi:http://dx.doi.org/10.1533/9780857093691.4.419