EVALUATION OF PLATELET–DERIVED AND EPIDERMAL GROWTH FACTORS ON SURGICALLY- INDUCED WOUNDS IN NEW ZEALAND WHITE RABBITS TREATED WITH METHYLPREDNISOLONE

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIAIN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY DEGREE IN VETERINARY SOFT-TISSUE SURGERY

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DECLARATION

I declare that the work in the thesis, entitled "EVALUATION OF PLATELET–DERIVED AND EPIDERMAL GROWTH FACTORS ON SURGICALLY-INDUCED WOUNDS IN NEW ZEALAND WHITE RABBITS TREATED WITH METHYLPREDNISOLONE." has been performed by me in the Department of Veterinary Surgery and Radiology of Ahmadu Bello University Zaria under the supervision of Prof. A. Z. Hassan, Prof. J. O. Ayo and Dr. E. O. Abidoye. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any tertiary institution.

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CERTIFICATION

This thesis, entitled "EVALUATION OF PLATELET–DERIVED AND EPIDERMAL GROWTH FACTORS ON SURGICALLY INDUCED WOUNDS IN NEW ZEALAND WHITE RABBITS TREATED WITH METHYLPREDNISOLONE**"** by Joel Kalang JAPHETH, meets the regulations governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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To the Lord Most High God.

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ABSTRACT

The study was designed to evaluate the influence of platelet-derived growth factor (PDGF-AB) and epidermal growth factor (EGF-AB) on modulating wound healing of rabbits. A total of twenty four (24) New Zealand White rabbits of nine months to one year old from both sexes were used for the study. The rabbits weigh between 1.5–2.0 kg body weights. The rabbits were randomly grouped into two major groups (1 and 2) of twelve rabbits each. The rabbits were treated parenterally with methylprednisolone (methylP), and compared with the control treated parenterally with normal saline (NS). They were subsequently placed randomly, into four sub-groups each 1A (methylP + EGF), 1B (methylP + PDGF), 1C (methylP + NS) and 1D (methylP + EGF + PDGF); 2A (NS + EGF), 2B (NS + PDGF), 2C (NS + NS) and 2D (NS + EGF + PDGF) of three rabbits each. The animals were pre-anaesthetised with atropine sulphate and chlorpromazine. Ketamine hydrochloride was used for anaesthesia. An area of wound that is 2 cm × 1 cm dimension on rabbit dorsum, was created on each site of the dorsum. Group 1 were treated parenterally with methylP 0.5 mg/kg and the sub-groups were treated topically with 1A (EGF), 1B (PDGF), 1C (Normal saline) and 1D (EGF + PDGF). Group 2 were parenterally treated with normal saline and topically with 2A (EGF), 2B (PDGF), 2C (Normal saline) and 2D (EGF + PDGF). Each wound designated for PDGF, EGF and NS were smeared with 2.92 µg of PDGF, 20.8 µg of EGF, and 0.2 mL of normal saline, respectively. The wounds were monitored until complete healing. Doppler ultrasound of the scar tissue was carried out at the frequency of 5 Hz using C5 sonostar laptop ultrasound. Blood, serum and wound biopsy were taken. Wound retraction was recorded in all the groups with highest peak in NS + PDGF (3.13 cm^2) at day 3 and 3.11

cm^2 in NS + NS at day 5. Onset of epithelialisation was recorded at day 7 except for groups treated with EGF + PDGF the recorded epithelialisation at day 5 post-wounding. Inflammatory cells influx were diffused in the NS + NS and NS + EGF + PDGF groups. However, synergy in anti-inflammatory activity was not recorded with the use of methylP + PDGF but was observed on methylP + EGF. The PDGF group showed superior anti-inflammatory activity compared to the EGF treated rabbits. Ultrasonography of NS + NS scar line showed hyper-echogenicity with least vascular perfusion but small scar lines in the EGF and PDGF treated rabbits. The methylP + PDGF and methylP + EGF rabbits had the strongest scar strength as recorded by the tensile strength of 76.8 N/cm^2 and 71.25 N/cm^2, respectively. In conclusion, the overall healing time of methylprednisolone-treated rabbits was delayed; however the scar had the best connective tissue fibre arrangement. Reduced period of wound retraction was achieved with the use methylprednisolone, EGF or PDGF, thus, reduced wound surface area for contamination and reduced chances of wound infection. Smallest scar size was achieved with the use of PDGF and methylP + PDGF, thus, maybe important in cosmetic surgeries. The administration of methylprednisolone at 0.5mg/kg in wound management to enhance collagen fibre arrangements in scar tissue. Tensile strength was best observed in methylP + PDF and methylP + EGF, thus, are relevant in management of wounds in a weight carrying regions like the ventral aspects of the abdomen. The place of proper wound dressing in wound healing management cannot be replaced.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Preamble

Injury generally induces loss of tissue integrity, with consequence physiological imbalance, that is commonly accompanied by loss of function or even death (Singer and Clark, 1999). Morphologic-functional disruption of the continuity of a tissue or structure is referred to as injury (Geethalakshmi *et al.,* 2013). Wound is defined as an injury to the body that result in the disruption of anatomical,physiological and cellular continuity of the normal body anatomy. Surgical processes involve creation of wounds and wound management. To be surgically successful, wounds should heal with minimum scarring, preservation of tissue, return of function and minimal healing time (Milne, 2008; Borena *et al.,* 2015). Wounds in both humans and animal pathology remains a clinical challenge, however, correct and efficient wound management is essential (Borena *et al.,* 2015). In the year 2014 United Kingdom spend £2,165 million (₦8.66 billion) on wound care services and this figure is expected to rise to £2,377 million (₦9.5 billion) in 2019 (Tricco*et al*., 2015; Morgan, 2015).

Improper wound management may result in increased duration of hospital stay and stress, increased cost of treatment, loss of function, poor aesthetic appearance and death of patients (Velner *et al.,* 2009; Fife *et al.,* 2012). The search towards more efficacious wound therapies for long-term relief and ultimately scarless wound healing is continuous;

in the light of this, cytokines provide encouraging results (Dudas *et al*., 2008; Borena *et al.,* 2015).Wounds are encountered frequently in veterinary and human practice; therefore, a veterinarian should be familiar with all processes involved in wound healing and the options for wound management (Borena *et al.*, 2015). Stuart and Price (2010) described wound healing as an evolutionarily complex and conserved, multi-cellular process that aims at barrier restoration. Wound healing process therefore consist of a series of cellular and biochemical events, ultimately resulting in tissue regeneration and repair (Traversa and Sussman, 2001; Lashkarizadeh*et al.,* 2015). The events involved in wound healing process include induction and resolution of inflammation, tissue formation, and remodel of the tissue formed, aimed at achieving complete reconstruction of the wounded area (Landén *et al.*, 2016; Hah and Ceilley, 2017).

Numerous processes, which occur during healing of wounds are grouped into four general phases; haemostasis; inflammatory; proliferative (tissue formation) and repair, and remodelling phase. A completely healed wound is one that has attained complete wound (skin) closure, without drainage or dressing required (Stuart and Price, 2010). Wound management effectively influences clinical outcome (Velner *et al.,* 2009; Wong *et al.*, 2011). A sound knowledge of the healing process and factors that influence wound are prerequisite for successful management of wound (Nalliappan, *et al.,* 2012; Borena *et al.,* 2015).

Primarily, wound management is targeted at enhancing wound repair, physiological aesthetics, and satisfactory scar tissue formation; thus, quality care is crucial in wound healing management (Borena *et al.,* 2015). Although, wound healing maybe easily observed grossly, it is better described and appreciated histologically (Werner and Grose, 2003; Reinke and Sorg, 2012; Han and Ceilley, 2017). Skin layer and tissue between species are unique, in functionality and structurally (Theoret and Wilmink, 2013), these variations also showed striking differences in wound healing processes, demostrated between and within species. Furthermore, differences also occur at gross, cellular and molecular levels (Westgate *et al.,* 2010; Borena *et al.,* 2015), as genes involved in the control of wound repair are partially been identified (Werner and Grose, 2003).

Inflammatory reaction is the response of the body to tissue damage, and is an integral component of the wound healing process. It involves leakage of plasma-like fluid from capillaries and migration of polymorphonuclear leucocytes into the damaged area. This is followed by healing through fibrous scar tissue formation. Increased quantity of glucocorticoids above physiological amount inhibits the inflammatory process and wound healing (Shaikh *et al.,* 2012; Trecco *et al*., 2015). Steroid therapy should not be maintained for period of more than 5 days, this is to ensure anti-inflammatory efficacy, maximize benefits and minimize risk of delayed healing and hypothalamic-pituitary-adrenal (HPA) axis suppression. Glucocorticoids should be used for complex, and in long surgeries, especially where significant amount of soft- and hard-tissue trauma is involved (Fleischmajer, 1961; Deshmukh, 2007; Barrientos*et al*, 2014). Mico-llorens *et al.* (2006), Tiigimae-Saar *et al*. (2010), and Chaurand-Lara and Facio-Umaña (2013),reported that steroid is well-suited for post-operative management in third molar surgery for pain, trismus, and swelling. Steroids should be used to reduce post-operative swelling of soft tissues (Wang*et al.*, 2013; Barrientos*et al*, 2014). They stabilize lysosomal membranes, decrease capillary permeability, and influx of neutrophils and monocytes that transform into macrophages at the site of inflammation. They also reduce the production of inflammatory mediators (interleukin-1, prostaglandins, and leukotrienes) by inhibiting the release of arachidonic acid from phospholipids. Hence, it is important to study substances that may influence inflammatory process, like the steroids to explore their beneficial effects on wound healing (Shaikh *et al.*, 2012; Wang *et al*., 2013).

Steroids are a group of lipids with a complex molecular structure of four interlocking carbon atoms, forming a hydrogenated cyclopentophenanthrene-ring system. The first 3 are rings of six carbon atoms each, and the fourth ring has five carbon atoms (Nikitina*et al.*, 2016). Glucocorticoids increase blood pressure, in the vascular smooth muscle, they increase sensitivity to pressure agents such as catecholamines and angiotensin II, while regulating endothelial dilatation due to nitric oxide activities (Mohammed *et al.*, 2015). Steroids can be classified based on their duration of action. The short-acting ones include cortisol and cortisone; intermediate-acting steroids are triamcinolone and methylprednisolone, while the long-acting include dexamethasone and beclomethasone (Werner and Grose, 2003; Wagner *et al*., 2008).

Corticosteroids and their biologically active synthetic derivatives differ in their metabolic (glucocorticoid) and electrolyte-regulating (mineralocorticoid) activities. Glucocorticoids inhibits inflammatory response; hence, they are recommended in diverse inflammatory and autoimmune conditions. This makes them one of the most frequently prescribed or over the counter classes of drugs (Bhanot and Mago, 2016). Steroids exert antagonistic effects on cytokines, and collagen fibre formation during wound healing. This could be relevant as a treatment option in a clinical setting (Wicke *et al.,* 2000). The interacting molecular signals in wound healing process is mainly mediated, primarily by cytokines, which motivate and orchestrate numerous cellular activities underlying inflammation and repair (Gillitzer, and Goebeler, 2001; Geethalakshmi *et al.,* 2013). Continuing progress in deciphering the basic and complex role of cytokines in wound healing, provides opportunities to explore pathways in modulating wound healing process to either inhibit or enhance appropriate cytokines (Gillitzer, and Goebeler, 2001; Geethalakshmi *et al.*, 2013; MacLeod and Mansbridge, 2016).

The term "cytokine" encompasses a wide range of low weight molecular proteins that regulate various aspects of the immune response, like the members of the growth factors family, chemokine family, and the adipokines (Ananya, 2006). They are released by cells, and affect the behaviour of other cells or the cell that produces them (Werner, and Grose, 2003). Cytokines are polypeptides (~5–20 kDa) that bind to cell-specific receptors present in cell membranes of target cells, and triggers cell-specific response. Nearly all nucleated cells are known to be activated by these proteins, and have the ability of synthesising them (Werner and Grose, 2003; Gabriel *et al.,* 2009). Experimental and clinical studies have shown varied outcomes, but often beneficial effects of exogenous cytokines on wound healing process (Edmonds *et al.,* 2000).

1.2 Statement of the Problem

The evaluation of wound healing is gradually moving from assessment of the influence of administration of antibiotics, or plant extracts on wound healing to the determination of the extent of involvement and the beneficial effects of molecules released by cells themselves, especially those at the wound site. Wounds are unavoidable in life because of the jet-age, animal fight, animal cruelty, diseases, surgery and accidents, and they are, therefore, frequently encountered in veterinary practice (Borena *et al.,* 2015). Systemic steroids are known to result in incomplete granulation tissue and inhibit wound contraction. They also inhibit production of hypoxia-inducible factor-1 (HIF-1), a key transcriptional factor involved in healing of wounds, and increasing the risk of wound infection (Franz *et al.,* 2007; Barrientos *et al.,* 2008).

Glucocorticoids remain the agent of choice in the management of disease conditions such as anaphylactic reactions (Kumar*et al.,* 2015), reverse acute endothelial graft rejection (Tandon *et al*., 2009), and for the management of acute phase-response in patients undergoing surgery (Chen *et al.,* 2017). Steroids are justifiable for patients with advanced invasive thymoma (Yokoi*et al*., 2007), and for patients undergoing laparascopy (Contini and Scarpignato, 2013). Injection of methylprednisolone reduces catabolic response after laparotomy and major abdominal surgery (Tajiri*et al.,* 2002; Bugada *et al*., 2015). Indications of steroids therefore, include perioperative replacement therapy, anti-inflammatory uses and hyper-reactive airway, post-operative nausea and vomiting, analgesia adjunct, day care surgery, anaphylaxis, and septic shock. Other indications are cerebral oedema, spinal cord injury, and various surgical causes (Grover *et al*., 2007).

Steroids are used in long and complex surgeries, that involves significant soft- and hard-tissue trauma (Barrientos*et al.*, 2014). They are well known to delay wound healing (Godbout and Glaser, 2006; Anderson and Hamm, 2012).

Cytokines are known to be produced by cells at the wound site and play an important role in wound healing. Animal studies have shown that exogenously administered cytokines, enhance the normal healing process (Werner andGrose, 2003; Dogan *et al.,* 2009). Therefore, there is the need to investigate the cytokines that, when topically applied on wound, may give a near normal tissue restoration and speedy wound healing, thereby reversing the effect of the systemically administered steroid. There is a dearth of information on the use of topical cytokines in wound healing. Systemic application of steroids to control inflammation and stress has not been evaluated in relation to wound healing. Since steroids have both glucocorticoid and mineralcorticoid activities, they are potent agents commonly used in inflammatory conditions, cell stabilisation and in reduction of stress (Werner and Grose, 2003).

1.3 Justification of the Study

Wound healing is a complex activity (Driver *et al.,* 2013), which are frequently modelled in the skin. Species with relatively loose skin organ like the laboratory animals, wound healing occurs relatively rapid as a result of wound contraction, initiated by *musculus panniculus carnosus* in their subcutaneous tissues. Unlike in “tight-skinned” animals such as humans and porcine that lack this muscle, thus, wound contraction is not rapid, and skin heals mainly through epithelialisation with little wound contraction (Westgate *et al.,* 2010; Theoret and Wilmink, 2013). Striking differences in wound healing may be exhibited within the same species, as can be observed at gross, cellular and molecular levels. For example, an earlier start and termination of wound inflammation leading to an earlier wound contraction and epithelialization was reported in ponies in comparison to horses (Wilmink and Van Weeren, 2005; Westgate *et al.*, 2010). Local inflammatory activities accounts for the unique pattern in wound healing where leucocytes in ponies were known to produce more inflammatory mediators. The inflammatory mediators are responsible for the local immunological activities, for enhanced cellular debridement, transition to regenerative phase, and enhanced wound contraction (Wilmink and Van weeren, 2005). Contrary to this, foetal wounds heal rapidly with a paucity of inflammation (Ramlet*et al*., 2009; Larson*et al*., 2010; Rolfe and Grobbelaar, 2012). Repair of wounds is mainly attained with fibrosis resulting in a scar tissue formation, which is fragile and less elastic because of its disorganised extracellular matrix (Soo, *et al.,* 2003; Gurter *et al.,* 2008; Borena *et al.,* 2015). This is not desirable in wound healing. The use of cytokines along with steroids may result in near foetal wound healing (Leung *et al.,* 2012; Yates *et al*., 2012), and the delay effect of steroids are likely antagonised. The application of exogenous growth factors on wounds may enhance wound healing process in all species (Longaker *et al*., 1991; Yagi*et al*., 2016). Examples of the growth factors are platelet-derived growth factors (PDGFs), epidermal growth factors (EGF), and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Nath and Gulati, 1998; Edmonds *et al.*, 2000; Harding *et al.*, 2002). Continuing progress in deciphering the basic and complex role of cytokines in wound healing provides opportunities to explore pathways in modulating wound healing process to either inhibit or enhance appropriate cytokines. Steroids are important in body chemistry, and consequently, it is important to evaluate their activities in wound healing, especially in relation to topically-applied cytokine–modulated wound healing. Results to be obtained in this study may be of value in adopting more efficient therapeutic management of wounds in domestic animals, aimed at reducing cost and increasing the rate and quality of wound healing.

1.4 Aim of the Study

To evaluate the influence of platelet-derived growth factors (PDGF) and epidermal growth factors (EGF) on modulating wound healing in New Zealand rabbits treated parenterrally with methylprednisolone.

1.5 Objectives of the Study

i. To assess grossly and histologically, the influence of PDGF and EGF on wound healing process in New Zealand rabbits parenterally administered with methylprednisolone.

ii. To compare the effects of methylprednisolone administration on specific topical cytokine influence on modulation of wound healing.

iii. To compare methylprednisolone wound healing to non-methylprednisolone wound healing process.

iv. To compare cytokine-modulated wound healing to non-methylprednisolone wound healing process.

v. To assess the impact of wound, methylprednisolone and cytokine on systemic cytokines.

vi. To assess the impact of methylprednisolone and topical cytokines on haematological values in New Zealand rabbits induced with wounds

1.6 Research Questions

i. Which cytokines is most beneficial to the wound healing in the methylprednisolone-treated New Zaeland rabbits?

ii. Which specific cytokine-modulate wound healing is influenced by methylprednisolone administration?

iii. Are there similarities between methylprednisolone-treated wound and non-methylprednisolone treated wounds?

iv. Do similarities exist between cytokine-treated and non-cytokine treated wound healing?

v. Do wounding, parenteral methylprednisolone or topical cytokine influence systemic cytokine?

vi. Do wounding, parenteral methylprednisolone or topical cytokine influence haematological parameters?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Wound

A wound is a disruption in the normal continuity of structure of an organ or tissue caused by an external agent (Martin, 2003; Rabess, 2015). The disruption could be superficial, limited to the integuments or deep, extending even to the bone as described by Wild*et al.* (2010). Traumatic injuries in animals can result from fight between animals, natural disaster, a foreign body, accident, surgical wound dehiscence and disease conditions (Pavletic and Trout, 2006; Gower *et al.,* 2009; Quist *et al.*, 2012; Lew *et al.*, 2015).

2.2 Classification of Wounds

There is no standard classification for wounds. However, there are numerous forms of wounds classification that are helpful in describing and grouping wounds with a view to aid their management and ultimate healing (Percival, 2002). Wounds can be classified based on, aetiology (that is the cause of the wound), level of contamination (microbial load), morphological characteristics and associations with hollow or solid organ (severity of injury in relation to its depth) and based on time (clinically categorised as acute or chronic non-healing based on the time taken for complete healing) (Daigle *et al.,* 2001; Velnar *et al.*, 2009; Lazarus*et al.,* 2014).

2.2.1 Classification based on time or duration

2.2.1.1 Acute wounds

These are wounds that progressively followed the timely and orderly wound healing pattern, resulting in the restoration of structural and functional quality of the damaged tissue. Maximal of thirty days is the normal time frame of healing, and surgical procedure and trauma are the common examples of acute wounds (Diegelmann, 2004; Velnar *et al.*, 2009). The proteases in acute wounds are closely orchestrated by their inhibitors while in chronic wounds; the concentration of their inhibitors at wound site is at minimal relative to abundant protease. This inbalance results in the degradation of the extracellular matrix, growth factors and their receptors (Stanley and Osler, 2001; Dhall *et al.*, 2014).

2.2.1.2 Chronic wounds

These are wounds that failed to follow through an orderly stage of healing in an organisedand time frame manner, exceeding 3 months. It is associated with incomplete healing process, interfered by extrinsic or intrinsic factors, thus, elongating one or more stages in the wound healing phases. These factors include disease conditions or foreign body (Diegelmann, 2004; Goldman, 2004; Velnar *et al.*, 2009; Tiwari*et al.*, 2014).Often the inflammatory phase of healing in chronic wounds is stalled. Chronic wounds share common features despite differences in aetiology, like excessive levels of pro-inflammatory cytokines, proteases (Rodriguez-Menocal *et al.*, 2012; McCarty *et al.*, 2013), and senescent cell populations that are not stimulated or activated by typical wound healing signals with consequence impaired cellular proliferation or secretory abilities (Stanley and Osler, 2001; Lobmann *et al.*, 2002; Telgenhoff and Shroot, 2005;

Wall *et al.,* 2008). Chronic non-healing wounds or draining wounds in animals can be life-threatening, problematic to treat, or even zoonotic (Fitoz *et al.,* 2001). Diagnostic steps include biopsy, cytology and histopathology, microbial culture and susceptibility test, serology, and diagnostic imaging, particularly after surgical intervention, trauma, or history of a wound in the region and patients complete health status (Kim *et al.,* 2003*;* Atiba*et al.,* 2014; Staudte *et al.,* 2004*;* DanielandMathieu,2006).

2.2.2 Aetiologic classification (that is the cause of the wound)

Wound classification based on aetiology considers the causative agent or event, including surgery, bite, trauma, burns, pressure, radiation, and proliferative scars. The nature of the wound and its severity depends on the wound formation, that is, the causative agent or event. Like surgical wounds are usually sharp wounds which are characterised by narrowed wound corners, can easily be apposed for healing by primary intention. Bite wounds are mostly deep into the muscles with usually small round openings characterised with two or more of such openings. Bite wounds heals poorly. Trauma wounds are mechanical, they can be bruises, that is, affecting only the epidermal layer, or it can be deeper causing deep wounds. Trauma wounds are normally irregular in pattern or shape. Others are burns, these can be due to fire or hot objects like hot water or metal, burns are usually wide spread and can be superficial that is affecting only the epidermal layer or deeper into the under-laying tissues. Pressure ulcers are caused due to constant pressure applied to a certain part of the body leading to sloughing off of the body part due to denervation and inadequate blood supply to the body part leading to the. Wounds due to radiation can be mild (erythema or dermatitis), it can also be severe like fibrosis or ulcers depending on the amount of radiation, length of exposure and the body part that was exposed. Symptoms may occur immediately after few days or may even take months (Gardner *et al*., 2001; Kupper *et al*., 2004).

2.2.3 Classification based on degree of contamination (microbial load)

Wounds generally are known to acquire microbes from the environment or normal flora of the body. Persistence of microbe in wound will result in continous, and progressive tissue degradation, wound infection and delay healing (Anderson and Hamm, 2012; Edwards and Harding, 2004).

2.2.3.1 Contamination

Wounds that acquire micro-organisms are regarded as contaminated; however, in the absence of nutrient and favourable environment for each microbial species, they are unable to successfully evade host defences. Hence, are unable to replicate or persist in the wound bed. Their occurrence at the wound site is therefore only short-lived with unhindered wound healing, although investigations showed that wound colonisation by microflora enhances healing (Edwards and Harding, 2004; Anderson and Hamm, 2012).

2.2.3.2 Colonisation

A wound is said to be colonised when the microbial species or wound contaminants productively replicate without causing damage to the host. Bacterial colonisation, is also known as bioburden. Research in wound care showed that, bacteria live in communities in the wound bed called biofilms (Edwards and Harding, 2004; Anderson and Hamm, 2012)

2.2.3.3 Infection

The development of colonisation into infection is a multifactorial process encompassing many host and microbial constituents. The progression of colonisationinto infection can be due to immunological reactions like in immunodeficiencies, local factors such as reduction in tissue perfusion. The microbes involved like the commonly isolated microbes in infected wounds include *Staphylococcus aureus*, Coagulase-negative *staphylococci*, *Enterococci*, *Escherichiacoli*, *Pseudomonasaeruginosa*, *Enterobacter* species, and *Proteusmirabilis.* Here, there is microbial growth, replication and invasion into host tissue that progresses into cellular damage with subsequent delay in wound healing. (Gardner *et al.*, 2001; Kupper *et al.*, 2004).

2.2.4 Classification based on morphology (severity of injury in relation to its depth)

This classification differentiates wound types and classifies them as partial thickness when only the skin is involved, deep partial thickness when muscles were also affected, and full thickness if the bones were involved. Based on the degree of epidermis, dermis, subcutaneous tissue, fascia, muscle and bone damaged. The degree of tissue damage determines the therapeutic options necessary, management options healing time, functional impairment and the amount of scarring (Gardner *et al.*, 2001; Kupper *et al.*, 2004; Tiwari*et al*., 2014).

2.3 Wound Healing

2.3.1 Introduction

The wound healing process is a complex mechanism. Understanding the processes involved in wound healing is important to making well-founded choice in the management of wounds (Rivera and Spencer, 2007;Rozman and Bolta, 2007).Wound healing targets the re-establishment of the integrity of the damaged tissue and consists of sequence of dynamic events. It involves a complex and orderly progression but superbly orchestrated events, leading to the repair of injured tissues. A completely healed wound is one that does not necessitate further drainage or dressing and has returned to its typical anatomical structure, physiology and form (Broughto *et al.*, 2006; Demidova-Rice*et al.*, 2012). The four provisionally and spatially intersecting phases through which physiological course of wound healing is realised include, coagulation and haemostasis phase, inflammation phase, proliferation and repair phase, and remodeling or maturation phase (Nadeem *et al.*, 2004; Falanga, 2005; Rozman and Bolta, 2007; Velnar *et al.*, 2009; Eming *et al.*, 2014).

2.3.2 Coagulation and haemostasis phase

Immediately after injury, haemostasis is initiated by exposed collagen to blood cells. The contact between blood components, collagen and other extracellular matrix components activates the coagulation cascade, which leads to platelet clumping together and clot formation to prevent haemorrhages (Nadeem *et al.*, 2004; Velnar *et al.*, 2009; MacCornick *et al.*, 2014).Platelets are well-known for their role in homeostasis, as they facilitate the inhibition of haemorrage at the sites of vascular damage. Platelets achieve this function by adhering, aggregating and forming pro-coagulant surface. This stimulates strong vasoconstrictor reaction through the release of thromboxane and prostaglandin to the wound milieu. Furthermore, contraction of vascular smooth muscle cells in the circular muscle layer of injured vessels occurs rapidly due to concurrent neuronal reflex. The neuronal reflex mechanism also stimulate the release of clotting factors and cytokines from the platelets to activates and attract neutrophils, macrophages, endothelial cells and fibroblasts (Velnar *et al.,* 2009; Bin and James, 2011).

The fibrin plug and vasoconstriction are the finish-product for accomplishing physiological hemostasis initiated by the coagulation cascade and neuronal reflex mechanism. The fibrin fibres in the plug become a temporary wound matrix and form the framework on which more platelets aggregate. The reservoir of cytokines, activated platelets are the most profuse cells in the wound site during the early post-wounding period, releasing cytokines to regulate the cellular interplay in wound healing (Rozman and Bolta, 2007). Moreover, the initial hemostatic response is improved with the leakage of blood constituents for the formation of the blood clot. This helps to minimise blood loss and provide an initial extracellular matrix for cellular migration. Thus, factor VII is activated to initiate the extrinsic coagulation cascade. The activation is via the discharge of tissue factor and intracellular calcium at the insult of wound or damage to the vessels. Concurrently, the coagulation cascade, the arachidonic acid pathways, and the release of cytokines, organised to initiate and maintain the inflammatory phase and the order of cells involved in the process. Fibrin mesh that traps platelets are synthesised from activated fibrinogen as a reaction to the damaged epithelium (Rozman and Bolta, 2007;MacCornick *et al.,* 2014). Injury to vessels stimulates alterations in the conformation of surface adhesion molecules; hence, neutrophils become sticky and stick to the endothelial cells in the post-capillary venules, surrounding the wound area. Consequently, the sticky neutrophils are pushed forward by blood flow and integrins, secreted by endothelial cells, forcing the neutrophils to roll along the surface of the endothelium. On reaching the wound milieu neutrophils phagocytise necrotic cells, debris and simultaneously release, proteolytic enzymes and reactive oxygen species (ROS). The cell remnants and apoptotic bodies are then phagocytosis by macrophages (Linehan *et al.,* 2005; Broughto *et al.,* 2006). Platelets, on interaction with the damaged extracellular matrix, stimulate the clotting factors, leading to the formation of blood clot. Platelet in the wound stroma release essential elements that promote wound healing and sensitised vascular and other blood cells in angiogenesis and inflammation. Platelets also serve as reservoir of cytokines like the EGF and PDGF (Falanga, 2005; Barrientos*et al.,* 2008; Eming *et al.,* 2014; MacCornick *et al.,* 2014).

2.3.3 Inflammatory phase

The inflammatory phase aims at creating an immunological line of defence against invading bacteria. This phase is further sub-divided into early inflammatory and late inflammatory sub-phases (Velnar *et al.,* 2009).

2.3.3.1 Early inflammatory phase

The insult of wound activates the complement cascade and stimulates cellular and molecular events, resulting in the infiltration of the wound site by neutrophils within 36 hours of injury. The early inflammatory phase is characterised by the arrival of phagocytic neutrophils at the wound site (Hart, 2002; Loning *et al.*, 1983; Barrientos*et al.*, 2008). The phagocytic neutrophils destroy bacteria and cleanse the wound site of foreign debris. The phagocytic cells, neutrophils and macrophages are the major cells at work in this wound healing phase. Neutrophils are known to release ROS and proteases that decontaminate the wound of cellular debris and inhibit microbial contamination. Monocytes from the vessels on reaching the wound site, differentiate into tissue macrophages to release cytokines and decontaminate the site, of microbes and non-viable tissue (Falanga, 2004; Rozman and Bolta, 2007; Eming *et al.*, 2014). Inflammatory process is initiated by the influx of neutrophils in the first two days of wound healing. These are then replaced by macrophages. Macrophages release various cytokines and also change the relatively acellular wound area into a highly cellular environment (Deuel*et al.*, 1991; Mustoe *et al.*, 2002). The secretions stimulate fibroblasts to produce collagen and promote angiogenesis; these secretions include collagenases and cytokines. Next, they increase in population to become the dominant cells of the reparative phase, recruiting additional fibroblasts, endothelial cells, and keratinocytes, which are the next type of cells attracted to the wound site. This step results in the shift from the inflammation into tissue reconstruction; that is, the proliferative phase (Mustoe *et al.*, 2002; Rozman and Bolta, 2007; Landén *et al*., 2016).

2.3.3.2 Late inflammatory phase

During the late inflammatory phase, 48 – 72 hours post-injury clotting factors, complement components and cytokines, as well as elastin and collagen break-down products are phagocytise by monocyte in the wound milieu that undergo phenotypic changes to macrophage. Depletion of monocytes and macrophages at the wound site leads to severe healing disturbances. Lymphocytes are the last cells to enter the wound site in the late inflammatory phase, and their influx occurs 72 hours post-injury (Hart, 2002; Daniel and Mathieu, 2006). Capillary vasodilatation happens, sequel to local histamine release, enhancing the migration inflammatory cells into the wound bed (Ueno *et al.,* 2006; Rowan*et al*., 2015). Lymphocyte may also be involved in collagen and extracellular matrix remodelling (Gabriel *et al.,* 2009; Landén *et al*., 2016). As wound healing progresses, the population of macrophages and fibroblasts is depleted by apoptosis (Rowan*et al*., 2015; Landén *et al*., 2016). The time-line for cell migration in an orderly wound healing process at the wound site is predictable (Fig. 2.1) (Gabriel *et al.*, 2009).

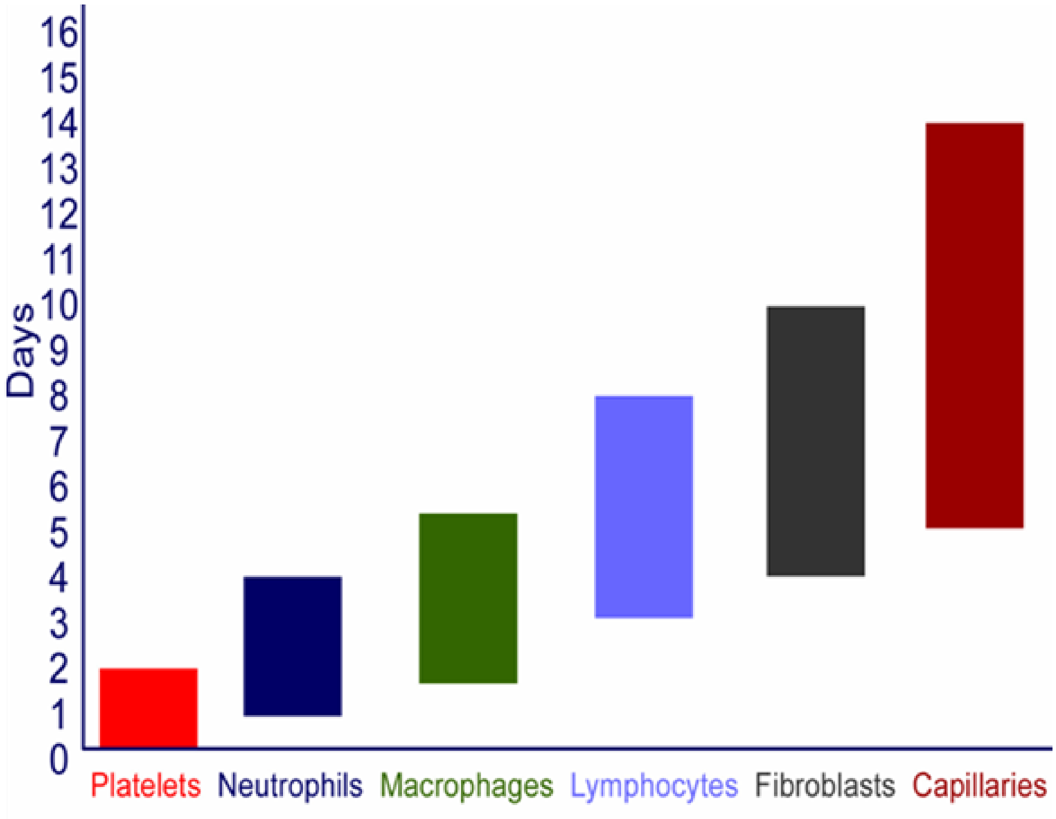


Figure 2.1: Blood-Related Factors Involved in Wound Healing and their Time of Action

(Source: Gabriel *et al.*, 2009)

2.3.4 Proliferative phase

The fibronectin–fibrin matrix are progressively been replaced by collagen synthesised by fibroblasts. The new wound structure of collagen, fibroblast and keratinocytes are maintained by angiogenesis, thus, initiating epithelialisation of the wound (Mustoe *et al.*, 2002).The clearance of the immune cells basically lymphocytes marks the end of the inflammatory and introduces the proliferation phase (Schultz *et al.*, 2003; Sun *et al.*, 2014). Proliferative phase commences from day 3 post-wounding.The secreted cytokines along with extracellular matrix molecules stimulate the differentiation of fibroblasts to produce ground substance and collagen. Fibroblasts play key role in providing strength and ground substance of the wound through the production, deposition, and remodeling of the extracellular matrix, (Broughto *et al.*, 2006; Longaker *et al.*, 1991; MacCornick *et al.*, 2014). The reparative phase is primarily characterised by the formation of granulation tissue, neovascularisation, and epithelialisation (Schultz *et al.*, 2003; Sun *et al.*, 2014).

2.3.4.1 Fibroblast migration and collagen synthesis

Fibroblasts proliferations from the surrounding in-tacked tissue are initiated for the first 3 days post-injury by wounding. Once the fibroblast are in the wound and on the third day, they synthesis the matrix proteins hyaluronan, fibronectin, proteoglycans and type 1 and type 3 procollagen. By day seven, the matrix protein accumulates for cell migration, and fibroblasts change to myofibroblasts that helps to approximate the wound edges (Tunçcan *et al*., 2016; Calfee and Manning, 2002). Collagen impart integrity and strength to all tissues, as well as a base for the extracellular matrix laying within the wound bed. Unwounded dermis contains 75 – 80% type 1 and 20 – 25% type 3 collagen while newly formed granulation tissue contains average of 40% type 3 collagen (Hart, 2002). The formation of new vessels and fibroplasia after day 3, marks the unset of collagen production on days 3–5 (Rozman and Bolta, 2007).

2.3.4.2 Angiogenesis

Four days post-injury newly formed vascular buds becomes noticeable within the wound milieu. This is essential to facilitate an entry point for cells, like the macrophages and fibroblasts to migrate into the wound bed. The influx of macrophages is essential, to enhance formation of new blood vessels and fibroplasia via synthesis and secretion of cytokines (MacCornick *et al.*, 2014). Cocktail of cytokines in the wound bed, excites surrounding healthy tissue, endothelial cells and the vessels, to secrete proteases such as matrix metalloproteinases. This degrades the basement membrane, allowing endothelial cells to escape the confines of their host vessel. Cytokines elicits an orderly re-arrangement of endothelial cells from surrounding healthy blood vessels. The endothelial cells elongate and align becoming a capillary sprout, spread out away from the host vessel. This indicates the unset of the formation of new vessels (Barrientos*et al.*, 2008;Gurtner *et al*., 2008).

The formation of new blood vessels from pre-existing ones is termed as angiogenesis, this process is reliant on oxygen. Oxygen is utilised in the synthesis and deposition of collagen essential as the scaffold on which new vascular buds are formed. Hypoxia-inducible factor (HIF) stimulated an increase synthesis of cytokine to elicits endothelial cell multiplication, thus, aiding the formation of new blood vessels throughout the repair phase. Extension of new vascular buds is aided by endothelial cell multiplication and advances their migration toward the chemical attractant. The joining of 2 new vascular buds within a healing wound forms a loop through which blood can flow and new vascular buds develop, propagating neovascularisation (Diegelmann, 2004; Eming *et al.*, 2014).

The extension of a vascular sprout into the wound permits oxygen and other essential nutrients to reach further into the injured site. Also transported are fibroblast and macrophage, advancing the healing into the injured site. When the unit has totally swept through the wound bed, blood vessels then networked over the entire space, oxygen concentrations are resumed to normal, and the angiogenesis process ends. Progenitor cells differentiate into early endothelial progenitor cells in the bone marrow and further differentiate into late endothelial progenitor cells in the vasculature system before arriving at the site of angiogenesis (Diegelmann, 2004; Eming *et al.,* 2014). The establishment of blood vessels from the differentiated progenitor cells is called vasculogenesis, Barrientos*et al.* (2008) showed that significant contribution to new blood vessel growth at the wound bed is accomplished by the process of vasculogenesis. In the proliferative phase, modelling and establishment of new blood vessels which is necessary in wound healing occurs simultaneously (Gurtner *et al*., 2008; Bryden, 2015). In addition to the phagocytic activities of neutrophils and macrophages, these cells also stimulate angiogenesis throughout the haemostatic phase by producing angiogenic factors. Surrounding endothelial cells are responsive to angiogenic factors, like the angiogenin. For check and balances angiogenesis is kept in check by the action of inhibitory factors, such as angiostatin and steroids. These inhibitory and stimulatory agents act on propagating endothelial cells, by stimulating mitosis, promoting locomotion and by eliciting the host cells to synthesis cytokines (Nataranjan *et al.,* 2000). During angiogenesis, vascular supply in the middle of the wound is the last. As viable tissue, which is limited to the wound margins, is perfused by sorrounding vessels and by diffusion through intact interstitium. New vascular buds from the surrounding edges invade blood clot, and within limited days a micro-vascular linkage composed of many new capillaries is formed (Barrientos*et al.,* 2008; Bryden, 2015).

Chemotaxis allows cells to react appropriately to environmental signals, which regulate proliferation, migration and differentiation. Chemotactic agents act on receptors on the cell membrane to direct the cell movement, which is involved in the formation of new blood vessels. The contributing factors act as intermediaries for angiogenesis, and vessel repair at the wound bed. They are also significant modulators of cell growth and specialisation (Nataranjan *et al.,* 2000; Bryden, 2015). The process of neovascularisation is closely connected to the establishment of granulation tissue (pro) angiogenic and angiostatic factors (Gillitzer, and Goebeler, 2001; Broughto *et al.,* 2006).

2.3.4.3 Granulation tissue formation

Granulation tissue is so termed, because of the pink granular appearance of abundant capillaries that enter, and are present in the wound bed (Hunt *et al.,* 2000). The process of neovascularisation occurs simultaneously, all through the phases of the healing process. As collagen accumulates in the granulation tissue, the density of vascular buds reduces, and disruption of this dynamic course could result in chronic wounds (Gabriel *et al.*, 2009). Fibroblasts produces collagen to provide strength and integrity, in wounds and all tissues in the body. They propagate and start forming new extracellular matrix when inside the wound bed. Collagen is accepted as the bases for wound extracellular matrix. While granulation tissue is primarily made up of propagating fibroblasts, new capillary buds and tissue macrophages in a matrix of collagen, glycosaminoglycans, including hyaluronan, and the glycoproteins, fibronectin and tenasci. Granulation tissue formation is apparent as early as 48 hours post-wounding and by 96 hours post-wounding fibroblasts become the major cells at the wound bed, since they are indispensable cells in the formation of granulation tissue. Fibroblast does not only produce collagen from its rough endoplasmic reticulum, but also synthesises elastin which is essential in wound remodelling. Immediately after wounding, sorrounding skin fibroblasts and perivascular mesenchymal cells specialises into a phenotypically changed cell, the "myofibroblast". Granulation tissue formation is particularly important in wound healing by secondary intention. When collagen production and break-down are in equilibrium, the next phase of wound healing, epithelialisation commences (Gabriel *et al.*, 2009).

2.3.4.4 Epithelialisation

The proliferative phase is marked by angiogenesis, formation of granulation tissue, collagen deposition and epithelialisation. Unset of epithelialisation starts within hours post-injury in the wound repair. Normal epidermis is restored within 3 days where the basement membrane is intact, as epithelial cells migrate above the intact progenitor cells hours after injury (MacCornick *et al.*, 2014). These cells lose their normal strong connections to the underlying tissue, permitting them to migrate in a leap-frog fashion across the temporary matrix (Wild*et al.*, 2010). A layer of cells is initially formed over the damage area, accompanied by proliferation of epithelial cell, dividing actively around the wound edges. Migrating cells across them becomes attached to the temporary matrix below. When migrating epithelial cells meet, mitosis stops and the synthesis of the basement membrane is initiated (Robson *et al.*, 2001). The migration of epithelium seeks a plane of critical humidity. The water content of the wound stroma partly determines the plane of movement of epidermal cells. Occluded wounds epithelialises faster when compared with open, or desiccated wound. Occlusive and semi-occlusive dressings, optimally enhances epithelialisation (Hunt *et al.*, 2000).

The contributions of keratinocytes in the process of epithelialisation include the synthesis of certain factors such as fibronectin, collagenases, plasminogen activator, neutral proteases and collagen. These factors promote linkage of keratinocytes, and contribute in their migration across the wound bed, they are also essential in the digestion of necrotic tissue (Wojtowicz *et al.*, 2014). Additionally, the use of dressings can be employed to promote debridement in an autolytic manner. Low-oxygen tension between the wound and bandages results in an acidic pH, which, is not favourable for bacteria growth but enhances collagen synthesis, neovascularisation and immune response. Bandages can be left in place longer between replacement with progress in healing (Hunt, 2000; Davidson, 2015).

2.3.4.5 Wound contraction

The term wound contraction can be defined as the centripetal movement of wound edges in order to simplify closure of the defect. Wound contraction occurs, when the underlying contractile connective tissue reduces in dimension to bring wound edges together. Myofibroblasts is a transformed and specialised fibroblasts within the wound stroma, that is responsible for tissue contraction, during the repair phase of wound healing. Wounds progressively contracts throughout this phase of wound healing, simultaneously with epithelialisation. Maximal contraction takes place between 5th and 15th day post-wounding, during which myofibroblast plays key role. In an open wound, networks of granulation tissue and myofibroblasts facilitate the forces of contraction in a unified fashion (Gabriel *et al.,* 2009). Contraction, thus, occurs throughout the interactions between myofibroblasts and the adjoining extracellular matrix. In addition to reduction in the size of wound, early deposited collagen are reorganised (Gabriel *et al.,* 2009).

2.3.5 Remodelling phase

The last phase of wound healing occurs immediately wound closure is achieved, which may last for more than 1 years. In the course of this phase, the temporary matrix is remodelled into orderly planned collagen bundles (Falanga, 2004; Schreml *et al.,* 2010; Tsourdi *et al.,* 2013). Fibroblasts organise and cross-link the collagen they produced, thus, wound strength progressively increases. Reduction in wound size still progresses, with the wound losing its pink or purple colour due to decline in capillary and fibroblast. The various stages in wound healing may vary in length, because of differences in host intrinsic and extrinsic factors (Mustoe *et al.,* 2002). Decline in the vascular density and cellular apoptosis is enhanced with adequate oxygenation in the course of the remodellingstage. Contraction is also finalised, collagen matrix is remodelled, and wound strength is improved from about 20% of the skin tensile strength (Barrientos*et al.,* 2008).

The initial arrangement of collagen deposition in a scar appears as densely packed fibers, in place of the reticular pattern seen in normal skin dermis. The extracellular matrix is active and constantly being remodelled, and can be conceptualised as the stability between synthesis, deposition, and degradation. A scar connects the space between wounded tissue with a maximum tensile strength of 70 – 80% of the normal skin (Gabriel *et al.,* 2009; Young and McNaught, 2011). After the secretion of collagen molecules, fibroblasts then assemble collagen extracellularly into collagen fibres. These fibres are then cross-linked and organised into bundles. The increasing content of wound collagen correlates with increasing tensile strength, which is the most important wound-healing parameter of surgical wounds, followed by epithelialisation, and ultimately wound remodeling (Rozman and Bolta, 2007; Yang *et al*., 2015). Along with intracellular matrix maturation, collagen bundles increase in diameter and hyaluronic acid and fibronectin are degraded. The tensile strength of the wound increases progressively in parallel with collagen collection (Robson *et al.,* 2001; Hart, 2002; Velnar *et al.,* 2009).

The initial degradation of collagen is achieved by matrix metalloproteinase enzymes, secreted by neutrophils, macrophages and fibroblasts in the wound (Toy, 2005). The activities of matrix metalloproteinase enzymes is firmly controlled and harmonised by inhibitory factors, thereby enhancing novel matrix accumulation. The initial deposition of collagen bundles is extremely disorganised, but its organisation is achieved during the final stages of the remodelling phase. The density of fibroblasts and macrophages is reduced by apoptosis, angiogenesis halts, blood flow to the area decreases and metabolic activity at the wound site drops. The outcome is a completely matured scar with a diminished number of cells, blood vessels and a high tensile strength (Hart, 2002; Velnar *et al.*, 2009; Yang *et al.*, 2015). This phase is categorised by decline in neovascularisation, increase in collagen synthesis, and increase in collagen degradation to equals the skin proportion (Gabriel *et al.*, 2009). Collagen I and II in this phase of wound healing substitutes the initial collagen III synthesised by fibroblasts, reshaping the wound and enhancing the strength of tissues in the wound area. In the elderly, the body's capacity to heal reduced owing to functional changes, like declined mitotic ability of the epidermal cells and flattened dermo-epidermal junction (Gabriel *et al.*, 2009; Wild*et al.*, 2010; Stuart and Price, 2010).

2.4 Wound Management

The prime objective of wound management is to achieve healing as quickly as possible in the simplest way, with the secondary aim of reducing resultant scar formation (Nicholas, 2002).

2.4.1 Wound cleansing/commonly used antiseptic compounds

Antiseptics are used for the preparation of the patient, personnel and some surgical instruments for surgery. Antiseptics are also used postoperatively to prevent infection or, on a healing infected wound, thus giving the body a chance to repair itself (Raut *et al.*, 2015). They are widely used in health care centres and veterinary settings, for topical and hard-surface applications. A wide variety of active chemical agents (biocides) are found in these products. Most accepted wound-cleansing solutions have been demonstrated to be toxic to fibroblasts and lymphocytes. These solutions include alcohol, chlohexidine, povidone-iodine, acetic acid, iodophor, hydrogen peroxide and Dakin's solution (sodium hypochlorite). Normal saline solution (0.9% sodium chloride) from research studies is the only known non-detrimental, and acceptable wound-cleansing solution that is not toxic to cells. Normal saline solution effectively removes contaminants, and has the same salt concentration as the fluid in cells. In addition, normal saline is inexpensive and readily available with close to zero chances of inducing hypersensitivity reactions. Removing the delicate granulation tissue or establishing epithelium in the wound stroma during wound management has also been shown to interfere with wound healing (Raut *et al.,* 2015; Landén *et al*., 2016).

2.4.1.1 Iodine

Iodine has been regarded as one of the most efficacious antiseptic. Several forms of iodine include, Lugol's solution, which is regarded as irritating with caustic properties; tincture of iodine (2% iodine) used for preoperative skin preparation; and the iodophors which are the most common form of topical iodine. The different forms increase solubility and allow sustained-release of iodine. The spectrum activities of iodines and iodophors are wide and include gram-positive and gram-negative bacteria, fungi, viruses and protozoa. The potentials of povidone-iodine absorption limit its use in pregnant and lactating mothers, because of its likelihood of inducing transient hypothyroidism. Cadexomer-iodine is a hydrophilic modified-starch polymer bead, containing immobilised molecules of iodine. The beads absorb wound exudates to increase in size, thus, gradually releasing the incorporated iodine. Its indication is for the management of infected ulcers (Atiyeh *et al.,* 2009).

2.4.1.2 Biguanides (Chlorhexidine gluconate, polyhexanide and polyhexamethylene)

The most common and widely used biguanide antiseptic is chlorhexidine. It is produced in three preparations; a 0.05%-dilution for wound cleansing, 2%-solutions for surgical skin preparation, and a 4%-solution for surgical skin preparation and hand scrub. Chlorhexidine has a high affinity for the mucous membranes and skin with low toxicity and strong antimicrobial properties. It exert its antimicrobial activity by triggering leakage via destroying the outer and inner membranes of the bacteria, thus, disrupting membrane potentials critical for adenosine triphosphate generation. It disrupts the microbial cell membrane to precipitates the cell contents. Except at increased temperature chlorhexidine (0.5–4%) has a limited effect against fungi, tubercle bacilli, and no effect against bacteria spores. It must not be used in contact with the eyes, middle ear or the meninges (McDonnell and Russell, 1999; Yakubu *et al*., 2010; Raut *et al.,* 2015).

2.4.1.3 Hydrogen peroxide (H_2O_2)

Hydrogen peroxide is a clear, colourless liquid with characteristic odour that is produced in large quantity available in a variety of concentrations ranging from 3% to 90%. This biocide is widely used for disinfection, decontamination and antisepsis. Hydrogen peroxide is a readily available oxidant that is rapidly converted to highly reactive hydroxyl radical. It is inoffensive and environmental friendly, because of the considerable catalase activity of some disease causing microbes, high concentrations is needed (Atiyeh *et al.*, 2009).

2.4.1.4 Alcohol.

Alcohols like ethanol, isopropanol and n-propanol are widely used as surface disinfection and skin antisepsis. More than 50% concentration is required for its' antimicrobial activities, most preparations are range of 60-90%. These alcohols have excellent in vitro bactericidal activity against most gram-positive and gram-negative bacteria. They also kill *Mycobacterium tuberculosis*, various fungi and certain enveloped viruses; however, they are not sporicidal and have poor activity against certain non-enveloped viruses. The mechanism of action of alcohol is via membrane damage, protein coagulation and rapid denaturation of proteins, with subsequent interference with metabolism and cell lysis (Fernandez *et al*., 2001; Halim *et al*., 2012).

2.4.1.5 Sodium Hypochlorite (Dakin Solution)

It is biocide against most microbes commonly found in wounds and are frequently used in pressure ulcers with necrotic tissue to help control infection. It is also cytotoxic to healthy cells and granulating tissues, occasionally used over cancerous growths to control bacteria and minimise odor, however it should be used for maximum duration of 10 days. Since antiseptics are not effective at killing bacteria at concentrations that are harmless to healing tissue, like the use of povidone iodine, hydrogen peroxide, or sodium hypochlorite. Their use should be reserved for highly contaminated or infected wounds (Fernandez *et al*., 2001; Halim *et al*., 2012).

2.4.1.6 Normal Saline

Sterile normal saline in view of its physiologic nature is safe to be use in wounds and is the most widely used wound irrigating solution. However, it does not have residual effect as found in commercial wound cleansers which makes them more potent in lasting effect on microbes and debris. Normal saline should be discarded after 24 hours of opening, because of the absence of preservatives to prevent microbial growth. To utilise normal saline for wound irrigation a 30 mL syringe is recommended with an 18 or 19 gauge needle. A 4 to 15 pounds per square inch (psi) pressures range that is, safe and effective for wound irrigation is conveniently achieved with the syringe. Pressures lower than 4 psi is ineffective and inadequate, while pressures greater than 15 psi dislodges fragile tissue and only forces microbes deeper into the wound stroma. Ensure sharp tips of the needle or devices do not come into touching base with the wound bed (Fernandez *et al*., 2001; Halim *et al*., 2012).

2.4.2 Wound contamination and wound infection

The vicinities in which injury occurred are prognostic of the number of pathogenic microbes in the wound and the possibility of emerging wound infection. Generally, for centuries it has been acknowledged that, bacterial infection often develops in wounds that are not clean (Atiyeh *et al.,* 2009). Specific infection-potentiating factors have been identified in the soil, which include its organic components as well as its inorganic clay fractions. For wounds contaminated by these fractions, only 100 bacteria are necessary to elicit infection. Their ability to enhance the incidence of infection seems to be related to their damage to host immune system (Atiyeh *et al.*, 2009).

2.4.3 Wound bed preparation

Wound bed preparation is a concept which tries to systematise the management of wound, with a view to enhance endogenous healing, and to promote the potency of other therapeutic measures. The 3 mainstays of wound bed preparation are controlling necrotic burden by debridement, controlling bacterial burden and conserving moisture balance. Furthermore, wound bed preparation aims at improving cellular dysfunction and attaining biochemical balance (Daunton *et al.,* 2012; Mayet*et al.,* 2014). Debridement is usually accomplished by surgical, mechanical, biological, or via enzymatic means. Mechanical debridement refers to a variety of techniques like wet-to-dry dressings, which cause separation of eschar and its removal; wound irrigation, which ideally is irrigation of the wound with normal saline using 18-gauge catheter or needle. Biological debridement involves the use of maggots. Enzymatic debridement denotes the topical use of bacterial collagenase, protease, DNAse/fibrinolysin, papain/urea combination or trypsin to digest necrotic tissue and debris (Lauterbach *et al.,* 2010; Daunton *et al.,* 2012).

Microbial contamination can be controlled by a combination of debridement; correcting underlying aetiologies like immunosuppression, ischemia, topical antimicrobial delivers through dressings like silver based dressings, and antibiotic impregnated gauze (Jian-dong and Chen, 2005; Mayet*et al.,* 2014; Dhivya*et al*., 2015). Conserving moisture balance on the other hand consist of the control of exudate, while maintaining a moist wound environment that is conducive to healing. Exudate may be controlled using fluid (exudate) absorbent dressings or mechanical devices such as topical, negative pressure therapy (Lauterbach *et al.,* 2010; Daunton *et al.,* 2012). A moist wound environment tends to have an advantage; it prevents tissue dehydration, which helps to preserve the viability and proliferative potential of the cells. This is associated with earlier epithelialisation; it accelerates angiogenesis; increases breakdown of dead tissue and fibrin, contributing to autolytic debridement; it potentiates interaction of growth factors with their target cells; is associated with less pain and reduces the incidence of infection. In contrast, exposure of the wound to air causes wound desiccation and scabbing, which delay epithelialisation and interfere with proliferative potential of the tissue. Studies have shown that, the healing time of deep or superficial cutaneous wounds is enhanced and less painful, when treated with a moisture-retaining dressing as against a simple gauze dressing (Lauterbach *et al.,* 2010).

2.4.4 Wound dressing

No single dressing or wound treatment is suitable for all types of wounds. During the healing process of a single wound a number of dissimilar types of dressings will be required (Tricco *et al*., 2015). The following functions are expected to be performed by wound dressing and treatments: preservation of moist environment at the wound/dressing boundary; clearance of excess exudate without leakage to the surface of the dressing and establishment of thermal insulation and mechanical shield; others are microbial defence; gaseous and fluid exchange; clearance of wound odour; non-adherence to the wound and easy removal without trauma; some debridement activity; optimal gaseous exchange; sterility, non-toxic and non-allergenic functions (Jian-dong and Chen, 2005; Lauterbach *et al.,* 2010; Mayet*et al.*, 2014).

2.4.4.1 Hydrocolloids

A hydrocolloid is defined as a dressing, typically consisting of an absorbent layer of gel on a semi-permeable film or foam that promotes rehydration and debridement of wounds. A hydrocolloid is an adhesive dressing consisting of natural or synthetic polymers, for example gelatine or pectin. When in contact with wound exudate they form a gel, which may be cohesive and/or hydrophilic. The acidic pH created by the hydrocolloid is effective against microbes, moreso, hydrocolloids are occlusive to provide bacterial barrier, re-hydrate necrotic and sloughy wounds, thereby facilitating debridement. They also influence healing by creating an environment. They the biological process of healing occurs in a more ordered fashion, they are waterproof, so patients can bath or shower; they however, have a particular odour. Examples are Duoderm® thin and Comfeel® plus. The Duoderm® thin should be used for healing and granulating or epithelialising wounds with mild exudate while Comfeel®plusissuitable for chronic, necrotic, sloughy wounds with mild exudate. Hydrocolloidscan stay on wounds for 7 days, however, they should be avoided in patients with heavily-exuding wounds to prevent maceration, like in patients with infected or non-healing wounds. Note hydrocolloids also have likelihoods of stimulating excessive granulation tissue formation (Wellerand Sussman, 2006; Ousey *et al*., 2016).

2.4.4.2 Hydrogel

Hydrogel products constitute a group of polymeric materials, with a hydrophilic structure designed to hold large amount of water in their three-dimensional networks. Hydrogels are amorphous, water based gels or sheets that promotes debridement via re-hydrating dry and necrotic tissue to provide a moist wound healing environment. Hydrogel is capable of cooling wound surface and bathing the nerve endings, hence, providing a painless wound. Performance varies according to gels constituents for example Intrasite® has less absorptive capacity when compared with Purilon® gel (Wild, *et al*., 2010). Purilon gel contains an alginate, carboxymethylcellulose and 90% water. Primarily, they are designed for dry or necrotic wounds, however, they necessitate a secondary bandage with film or hydrocolloid and they do not increase in size into the wound. Dressing should be avoided where anaerobic infection is suspected and should be replaced every 1-3 days (Weller and Sussman, 2006; Ahmed, 2015; Chen*et al.,* 2015).

2.4.4.3 Alginate

The alginate powder contains sodium alginate, calcium sulfate, trisodium phosphate, diatomaceous earth, zinc oxide, and potassium titanium fluoride. On mixing the powder with water, a sol is formed, a chemical reaction takes place, and a gel is formed.Alginate like sorbsan® is composed of alginic acid (basically mannuronic and guluronic acid residues), the mannuronic acid forms a soft flexible gel which is soluble with 0.9% sodium chloride and can easily be removed or rinsed away. Calcium is incorporated to promote healing. This gel permits pain-free dressing changes and can be used on bleeding wounds, infected or diabetic wounds. The flat dressing is appropriate for superficial wet wounds and ulcers, while the ribbon had been designed for cavity wounds, hence are neatly packed into the cavity, and wound dressing should be replaced after 3 – 7 days (Boateng*et al.,* 2008; Nandini*et al.,* 2008; Sood *et al.,* 2014).

2.4.4.7 Low adherents

Tricotex® is a low adherentdressing that does not provide a moist wound healing environment. Tricotex® is designated to inhibit secondary absorbent dressing stick to the surface of wound likein lacerations. Furthermore, low adherents dressing requires to be fortified with a bandage or adhesive tape. Another low adherent dressing is the urgotul®, the dressing consist of a fabric net impregnated with hydrocolloid particles, paraffin and petroleum jelly. The petroleum gel and hydrocolloid particles, forms a lipido-colloid on touching base with wound exudate. It is stress-free to apply and allows pain free removal; comfortable, conformable and has low-adherent property. Urgotul® tends to be more effective relative to other non-adherent dressings. It promotes fibroblast proliferation at the wound site, hence, recommended for venous leg ulcers in combination with compression bandaging. Urgotul® also enhances wound debridement, where only small amount of sloughing is required. It is an expensive dressing and is reserved for patients with painful wounds or neuropathic pains. It can be cut to shape of wound, but requires secondary dressing. Dressings should be replaced every 2 – 3 days. Urgotul® due to its nature adheres to gloves, however, pre-moistening gloves with normal saline can avoid this, hence, simplify handling and application (Fong *et al.*, 2005; Fong and Wood, 2006; Dhivya*et al*., 2015).

2.4.4.9 Inadine

Inadine®is a low adherent-knitted viscose dressing, impregnated with 10% povidone iodine in a water-soluble polyethylene glycol base. This medicated tulle is able to release antimicrobial agent than other medicated tulles.It is recommended in infected superficial wounds but not in dry wounds. Dressing should be changed when the orange-brown colour changes to white within 2 - 3 days. Secondary dressing is necessary however, the nature of the wound will determine the secondary dressing type (Weller and Sussman, 2006; Barnea*et al.,* 2010).

2.5 Advances in Wound Managements

2.5.1 Platelet rich plasma

Platelet rich plasma (PRP) therapy is more widely in used in the management of wounds in diverse specie, including non-healing wounds. Platelet rich plasma synergises very well alongside doses of anti-inflammatory medication and antibiotics (Knighton *et al.*, 1986; Veves*et al.*, 2001; Falanga, 2005; Jee *et al*., 2016).

2.5.2 Bioengineered skin

keratinocyte sheets was the first bioengineered skin product, however, a number of bioengineered skin products or skin equivalents are now available for the management of acute or chronic wounds as well as burns (Quesenberry *et al.,* 2002; Falanga, 2005). Furthermore, complex constructs have been developed and tested in human wounds, with the skin equivalents containing living cells, such as fibroblasts or keratinocytes, or both (Natarajan *et al*., 2000; Badiavas *et al.,* 2003); while the skin products were generated from acellular materials or extracts of living cells (Veves *et al.,* 2001). The clinical outcome with the use of these constructs is 15-20% better than conventional 'control' therapy (Sosa *et al.,* 2008; Ramelet *et al.,* 2009).

2.5.3 Gene therapy

This technology involves the introduction of certain or desired genes into wounds, by a variety of physical means. The manipulated cells or genes are being introduced into the wound using a simple injection, gene gun or biological vectors. The introduction of the gene, in gene therapy rather than its product for example a growth factor, is seen as a less expensive and possibly a more efficient delivery method (Fan et al., 2010; Lee *et al*., 2016). Furthermore, regenerative healing may be achieved with the understanding of foetal wound healing. It may also lead to wound management approaches to prevent scarring and fibrosis. It has the potentials in future of been the main wound management approach, for enhanced wound healing (Sabolinski *et al*., 1996; Petrie and Eriksson, 2003; Hodde, 2006; Peng*et al.*, 2016).

2.5.4 Stem cell therapy

The pluripotential stem cells are precursors to all cells of all tissues. Stem cells are potential desired cell type of choice. They differentiate into any cell types, hence their use in enhancing wound healing and the potential of achieving a regenerative, scarless wound healing (Quesenberry *et al*., 2002). Research studies have shown the prospective skeletal muscle-derived stem cells and the adipose-derived stem cells. They are established to have same tendencies to differentiate as the cells and tissues of mesodermal origin, like the bone marrow-derived mesenchymal stem cells, hence, are explored for re-epithelisation in soft-tissue wound healing (Wai-Ping *et al*., 2010).

2.5.5 Hyperbaric oxygen

Hyperbaric oxygen therapy is used in the management of severe wounds. A pressurised chamber of 100% oxygen is used, where the patient breathes oxygen for 90-120 minutes. Here, adequate or extra oxygen is delivered to the cells at the wound site, via dissolved oxygen distributed throughout the body. This wound management practice has been shown to increase the rate of collagen deposition, enhance angiogenesis, microbial clearance and wound contraction (Boykin and Baylis, 2007).

2.5.6 Ultrasound

Ultrasound wound management uses sound energy above the range of human hearing and its mechanical vibration achieved at the frequency. The wound area is covered with hydrogel film as reported by the physical therapists, the probe is applyed during the inflammatory and proliferative stages. Thus, providing warm to the tissue, activating the cells involved in wound healing and also, enhancing healing by improving blood flow and circulation (Young and Dyson, 1990).

2.5.7 Electrical stimulation

Electrical stimulation influences wound healing by attracting repair cells, mimicking bioelectric phenomena in the body. This influences the permeability of cell membranes and enhances secretions and re-orientations of cell and cellular structures. Electrical charges are emitted through electrodes that are placed around the wound area. It can be used at any stage of the wound healing phases. Electrical stimulation is known to support, speed-up, and even enhances all phases of wound healing. The use of this therapy results in a smoother and thinner scar (Sumano *et al.,* 2002; Sosa *et al.,* 2008; Ramelet *et al.,* 2009).

2.5.8 Cytokines

The discovery of cytokines with regards to wound healing has raised expectation as to the use of these potent low-weight polypeptides and speculation to attaining a scarless wound healing. This heterogeneous group of low weight molecular peptides, exert their activities on target cells via precise interactions with cell-surface receptors (Steenfos, 1994). In vitro research studies disclosed that, cytokines were very effective in regulating cell multiplication, chemotaxis, and extracellular-matrix formation. The activities of cytokines is not limited to wound repair, regeneration or scar-tissue remodelling, but are crucial players in the preservation and sustenance of tissue and cellular integrity and, cell-to-cell communication (Traversa and Sussman, 2001; Nadeem *et al.,* 2004).

The epidermal growth factor is a family of mitogens that is secreted in the wound fluid with a well-known influence in promoting wound healing. This growth factor has been shown to promote overall wound healing. It speeds-up epithelialisation; enhances cellular multiplication and tensile strength of healed dermis (Robson *et al.,* 2001; Lin and Wang, 2011; Dogan *et al.,* 2009). Evidence for the role of epidermal growth factor and transforming growth factor in wound repair is seen through their impact by increasing the speed of keratinocyte multiplication to accelerate re-epithelialisation and, it is also a mitogen for fibroblasts (Lin and Wang, 2011). Reduced platelets derived growth factor in wounds, due to steroid treatment or diabetes, impairs wound healing, indicating that a certain expression level of platelets derived growth factor and their receptors, is essential for organised repair. On the other hand, amplified platelet-derived growth factor (PDGF) production could ultimately cause a hypertrophic scar and keloids, as suggested by its strong effect on fibroblast and keratinocyte multiplication, and extracellular matrix production. A study demonstrated that, platelets-derived growth factors of haematopoietic origin is not essential for the establishment of granulation tissue and that, its non-appearance even improves vascularisation (Werner andGrose, 2003).

The initial indications suggesting the role of epidermal growth factor receptor ligands in wound repair came from an investigation of wound secretions in rats. Epidermal growth factor was observed to act in synergy with insulin-like growth factor, in stimulating keratinocyte proliferation. *In-vitro* source of these epidermal growth factor ligands in wounds are macrophages, eosinophil, keratinocytes from the epidermal wound edge, hair follicle, epithelial cells, inflammatory cells and wound fibroblasts (Werner andGrose, 2003; Lin and Wang, 2011; MacCornick *et al.*, 2014). In sweat glands, the growth factor was also identified in bordering superficial keratinocytes and hair follicle epithelial cells, with peak concentrations recorded throughout the keratinocyte proliferation period. During neovascularisation not only new blood vessels are formed, but also lymphangiogenesis in skin wound healing (Robson *et al.*, 2001; Werner andGrose, 2003; Lin and Gyenge, 2008). Lymphangiogenesis is vital in wound healing as its non-appearance was establish in chronic human wounds, which might be one of the explanations for their compromised healing. Thus, nerve growth factor due to their role in lymphatic vessels re-generation has been suggested to be involved in skin wound healing.

Indeed, exogenous nerve growth factor topical administration was revealed to be protective against experimental diabetic sensory neuropathy, as well as supportive in the management of difficult-to-heal ulcers. The occurrence of nerve growth factor in greater concentrations at wound site in neonates, compared with adult wounds, is proposed that, this growth factor is in part partially accountable for the faster healing detected in neonatal wounds (Werner andGrose, 2003; MacCornick *et al.*, 2014).

There are several reports displaying the accelerative influence of endogenous epidermal growth factor or exogeneously administered epidermal growth factor, on wound re-epithelialisation. Rapid wound closure induced by epidermal growth factor treatment, is due to increase in epidermal cell multiplication and granulation tissue formation. In addition the rate of wound contraction, it increases the activities of myofibroblasts in the intermediate stages of the wound healing process (Young and McNaught, 2011). Epidermal growth factor reduces cutaneous scars by inhibiting inflammatory responses, lessening expression of transforming growth factor, and mediating the formation of collagen (Young and McNaught, 2011). Epidermal growth factor is observed in the epidermis, sebaceous glands, and hair follicular epithelium. It is essential and significant in the sustenance of systematic skin health, including regulation of cell differentiation, fortification against harm prompted by ultraviolet radiation, inhibition of inflammation, and acceleration of wound healing. Although the exact mechanism of skin rash mediated by epidermal growth factor-targeted monoclonal antibodies is incompletely understood, inhibition of epidermal growth factor may cause follicular occlusion and rupture, in the expression of genes that excite inflammation, apoptosis, and cell attachment; hence, anti-epidermal growth factor/epidermal growth factor receptor can be medicinal as anti-cancer drug (Efron and Moldawer, 2004; Casacó *et al.*, 2012).

Afshari*et al.* (2005) demonstrated the prospective influence of topical epidermal growth factor in significantly enhancing speedy wound healing. Epidermal growth factor plays a fundamental role by acting on epithelial cells and fibroblasts in wound healing, as well as stimulating the re-establishment of damaged epithelium. Application of epidermal growth factor in addition to standard treatment in topical or intralesional injection is able to prevent foot amputations (Tiaka*et al*., 2012).

Wound healing process requires cellular (immune cells, fibroblasts, keratinocytes, endothelial cells, smooth muscle cells, and myofibroblasts) interactions. These interfaces are facilitated by cytokines. Cytokines at the wound site include, epidermal growth factor, fibroblast growth factor, insulin-like growth factor, keratinocyte growth factor, platelet-derived growth factor, transforming growth factor and vascular endothelial growth factor. At present, only platelet-derived growth factor was approved for use in the medical field. Platelet-derived growth factor is a positively charged protein, which is not denatured by heat. Circulating platelets are reservoir of these low-weight polypeptides, they are contained in the granules of platelets and released from platelets into the serum during heamostasis phase of wound healing (Amendt*et al.,* 2002; Grazul-Bilska *et al.,* 2003; Jaiswal*et al.,* 2010). The vital and explicit activities of platelet-derived growth factor include mitogenesis (increase in the cell populations of healing wounds), angiogenesis (endothelial mitoses of functioning capillaries), and macrophage activation (debridement of the wound site and a second phase source of growth factors for continued repair and bone regeneration). Thus, platelet-derived growth factor can be employed in wounds with bone defect (Shah, 2014). The addition of partially purified platelet-derived growth factor, give rise to a significant dose-dependent growth in the size of the newly synthesised connective tissue and epidermal layers. The administration of epidermal growth factor alone or alongside with platelet-derived growth factor give rise to the thickening only of the epidermal layer. This result point toward the synergistic actions of other factors with platelet-derived growth factor are essential in the modulation of wound healing process (Foey *et al.*, 2001;Nadeem *et al.*, 2004).

Since platelet-derived growth factor can chemotactically attract, activate, and initiate new expression of quiescent genes, like the small inducible gene, it satisfies the expectations of a candidate, wound hormone. Platelet-derived growth factor added directly to experimental wounds in animals enhances wound healing (Rozman and Bolta, 2007).Cytokines can accelerate wound healing, when applied topically. Hence, in patients with burn wounds it permits early re-harvest of skin by clinicians (Gillitzer, and Goebeler, 2001; Broughto *et al.,* 2006).

In wound healing immunological functions are exhibit by macrophages which are important for well-ordered wound repair. They function as antigen-presenting cells, as phagocytes and are in particular an essential reservoir of growth factors, their influx into the wound site is after 24 hours. Endothelial cells perform two major roles during wound healing: First, they mediate and regulate the recruitment of leukocytes and, second, they form new buds during wound repair, which ascend from the pre-existing micro-vasculature (Gillitzer, and Goebeler, 2001; Broughto *et al.,* 2006).In the heamostasis phase during the formation of the initial blood clot, platelet-derived growth factor (PDGF) is released by platelets. This low-weight molecular protein attracts, recruit, and activate additional macrophages which arrive at the wound site to orchestrate wound healing. (Mustoe *et al.*, 2002; MacCornick *et al.*, 2014). The use of serum measurements of cytokines and chemokines is not reflective of confined surgical wound biochemical events; rather only specify a comprehensive systemic reaction to surgical injury or procedure (Carvalho *et al.*, 2012).

2.6 Factors Affecting Wound Healing Process

The factors affecting wound healing process can be generally classified into two, the local and systemic factors. Those factors that directly influences the immediate wound environment are classified as the local factors, while the general health of the individual distracting his capability to heal are classified as the systemic factors (Guo and DiPietro, 2010; Anderson and Hamm 2012; Gupta and Kumar, 2015).

2.6.1 Local factors that influence wound healing process

2.6.1.1 Oxygenation

Oxygen is an essential element needed for cell metabolism, particularly in the production of energy by means of adenosine triphosphate (ATP), and is critical for virtually all wound-healing processes. It inhibits wound infection, induces and regulates angiogenesis, enhances keratinocyte differentiation, migration, and re-epithelialisation, enhances fibroblast multiplication and collagen production, and also promotes wound-contraction

(Bishop, 2008;Rodriguez*et al.,* 2008; Anderson and Hamm, 2012). In addition, the level of superoxide production is dependent on the degree of oxygen supply to the polymorphonuclear leucocytes. The early stage of wound healing is depleted of oxygen at the wound site and is quite hypoxic due to vascular disruption and high oxygen consumption by metabolically active cells in the microenvironment. Therefore, healing is impaired in wounds where oxygenation at such site is not restored (Labler *et al*., 2006; Anderson and Hamm, 2012).

2.6.1.2 Infection

Injuries to the skin offers an unhindered accessed for micro-flora and other micro-organisms resident on the skin surface to gain right of entry to the underlying tissues. The state of micro-organisms determines, whether the wound is classified as having contamination, local infection/critical colonisation, and/or spreading invasive infection. Contamination is the existence of non-multiplying micro-organisms on a wound. Local infection/critical colonisation is an in-between stage, where multiplication of micro-organism is taking place with an unset of limited tissue reactions. Invasive infection is defined as the occurrence of multiplying microbes contained by a wound with succeeding tissue injury (Edwards and Harding, 2004; Anderson and Hamm, 2012). The contaminating micro-organisms are commonly controlled or removed during one of the most important phases of wound healing, the inflammatory phase. However, inflammatory phase may be protracted in the absence of operational decontamination, in the event of such, acute wound advances to chronic state (Edwards and Harding, 2004; Bjarnsholt*et al.,* 2008). Wound infection naturally will impede the wound healing process. Although, no sterile wound exists, all wounds hold some level of microbes that usually does not influence the healing process. Infected wound is generally characterised by reddening of the skin around the wound, discharges containing pus, swelling, warmth, foul odour and fever.The aged are more at risk of wound-related infections (Hunt *et al.*, 2000; Wild*et al.*, 2010).

2.6.2 Systemic factors that influence wound healing process

2.6.2.1 Age

In the aged and healthy, a temporal delay in wound healing is experienced due to aging, but not an actual diminishing of wound healing in terms of the superiority of healing (Gosain and DiPietro, 2004;Keylock*et al.*, 2008). In the aged, it has been established that, there is altered platelet aggregation, increased secretion of inflammatory mediators, delayed infiltration of macrophages and lymphocytes, reduced macrophage function, decreased secretion of growth factors, hindered re-epithelialisation, delayed neovascularisation and collagen deposition, reduced collagen turnover and remodelling, and decreased wound strength (Gosain and DiPietro, 2004).

Interestingly, exercise has been reported to improve cutaneous wound healing in the aged, and the improvement is accompanied by decreased concentration of pro-inflammatory cytokines like IL-1, IL-6, and tumour necrosis factor (Emery*et al.*, 2005; Keylock*et al.,* 2008). Fibronectin accumulates in the extracellular matrix environs owing to an increase in the rate of its production with age, its physical nature is also altered, its' cell-adhesive properties decreased so also skin's flexibility and Langerhans cells. This deters the development of granulation tissue formation as a result also a decline in fibroblast population and collagen deposit (Hunt *et al.,* 2000; Enoch and Price, 2004). Some authors, however, reported that ageing does not affect collagen synthesis (Holt *et al.,* 1992; Zulkowski and Albrecht, 2003; Stuart and Price, 2010).

2.6.2.2 Stress

Stress had been established to have a great influence on health and social behaviour of all species. Psychological stress causes a significant delay in wound healing, owing to some research studies conducted in both humans and animals. Under stress conditions glucocorticoids is up-regulates, while pro-inflammatory cytokines IL-1β, IL-6, and TNF-α concentration at the wound site is reduced (Boyapati and Wang, 2007; Godbout and Glaser, 2006). Furthermore, significant delay in healing process as a result of an impaired systematic cell-mediated immunity, caused by psychological stress was also documented (Godbout and Glaser, 2006; Anderson and Hamm, 2012).

2.6.2.3 Medications

Wound healing is negatively affected by the administration of some medications, especially those which interfere with clot formation, platelet function, inflammatory responses, or cell proliferation. The use of steroid parenterally interferes with wound healing resulting in an incomplete granulation tissue formation and reduced wound contraction. They also interfere with the synthesis of hypoxia-inducible factor-1 (HIF-1), a vital transcriptional factor in healing wounds, and were known to increase the chances of wound infection (Franz *et al.,* 2007; Wagner *et al.,* 2008). Studies have shown that low-dosage of steroid applied topically in wound management accelerate healing, reduce pain, exudate, and suppress hyper-granulation tissue formation. Although positive effects are striking and encouraging, extensive administration of steroids must be avoided (Hofman *et al.,* 2007; Anderson and Hamm, 2012). The use of non-steroidal anti-inflammatory drugs (NSAIDs) are associated with non-replicative effect of cells in wound healing, resulting in diminished numbers of fibroblasts, weakened tensile strength, reduced wound contraction, impaired epithelialisation and decreased neovascularisation (Krischak *et al.,* 2007; Price *et al.*, 2007; Anderson and Hamm, 2012). Most chemotherapeutic drugs inhibit cellular metabolism, rapid cell division, and angiogenesis, thus, inhibit many pathways that are critical for appropriate wound repair. These medications inhibit protein synthesis, resulting in decreased fibroplasia, neovascularisation, collagen production, contraction of wounds, delay wound matrix formation and cell migration into the wound site (Franz *et al.,* 2007). In addition, these agents weaken the immune functions and increase the chances of wound infection (Scappaticci *et al.,* 2005; Scott, 2007; Lemmens *et al.,* 2008; Anderson and Hamm, 2012).

2.6.2.4 Obesity

Obesity is well-known from studies to deteriorate many diseases and health conditions; it interferes with wound healing and increase incidence of complications in wound healing (Greco *et al.,* 2008; Momeni *et al.*, 2009). Relative hypoperfusion and ischemia occurs in subcutaneous adipose tissue of the obese, hence, account for the complications obtainable in the obese (Wilson and Clark, 2004; Anaya and Dellinger, 2006). Furthermore, reduced immunological response, stress, anxiety, and depression, all of which are associated or connected with obesity (Juge-Aubry *et al.,* 2005; Fontana *et al.,* 2007; de Mello *et al.,* 2008; Wozniak *et al.,* 2009).

2.6.2.5 Nutrition

Wound management is a multidisciplinary field of medicine of which, one of the fundamentals is nutrition. The role played by nutrition can be obvious even with a superficial wound. Wound healing process is naturally delayed, inhibited or even complicated in the event of malnutrition. The important and necessary nutritional constituents with regards to wound healing include, protein, carbohydrates, fat, and vitamins (Arnold and Barbul, 2006; Woodward *et al.,* 2010; Anderson and Hamm, 2012; Chamanga, 2015; Johnson, 2015).

2.6.2.5.1 Protein: Inadequate or depleted protein influences wound healing negatively (Arnold and Barbul, 2006; Campos *et al.,* 2008). A deficiency in this polypeptide impairs neovascularisation, fibroblast multiplication, proteoglycan production, collagen production, wound remodelling phase and immunodeficiency. For example in the absence of a polypeptide arginine, modulation of immune function, wound healing, hormone secretion, vascular tone, and endothelial function are affected since it is necessary in stress conditions. Collagen deposition is directly dependant on the availability of proline, of which the precursor of proline is the amino acid, arginine (Shepherd, 2003; Tong and Barbul, 2004; Campos *et al.,* 2008). Monitoring of serum protein is vital in wound management, specifically with frequent change of dressing since protein is lost via wound exudate. In cases where consistent wound dressing is obligatory, protein supplementation or replacement must be prepared for optimal maintenance and repair of wounds (Woodward *et al.*, 2010; Chamanga, 2015; Johnson, 2015).

2.6.2.5.2Carbohydrates and fats: These nutrients with regards to wound healing process are the main-stay and supply energy for ideal cellular response (Shepherd, 2003; Heyman *et al.,* 2008; Anderson and Hamm, 2012). Adenosine triphosphate derived from glucose is the fuel needed for day-to-day cellular activities and in tissue repair. In the case of unsatisfactory carbohydrate supply, gluconeogenesis is initiated to provide energy through non-glucose source for cellular activities. Collagen production is the most important energy demanding activities in wound healing process (Chamanga, 2015; Johnson, 2015; Rabess, 2015).

In view of this, adequate supply of fat is required to avoid the host from utilising protein for energy (Woodward *et al.*, 2010). Essential fatty acids play key role in wound repair, for the reason that they are the key constituents of cell-membranes, therefore, fatty acid demand increases after injury. In surgical or critically-ill patients fatty acids supplements are administered, to help meet energy demands and provide essential building blocks, for wound healing and tissue repair. The supplement maybe necessary in view that, some fatty acids like the omega-3 fatty acids cannot be synthesised by mammals, hence must be supplemented (da Costa *et al.*, 2003; Heyman *et al.*, 2008; McDaniel *et al.,* 2008; Shingel *et al.,* 2008; Anderson and Hamm, 2012).

2.6.2.5.3 Vitamins and micronutrients: Vitamins C (L-ascorbic acid), A (retinol), and E (tocopherol) show potent anti-oxidant and anti-inflammatory effects. Vitamin C plays vital roles in the repair and regenerative phase of wound healing. It also plays key role in the synthesis of collagen, their subsequent crosslinking and replacement, fibroblast multiplication, neovascularisation and prevention of capillary breakability. Deficiency of vitamin C leads to an impaired immune response, thus, increase chances of wound infection (Arnold and Barbul, 2006; Campos *et al.,* 2008; Minka and Ayo, 2011). Vitamin C also has important antioxidant properties that enhances the function of the immune system, reduce susceptibility to wound infection as well as increases the absorption of iron, which is important as a cofactor in collagen synthesis (Minka and Ayo, 2011; Chamanga, 2015; Johnson, 2015; Rabess, 2015). Vitamin A is also an anti-oxidant with the following biological activities with regards to wound healing, increased fibroblast multiplication, modulation of cellular differentiation and replication, increase collagen production and its cross-linking, hyaluronate production, and decreased matrix metalloproteinase-mediated extracellular matrix digestion (Witte and Barbul, 2002; Burgess, 2008; Woodward *et al.*, 2010).

B-Complex vitamins are major co-factors or co-enzymes in the energy production cycle release from carbohydrates (Chamanga, 2015; Johnson, 2015; Rabess, 2015). Vitamin E, is also an anti-oxidant, that function majorly in the maintenance and stabilisation of cell membrane integrity. It also plays a role as an anti-inflammatory agent and has been suggested to function in reducing excessive scar tissue formation. (Arnold and Barbul, 2006; Khoosal and Goldman, 2006; Burgess, 2008). Vitamin K is a major component in the formation of thrombin which is essential in clot formation, its deficiency in the presence of wounds could lead to haematoma (Chamanga, 2015; Johnson, 2015).

Several micronutrients have been shown to be essential in wound healing and for optimal tissue repair. Magnesium functions as a co-factor for numerous enzymes, implicated in protein and collagen production, while copper is a co-factor for cytochrome oxidase, for cytosolic anti-oxidant superoxide dismutase, and for optimal cross-linking of collagen. Zinc is a co-factor for both RNA and DNA polymerase. Iron is obligatory for the hydroxylation of proline and lysine (Shepherd, 2003; Arnold and Barbul, 2006; Campos *et al.,* 2008; Anderson and Hamm, 2012).

2.6.2.6 Hydration

Water intake for rehydration is imperative in wound healing to prevent dehydration. Dehydration is usually reflective on the skin tissue. It results in skin that is less elastic, more delicate and more susceptible to breakdown. Dehydration also reduces effectiveness of blood circulation, which will interfere with the supply of oxygen and nutrients to the wound site. The major risk factor for dehydration in patients is poor oral fluid consumption (Woodward *et al.,* 2010).

2.7 Steroids

2.7.1 Definition of steroids

Steroid is a collection of naturally occurring or synthetic fat-soluble organic compounds (lipids), whose structure is chemically based on a steroid nucleus, that is, a hydrogenated cyclopentophenanthrene ring nucleus of 17 carbon atoms arranged in four rings, composed of three 6-member rings and one 5-member ring. The substitution of the hydrogen molecule in cyclopentophenanthrene ring produces generates a different drug activities. Furthermore, a number of drugs, body constituents and most hormones are steroids (Shaikh *et al.*, 2012; Han, 2015).

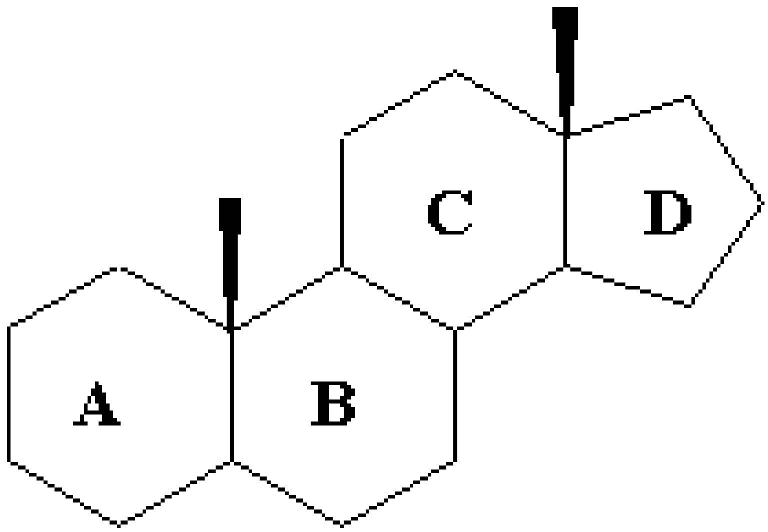


Figure 2.2: Basic Structure of the Steroid Ring

(Source: Shaikh *et al.*, 2012)

2.7.2 Steroids and wounds

Busti *et al.* (2005) stated that, in the perioperative setting drugs like the steroids thatmodifies inflammation and immune responses are essential for appropriate wound healing process. Furthermore, side effect of propofol injection, that is pains during synovial effusion and joint pains can be ameliorated using methylprednisolone (de Souza, 2016; Shivanna *et al*., 2016).Methylprednisolone is recommended for the control of oedema, increase in collagen fibers and intact nerve integrity, in the healing of traumatic facial nerve paralysis (Tuncay *et al.*, 2016; Tunçcan *et al*., 2016). It inhibits post-surgical stress (nausea and fatigue), augments outcome with less need for later analgesic and convalescence (Sauerland *et al.,* 2000; Bjerregaard*et al*., 2017). For surgical deletion of jammed mandibular third molars, sub-mucosal injection of methylprednisolone pre-operatively is simple, safe, and effective in the control of swelling, pain, and trismus (Micó-Llorens *et al*., 2006; Chugh *et al.,* 2017).

Methylprednisolone is the standard pharmacological care in the management of acute spinal cord injury (Hurlbert, *et al.,* 2013; Chikuda *et al.*, 2014; Toma *et al*., 2014) it also prevents post-pericardiotomy syndrome by controlling pericardial and pleural oedema (Seyuk *et al*., 2016). Steroid therapy results in an increase in the quantity of circulating neutrophils, but a decrease in the number of eosinophil (Haul, 2010). In patients that undergo total laryngectomy, neutropenia and neutrophil dysfunction have been observed to play a role in poor healing (Bosanquet*et al.,* 2013). However, Colin *et al*. (2017) reported that, methylprednisolone does not reduce delirium nor enhances the quality of recovery in cardiac surgeries. Woodward *et al.* (2010) observed a significant decrease in wound fluid, cytokine and hydroxyproline content of tissue, in patients receiving steroid treatment. Steroids and retinoids have antagonistic effects on cytokines and collagen deposition. This effect can be relevant for treatment options in a clinical setting (Wicke *et al.,* 2000). For instance, increased collagen expression by humandermal fibroblasts occurs in response to steroid treatment in a dose- and time-dependent fashion of up to 8-fold increase in collagen synthesis (El-Okdi*et al.,* 2008). It is well known that corticosteroids inhibit collagen synthesis, neo-capillary budding, thereby retard wound healing when given prior to, or soon after, injury. Vitamin A may reverse this potentially serious side-effect of corticosteroids, while preserving their beneficial action.Wound healing can be impaired by a single subcutaneous injection of 6 mg of methylprednisolone acetate (Busti *et al.*, 2005).

Steroids interfere with inflammation, fibroblast multiplication, collagen production and degradation, deposition of connective tissue ground substances, neovascularisation, wound contraction, and epithelialisation. Consequently, they cause dehiscence of surgical incisions, increased risk of wound infection and delayed healing of open wounds. Anabolic steroids reportedly play crucial role in the body's capability to heal. This is a very interesting observation for people at the marginal end of health and the elderly, since anabolic steroid therapy combined with increased protein intake has been successful in promoting weight gain, reversing catabolism, and increasing the rate of wound closure (Salcido, 1999; Ani, 2010). Steroids effectively decrease the incidence of nauseaand vomiting with a single dose and significantly reduce collagenisation, epithelisation, and fibroblastcontent (Durmus*et al.,* 2003).In females, the decline in the effectiveness of skin repair mechanisms follows menopause, and a series of clinical studies has identified oestrogens as an endogenous enhancer of the healing processes (Gilliver and Ashcroft, 2007).

2.7.3 Physiological and clinical uses of steroids

2.7.3.1 Physiological role of steroids

The body naturally inclines to adjust to stress, whether physical or neurological through an immediate and noticeable increase in adrenocorticotrophic hormone secretion. One possible reason for the secretion of steroids, is that, glucocorticoids enhances immediate mobilisation of amino acids and fat from their cellular stores, making them readily available both for energy and for synthesis of other biological compounds, like the glucose needed by different tissues of the body. When steroid is produced or administered into the body, it has two basic anti-inflammatory effects: (1) it can inhibits the early phase of inflammatory process before the onset of the inflammation; and (2) if the inflammatory process is in progress, it leads to speedy resolution of the inflammation (Shimada*et al.*, 2000; Charmandari *et al.*, 2004; Grover *et al.*, 2007). Steroids hence, prolong the latent phase of wound healing, slow the rate and decrease the total amount of contracture: corticosteroids impair collagen turn-over, disrupt dermal–epidermal junctional interactions, and decrease the tensile strength of cutaneous wounds by reducing collagen accumulation (Wang *et al.*, 2013). Specifically, steroids: (1) stabilise the lysosomal membranes; (2) reduces permeability of the capillaries; (3) inhibits both migration of white blood cells into the inflamed area and phagocytosis of the damaged cells; (4) suppress the immune system, causing decline in lymphocyte production; and (5) inhibits fever mainly because they reduce the release of IL-1 from the white blood cells (Grover *et al*., 2007).

2.7.3.2 Clinical uses of steroids

The effects of steroids are numerous not excluding alterations in carbohydrate, protein and lipid metabolism; maintenance of fluid and electrolyte balance; and preservation of normal function of the cardiovascular system, the immune system, the kidneys, skeletal muscle, the endocrine system, and the nervous system (Shaikh *et al.,* 2012). Steroids are recommended in reduction or prevention of cerebral oedema. Generally vasogenic oedema can be reduced or prevented with the use of steroids which likely functions through the following mechanism: (i) stabilisation of cerebral endothelium; (ii) increase in lysosomal activity of cerebral capillaries; (iii) inhibition of release of potentially toxic substances such as free radicals, fatty acids, and prostaglandins; (iv) electrolyte shifts favouring transcapillary efflux of fluid; and (v) increase of local and global cerebral glucose use, leading to improved neuronal function (Shaikh *et al.,* 2012). Post-surgical complications can be reduced with the administration of steroids like methylprednisolone which is safe and effective (Chappi*et al.,* 2015).

In a stressful circumstances steroids are known to endow the individual with the ability to resist stress. The amplifying and maintenance of pain perception via production of a number of inflammatory mediators in stress is decreased, by the anti-inflammatory activities of steroids. Steroids are also used to prolong the duration and action of local anaesthetic. Their use enhances the activities of vasopressor drugs to sustain cardiovascular homeostasis, thereby, reducing vasopressor dosage in stress. In mild forms of allergy, antihistamines are the first choice of drugs. In very severe forms, steroids like methylprednisolone are recommended (Shaikh *et al.*, 2012). With the advances in surgery the application of steroid therapy, during perioperative period for various purposes is growing. Some of the recent indications are: 1. perioperative replacement therapy in adrenal insufficiency; 2. anti-inflammatory uses and hyper-reactive airway; 3. post-operative nausea and vomiting; 4. analgesia adjunct; 5. Dental surgeries; 6. Day-care surgery; 7. anaphylaxis; 8. Ophthalmic surgeries; 9. septic shock; and 10. other indications, which include cerebral oedema, and spinal cord injury (Carlos, 2007; Spiriev *et al*., 2014).

Steroids are one of the most commonly used drugs and efficient treatments for the management of inflammatory and autoimmune conditions. They are also used in the management of various dermatologic, ophthalmologic, rheumatologic, pulmonary, hematologic, and gastrointestinal disorders. Despite their beneficial effects, prolonged oral or parenteral administration is discouraged due to its immunosuppressive activities (Liu *et al.*, 2013). Steroids decrease the incidence of post-operative nausea and vomiting, postoperative pain and, enhances early oral intake. They produce euphoric effect by decreasing level of prostaglandins, and elevating those of endorphins. Steroids are recommended for the management of hypoglycaemia and hypercalcaemia. They induce cell growth, cell specialisation and even cell death; thus allowing their use in the treatment of tumors, especially haematopoietic lineage tumors. They also play central roles in the management of diseases involving immune and inflammatory mechanisms (Carlos, 2007). Steroids are recommended in the management of idiopathic facial nerve paralysis (Sevuk*et al.,* 2014; Tunçcan *et al*., 2016). However, Póvoa*et al.* (2015) observed that the administration of intravenous steroids for the management of septic shock is not advantageous.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Experimental animals

Twenty four (24) New Zealand White rabbits of nine–months to one-year old of both sexes were used for the study in the month of October, 2016 to May, 2017. The rabbits were purchased from National Animal Production and Research Institute (NAPRI), Shika Zaria, Nigeria. The rabbits weighed between 1.5–2 kg.

3.1.2 Drugs and consumables

The drugs used in this study included: steroid [methylprednisolone (Pfizer)]; epidermal growth factor (lyophilised powder) Abcam ab9697; platelet-derived growth factor (lyophilised powder) Abcam ab61903; pre-aneasthetic agents [atropine sulphate (Pauco pharmaceutical Anambra, Nigeria) and chlorpromazine hydrochloride (Hubei Tianyao pharmaceutical limited Hubei, China)]; anaesthetic agents [ketamine hydrochloride (Rotex medica Trittau, Germany) and lidocaine hydrochloride (El-salmat pharmaceutical Kwara, Nigeria)]; chlorhexidine [purit® (Saro lifecare limited Ibadan, Nigeria)] and methylated spirit (El-salmat pharmaceutical Kwara, Nigeria). Others were epidermal growth factor enzyme-linked immunosorbent assay (ELISA) kit abcam 100504; platelet derived growth factor enzyme-linked immunosorbent assay (ELISA) kit abcam ab100623 (Abcam manual, 2017). Others were as shown in Plates I.

Plate I: Materials used for Enzyme-Linked Immunosorbent Assay (ELISA) in the Study

Key: A = Serum bottle; B = Washing bottle; C = Examination glove; D = Platelet derived growth factor ELISA kit; E = Epidermal growth factor ELISA kit; F = Multi-channel micro-pippete litre; G = Distilled water; H = Measuring cylinder; I = Micro-pippete litre; J = Multi-channel micro-pippete litre bath

3.1.3 Surgical materials

The materials used in creating the wounds on the experimental animals included: wound template (autoclaved exposed X-ray film); syringes and needles [1 mL, and 2 mL syringes with 23 and 21 gauge needles (El-salmat pharmaceutical Kwara, Nigeria)] for drug administration and indelible marker. Other surgical materials used were, surgical gloves (Agary pharmaceutical limited Hubei, China), scalpel blade (Divine care, Anambra, Nigeria), scalpel holder, needle holder, thumb forceps and haemostatic forceps, towel clamp, rat-tooth forceps, sterile gauze (Yangzhou super union medical material limited Jiangsu province, China) and drape-set.

3.1.4 Dressing materials

The dressing materials for the study were: normal saline (Unique pharmaceutical limited Ogun, Nigeria), adhesive gauze (Yangzhou super union medical material limited Jiangsu province, China), vaseline gauze (Faisal pharmaceutical industries Faisalabad, Pakistan), absorbent cotton (Shaoxing fuqing health products limited Shaoxing, China), crêpe bandage (El-salmat pharmaceutical Kwara, Nigeria), disposable gloves (Supermax glove manufacturing SDN BHD Selangor, Malaysia), adhesive plaster (Anji Jixiang medicals limited Zhe Jiang China), sterilised gauze (El-salmat pharmaceutical Kwara, Nigeria), purit® (Saro lifecare limited), thumb forceps and micro-pippite litre. Others were as shown on Plate II.

Plate II: Wound Dressing Materials used in the Study

Key: A = Exercise book; B = Normal saline; C = Crĕpe bandage; D = Adhesive plaster; E = Vaseline guaze; F = Sterile guaze; G = Crêpe bandage on experimental animal; H = flexible meter rule; I = kidney dish with diluted purit; J = Towel for restrain; K = Dressing table; L = Pen; M = Exammination gloves; N = Ruler.

3.2 Methodology

3.2.1 Pre-surgical checks

Twenty four rabbits aged between nine months to one year old were purchased from NAPRI and housed in the laboratory animal unit of the Department of Veterinary Parasitology and entomology Ahmadu Bello University (ABU), Zaria. The rabbits were evaluated clinically, blood samples were evaluated in the Veterinary Clinical Pathology and Veterinary Protozoalogy Laboratories. Results from the laboratories showed that the animals' blood picture were within normal haemogram, and no blood parasite was seen. Faecal samples were also screened in the Veterinary Helminthology Laboratory, and no helminth egg or oocyte was seen.

The New Zealand rabbits were allowed to acclimatise for two weeks, where they were provided with feed and water *adlibitum,* throughout the period of the experiment. The feed used in this experiment was a 'Grand Cereals' product, Pelletised Grower Vital® Feed, Jos, Nigeria containing cereals/grains, vegetable protein, premix (vitamins/minerals), essential amino acids, salt, antioxidant, antitoxins, prebiotics and enzymes. Its composition was as follows: crude protein 13% (minimal), fat 8% (maximal), crude fibre 18% (maximal), calcium 0.9% (minimal), available phosphorus 0.35% (minimal), and metabolisable energy 2600 kcal/kg (minimal). The rabbits were randomly grouped into two major groups (1 and 2) of 12 rabbits each; and, subsequently, into four sub-groups each (1A, 1B, 1C and 1D; 2A, 2B, 2C and 2D) of three rabbits each; that is, a total of eight sub-groups were used in the study.

3.2.2 Pre-surgical procedures

The New Zealand rabbits were deprived of feed and water for 12 and 6 hours, respectively before surgery. The dorsum (site of wounding) was shaved on the same day of the surgery. The animals were pre-anaesthetised with atropine sulphate at 0.2 mg/kg IM and chlorpromazine at 10 mg/kg IM. The drugs were allowed to take effect in the animals for 5 minutes. Ketamine hydrochloride was used to anaesthetise the animal at the dose rate of 40 mg/kg IM. The dorsum of the rabbits were cleaned and aseptically prepared using Purit®(Chlorhexidine gluconate B.P 0.3%w/v, Cetrimide B.P 3.05 w/v) and alcohol at 1:6 ratio as recommended by the manufacturers. The animals were scrubbed, placed on the surgical table on lateral recumbency and rectangularly draped as presented in Plate III.

3.2.3 Cytokine reconstitution

The cytokines used in this study were acquired from the University of Cambridge in England, Britain (Abcam). Seventy micro-gram of PDGF protein and 500 microgram of EGF protein were purchased which were packaged as lyophillised powder. They were subsequently reconstituted with 720 µL of normal saline to obtain an appropriate volume needed for the study. The PDGF protein was reconstituted to a concentration of 0.09722 µg/µL, which is equivalent to 97.22 mg/ml. Each wound on an animal in group 1B, 1D, 2B and 2D were treated with 2.92 µg of PDGF, that is, 5.84 µg per animal. Subsequently, EGF was also reconstituted to 0.69444 µg/µL, which is equivalent to 694.44 mg/mL. Each wound on an animal in group 1A, 1D, 2A and 2D were treated with 20.8 µg of EGF, that is, 41.6 µg per animal. A micro-pippite litre was used for the reconstitution.

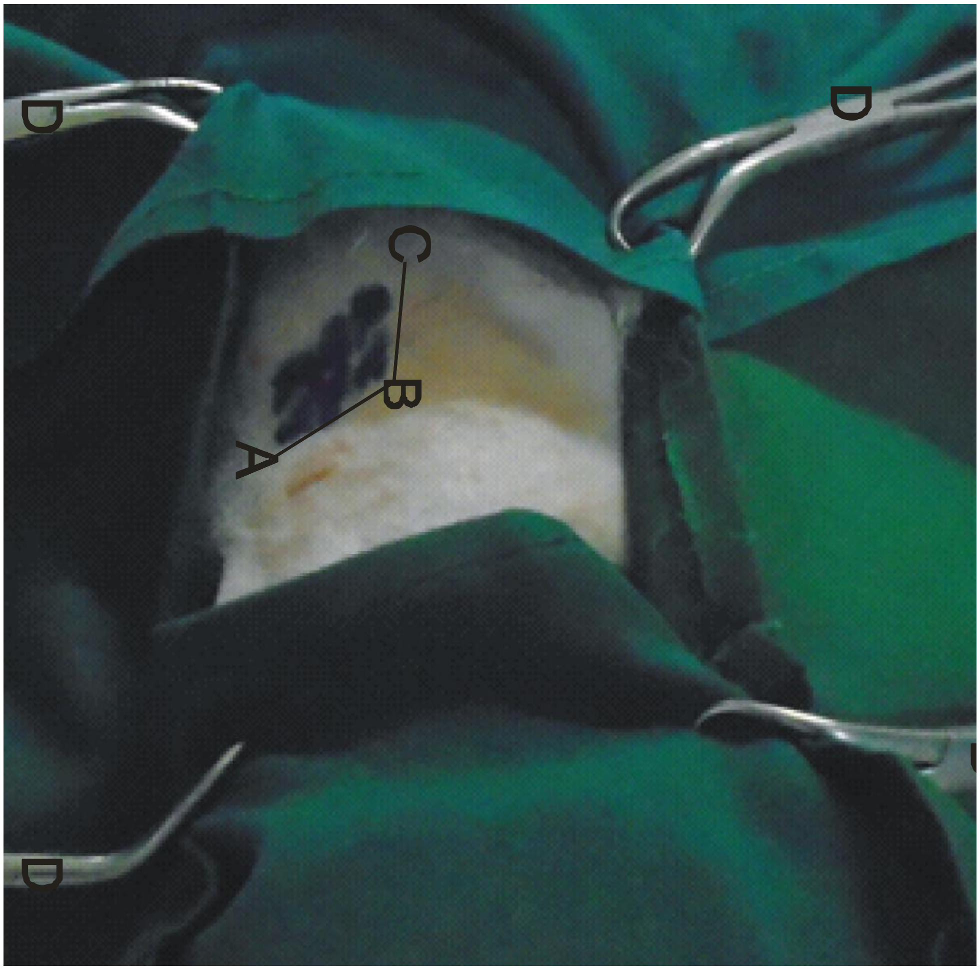


Plate III: A Rabbit on Lateral Recumbency, Draped Rectangularly, and Wounding site Shaded with an Indelible Marker

Key: AB = 2cm; BC = 1cm; D = Towel clamps

3.2.4 Wounding procedure

A wound template for creation of 2-cm × 1-cm wound area was prepared using an exposed X-ray film, sterilised in an autoclave. The rabbits were then taken for wounding in the surgery room, in the research and teaching wet-lab of the Department of Veterinary Surgery and Radiology ABU Zaria. Placing the wound template on the anaesthetised animal, an indelible marker was used with the template to mark a 2-cm by 1-cm wounding area. The full skin excision was achieved using scalpel blade and a sharp–blunt scissors with the aid of a rat-tooth forceps. A 2-cm × 1-cm full skin thickness was then excised as already marked on each side of the dorsum (Plate IV). Bleeders were controlled by applied digital pressure using sterile gauze. Two wounds of 2-cm × 1-cm were created, one on each side of the dorsum. This was done for all the animals used in this study. The wounds were then treated and dressed according to the grouping (Table 3.1).

3.2.5 Treatment groups

Group 1 and 2 were treated accordingly; group 1 methylprednisolone (methylP) group were injected with methylprednisolone at dose rate of 0.5 mg/kg intramuscularly, while the group 2, the normal saline (NS) group, were given 0.5 mL of normal saline intramuscularly respectively, immediately after wounding as shown in Table 3.1. The wounds were treated as grouped. The epidermal growth factor (EGF) groups were treated topically with epidermal growth factor protein (20.8 µg) while the platelet–derived growth factor (PDGF) group were topically treated with platelet–derived growth factor protein (2.92 µg). The normal saline group were treated with normal saline topically, all at the sub-group units, immediately after wounding. Each wound was smeared topically and appropriately using a micro-pipette litre, the treatment groups and experimental design were as shown in Table 3.1.

Table 3.1: Treatment groups (Experimental design)

Groupings (PT)	Sub-groupings	Treatment (TT)	Number of animals n=3
Group 1	1A	EGF (20.8 µg)	3
(Methylprednisolone	1B	PDGF (2.92 µg)	3
0.5 mg/kg. IM)	1C	Normal saline (30 µL)	3
	1D	EGF (20.8µg) + PDGF (2.92 µg)	3
Group 2	2A	EGF (20.8 µg)	3
(Normal saline	2B	PDGF (2.92 µg)	3
0.5 mL. IM)	2C	Normal saline (30 µL)	3
	2D	EGF (20.8 µg) + PDGF (2.92 µg)	3

KEY
PT= Parenteral treatment; TT.= Topical treatment; IM= Intramuscular; EGF= Epidermal growth factor; PDGF=Platelet-derived growth factor.

3.2.6 Wound dressing

Immediately after wounding, topical and parenteral treatments were administered accordingly. Vaseline gauze was applied on the wound as indicated in Plate IV, followed by adhesive gauze and all were then held in place with crêpe bandage. To avoid unwinding of the bandage, adhesive plaster was used to keep the crêpe bandage in place. All these were done to avoid interference of the healing process by the animal through scratching or licking and to minimise contamination. Aseptic procedures were adhered to during post-operative wound dressing, and sampling. Wound dressing was done daily for the first seven days post wounding and subsequently on alternate days to reduce interference with the healing process until healing was completed in all the groups on day 16 post-wounding. The wounds were cleaned three to four times at each dressing with sterilised gauze and diluted chlorhexidine (purit®) by gentle damping to avoid bleeding using thumb forceps, this was done for all the wounds, in all the groups. Wound dimensions were taken using measuring tape and camera for documentation.

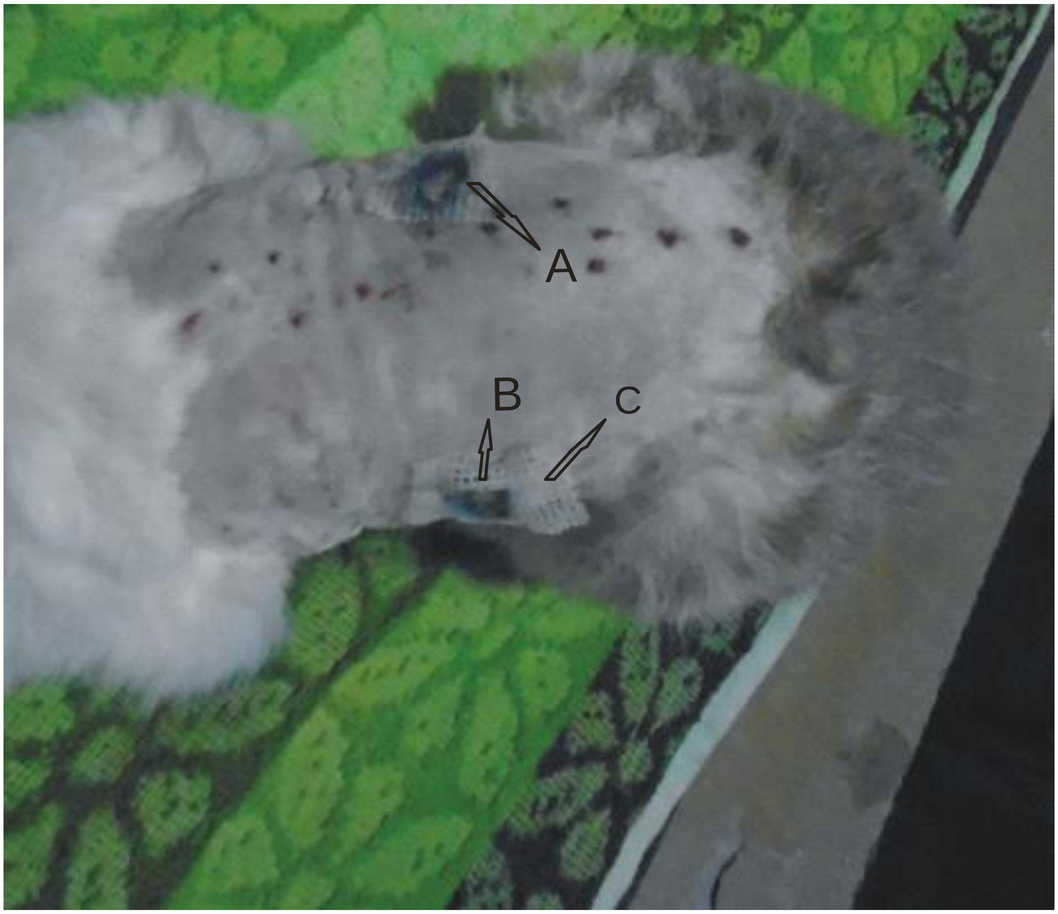


Plate IV: Created Wound on the Dorsum of the New Zealand Rabbits Covered with Vaseline Gauze .

Key: A = 2×1 cm wound on right side of the dorsum; B = 2×1 cm wound on the left side of the dorsum; C = Vaseline gauze

3.2.7 Sample collection

The middle ear-vein was used for blood sampling on days 0, 3, 5, 7, 10 and 12. Five millilitres of blood samples were collected from each animal, for each sampling day using sterile syringe and needle, 2 mL of the blood in heparinised sample bottles for packed cell volume and differential leucocytes count in the haematology laboratory unit of the ABUTH, Zaria, using an auto-analyser, while 3 mL was emptied in non-heparinized (plain) sample bottles, which was slanted on an ice pack for two hours for serum collection. The serum was then decanted into a sterile serum bottle and stored at –30^{0}C for serology (ELISA). The serological analysis was carried-out in the Biotechnology Center of the ABU, Zaria, as described by abcam ELISA kit instruction manual kit human ab100623-PDGF-AB and human ab100504-EGF-AB ELISA kit (Abcam, United kingdom).

Skin biopsy was taken for histopathological evaluation of the wounds on days 0, 3, 7, 12, post-wounding and the scar after complete healing. For skin biopsy, the wound site was anaesthsised using 0.5 mL of lidocaine. A 1-cm × 0.5-cm skin biopsy was taken, 0.5 cm into the wound and 0.5 cm of the surrounding apparently normal skin. The skin biopsy was fixed in sample bottles containing 10 mL of 4% formaldehyde. The samples (skin biopsy) were trimmed; sectioned and histological slides were prepared in the histology laboratory of the Department of Human Anatomy, Ahmadu Bello University (ABU), Zaria as described by Smith and Bruton (1978). Sampling days are summarised in Table 3.2.

Table 3.2: Sampling Days for the New ZealandRabbits used in the Study

Parenteral treatment	Number of rabbits	Days of sample collection	
		Blood sample	Skin biopsy
Steroid (group 1)	n = 12	0, 3, 5, 7, 10 and 12	0, 3, 7, and 12
Normal saline (group 2)	n = 12	0, 3, 5, 7, 10 and 12	0, 3, 7, and 12

3.2.8 Gross evaluation of the wounds

The wounds were assessed for epithelialisation until the wound area was completely covered; and wound contraction was also measured, using a measuring tape until complete healing. Pus formation was also assessed as mild (that is pus present), moderate (pus covered the wound stroma) or high (pus pouring off the wound stroma). Granulation tissue formation was also assessed as mild (slightly pinkish with still some brownish colouration), moderate (slightly pinkish), or severe (pink in colour).

3.2.9 Histological evaluation of wounds

Histological slides were prepared as described by Alturkistani *et al*. (2016). Slides were viewed at magnification of × 250; the wounds were evaluated for severity of the inflammatory cells present as absent, mild (with few inflammatory cells), moderate (average) or severe (diffused). The degree of oedema was also evaluated and scored as mild, moderate or severe. Other variable assessed were neovascularisation, granulation tissue formation, fibroblast, connective tissue like elastin fibre, and collagen deposition.

3.2.10 Wound breaking strength and tensile strength

Tensile strength were measured as described by Lazarus*et al.* (2014) however, with few modification as described below. The scar biopsy was taken alongside with 1 × 0.5 cm normal skin on each side of the scar. The biopsy were kept in normal saline and were immediately assessed. A 2-mm diameter wire was attached at the two ends of the biopsy, 0.5 cm away from the scar line on both ends. One side of the wire was hanged on a drip stand while the other end was attached to the device (algometer). Weight was applied on the algometer until sample breaks and the readings taken. This was also carried-out in the research and teaching wet-lab of the Department of Veterinary Surgery and Radiology ABU Zaria. The tensile strength was calculated as weight required for breaking the sample divided by the scar area (Wen *et al*., 2015).

3.2.11 Ultrasonography

The site surrounding the scar tissue area were shaved and transported to the Diagnostic imaging centre of the Department of Veterinary Surgery and Radiology ABU Zaria where the animal restraint for ultrasonography. The ultrasounds of the scar were evaluated using an ultrasound machine (Sonostar technologies, C5 model for veterinary) at a frequency of 5 HZ in the tissue harmonic mode and doppler, the ultrasonography were recorded.

3.3 Documentation, Analysis and Presentation of Result

Mean values of wound dimensions, rate of epithelialisation, haematology, tensile strength and systemic EGF-AB and PDGF-AB concentrations were subjected to statistical analysis using Graph Pad prism, version 5.0, One-way analysis of variance (ANOVA) was obtained to determine the significance of the result at probability value of 0.05% (95% confidence interval) Probability values of P □ 0.05 were considered significant. Data were presented, using Tables, Line graphs and Bar charts.

CHAPTER FOUR

4.0 RESULTS

4.1 Gross Evaluation of Wound Healing

4.1.1 Wound contraction

The wound contraction monitored as wound healing progressed is shown in Appendix (1). However, Figure 4.1.1 summarises wound contraction as healing progressed. The wounds created on all the animals progressively increased in dimension within the first five days post-wounding. The steroid-treated group recorded peak of values at day 3 post-wounding as follows in a descending order of wound retraction methylprednisolone (methylP) + normal saline (NS) (2.59 cm^2), methylP + EGF + PDGF (2.57 cm^2), methylP + PDGF (2.54 cm^2) and methylP + EGF (2.45 cm^2) respectively. However, within the non-steroid treated groups, peak values for wound retraction were recorded by day 3 in NS + PDGF (3.13 cm^2) and NS + EGF + PDGF (2.56 cm^2), respectively. On day 5, the dimension of wound retraction recorded were NS + NS (3.11 cm^2) and NS + EGF (3.08 cm^2) respectively. Wound contraction was observed from day 5 with the minimal wound size in rabbit treated with methylP + NS closely followed by methylP + PDGF and then methylP + EGF. However by day 7 except for NS + EGF + PDGF, NS + NS, and NS + EGF group, their wound size was less than the size of the initial wound, in descending sequence methylP + EGF + PDGF (1.88 cm^2), methylP + EGF (1.81 cm^2) methylP + NS (1.77 cm^2) and methylP + EGF + PDGF (1.76 cm^2).

Furthermore, wound contraction was more pronounced in all the treated (steroid and non-steroid) groups between days 7 and 12 post-wounding. Maximum wound contraction occurred immediately after complete epithelialisation, in NS + NS (0.39 cm^2) and NS + EGF (0.53 cm^2) at day 14 respectively, methylP + NS (0.25 cm^2) and methylP + EGF + PDGF (0.42 cm^2) at day 16, respectively. By day 18, the contraction size recorded were in the following increasing order, methylP + EGF (0.32 cm^2), methylP + EGF + PDGF (0.43 cm^2), NS + EGF + PDGF (0.54 cm^2), and NS + PDGF (0.56 cm^2) respectively. The wound size in this study recorded more than 70% reduction immediately after complete epithelialisation; as shown in Table 4.1.1, and summarised in Figure 4.1.1.

Table 4.1.1: Scar size and Percentage Wound Contraction inNew ZealandRabbits treated with Epidermal Growth Factor, Platelet-Derived Growth Factor with or without Methylprednisolone

Group	Initial Wound Size (cm^2)	Healed Wound Size (cm^2)	Percentage contraction (%)
MethylP + EGF	2	0.32	84
MethylP + NS	2	0.42	79
MethylP + EGF + PDGF	2	0.43	78.5
MethylP + PDGF	2	0.25	87.5
NS + EGF	2	0.53	73.5
NS + NS	2	0.39	80.5
NS + EGF + PDGF	2	0.56	72
NS + PDGF	2	0.54	73

Key: methylP = Methylprednisolone, NS = Normal saline, EGF = Epidermal growth factor, PDGF = Platelet-derived growth factor

4.1.2 Wound epithelialisation

Complete epithelialisation was achieved when epithelium covered the entire wound surface, from day 14 post-wounding for the NS + NS, NS + EGF and methylP + EGF + PDGF. By day 18 in all the groups, the wound stroma was completely covered with epithelium. The progression in the wound healing was observed to occur predominantly by wound contraction however re-epithelialisation did occurred. By day 5, epithelialisation was observed in methylP + NS, NS + EGF, NS + PDGF, and NS + EGF + PDGF, but it occurred in methylP + EGF, methylP + PDGF, methylP + EGF + PDGF, and NS + NS on day 7 post wounding. Graphical representation of epithelialisation is shown in Figure 4.1.2. The gross pictures were also presented in Plates V – X.

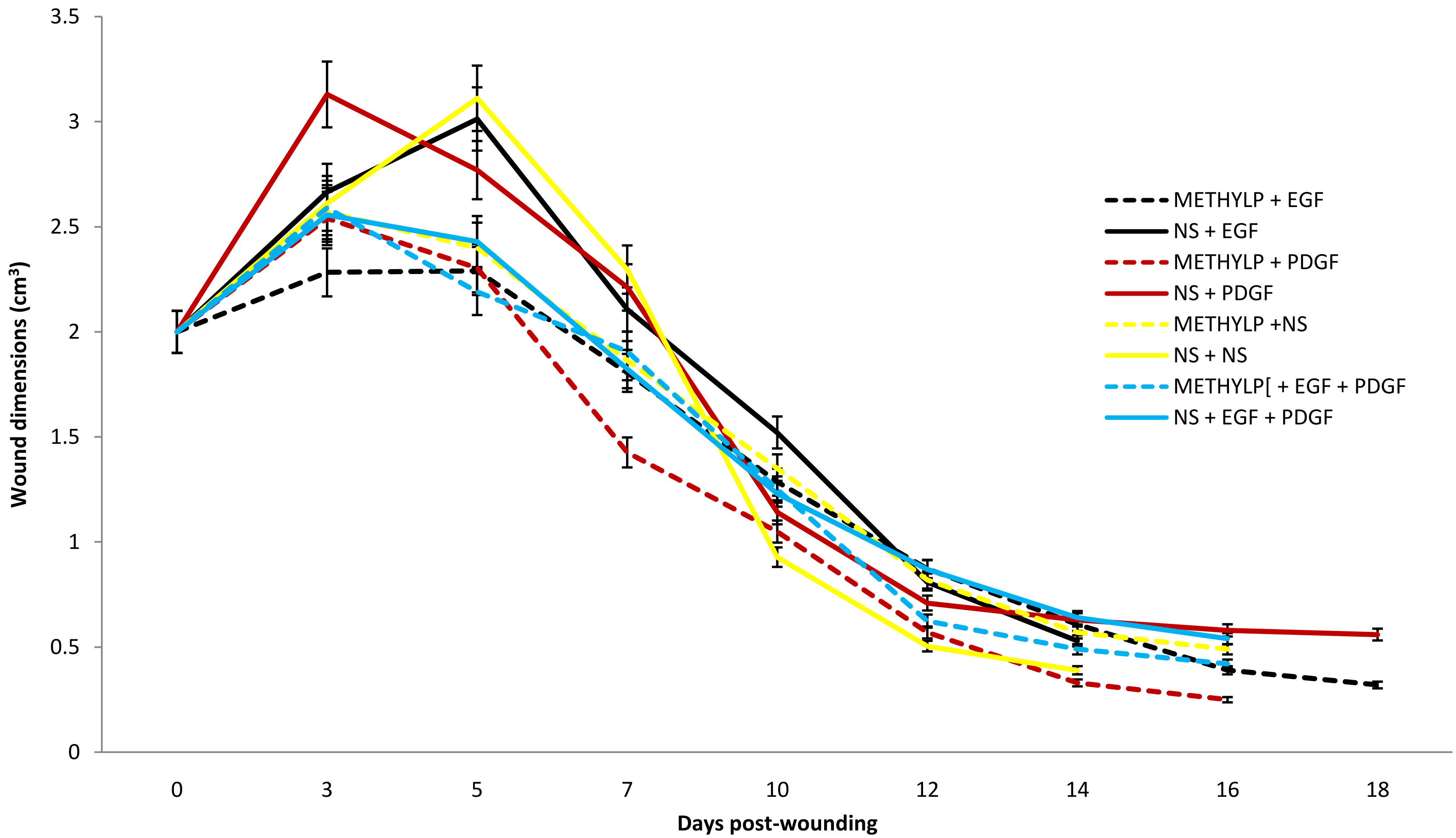


Figure 4.1.1: Effects of Epidermal Growth Factor (EGF-AB), Platelet-Derived Growth Factor (PDGF-AB) with or without Methylprednisolone on Wound Dimensions in New Zealand rabbits

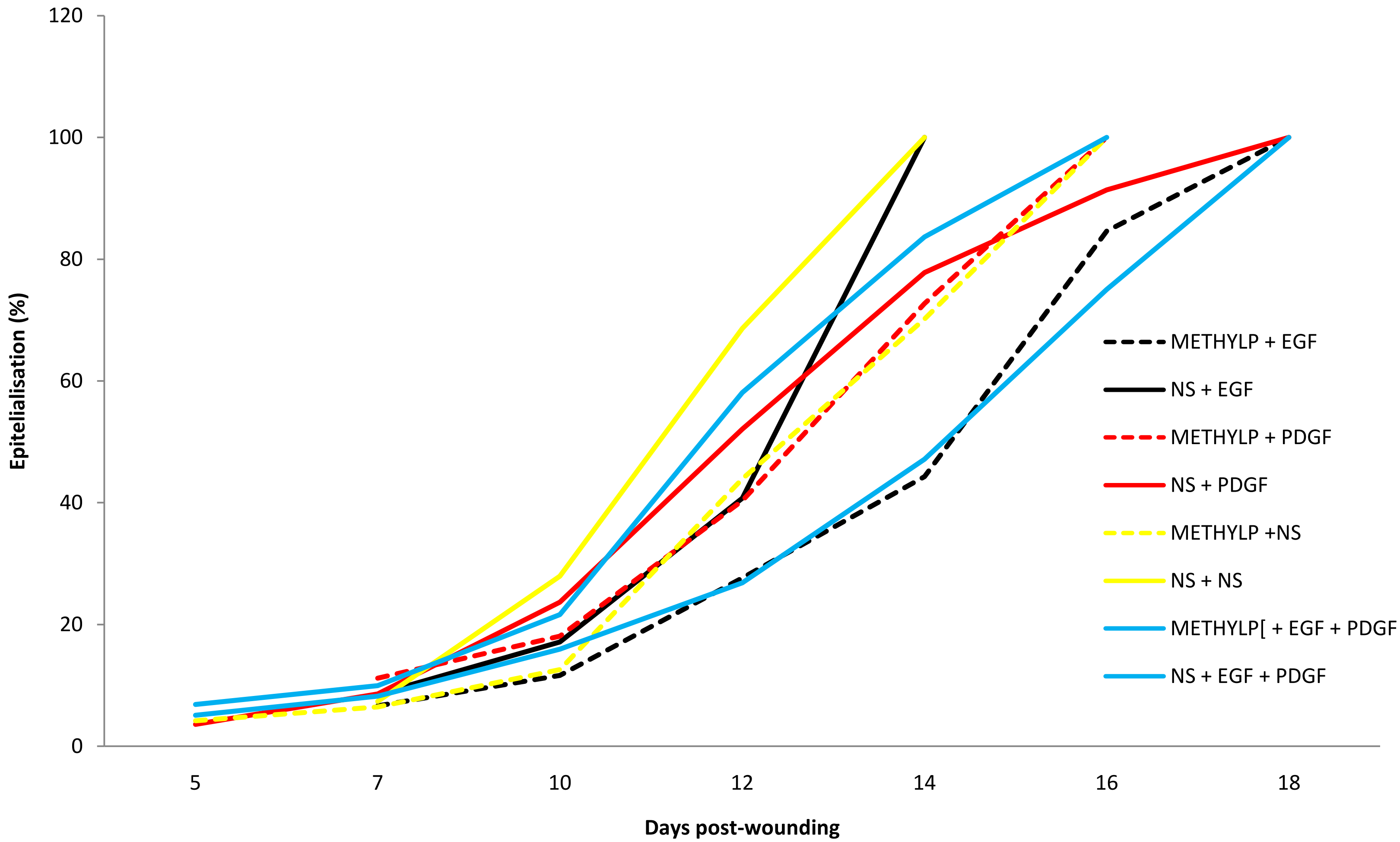
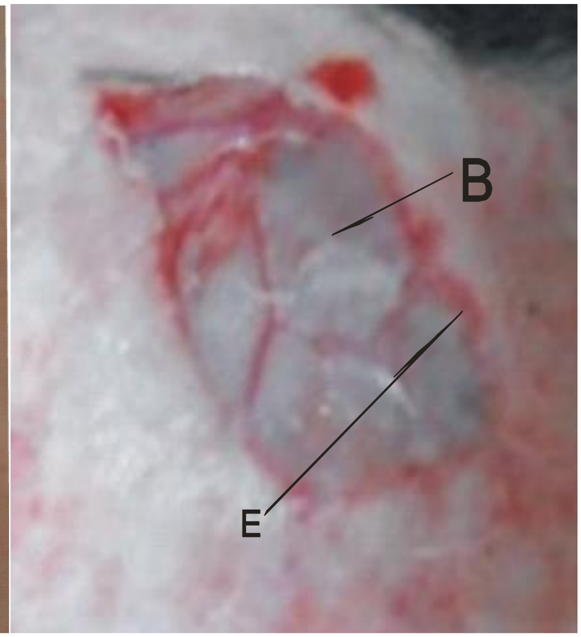
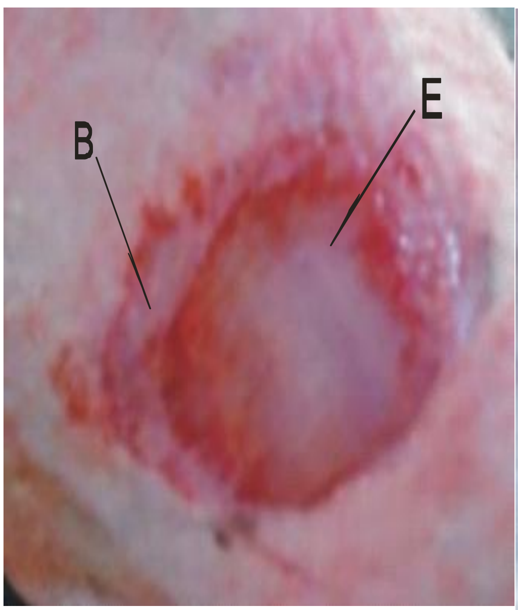


Figure 4.1.2: Effects of Epidermal Growth Factor (EGF-AB), Platelet-Derived Growth Factor (PDGF-AB) with or without Methylprednisolone on Percentage epithelialisation of wound area in New Zealand rabbits

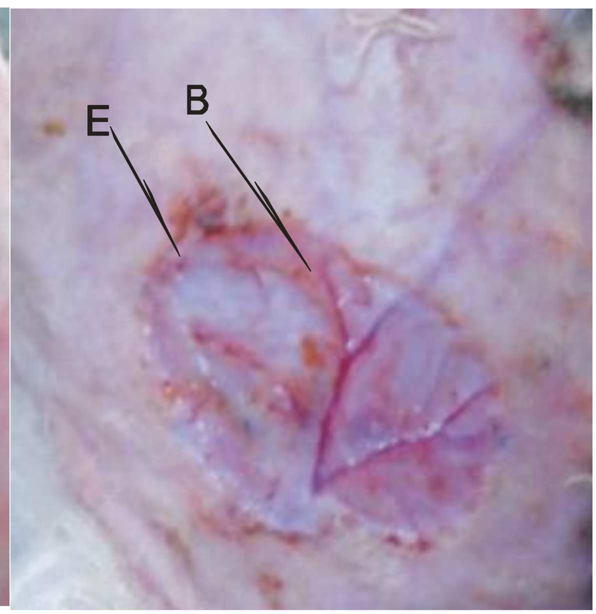
1C = MethylP + NS treated rabbits



2C = NS + NS treated rabbits



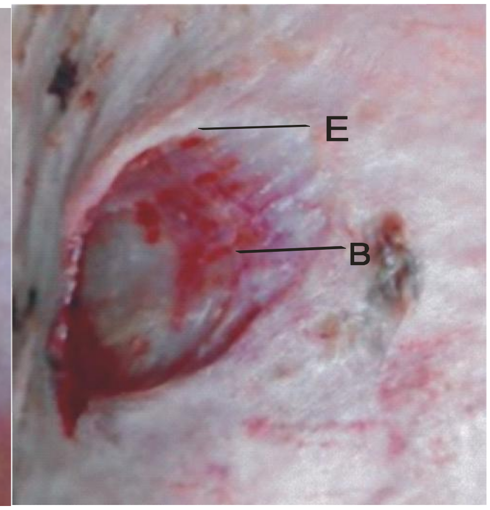
2A = NS + EGF treated rabbits



1A = methylP + EGF treated rabbits

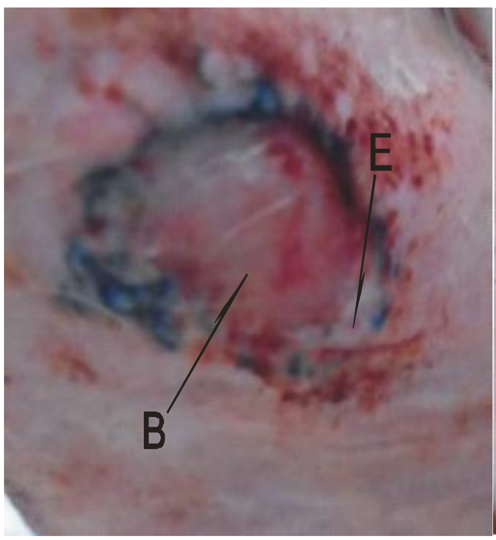
Plate V: Gross Picture of Wounds in New Zealand Rabbit at Day 3 Post-wounding Treated with Normal saline, Methylprednisolone and Epidermal growth factor (EGF-AB)

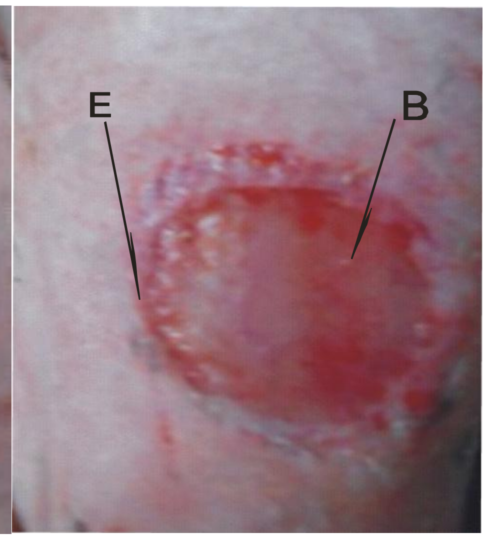
Note: Wound edge (E) with micro bleeders in 1C and 2A but was least in 1A. The wound bed (B) with intact muscle fascia; mild granulation at wound edge in 2A and 1C and least in 1A



2D = NS + EGF + PDGF treated Rabbits

1D = MethylP + EGF + PDGF treated rabbits





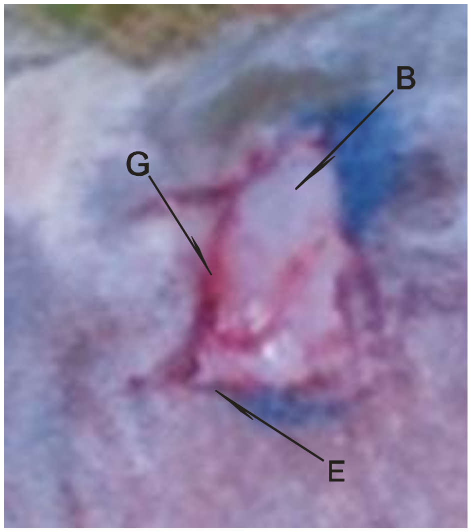
1B = MethylP + PDGF treated rabbits

2B = NS + PDGF treated rabbits

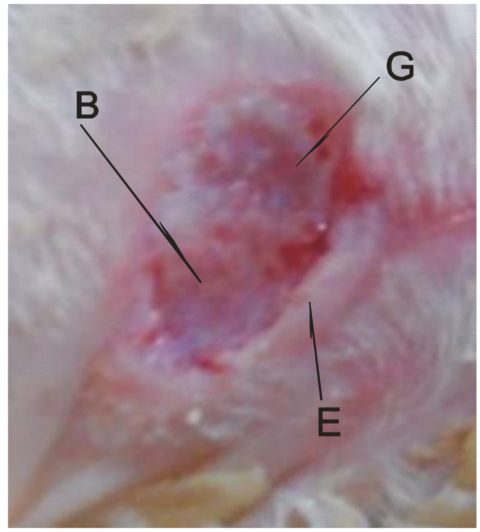
Plate VI: Gross Picture of Wounds in New Zealand Rabbit at Day 3 Post-wounding Treated with Normal saline, Methylprednisolone, Epidermal growth factor (EGF-AB) and Platelet-derived growth factor (PDGF-AB).

Note: Wound edge (E) with micro bleeders which was least in 2B and 2D. The wound bed (B) with intact muscle fascia; mild granulation at wound edge in 2B and 2D and least in 1B.

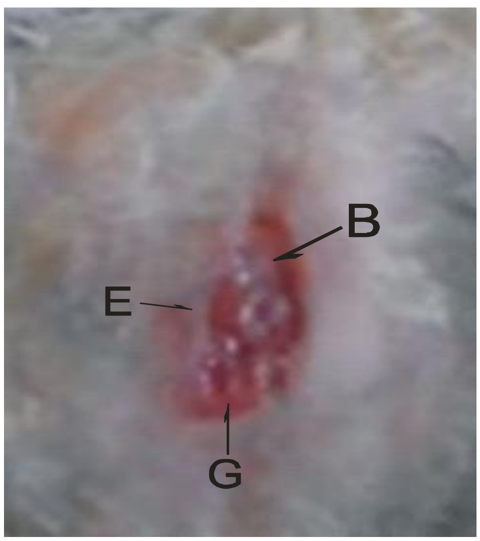
Key: NS = Normal saline;MethylP = Methylprednisolone; EGF = Epidermal growth factor; PDGF = Platelet-derived growth factor



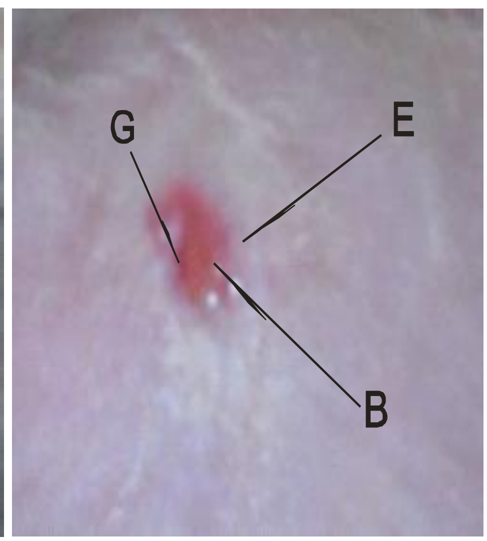
1C = MethylP + NS treated rabbits



2C = NS + NS treated rabbits



2A = NS + EGF treated rabbit

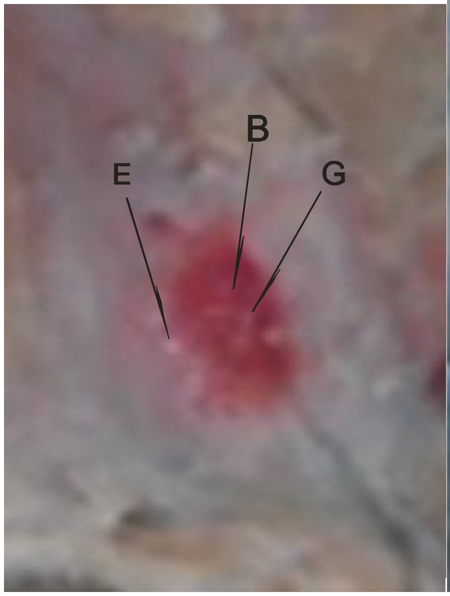


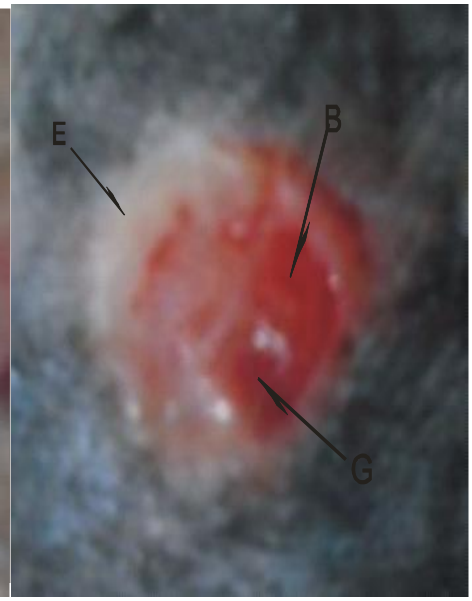
1A = MethylP + EGF treated rabbits

Plate VII: Gross Picture of Wounds in New Zealand Rabbit at Day 7 Post-wounding Treated with Normal saline, Methylprednisolone, and Epidermal growth factor (EGF-AB).

Note: Epithelialisation (E) was more in 1A but least in 1C and 2C. The wound bed (B) with mild pus but more observed in 1C. Granulation tissue (G) mildly formed in 1C and 2C but moderately observed in 1A and 2A.

Key:NS = Normal saline;MethylP = Methylprednisolone; EGF = Epidermal growth factor





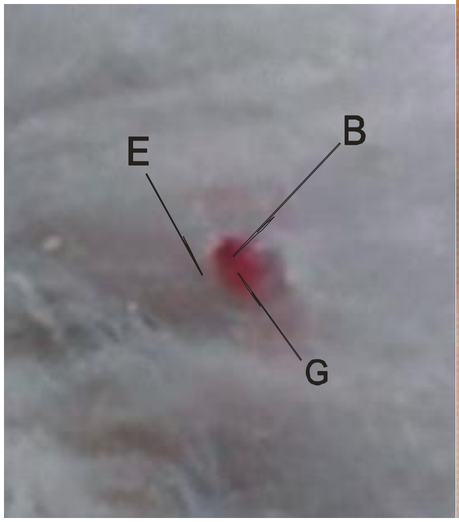
2B = NS + PDGF treated rabbits   1B = MethylP + PDGF treated rabbits

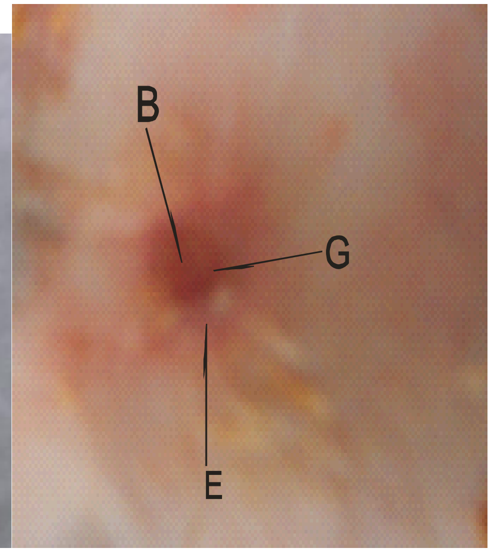
Plate VIII: Gross Picture of Wounds in New Zealand Rabbit at Day 7 Post-wounding Treated with Normal saline, Methylprednisolone, Platelet-derived growth factor (PDGF-AB) and Epidermal growth factor (EGF-AB)

Note: Epithelialisation (E) was more on one side in 2D, 1D, 1B and 2B. The wound bed (B) with mild pus but more observed in 1D and 1B. Granulation tissue (G) modrately formed.

Key: NS = Normal saline;MethylP = Methylprednisolone; EGF = Epidermal growth factor; PDGF = Platelet-derived growth factor

1C = MethylP + NS treated rabbits   2C = NS + NS treated rabbits





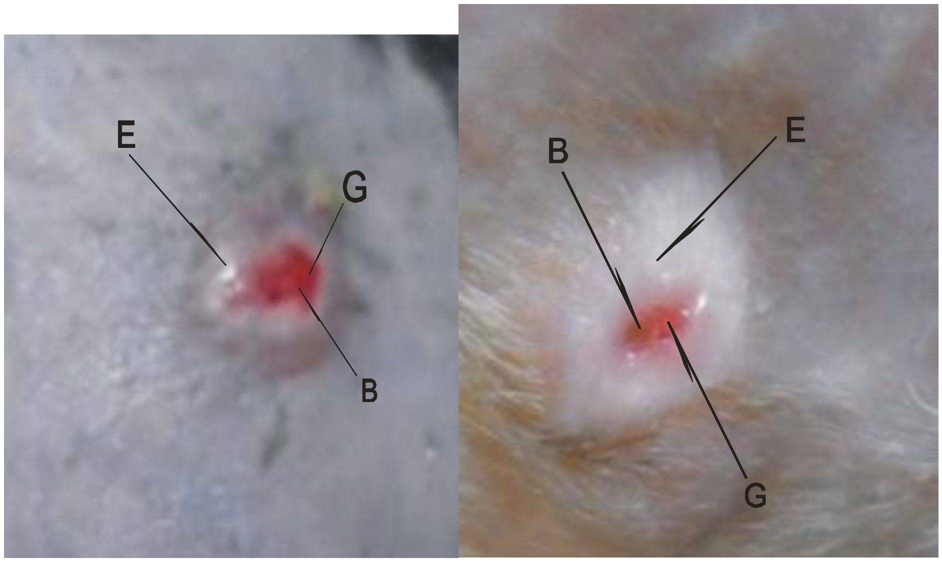
2A = NS + EGF treated rabbits **1A = MethylP + EGF treated rabbits**

Plate IX: Gross Picture of Wounds in New Zealand Rabbit at Day 12 Post-wounding Treated with Normal saline, Methylprednisolone and Epidermal growth factor (EGF-AB)

Note: Epithelialisation (E) was more on one side in 2C, 1C, 1A and 2A. The wound bed (B) with fully formed granulation tissue (G) showing the bright red colour.

Key: NS = Normal saline;MethylP = Methylprednisolone; EGF = Epidermal growth factor

2D = NS + EGF + PDGF treated Rabbits **1D = MethylP + EGF + PDGF treated rabbits**



1B = MethylP + PDGF treated rabbits **2B = NS + PDGF treated rabbits**

Plate X: Gross Picture of Wounds in New Zealand Rabbit at Day 12 Post-wounding Treated with Normal saline, Methylprednisolone, Platelet-derived growth factor (PDGF-AB) and Epidermal growth factor (EGF-AB).

Note: Epithelialisation (E) was more on one side in 1D, 1B and 2D. The wound bed (B) with fully formed granulation tissue (G) showing the bright red colour.

Key: NS = Normal saline;MethylP = Methylprednisolone; EGF = Epidermal growth factor; PDGF = Platelet-derived growth factor.

4.1.3 Granulation tissue formation

Granulation tissue formation was visible at the wound edges on day 3 post-wounding in all the groups (Plates V-VI). Plates VII-VIII shows the gross pictures of wound progression at day 7 post-wounding. In the starting, the granulation tissue formation was moderately formed, in the following descending order: methylP + PDGF, methylP + EGF + PDGF, NS + EGF, NS + PDGF, NS + EGF + PDGF, methylP + EGF, NS + NS, and methylP + NS, which had the lest progress. By day 10 post-wounding granulation tissue formation was formed considerably in all the groups with the bright red colouration. Plates IX-X show the gross pictures obtained at day 12 post-wounding.

Furthermore, signs of itching occurred in NS +NS, NS + EGF, NS + PDGF, and NS + EGF + PDGF between days 1–8 post-wounding. However, itching was not observed in the methylprednisolone treated groups; except in methylP + EGF + PDGF, which was topically treated with both growth factors. Hair growth after shaving was observed by day five post-wounding, especially in the steroid-treated groups (Plate V-X).

4.1.4 Wound suppuration

Plates V-VI and VII-VIII show the gross pictures of wound progression from day 3 post-wounding, and from day 7 post-wounding, respectively. Mild to moderate pus was observed in all the groups from day 3 post wounding, which persisted till day 8 post-wounding. Moderate pus persisted till day 6 in the NS + NS unlike in the other groups where mild pus was observed predominantly. The gross wound pictures with the pus are shown in Plate V-VIII.

4.1.5 Wound breaking strength and tensile strength

The result of scar breaking strength and tensile strength are presented in Figure 4.1.3. The wound breaking strength by day 28 post-wounding was less than 40 Newton in all the treated groups; and the highest values recorded were in an increasing order NS + PDGF (36.1 N), NS + EGF (37.5 N) and NS + EGF + PDGF (38.1 N). The least values of the breaking strength in a decreasing order were observed in methylP + EGF + PDGF (30.3 N), methylP + NS (29.7 N), methylP + EGF (22.8 N), and NS + NS (22.2 N). The least was observed in methylP + PDGF (19.2 N).

4.1.6 Vancouver scar scale

The individual scoring parameter and score points for scar were presented in Appendix 18. However, the best scar was obtained from methylP + EGF (3), and NS + EGF (3.5), followed by methylP + PDGF (3.5), and methylP + NS (4.5); while, the amount of scar tissue obtained in the groups were in the following descending order: methylP + EGF + PDGF (5), NS + EGF + PDGF (5), NS + PDGF (6.5), and NS + NS (9) (Figure 4.1.4).

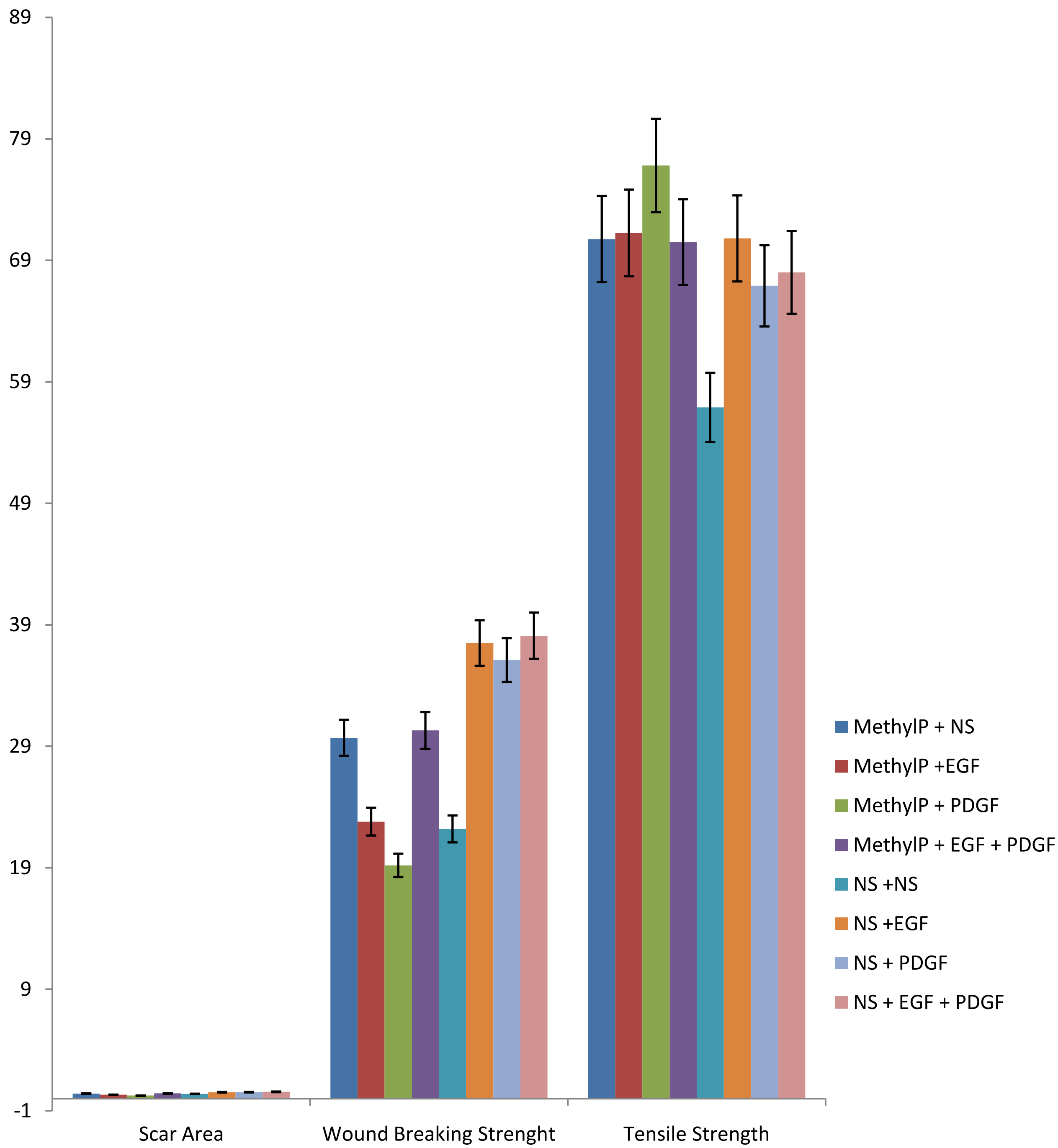


Figure 4.1.3: Wound Breaking Strength and Tensile Strength at day 28 post-wounding in New Zealand rabbits treated with Epidermal Growth Factor (EGF-AB), Platelet-Derived Growth Factor (PDGF-AB) with or without Methylprednisolone (methylP)

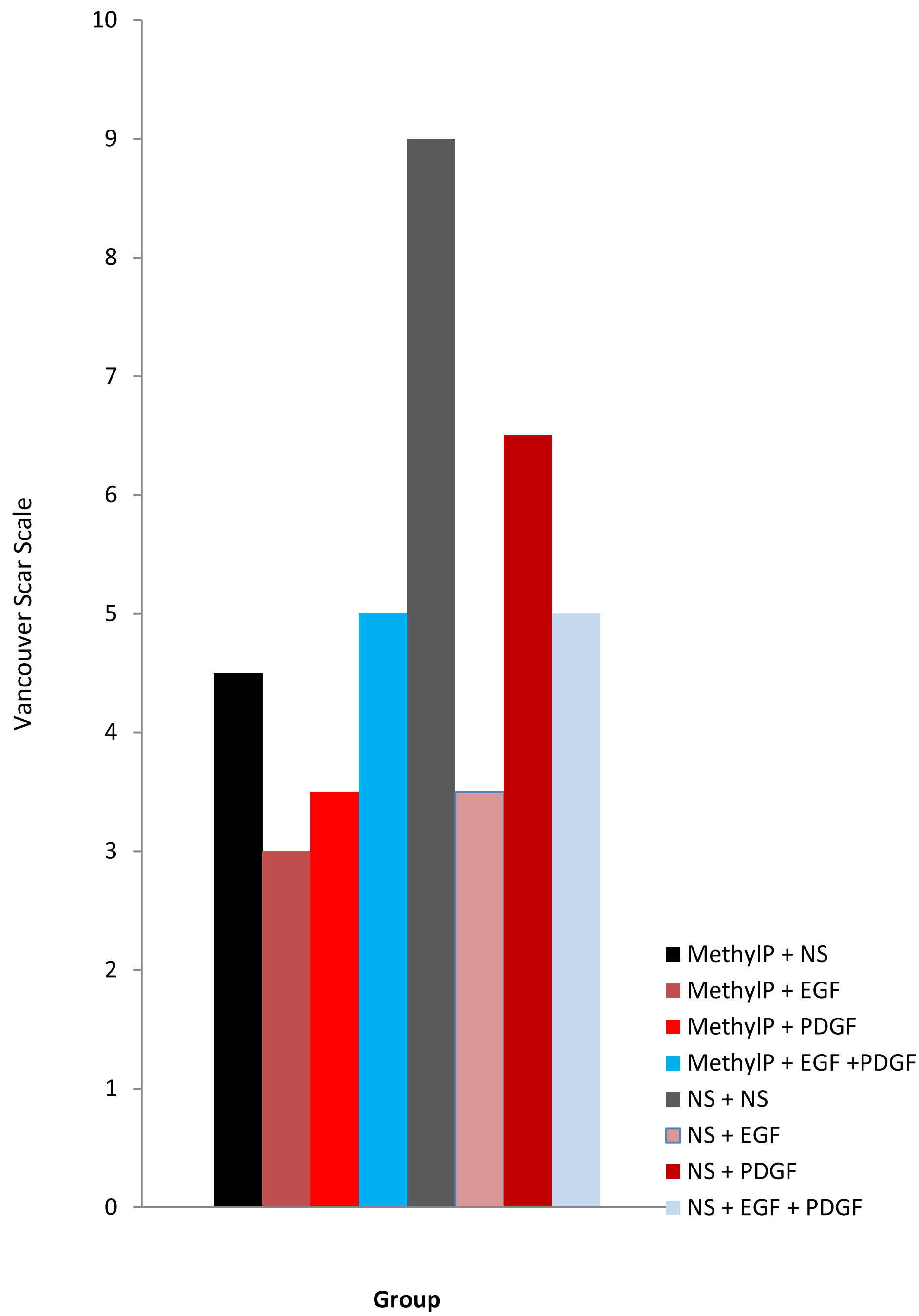
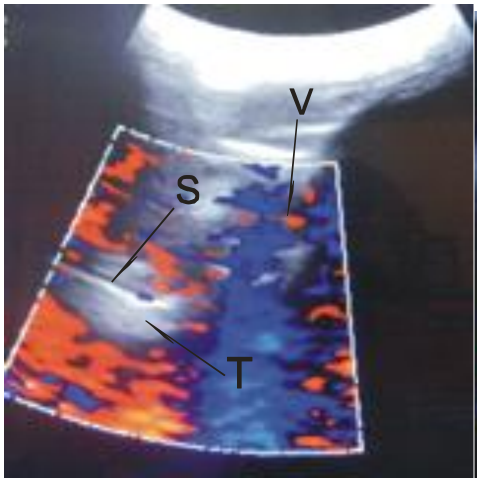


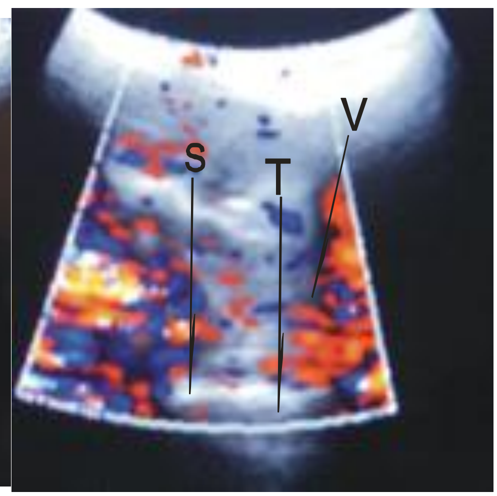
Figure 4.1.4: Vancouver Scar Scale at day 28 post-wounding in New Zealand rabbits treated with Epidermal Growth Factor (EGF-AB), Platelet-Derived Growth Factor (PDGF-AB) and with or without Methylprednisolone (methylP)

4.1.7 Ultrasonography of the scar tissue

The ultrasound images are presented in Plates XI – XII, the scoring in Appendix 16, and the ultrasound scoring is shown in Appendix 16. The vascularity observed from the doppler in the descending order were as followed NS + EGF, methylP + EGF, methylP + PDGF, NS + PDGF, methylP + NS, methylP + EGF + PDGF, NS + EGF + PDGF, and NS + NS. In terms of isoechogenicity in ascending order methylP + NS, NS + PDGF, methylP + EGF + PDGF, NS + EGF + PDGF and NS + NS were hyperecchogenic, while in a descending order methylP + EGF, methylP + PDGF, NS + EGF, were relatively isoecchogenic.



1C = MethylP +NS treated rabbits **2C = NS + NS treated rabbits**

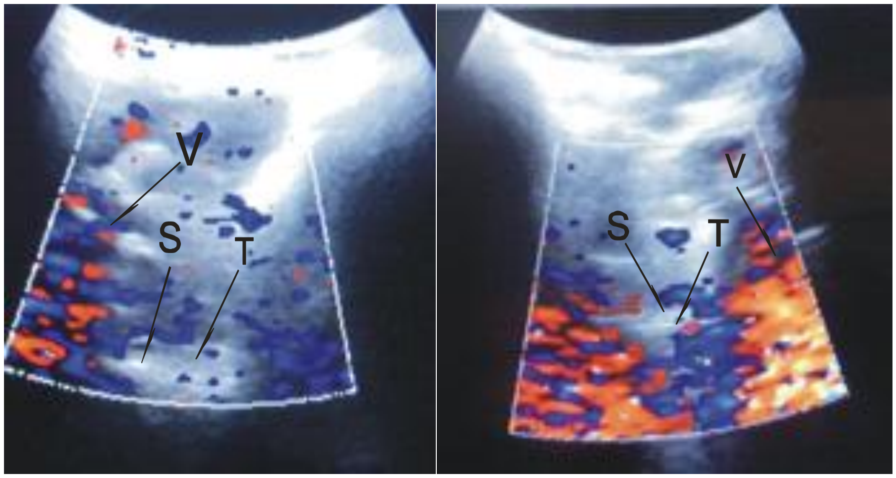


1A = MethylP + EGF treated rabbits **2A = NS + EGF treated rabbits**

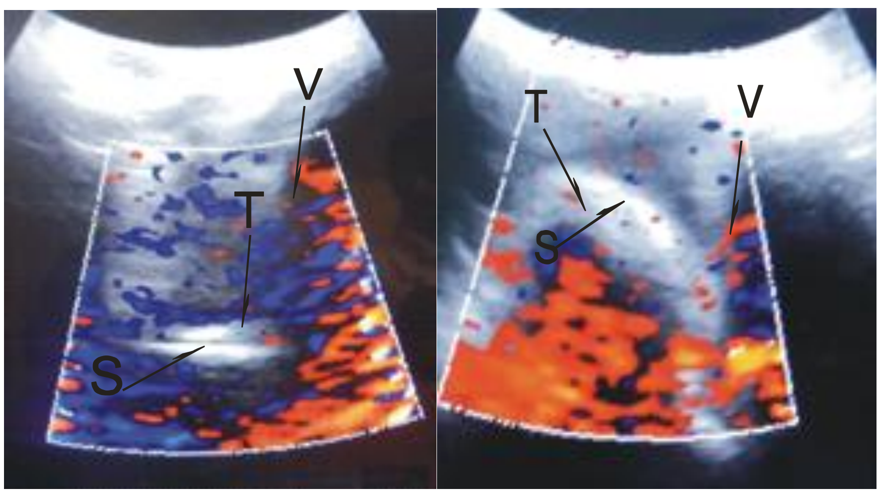
Plate XI: Doppler Ultrasonography of Scar in New Zealand Rabbit Treated with Normal saline, Methylprednisolone and Epidermal growth factor (EGF-AB) at Day 28 Post-wounding

Note: The scar line (S) was least obvious in the1A, and hyper-echoic in 2A, 1C and 2C. The scar tissue (T) was more in 1C and 2A but was largest in 2C while 1A recorded least scar tissue. Blood vessels (V) in 1A were more, compared to that in 2A, and in 1C relative to 2C.

Key: NS = Normal saline; MethylP = Methylprednisolone; EGF = Epidermal growth factor



1B = MethylP + PDGF treated rabbits   2B = NS + PDGF treated rabbits



1D = MethylP + EGF + PDGF treated rabbits   2D = NS + EGF + PDGF treated rabbits

Plate XII: Doppler Ultrasonography of Scar in New Zealand Rabbit Treated with Normal saline, Methylprednisolone, Platelet-derived growth factor (PDGF-AB) and Epidermal growth factor (EGF-AB) at Day 28 Post-wounding

Note: The scar line (S) was least obvious in the1B and 2B treated rabbits but was hyper-echoic in 1Dand 2D. The scar tissue (T) was more in 1D but largest in 2D while 2B recorded least scar tissue. Blood vessels (V) in 1B were more compared to that in 2B and, 2D also recorded more vessels than 1D.

Key: NS = Normal saline; MethylP = Methylprednisolone; PDGF = Platelet-derived growth factor; EGF = Epidermal growth factor (EGF)

4.2 Haemograms

Values of blood parameters obtained from samples are detailed and presented in Figures 4.2.1 to 4.2.7 and Appendix 3–9. There were minor fluctuations in the values of packed cell volume (PCV), haemoglobin concentration (Hb), white blood cell (WBC), platelet (PLT), lymphocyte (L), monocyte (M) and neutrophil (N) counts. Subsequently, eosinophils and basophils count were absent in the haemogram. However, except for the PCV, HB, and WBC, that had a P-value ($P > 0.05$), that is, PCV (0.157), Hb (0.113), and WBC (0.257), all others were of statistical significance as follows.

The PLT count was significant ($P < 0.05$) using ANOVA, and was further subjected to a Tukey's multiple comparison test to further compare between individual groups that were of statistical significance. NS + NS, and methylP + EGF were statistically significant, so also, NS + NS and methylP + NS, NS +NS and methylP + EGF + PDGF and methylP + EGF + PDGF and methylP + PDGF. Lymphocyte count (L) was significant ($P < 0.05$) using ANOVA and was further subjected to a Tukey's multiple comparison test, the following were statistically significant NS + NS and NS + EGF, NS + EGF and methylP + EGF, NS + EGF and methylP + NS, and NS + EGF and methylP + EGF + PDGF.

Furthermore, monocyte count (M) was significant ($P < 0.05$) using ANOVA, this was further subjected to a Tukey's multiple comparison test to compare between individual groups, that were of statistical significance NS + EGF + PDGF and methylP + NS. Subsequently, NS + EGF + PDGF and methylP + EGF + PDGF; NS + EGF and methylP + NS. Neutrophil count (N) was significantly ($P < 0.05$) using ANOVA and was also subjected to a Tukey's multiple comparison test, groups that were statistically significant were NS + EGF + PDGF and methylP + EGF, NS + EGF + PDGF, and methylP + NS, NS + EGF + PDGF, and methylP + EGF + PDGF, NS + NS and methylP + NS, NS + EGF and methylP + EGF, NS + EGF, and methylP + NS, NS + EGF and NS + EGF + PDGF, NS + EGF, and methylP + PDGF, and methylP + NS, and NS + PDGF.

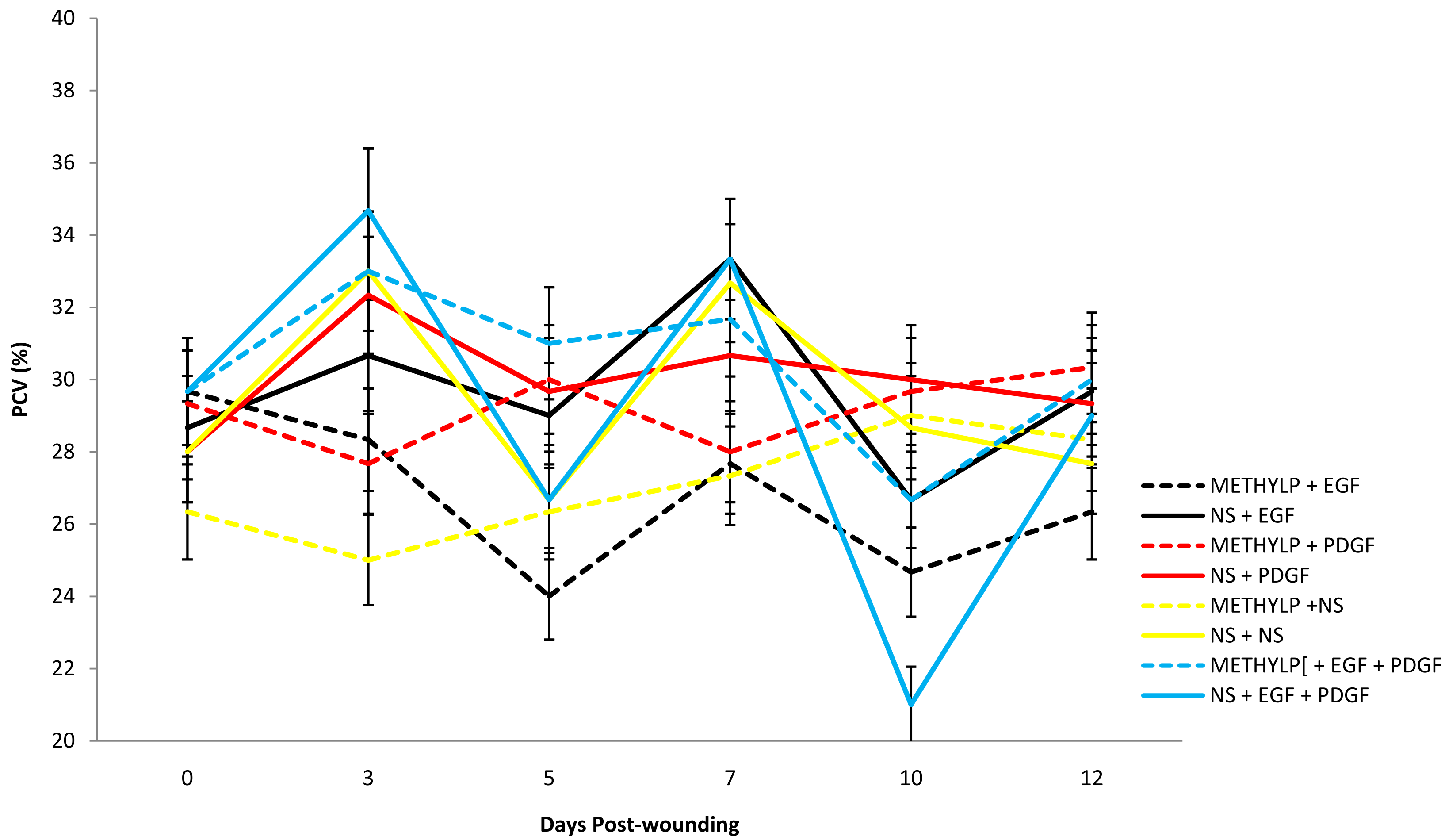


Figure 4.2.1: Effects of Epidermal Growth Factor (EGF-AB), Platelet-Derived Growth Factor (PDGF-AB) with or without Methylprednisolone on Packed cell volume (PCV) of the New Zealand rabbits subjected to Experimental studies

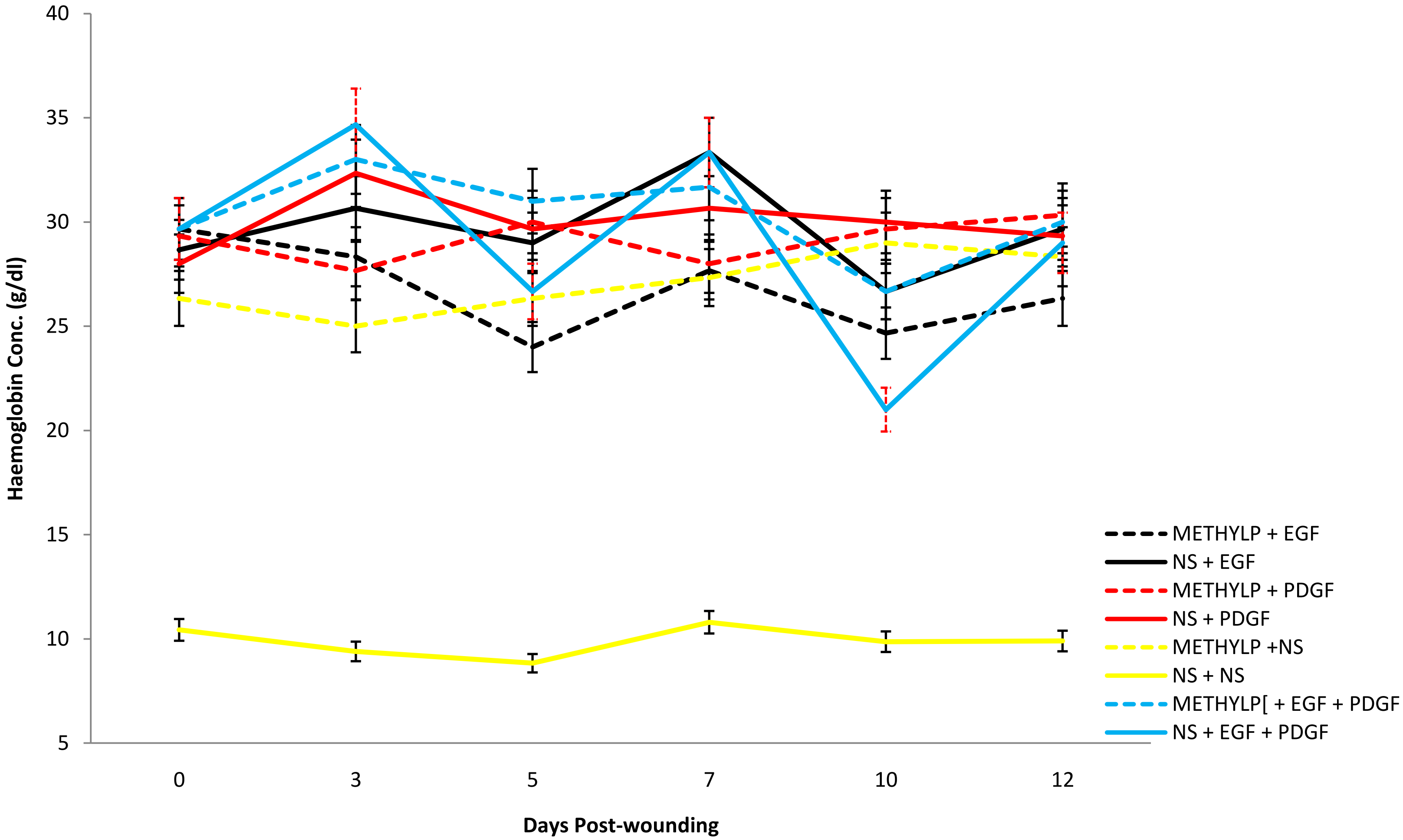


Figure 4.2.2: Effects of Epidermal Growth Factor (EGF-AB), Platelet-Derived Growth Factor (PDGF-AB) with or without Methylprednisolone on Haemoglobin (Hb) Concentration of the New Zealand rabbits Subjected to Experimental Studies

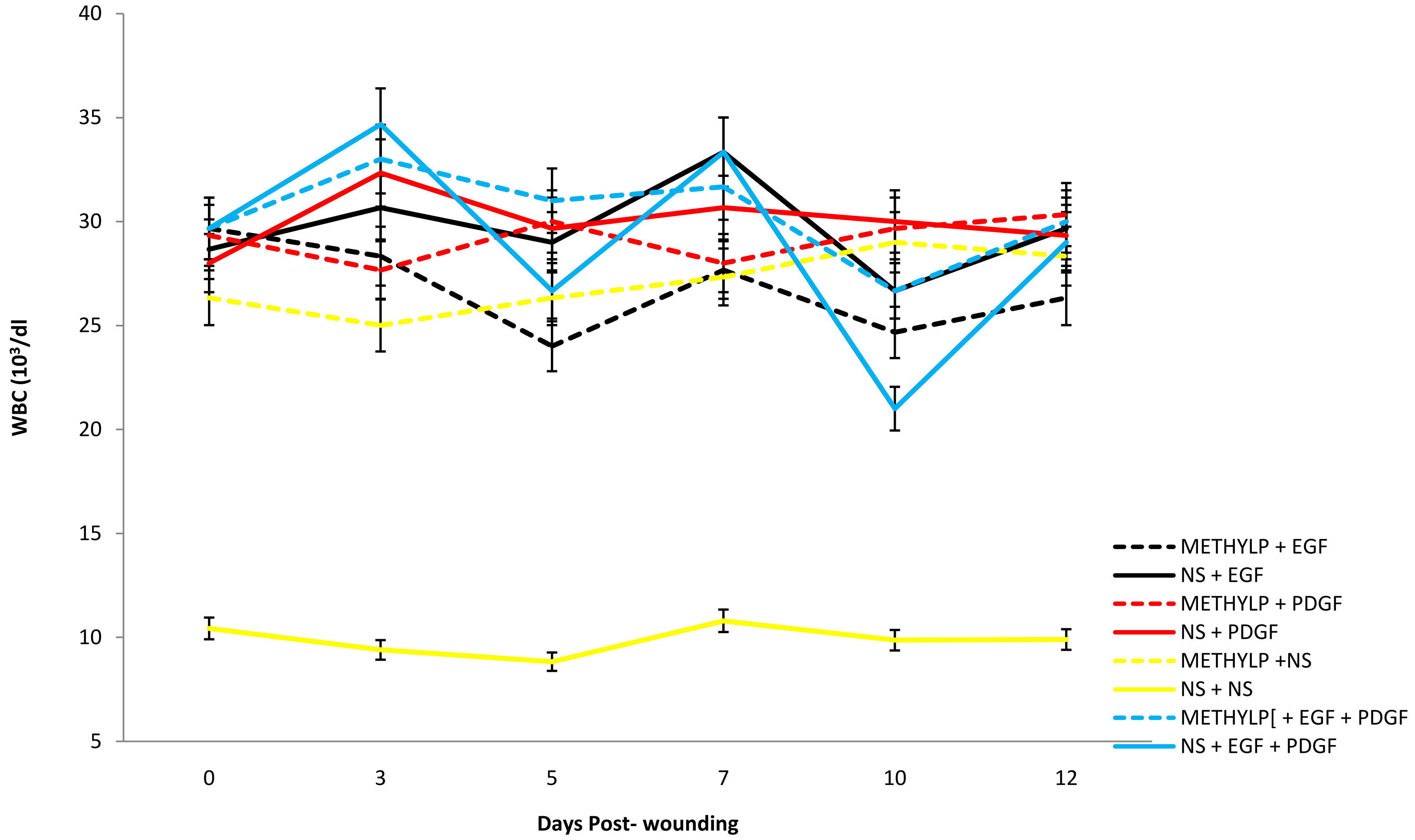


Figure 4.2.3: Effects of Epidermal Growth Factor (EGF-AB), Platelet-Derived Growth Factor (PDGF-AB)with or without Methylprednisolone on Total Leucocyte Count (WBC) of the New Zealand rabbits Subjected to Experimental Studies

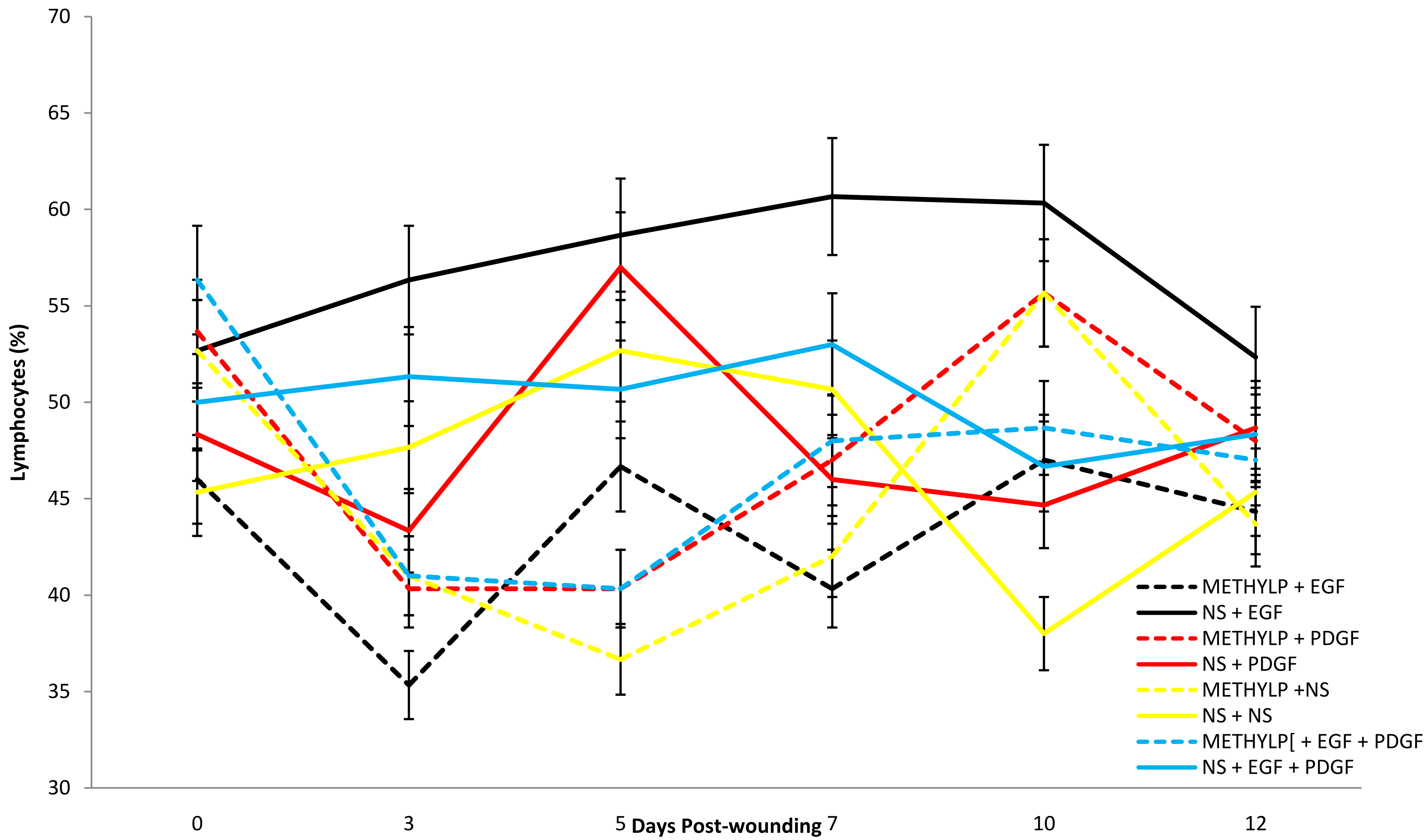


Figure 4.2.4: Effects of Epidermal Growth Factor (EGF-AB), Platelet-Derived Growth Factor (PDGF-AB) with or without Methylprednisolone on Lymphocyte Count (L) of the New Zealand rabbits Subjected to Experimental Studies

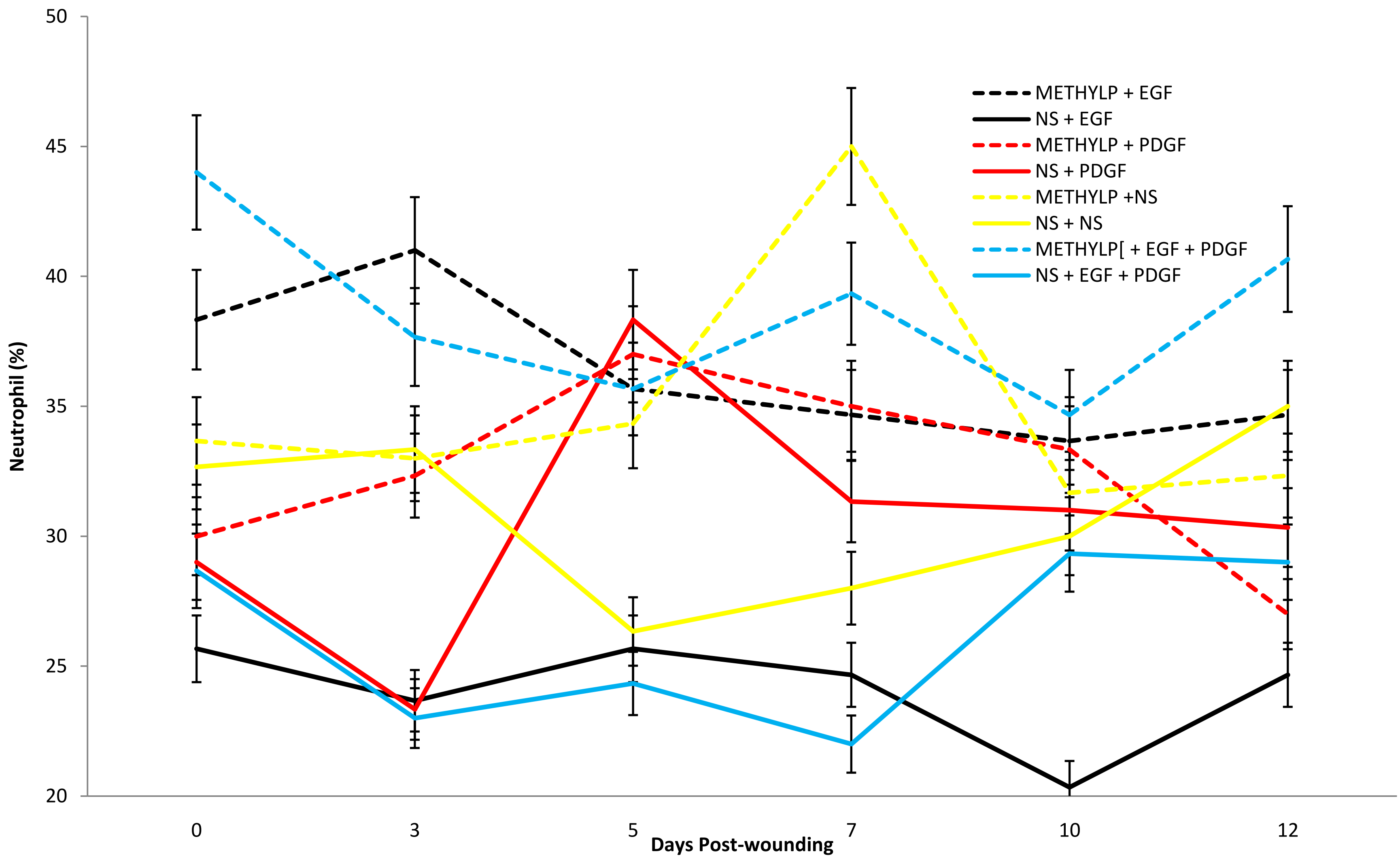


Figure 4.2.5: Effects of Epidermal Growth Factor (EGF-AB), Platelet-Derived Growth Factor (PDGF-AB) with or without Methylprednisolone on Neutrophil count (N) of the New Zealand rabbits Subjected to Experimental Studies

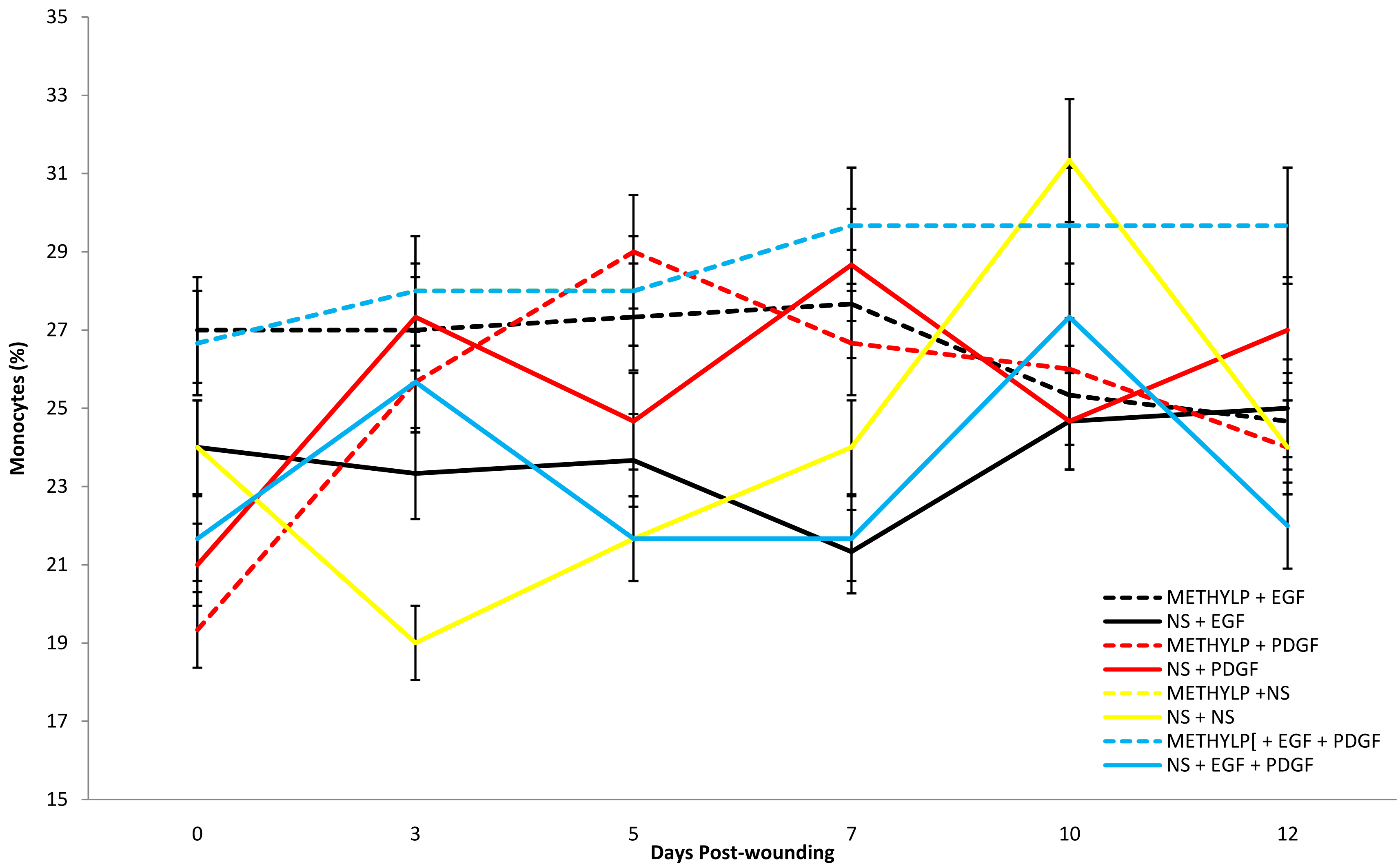


Figure: 4.2.6: Effects of Epidermal Growth Factor (EGF-AB), Platelet-Derived Growth Factor (PDGF-AB) with or without Methylprednisolone on Monocyte Count (M) of the New Zealand rabbits Subjected to Experimental Studies

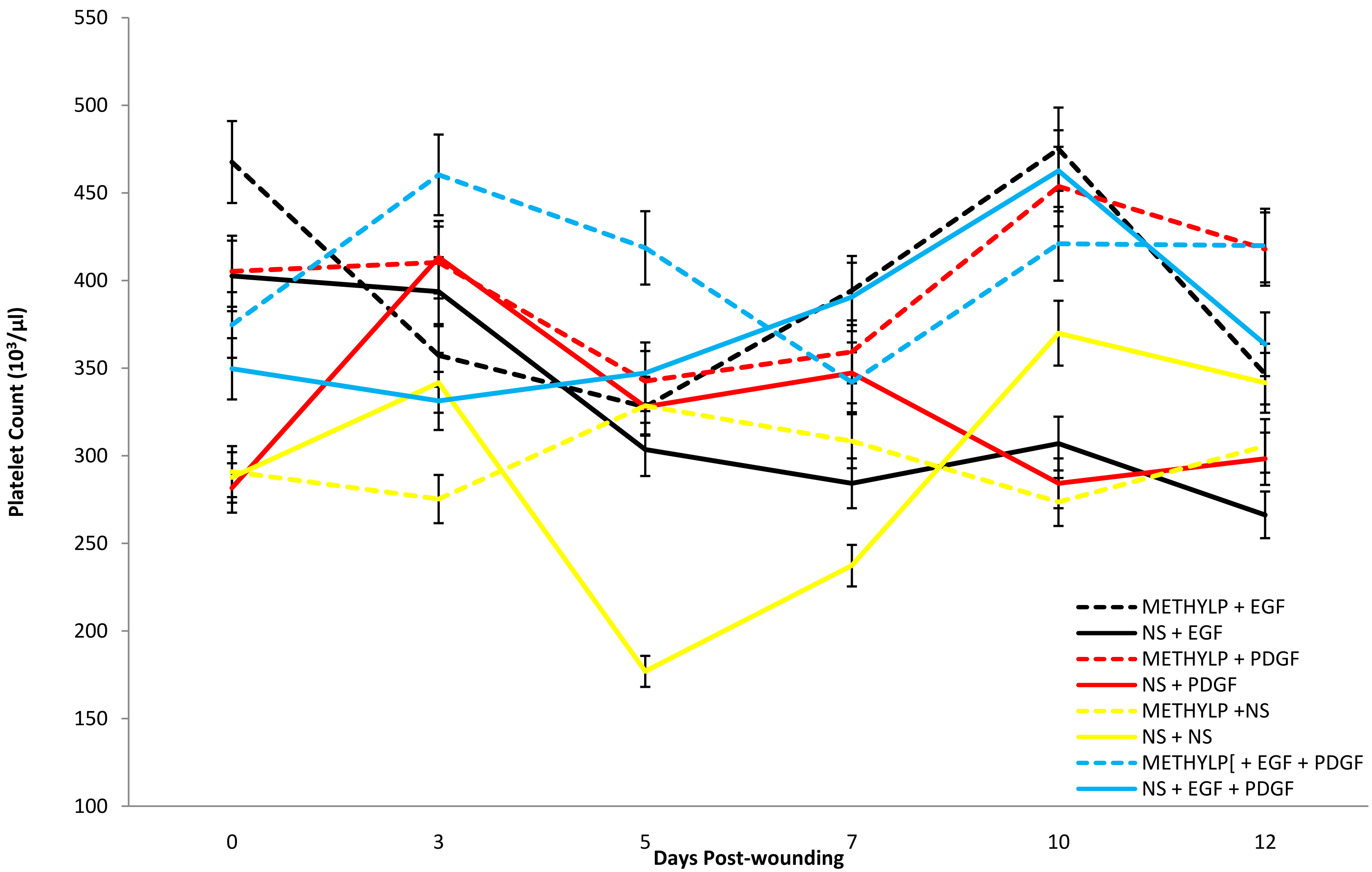


Figure 4.2.7: Effects of Epidermal Growth Factor (EGF-AB), Platelet-Derived Growth Factor (PDGF-AB)with or without Methylprednisolone on Platelet (PLT) Count in of the New Zealand rabbits Subjected to Experimental Studies

4.3 SEROLOGY

The effects on the EGF and PDGF concentration in the serum of the rabbits were shown in Figures 4.2.8 and 4.2.9 respectively. Statistically, P-value of 0.3517 was obtained for PDGF and P-value 0.3517 for EGF using ANOVA. The result for the EGF werestatistically not significant.

The serum concentration of EGF fluctuates between 0.50 pg/dl and 2.00 pg/dl, and this was more in methylP + EGF + PDGF, methylP + NS, methylP + EGF and NS + EGF + PDGF. Drop in the serum EGF was observed at day 5 and day 10 post-wounding in methylP + EGF + PDGF, methylP + NS, methylP + EGF and NS + EGF + PDGF but was relatively consistent at 0.5 ± 0.2 pg/dl in methylP + PDGF, NS + NS, NS + EGF and NS + PDGF.

Circulating PDGF obtained ranges between 50.00 to 80.00pg/dl in the study with minor fluctuations. Slight drop in serum concentrations were recorded at day 5 post-wounding in all the treated groups except NS + EGF + PDGF and NS + EGF. Furthermore, at day 10 post-wounding methylP + EGF + PDGF recorded 150.00 pg/dl and methylP + PDGF recorded 200.00 pg/dl at day 12 post-wounding.

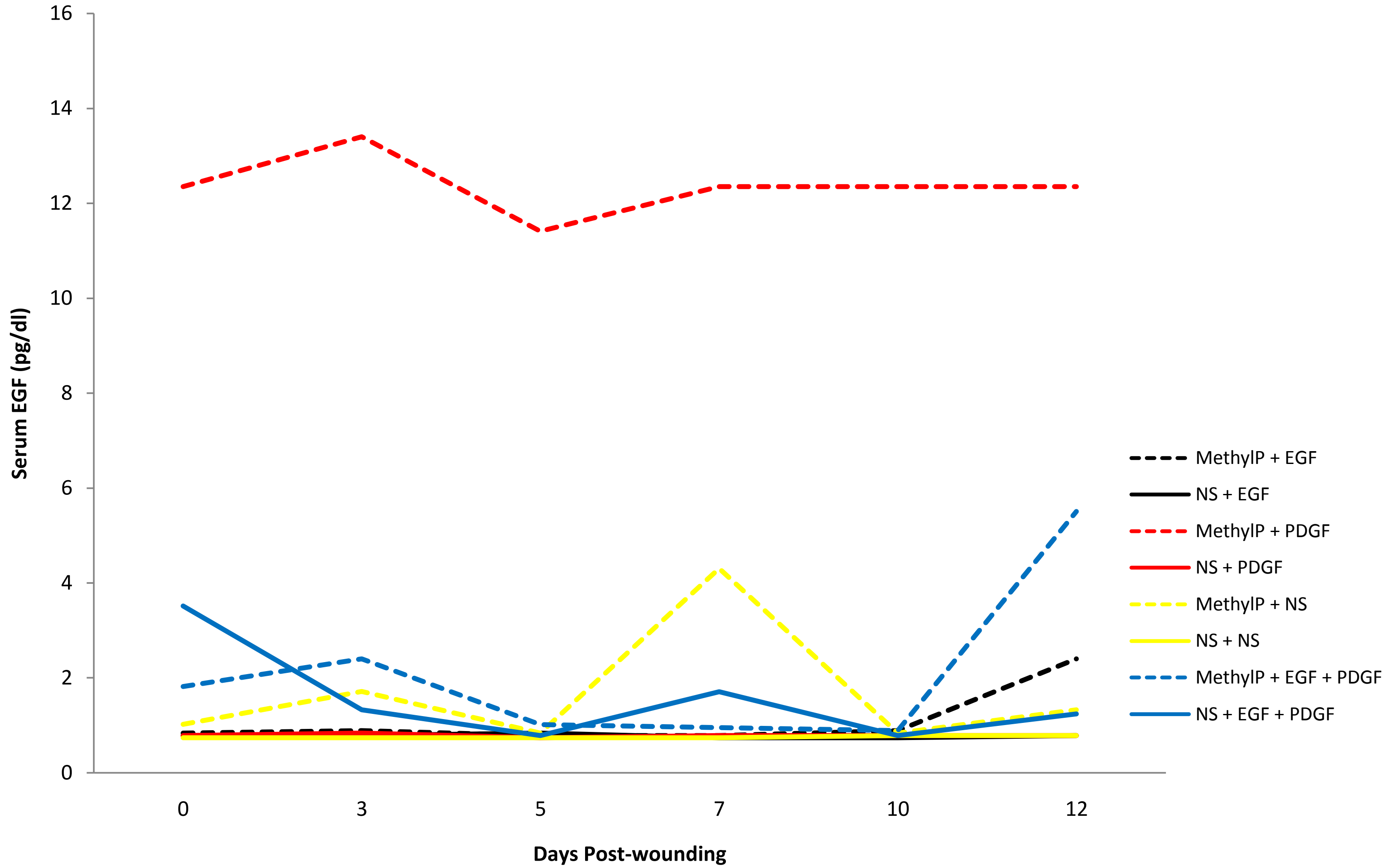


Figure 4.2.8: Effects of Epidermal Growth Factor (EGF-AB), Platelet-Derived Growth Factor (PDGF-AB) with or without Methylprednisolone on Serum Concentration of Epidermal Growth Factor of the New Zealand rabbits Subjected to Experimental Studies.

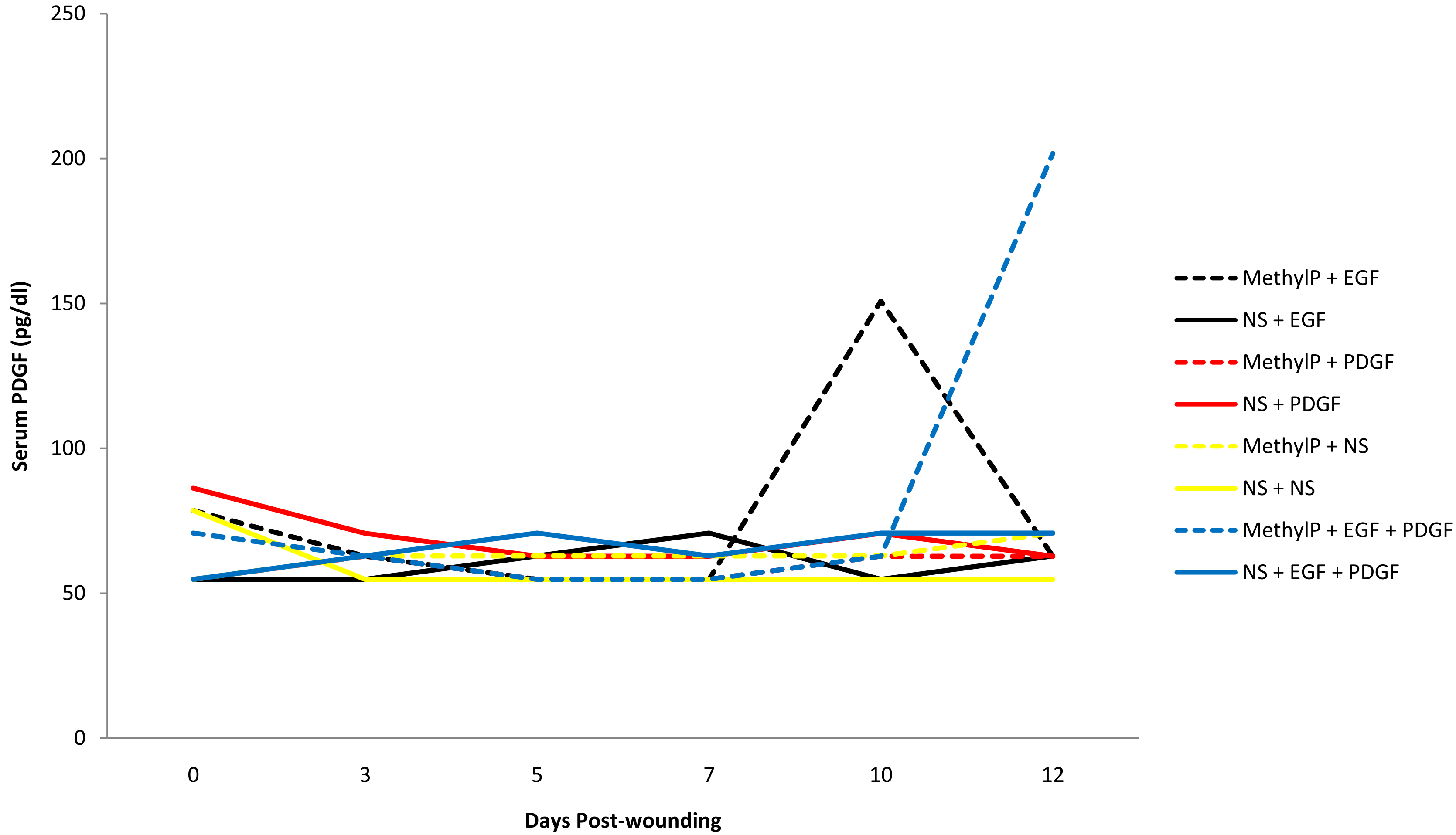


Figure 4.2.9: Effects of Epidermal Growth Factor (EGF-AB), Platelet-Derived Growth Factor (PDGF-AB) with or without Methylprednisolone on Serum Concentration of Platelet-Derived Growth Factor of the New Zealand rabbits Subjected to Experimental Studies

4.4. Histological progression of wound healing

Biopsy samples taken from the rabbits on the day of wounding, as the normal skin tissue were presented in Plate XIX and XX. Furthermore, the photomicrographs on days 3, 7, and scar tissue post-wounding were shown and summarised, indicating the progression of wound healing (Plates XIII – XIV).

Day 3 of methylP + EGF rabbit: The aggregation of the connective tissue fibres observed was numerous, and thicker but sparse, when compared to that in the normal skin tissue. Engorged small sized blood vessels were observed just as found in the normal skin, however, they were seen in clusters of vessels. Severe inflammatory cells were observed. The nuclei of the connective cells were round in shape with few flat nuclei Plate XIII.

Day 3 of methylP + PDGF rabbit: The connective tissue fibres were not uniformly distributed, unlike in the normal skin. The density progressively increased away from the re-epithelised site towards the wound stroma, but was sparse in arrangement, compared to that of the skin. Larger blood vessels were observed compared to that observed in the normal skin, and were few, with severe infiltration by inflammatory cells. The nuclei of the connective cells observed were round in shape with some flat nuclei Plate XIV.

Day 3 of methylP + NS rabbit: The distribution of the connective tissue fibres were relatively sparse with thinner strands, compared to that of the normal skin. The blood vessels were small in size, but mildly engorged with blood cells. Inflammatory cells, including flat, elongated nuclei of the connective cells were observed Plate XIII.

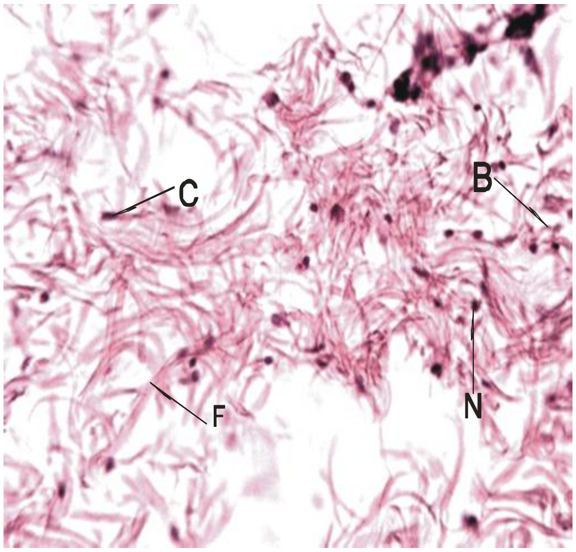
Day 3 of methylP + PDGF + EGF rabbit: The aggregations of the connective tissue fibres were similar to that of the normal skin; however, the strands of the connective tissue fibre were thinner. Blood vessels observed were mainly larger than that in the normal skin, moderately engorged with blood cells. Severely infiltrated with inflammatory cells. Connective cell nuclei were round in shape Plate XIV.

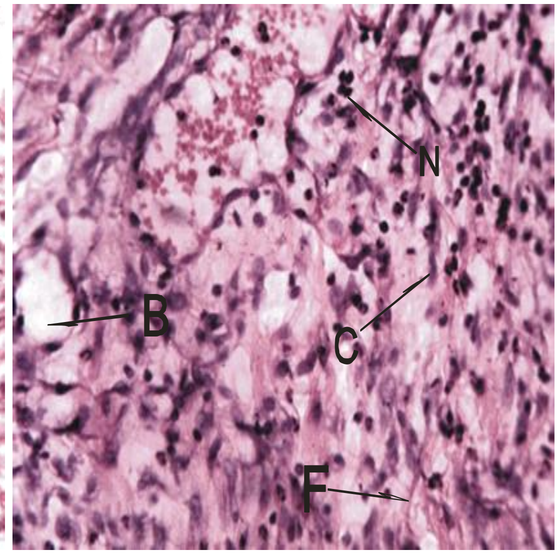
Day 3 of NS + EGF rabbit: The distribution of the connective tissue fibres were relatively of higher density, but of similar size with that of the skin. The blood vessels were small, few and mildly engorged with blood cells. Inflammatory cell infiltration was mild. The nuclei of the connective cells were round in shape Plate XIII.

Day 3 of NS + PDGF rabbit: There were moderately infiltrated inflammatory cells. The blood vessels were also larger than those observed in the normal skin, few and mildly engorged. The connective tissue fibres and cells were relatively sparse compared to that found in the normal skin, relatively thicker and absorbed more of the stain. The nuclei of the connective cells were round in shape Plate XIV.

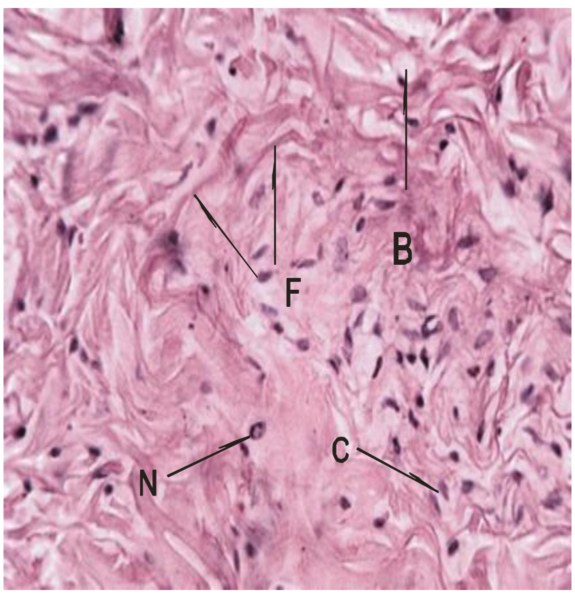
Day 3 of NS + NS rabbit: The connective tissue fibres were relatively sparse, and thinner in size, compared to that recorded in the normal skin. The blood vessels were large with few and small ones. The blood vessels were severely engorged with blood cells. Inflammatory cells were also severed. The nuclei of the connective tissue cells were round in shape Plate XIII.

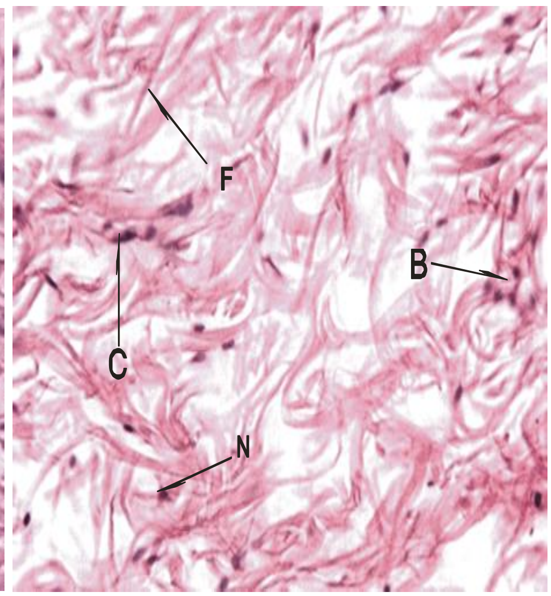
Day 3 of NS + PDGF + EGF rabbit: The connective tissue fibres were relatively of lower density, compared to that of the normal skin and were thinner in size. The blood vessels were small, numerous and mildly engorged with blood cells. Inflammatory cells were severely dense. The connective cell nuclei were round in shape Plate XIV.





1C = methylP + NS treated rabbits **2C = NS + NS treated rabbits**



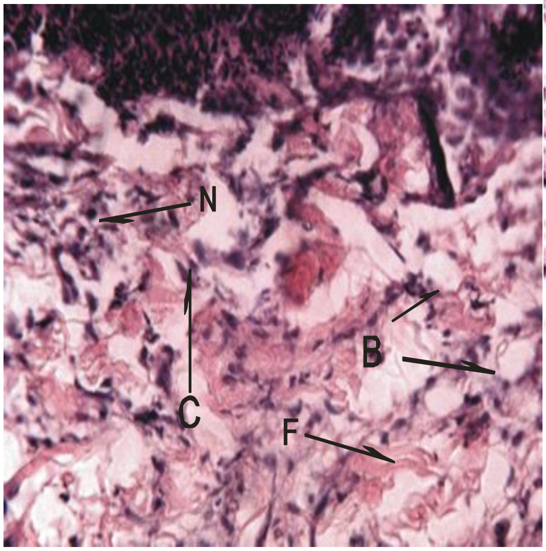


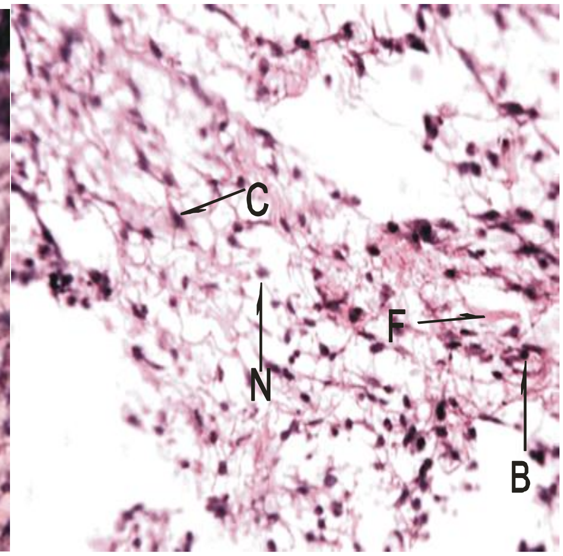
2A = NS + EGF treated rabbits **1A = methylP + EGF treated rabbits**

Plate XIII: Photomicrograph 3 Days Post-wounding in New Zealand rabbits Treated with Normal saline, Methylprednisolone and Epidermal growth factor (EGF-AB)

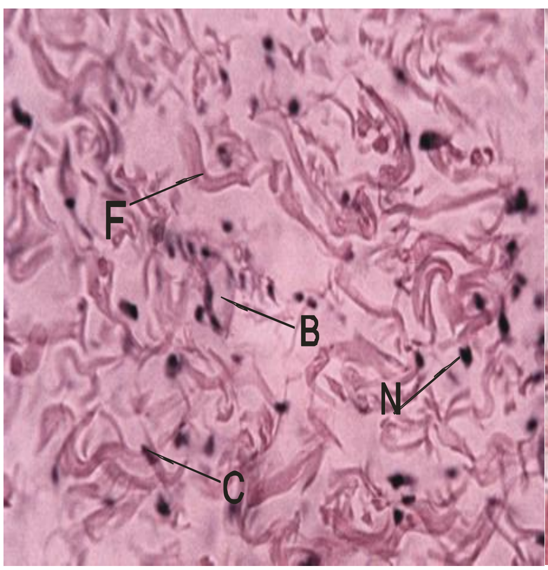
Note: Inflammatory cells (N) influx was least in 1A but were mainly myofibroblast (flattened cell nuclei), 1C and 2A recorded moderate influx of inflammatory cells while infiltration of inflammatory cells was severe in 2C. Blood vessels (B) were observed in all the treated groups. Connective tissue fibre (F) were more distinct in 1C and 1A but less obvious in 2A and least distinct in 2C. Collagen (C) deposit was more in 2C followed by 2A, and least in 1A and 1B.

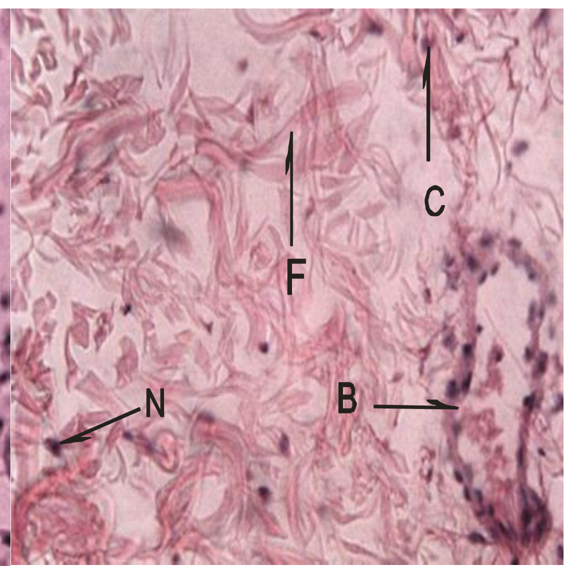
Key:NS= Normal saline; MethylP = Methylprednisolone; EGF = Epidermal growth factor; PDGF= Platelet-derived growth H & E ×250.





2D = NS + EGF + PDGF treated rabbits **1D = methylP + EGF + PDGF treated rabbits**





1B = methylP + PDGF treated rabbits **2B = NS + PDGF treated rabbits**

Plate XIV: Photomicrograph 3 Days Post-wounding in New Zealand rabbits Treated with Normal saline, Methylprednisolone, Platelet-derived factor (PDGF-AB) and Epidermal growth factor (EGF-AB)

Note: Inflammatory cells (N) influx was least in 2B but with relatively more myofibroblast (flattened cell nuclei) in 1B. The inflammatory cells (N) influx was severe in both 2D and 1D. Blood vessels (B) were also observed in all the groups. Connective tissue fibre (F) were more distinct in 2B and 1B but less obvious in 1D and least distinct in 2D. Collagen (C) deposit was more in 2D followed by 2B, and least in 1B.

Key:NS= Normal saline; MethylP = Methylprednisolone; EGF = Epidermal growth factor; PDGF= Platelet-derived growth H & E ×250.

Day 7 of methylP + EGF rabbit: The connective tissue fibres were not uniformly distributed as they were more numerous in the wound stroma, relative to re-epithelised site. The strands of the connective tissue fibres were thinner, and more compacted (higher density) compared to those in the normal skin. The severely-engorged blood vessels were numerous, and small, but some with scanty large ones were observed. Inflammatory cells were also severe. The nuclei of the connective cell were round with few flat in shape Plate XV.

Day 7 of methylP + PDGF rabbit: The connective tissue fibres were uniformly distributed, similar in strand size but more in density, compared to those of the normal skin. Moderately perfused by large blood vessels along-side with few small blood vessels, these vessels were moderately engorged with blood cells. Mild infiltration of inflammatory cells was also observed. Flat, elongated, connective cell nuclei were present Plate XVI.

Day 7 of methylP + NS rabbit: The distribution of the connective tissue fibres were uniform with similar density though, thinner strands compared to that seen in the normal skin. The blood vessels were larger than that found in the skin, numerous and moderately engorged with blood cells. The inflammatory cells were moderate. Flat, elongated connective cells nuclei were also seen Plate XV.

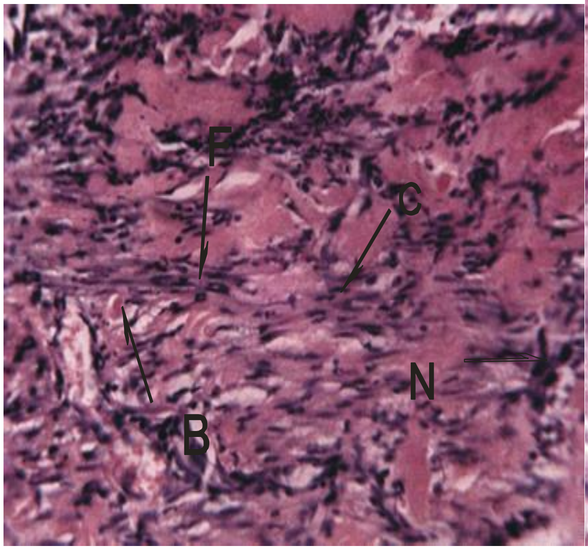
Day 7 of methylP + PDGF+EGF rabbit: The connective tissue fibres are relatively much in number (higher density) but similar in size to that seen in the normal skin. The blood vessels were small as seen in the normal skin and numerous in number. They are mildly engorged with blood cells. Inflammatory cells are numerous. Connective tissue cell nuclei were flat with few rounded in shape Plate XVI.

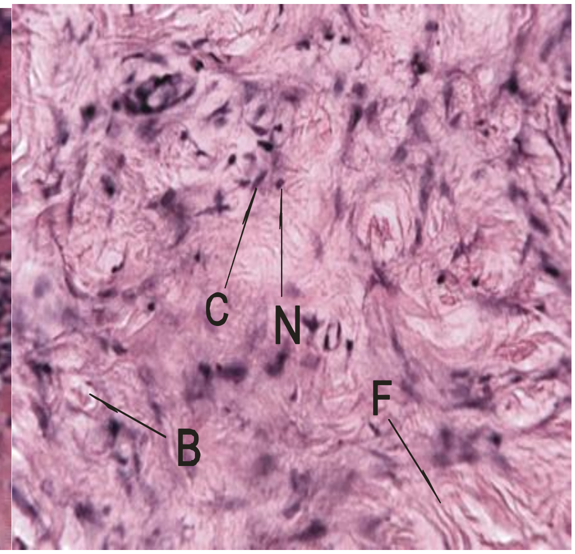
Day 7 of NS + EGF rabbit- The connective tissue fibres were relatively of similar density to those of the normal skin but were thicker in size. The blood vessels were relatively and equally distributed in size. Both small and large vessels on the average were 8 observed, and were moderately engorged with blood cells. Inflammatory cells were severe. The cells of the connective tissue were flattened and slightly elongated Plate XV.

Day 7 of NS + PDGF rabbit: The blood vessels were larger compared to those in the skin, and were mildly engorged with blood cells. The distribution of connective tissue fibres was relatively similar to those recorded in the normal skin based on thickness and density. The distribution was not uniform as higher densities of the fibres were observed from the re-epithelised site into the wound stroma. Infiltrated inflammatory cells, and were flat, elongated connective cell nuclei which were also observed Plate XVI.

Day 7 of NS + NS rabbit: The distribution of the connective tissue fibres were relatively of higher density, compared to those observed in the skin and were not uniformly distributed., More strands were observed in the wound stroma, to the re-epithelised site. The blood vessels were large, numerous and severely engorged with blood cells. Infiltrated inflammatory cells were also severe, and the connective cell nuclei were round in shape but with few flat nuclei Plate XV.

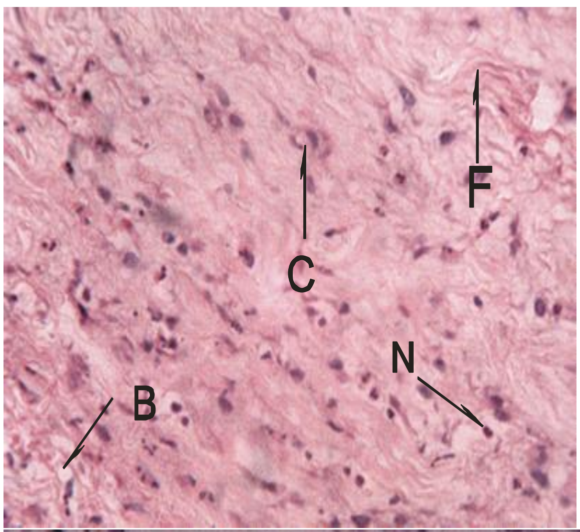
Day 7 of NS + PDGF + EGF rabbit: The distribution of the connective tissue fibres were relatively of higher density and thicker, but uniform when compared to that of the skin. The blood vessels were small, numerous, with few large ones, and they were severely engorged with blood cells. Inflammatory cell were severe. The connective cells nuclei were round in shape, with few flat and elongated Plate XVI.

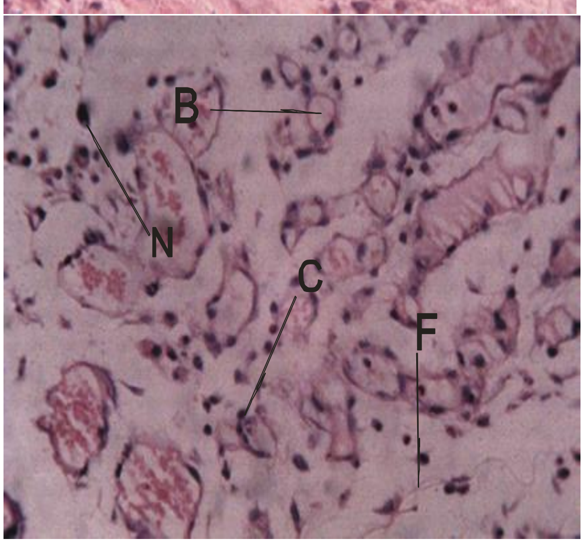




1C = methylP + NS treated rabbits

2C = NS + NS treated rabbits





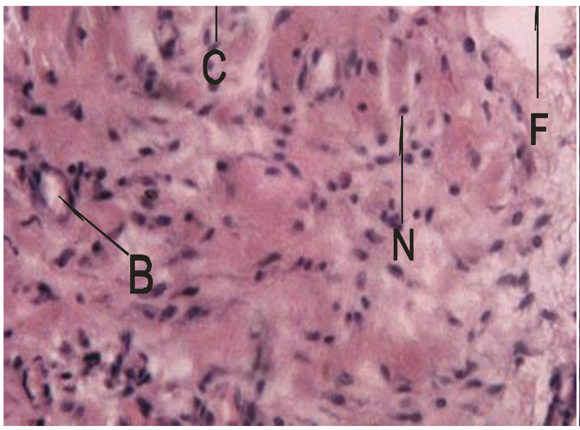
2A = NS + EGF treated rabbits

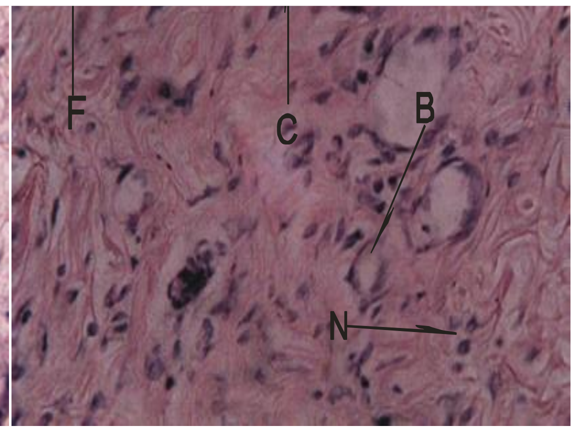
1A = methylP + EGF treated rabbits

Plate XV: Photomicrograph 7 Days Post-wounding in New Zealand rabbits Treated with Normal saline, Methylprednisolone and Epidermal growth factor (EGF-AB)

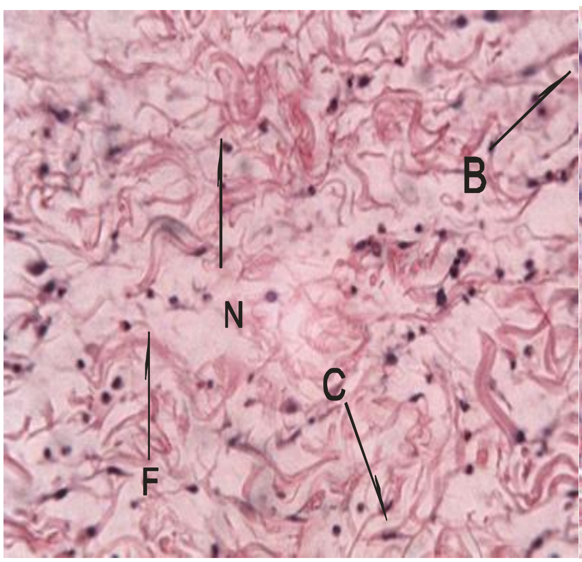
Note: Inflammatory cells (N) influx were severe in 1C but moderate in 2C, 2A and 1A. Blood vessels (B) were severely observed in 1A and mild in 2A, 1C and 2C. Connective tissue fibre (F) were almost absent in 1A, less distinct in 2A and 2C but least distinct in 1C. Collagen (C) deposit was observed severely in 1C followed by 2C and 2A.

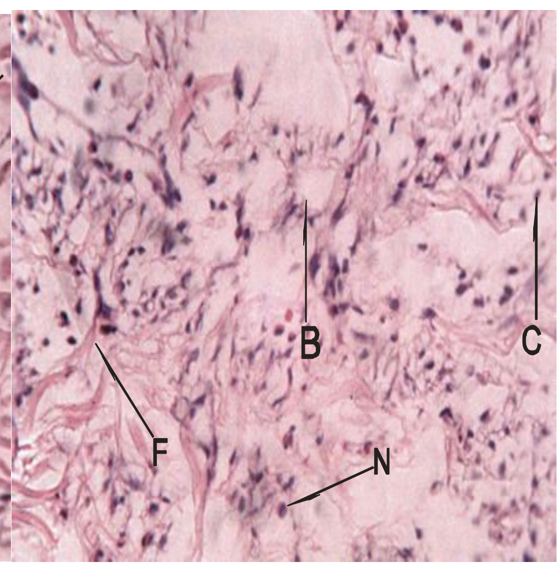
Key:NS= Normal saline; MethylP = Methylprednisolone; EGF = Epidermal growth factor H & E ×250.





2D = NS + EGF + PDGF treated rabbits **1D = methylP + EGF + PDGF treated**





1B = methylP + PDGF treated rabbits **2B = NS + PDGF treated rabbits**

Plate XVI: Photomicrograph 7 Days Post-wounding in New Zealand rabbits Treated with Normal saline, Methylprednisolone, Platelet-derived factor (PDGF-AB) and Epidermal growth factor (EGF-AB)

Note: Inflammatory cells (N) influx was severe in 2D, 1D, 1B and 2B. Blood vessels (B) were also observed in all the groups and were highly vascularised. Connective tissue fibre (F) were distinct in 1B and 2B followed by 1D but least distinct in 2D. Collagen (C) deposit was more in 2D followed by 1D, less in 1B and least in 2B.

Scar of methylP + EGF rabbit: Unlike the connective tissue fibres recorded in normal skin tissue that were pinkish in colour, the scar was purple and uniformly distributed from the re-epithelised site into the stroma. The strands of the connective tissue fibres were of higher density than those observed in the normal skin, but were similar in size. Small and numerous blood vessels were also observed, and most of the blood vessels were mildly engorged with blood cells. The connective cell nuclei were flat and elongated, with few rounded in shape Plate XVII.

Scar of methylP + PDGF rabbit: The distribution of the connective tissue fibres were relatively of higher density, and thinner but uniformly distributed as recorded in the normal skin. The blood vessels were small in size, numerous and mildly engorged with blood. Scanty cells were also observed. Flat and elongated connective cell nuclei were predominantly present Plate XVIII.

Scar of methylP + NS rabbit: The distribution of the connective tissue fibres were uniform with similar density and similar strand thickness, compared to that recorded in the normal skin. The numerous blood vessels were small and mildly engorged with blood, with scanty cells. Flat, elongated connective cells nuclei were also observed Plate XVII.

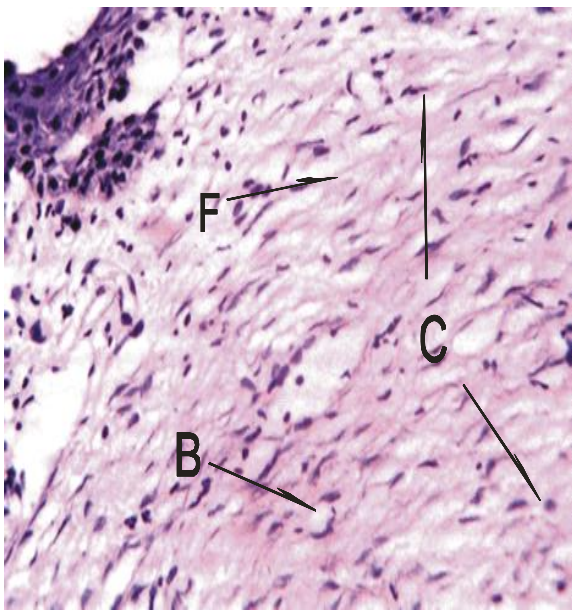
Scar of methylP + PDGF + EGF rabbit: Unlike the connective tissue fibres recorded in normal skin tissue, which were pinkish in colour, the fibres observed in the scar were purple. The connective tissue fibres were relatively more in number (higher density) than that recorded in the normal skin, the fibres were uniformly distributed from the re-epithelised site through the wound stroma. The strands of the connective tissue fibres were thinner, relative to those of the skin. The blood vessels were small similar to those found in the normal skin, and were mildly engorged with blood cells. Few connective cells were present, flat connective cell nuclei were also present and elongated Plate XVIII.

Scar of NS + EGF rabbit: The connective tissue fibres were uniformly distributed and relatively of similar density to those of the normal skin but were thinner in size. Moderately distributed small blood vessels with few large ones, moderately engorged were recorded. Few connective cell were observed. The connective cell nuclei were flat and elongated, with few rounded in shape Plate XVII.

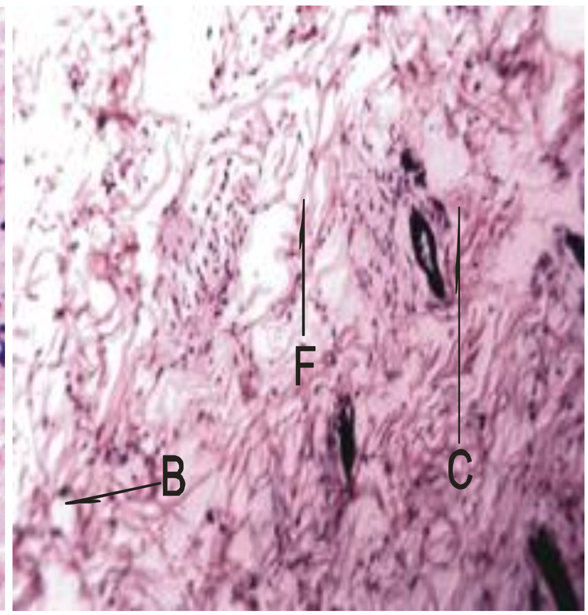
Scar of NS + PDGF rabbit: Unlike the connective tissue fibres observed in the normal skin tissue, pinkish in color, in the scar, the fibres were purple and uniformly distributed from the re-epithelised site into the stroma. The strands of the connective tissue fibres were of higher density to those found in the normal skin and were similar in size. Few small blood vessels mildly engorged with blood cells were recorded. Few connective cells were present. Few of the connective cell nuclei were flattened and elongated Plate XVIII.

Scar of NS + NS rabbit: The distribution of the connective tissue fibres were relatively higher in density compared to that of the skin, and the fibres were uniformly distributed with similar strand size. The blood vessels were small, few and mildly engorged with blood cells. Few cells were present. The connective cell nuclei were flat and elongated Plate XVII.

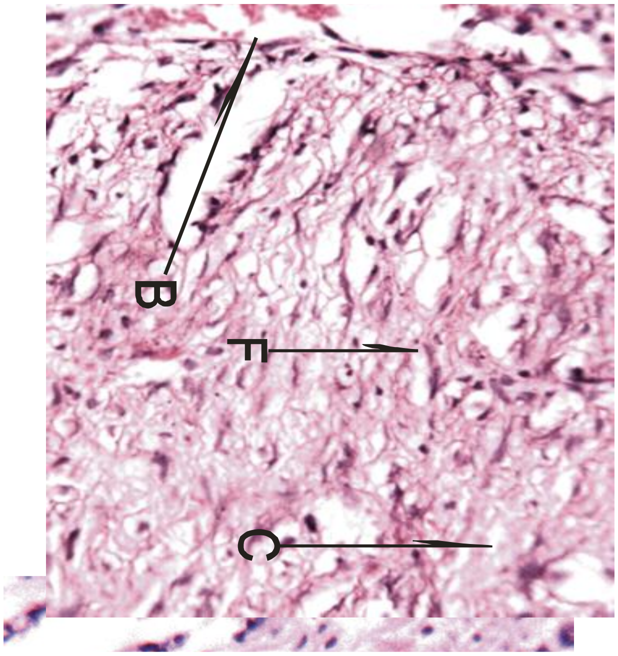
Scar of NS + PDGF + EGF rabbit: The distribution of the connective tissue fibres was relatively of higher density and mated, compared to that of the skin. The blood vessels were small, few and mildly engorged with blood cells. The nuclei of connective cell were flat and slightly elongated, with few round nuclei Plate XVIII.



1C = methylP + NS treated rabbits



2C = NS +NS treated rabbits



2A = NS + EGF treated rabbits **1A = methylP + EGF treated rabbits**

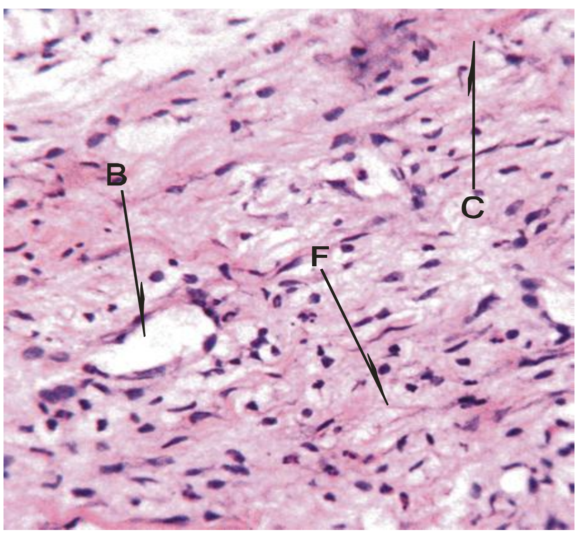
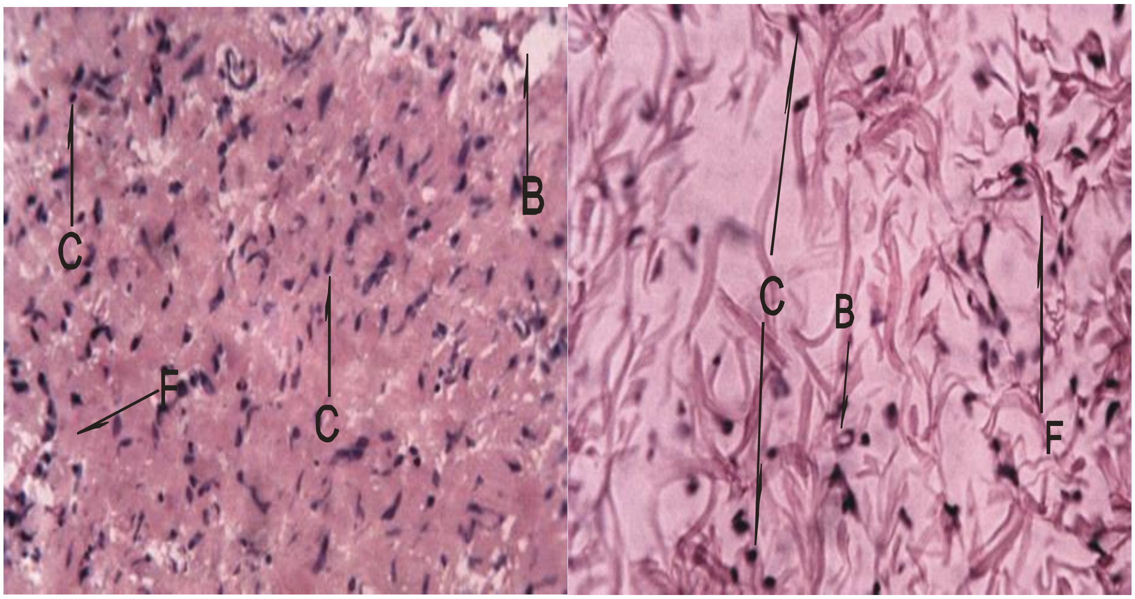


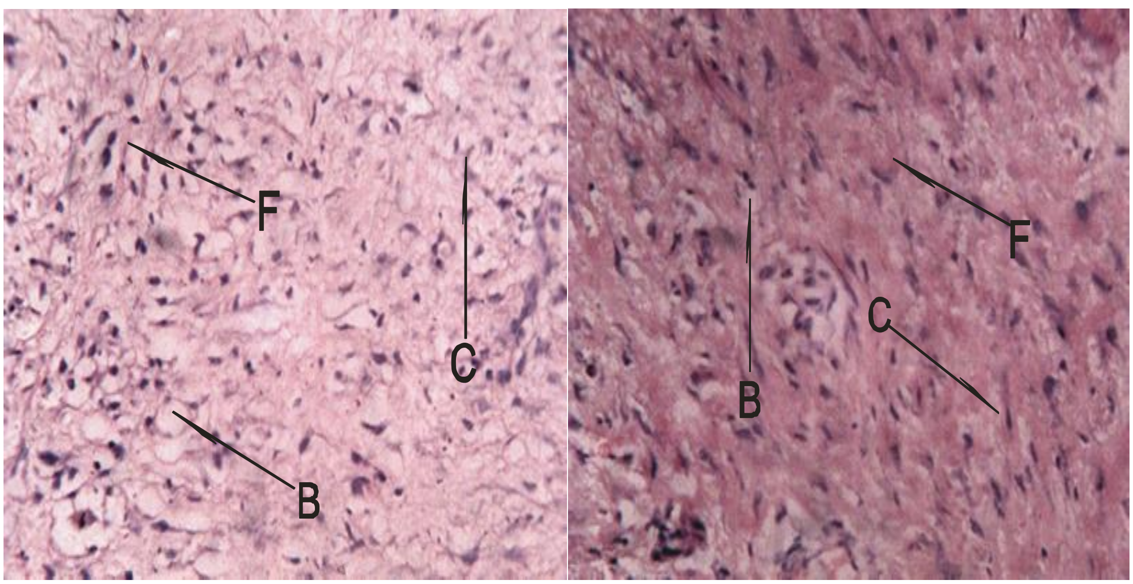
Plate XVII: Photomicrograph of Scar Tissue Post-wounding in New Zealand rabbits Treated with Normal saline, Methylprednisolone and Epidermal growth factor (EGF-AB)

Note: Blood vessels (B) were well distributed in 2A, 1A and 1C but were distributed in 2C. Connective tissue fibre (F) were distinct in all the treated groups but more organised in 1C and 1A. Connective tissue fibre (F) were least organised in 2C. Collagen (C) deposit was more in 2C followed by 2A, and less in 1A and 1C.

Key:NS= Normal saline; MethylP = Methylprednisolone; EGF = Epidermal growth factor H & E ×250.



2D = NS + EGF + PDGF treated rabbits **1D = methylP + EGF + PDGF treated rabbits**



1B = methylP + PDGF treated rabbits **2B = NS + PDGF treated rabbits**

Plate XVIII: Photomicrograph of Scar Tissue Post-wounding in New Zealand rabbits Treated with Normal saline, Methylprednisolone, Platelet-derived factor (PDGF-AB) and Epidermal growth factor (EGF-AB)

Note: Blood vessels (B) were well distributed in 1Band poorly seen in 2D, 1D and 2B. Connective tissue fibre (F) were distinct in 1D but less distinct in 1B, furthermore they were least distinct in 2D but were relatively more organised in 1B and least organised in 2D. Collagen (C) deposit was more in 2D followed by 2B, and less in 1B and 1D.

Key:NS= Normal saline; MethylP = Methylprednisolone; EGF = Epidermal growth factor; PDGF= Platelet-derived growth H & E ×250.

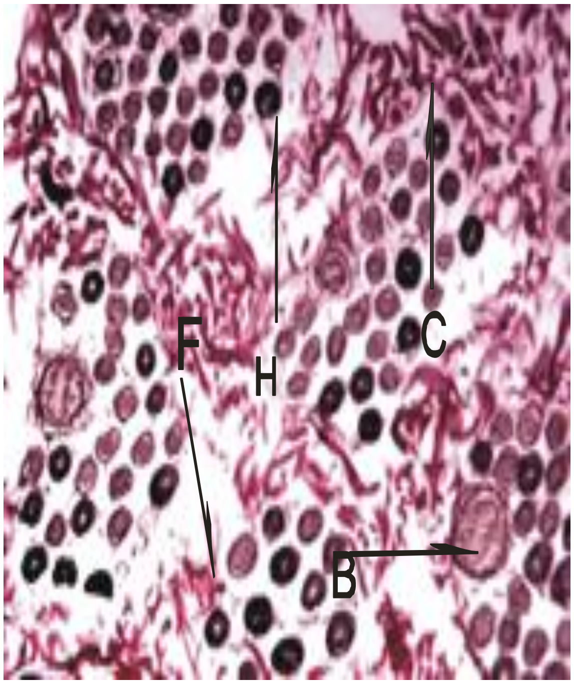


Plate XIX: Photomicrograph of the Normal Skin of a New Zealand rabbit H & E × 250.
Note: C= Connective tissue cells; F= Connective tissue fibre; B=Blood vessel.

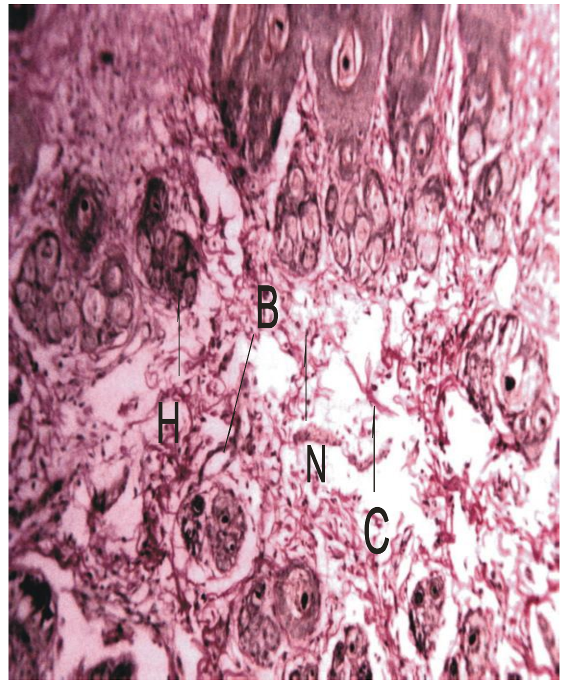


Plate XX: Photomicrograph of the Normal Skin of a New Zealand rabbit VVG × 250.
Note: N = Cell nuclei, H= Hair root, C = collagen; B=Blood vessel.

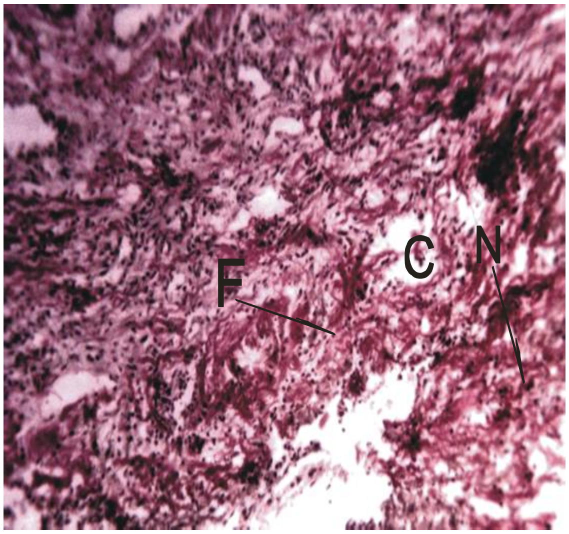
The above observations were made on slides stained with haematoxylin and eosin (H & E). To elaborate on the collagen deposition, a special stain was used, which was specific for collagen (Verhoeff-Van Giesen stain). It stained collagen red, nucleus black, elastic fibres black and muscles yellow. Collagen deposits in the scar tissue stainned with Verhoeff-Van Giesen stain (VVG) (Percival and Radi, 2016), and on day 3 post-wounding were shown in Plates XXIII – XXIV, and XXI – XXII, respectively.

Plate XXI (2B) day 3 and Plate XXII (2D) showed absence of clear collagen fibre strands, however, more collagen deposits were observed in NS + EGF + PDGF. Clear collagen fibre strands were observed in NS + EGF but were more in methyl + EGF.

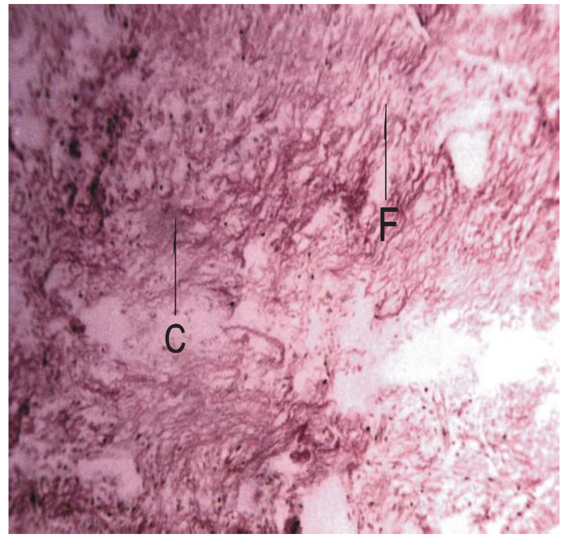
No clear collagen fibre strands recorded in NS + NS or methylP + NS treated rabbits but NS + NS recorded more collagen deposit relative to methylP + NS. Scanty collagen fibre strands were not distinct in NS + PDGF but were relatively more in methylP + PDGF.

Collagen fibre strands were scanty in rabbits treated with both cytokines. Collagen fibre strands arrangement was better recorded in methylP + EGF relative to NS + EGF.

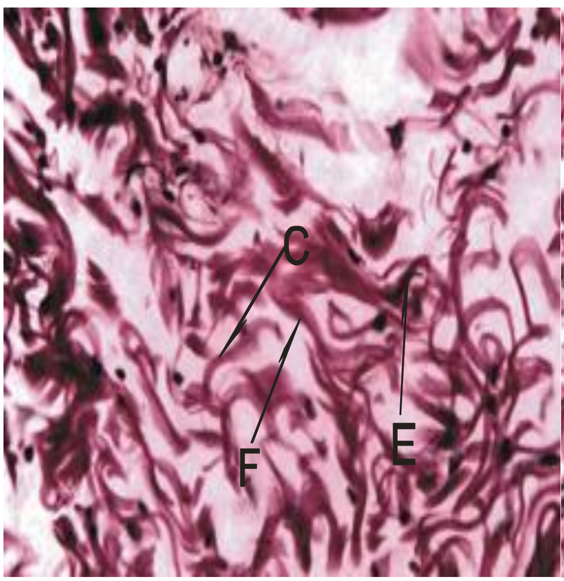
Distincted and organised collagen fibre strands were observed in methylP + PDGF to that observed in NS + PDGF.



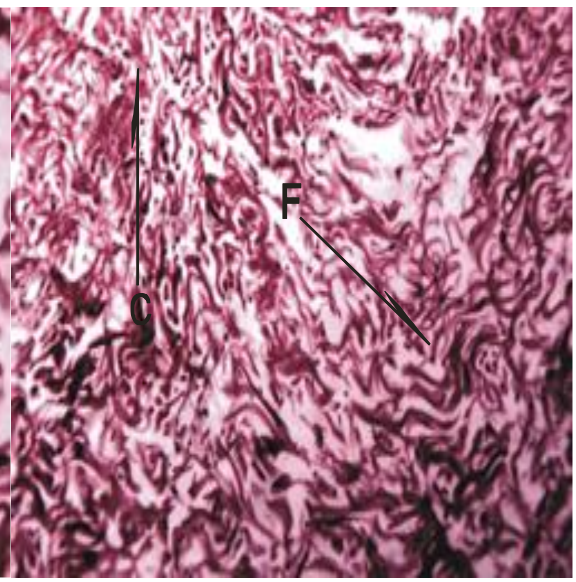
2D = NS+ EGF + PDGF treated rabbits



1D = methylP + EGF + PDGF treated rabbits



2A = NS+ EGF treated rabbits

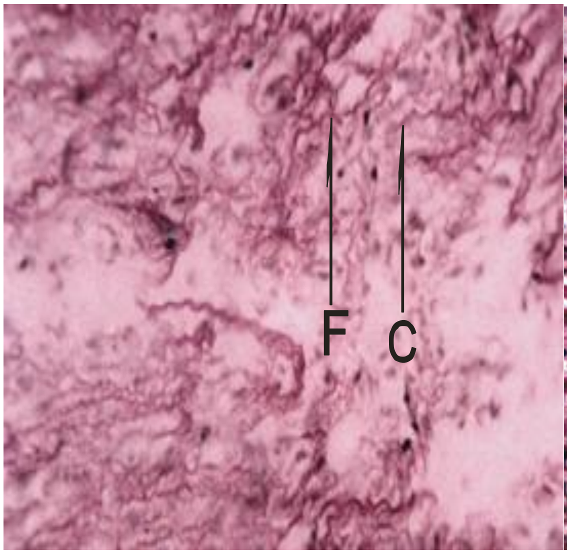


1A = methylP + EGF treated rabbits

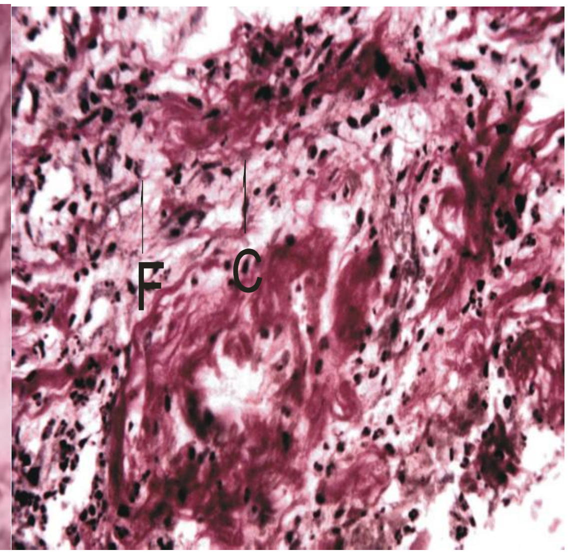
Plate XXI: Photomicrograph 3 Days Post-wounding in New Zealand rabbits Treated with Normal saline, Methylprednisolone, Platelet-derived growth factor (PDGF-AB) and Epidermal growth factor (EGF-AB)

Note: Collagen fibre (F) were distinct in 2A and 1A. Collagen (C) deposit was more in 2D followed by 1A, and less in 1D and 2A.

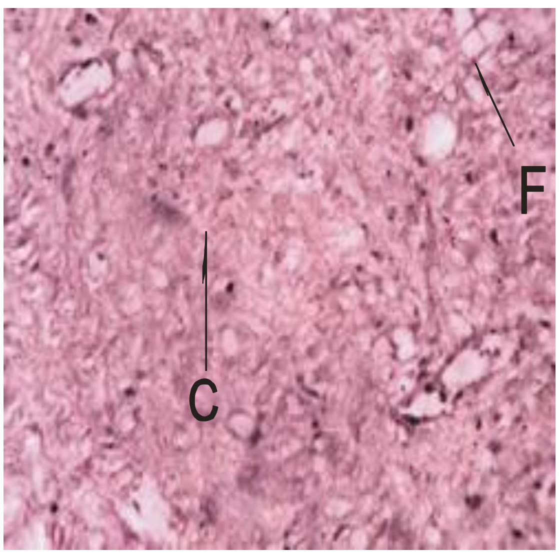
Key:NS= Normal saline; MethylP = Methylprednisolone; PDGF = platelet-derived growth factor; EGF = Epidermal growth factor VVG × 250.



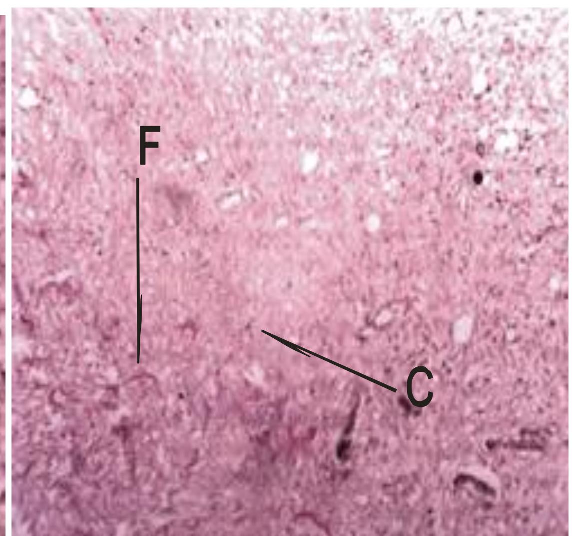
1C = methylP + NS treated rabbits



2C = NS + NS treated rabbits



1B = methylP + PDGF treated rabbits

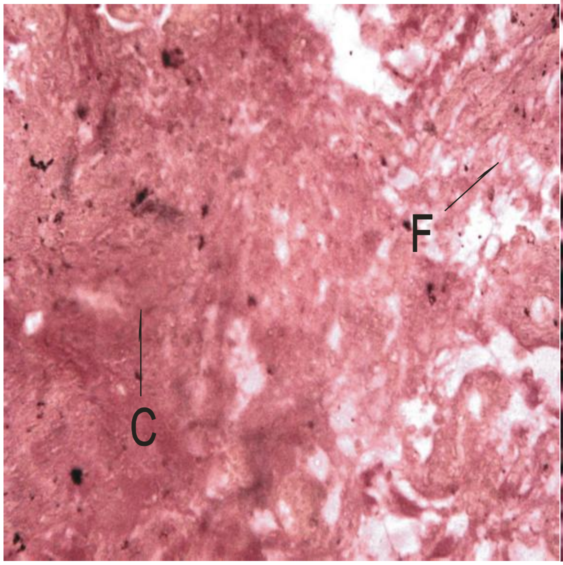


2B = NS + PDGF treated rabbits

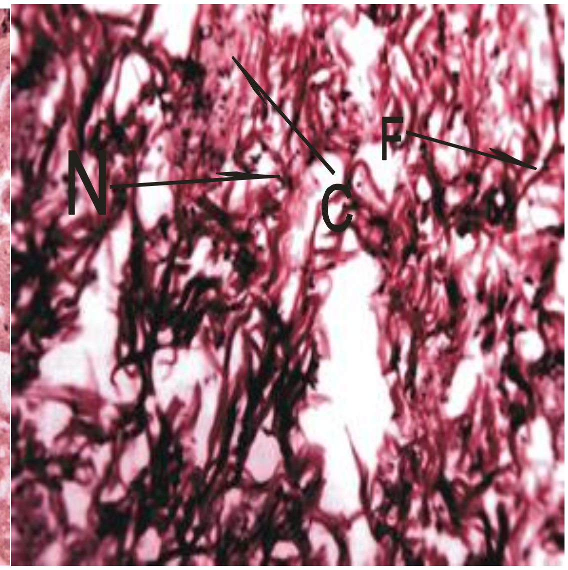
Plate XXII: Photomicrograph 3 Days Post-wounding in New Zealand rabbits Treated with Normal saline, Methylprednisolone, Platelet-derived growth factor (PDGF-AB) and Epidermal growth factor (EGF-AB)

Note: Collagen fibre (F) were poorly distinct in all the treated groups, however, few were distinct in 1B. Collagen (C) deposit was more in 2C and less observed in 1B and 2B, but was least in 1C.

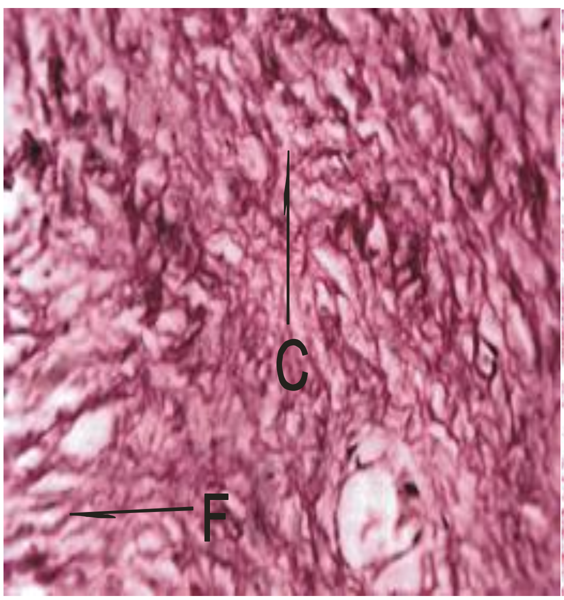
Key:NS= Normal saline; MethylP = Methylprednisolone; PDGF = platelet-derived growth factor; EGF = Epidermal growth factor VVG × 250.



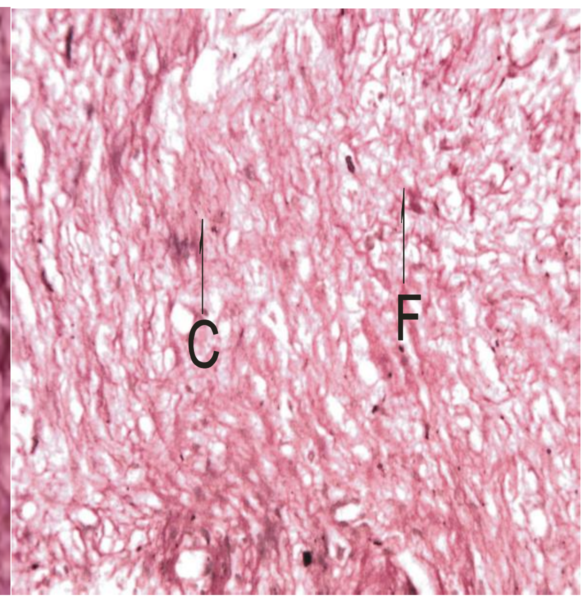
2D = NS + EGF + PDGF treated rabbits



1D = methylP + EGF + PDGF treated rabbits



2A = NS + EGF treated rabbits

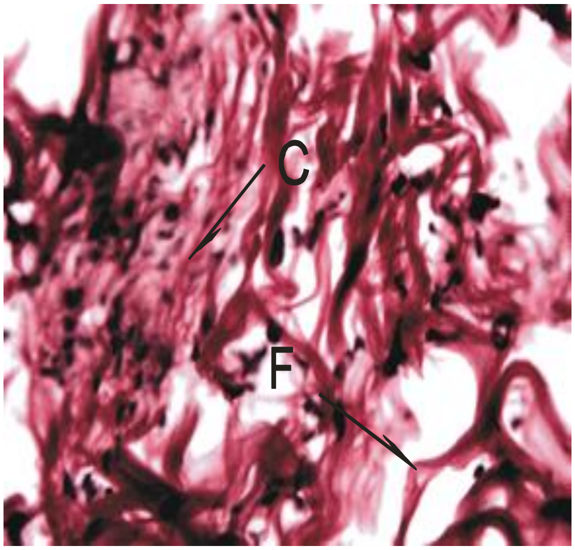


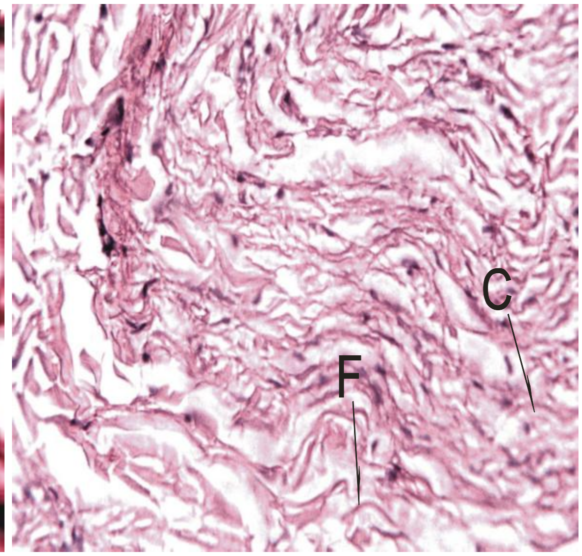
1A = methvlP + EGF treated rabbits

Plate XXIII: Photomicrograph of Scar Tissue Post-wounding in New Zealand rabbits Treated with Normal saline, Methylprednisolone, Platelet-derived growth factor (PDGF-AB) and Epidermal growth factor (EGF-AB)

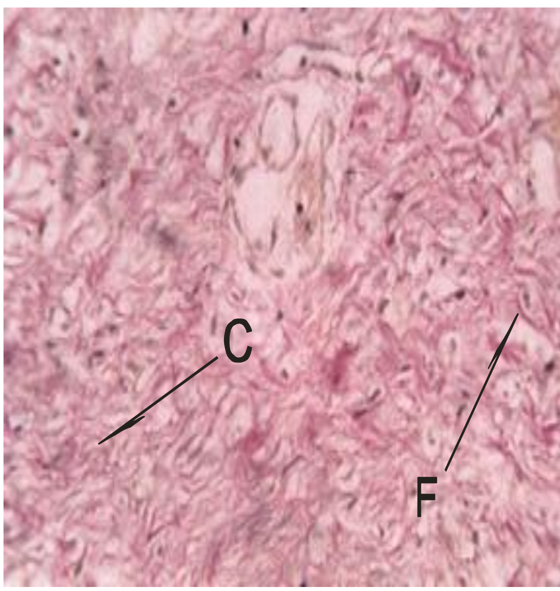
Note: Collagen fibre (F) were poorly distinct in 2D but were best observed in 1A. The collagen fibre were also distinct in 2A and 1D. Collagen (C) deposit was recorded in all the treated rabbits.

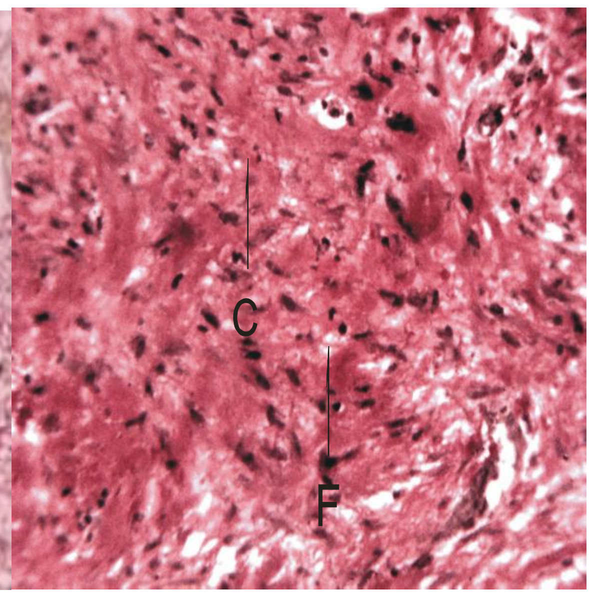
Key:NS= Normal saline; MethylP = Methylprednisolone; PDGF = platelet-derived growth factor; EGF = Epidermal growth factor VVG × 250.





1C = methylP + NS treated rabbits **2C = NS + NS treated rabbits**





1B = methylP + PDGF treated rabbits **2B = NS + PDGF treated rabbits**

Plate XXIV: Photomicrograph of Scar Tissue Post-wounding in New Zealand rabbits Treated with Normal saline, Methylprednisolone, Platelet-derived growth factor (PDGF-AB) and Epidermal growth factor (EGF-AB)

Note: Collagen fibre (F) were poorly distinct in 1C and 2B but were more observed in 2C followed by 1B. The collagen (C) deposit was more in 2B and 1C.

Key:NS= Normal saline; MethylP = Methylprednisolone; PDGF = platelet-derived growth factor; EGF = Epidermal growth factor VVG × 250.

CHAPTER FIVE

5.0 DISCUSSION

The wound dimension at the beginning of the study after wound creation was observed to be more than the initial dimensions after complete re-epithelialisation. Wound dimensions increased (that is wound retraction) from the day of wounding. Wound retraction was less in the methylP treated groups, apparently due to the anti-inflammatory activities of methylP, Shaikh *et al.* (2012) also reported the anti-inflammatory activities of steroids. In addition the early resolved wound retraction could also be due the anti-inflammatory activities of the methylprednisolone. The observed reduced and resolution of wound retraction 2 days earlier in the methylP group could be relevant in clinical practice. Thus, less contact with the environment to accommodate wound contaminants, for collinisation, wound infection, and subsequently delay wound healing. Secondly, such an enhancement in healing is of significant importance in terms of aesthetical purpose and acceptability by the patient, client and clinician by implication. The clinician will only have minimal wound retraction to manage. This early resolution of wound retraction was also observed in NS + PDGF and NS + EGF + PDGF, which is in line with the findings of Werner and Grose (2003) and MacCornick *et al.* (2014) who both shared that PDGF is an anti-inflammatory cytokine. MacCornick *et al.* (2014) and Salmerón-Sánchez and Dalby (2016) also reported that cytokines work in synergy. This finding suggests that PDGF or EGF + PDGF may be administered to replace methylprednisolone in order to obtain an early resolution of wound retraction. Furthermore, the use of PDGF or PDGF + EGF alongside with methylprednisolone in the current study did not exert synergic effects in the resolution of wound retraction. It was also observed that the use of PDGF had better anti-inflammatory effect in healing of acute wounds, compared to the use of EGF. Both agents EGF and PDGF are anti-inflammatory cytokines (Werner and Grose, 2003; MacCornick *et al.,* 2014). The result of the current study demonstrated that, topical administration of PDGF, with or without steroid induced relatively, early resolution of inflammatory activities. Therefore, PDGF exhibited anti-inflammatory activities which were not synergised by the administration of the steroid. It was observed in this study that wound contraction was highest between day 7-12. This finding was in line with the report obtained by Gabriel *et al*. (2009) and Eming *et al*. (2014) who reported that the highest contraction in wound healing occurred between days 5-15. The current study showed that wound contraction occurred from day 5 post-wounding, which agreed with the findings of Gabriel *et al*. (2009) and Eming *et al*. (2014) that wound contraction was maximal between 5^{th} – 12^{th} day of post-wounding. The initial increase in wound size could be due to infiltration by inflammatory cells and oedema. Though not statistically significant, the combination of steroid with PDGF may have some enhancing potentials on the formation of myofibroblasts.Wound contraction is the major pattern of wound healing in rabbit. Wound healing occurs relatively rapid as a result of wound contraction initiated by the *musculus panniculus carnosus* in the subcutaneous tissues of laboratory animals including the rabbit (Westgate *et al.,* 2010; Theoret and Wilmink, 2013). The smallest scar size was obtained in methylP + PDGF and methylP + EGF rabbits with the values of 0.25 and 0.32 cm^2 respectively suggesting that in order to achieve minimal scar size, aesthetic and quick return to function, the use of methylP +NS or methylP + EGF was beneficial. Consequently, the combination of NS + EGF + PDGF resulted in larger scar size. It was also observed that the groups treated with the combination of the cytokines

(EGF and PDGF) were the least to heal. The combination of cytokines as reported by Foey *et al*. (2001) and Nadeem *et al*. (2004) was synergic in wound modulation, but need other factors to be considered. Apparently, combinations of EGF and PDGF may be synergic in scar quality but not in reduction of the overall wound healing time. Furthermore, wound healing was achieved after 14 days in methylP + EGF, and NS +NS, hence the use of these cytokines or steroid may not enhance the healing time of wounds; although good management practice is the major factor affecting the time in wound healing (Franz *et al.,* 2007). MethylP + NS treated groups had the largest wound retraction; therefore, at the end, had the smallest scar size. This finding suggested that wound retraction is vital in wound healing, if good wound management is provided as reported by Franz *et al*. (2007), interference in wound healing at any stage of healing may retard wound healing resulting in chronic non-healing wound.

Epithelialisation were recorded in rabbits belonging to NS + EGF + PDGF and methylP + EGF+ PDGF groups on day 5, while on day 7 in NS + EGF and methylP + EGF rabbit groups post-wounding which was contrary to the finding of Sardari *et al*. (2006) and Pastar*et al*. (2014) who reported that, epithelialisation occurs from 72 hours post-wounding in dogs, and the result may be due to difference in animal species as rabbits were used in this studies. Similar findings were obtained by Westgate *et al*. (2010) and Theoret and Wilmink (2013), who reported that wound heals by contraction in laboratory animals rather than epithelialisation as observed in dogs or large animals. It was observed in the present study that epithelialisation started from wound edges and increased progressively until the wound stroma was completely covered. This observation was similar to the finding of Robson *et al*. (2001) who reported that epithelialisation occurs from wound edge. In line with Deodhar and Rana (1997) and Slavkovsky *et al.* (2010) epithelialisation may cover wound area of 9 cm^2, which is even more than the wound dimension (2 cm^2) created originally in this study. In addition, epithelialisation which was observed in the present study was irregular from all directions, but more from one direction (unilateral) which was contrary to the report that, epithelialisation occurs in all directions, as reported by Deodhar and Rana (1997) and Slavkovsky *et al.* (2010) that epithelialisation occurs in all directions. The non-uniform epithelialisation recorded in this study could be due to the arrangement of skin cells, blood supply or centripetal forces acting on the wound edges. From the result of the treatments, early stimulation of epithelialisation was observed from day 5 post-wounding in methylP + NS, NS + EGF, NS + PDGF, NS + EGF + PDGF and methylP + EGF + PDGF. This was earlier than that observed in rabbits treated with methylP + EGF, methylP + PDGF and NS +NS that demonstrated retarded epithelialisation. The administration of methylprednisolone with EGF or PDGF retarded epithelialisation by 2 days when compared with rabbits treated with either EGF or PDGF protein alone. However, the use of methylprednisolone enhanced early epithelialisation in methylP + NS and methylP + EGF + PDGF. Thus, the early epithelialisation achieved with the use of EGF, PDGF and EGF + PDGF was also recorded with the use of methylP + NS, and methylP +EGF + PDGF. Furthermore, an exception was obtained in the rabbits treated with methylprednisolone alone, in which most groups with early epithelialisation had larger scar relative to those with latter onset of epithelialisation. This finding strongly suggested that PDGF and EGF exerted each a relatively more pronounced effect on early epithelialisation than when either was combined with the steroid. Contrary to our findings Langer and Rajagopalan (2012) reported that no variations in wound healing between patients treated with PDGF or EGF, compared to those treated by conventional dressings, of which we observed earlier epithelialisation with the use of cytokines. Generally, wound healing was observed to be by wound contraction in the present study, apparently demonstrating that stimulation of early epithelialisation is undesirable in rabbits, but may be required in other animals where healing occurs mainly by epithelialisation.

The result of the present study demonstrated that granulation tissue formation was evident from wound edges 72 hours post-wounding, and this results agree with the findings of Dabiri *et al.* (2016) and Gonzalez*et al.* (2016) who reported that granulation tissue formation is apparent 2 days post-wounding from the wound edges. Granulation tissue formation was relatively or generally very poor in this species. This finding was likely due to the wound healing pattern of rabbits, which is by contraction, important for re-epithelialisation. By day 10, granulation tissue was fully formed in all the groups. Histologically on day 3, the quantity of blood vessels recorded was best observed in methylP + PDGF, and methylP + EGF + PDGF. Thus, the cytokines antagonised the effect of methylprednisolone in the inhibition of granulation tissue formation, especially in PDGF treated rabbits. Granulation tissue formation was fully formed at day 10 in all treated groups. On day 7 however, the formation occurred in the following descending order of vascularity: NS + EGF, methylP + EGF + PDGF, NS + EGF, methylP + NS, NS +PDGF, methylP + PDGF, NS + NS, and NS + EGF + PDGF. Collagenisation was progressive after the infiltration of fibroblasts into the wound site. In this study however, using Verhoeff–Van Giensen stain (VVG), a special stain for collagen, it was observed that collagen at the wound site was present in all the groups; and in some groups the strands of collagen were visible like in the methylP + PDGF, methylP + EGF, and methylP + NS rabbits, which was an evidence of the superior effect of metyhylP + NS or its combination with EGF or PDGF on day 3. The effect observed later with the administration of the cytokines agreed with the result of Carvalho *et al*. (2012), that cytokines display deferred activities. Furthermore, Langer and Rajagopalan (2012), did not observed any significant difference in wound healing between patients treated with PDGF, compared to those treated by conventional dressings. This was contrary to our findings where collagen strands were seen at day 3 in methylP + NS, methylP + EGF and methylP +PDGF. However at day 7, the methylP + NS rabbits had most collagen deposits, followed by methylP + EGF +PDGF, and NS + EGF +PDGF, suggesting the synergic effects of the cytokines. Clear strands were observed only in methylP + PDGF and NS + PDGF, suggesting that PDGF exerted superior effects in early organised deposition of collagen. Though the clear strands continued in methylP + PDGF to day 7, it was not recorded in methylP + NS and methylP + EGF. Overall, the result of the study has demonstrated the superior effect of the methylP + PDGF and the cytokine synergy over single use.

Eosinopenia was, not observed in this study even with the use of steroids, and this finding disagreed with the finding of Kreindler *et al.* (2016) who used steroid and recorded eosinopenia in their study. The lack of eosinopenia in the current study may be due to animal species (rabbit) used in this study as eosinophils are not common circulating blood cells in rabbits (Etim *et al.,* 2014). Furthermore, the regiment of the steroid (methylprednisolone) in this study was not prolonged, but rather short-term and at therapeutic dose. Much attention was observed to the dressing applied on the rabbit treated without methlP in NS + NS, NS + EGF, NS + PDGF, NS + EGF + PDGF groups, which may be due to mast cell degranulation as reported in dogs by Misdorp (2004). Steroids are recommended in hypersensitivity reactions where histamine plays vital role. Mast cell activities like the degranulation of mast cells leads to the release of histamine and are responsible for itching (Misdorp, 2004). MethylP was expected to stabilise mast cells and, hence, prevent degranulation. Hair growth after shaving was recorded within 10 days in the methylprednisolone-treated rabbits, however, earlier than that observed in the non-methylP treated 14 days post-wounding, apparently hair regrowth maybe due to the activities of the methylprednisolone as involved in the enhancement of hair growth, psychological comfort, or distraction induced on the non-steroid treated groups that concentrated on itching of the body. The use of steroids at therapeutic dose in this study was to promote cell stability and reduce stress, but the blood cell picture showed significant differences in lymphocyte, neutrophil, monocyte and platelet counts. Contrary to the findings of this study, Kim and Kang (2000) reported marked increase in total WBC and neutrophils, decrease in lymphocytes, and no changes in monocytes. In the present study, WBC count was not significant. This was similar to the findings of Rana *et al*. (2014) who reported that RBCs, Hb, and PCV increased significantly, but WBC count showed no significant difference. The considerable differential WBC counts may be as a result of immunological response to possible surgical procedure or wound healing. It may also be due to the normal wound healing dynamics that favoured the interplay between the cells to achieve complete healing, as lymphocytes, neutrophils, monocytes and platelets are all essential at the wound site for wound healing to be achieved. Methylprednisolone had significant effect on circulating platelets in exerting suppressive effect on platelet counts which were low in NS + EGF + PDGF, and methylP + EGF + PDGF rabbits. Lymphocyte count was also suppressed significantly in methylprednisolone treated group, and was more pronounced in EGF-treated groups, that is, methylP + EGF and NS + EGF. Similarly, monocyte counts were low in methylP + EGF + PDGF, and NS + EGF + PDGF. The neutrophil counts was also suppressed in all steroid-treated groups compared to the normal saline-treated, an exception was the group administered with PDGF. Methylprednisolone is recognised to be WBC suppressant (Shimada*et al*., 2000; Grover *et al*., 2007) and was observed significantly on lymphocyte, monocyte, neutrophil and platelet. However, PDGF alone did not significantly decrease the blood cells count in the treated animals, except in the EGF + PDGF group. Methylprednisolone suppressed circulating platelets, monocytes and neutrophils especially in methylP+ EGF + PDGF and NS + EGF + PDGF. However, the suppression of these circulating cells may be responsible for the delay in epithelialisation as observed in NS + EGF + PDGF, NS +NS, methylP + EGF and NS + EGF rabbits, but could also be responsible for the similar scar size. This finding may be due to stress caused by the wounds on the rabbits, or their attraction to the wound site where they formed a pool.

Wound healing is a complex process that involves interactions among a large number of distinct cell populations, whose regulation is tightly controlled. In this study the concentration of EGF and PDGF in the serum were unaffected or altered with wound creation and wound healing process. There was no significant difference between the measured serum cytokines level after surgery and the values obtained before surgery. There was also no correlation among measured cytokines levels and wound healing rate in the present study which agreed with Mohammad *et al*. (2015) who demonstrated absence of significant correlation between systemic cytokine concentrations and progress in healing. Gohel *et al*. (2008) also showed great differences occurred between wound and serum cytokine intensities, which largely inhibit their usefulness as markers of wound healing. Furthermore, topically administered EGF and PDGF in the present study had no effect on the serum cytokine, this was apparent because the little concentration applied at the wound site might be poorly absorbed or inadequate to cause any systemic effects, or they were readily denatured by the matrix metalloproteinase. Bae *et al*. (2014) and Hong*et al*. (2014) also reported that, the set-back in the administration of topical cytokines is their digestion by matrix metalloproteinase. Cavalho *et al*. (2012) reported that systemic quantification of cytokines may not be a mirror of biochemical events taking place at surgical site. Cytokines are within the granules of circulating platelets, and tissue cells which are only released during clotting, therefore lack of clotting make circulating cytokines to be unaffected in surgical procedure and wound healing (Casacó *et al*., 2012).

On day 3 post-wounding the connective tissue fibres were numerous(of high density) in methylP + EGF, methylP + PDGF, methylP + NS and NS + EGF groups and they were of similar density with that of the normal skin in methylP + EGF + PDGF group. The connective tissue fibres, however, were sparse (low density) in relation to that observed in the skin for NS + PDGF, NS + NS, and NS + EGF + PDGF rabbit groups. It was possible here that, methylP enhanced infiltration of fibroblasts into the wound site, or the anti-inflammatory effect of methylP gave a false impression of dense population of the connective tissue fibres. The nuclei of the connective tissue in methylP + NS rabbits demonstrated early transformation of fibroblast to myofibroblasts, as evidenced by the presence of flattened and elongated cell nuclei. The blood vessels were smaller than those in the skin in groups methylP + EGF, methylP + NS, NS + EGF, and NS + EGF + PDGF: thus, early neovascularisation were enhanced by the steroid and the EGF treated groups. Thus, the addition of EGF in wound treatment enhanced early neovascularisation and was unaffected with the use of methylprednisolone.Fibres distribution on day 7 appeared similar to that in the skin in methylP + NS, NS + EGF and NS + PDGF groups, but was denser (more compact) in other groups. The fibres were thinner in group methylP + EGF, and methylP + NS, similar to that on the skin in methylP + PDGF, methylP + EGF + PDGF, NS + PDGF and NS + NS and thickened in NS + EGF and NS + EGF + PDGF rabbits. Overall, the effect of PDGF at day 7 showed that PDGF modulated fibres in density and thickness better than EGF or its combination with EGF, and methylP or its combination with PDGF. Furthermore, the addition of PDGF to methylP did not influence the fibre density but result in a similar fibre thickness. Round nuclei persisted in methylP + EGF, NS + NS and NS + EGF + PDGF, but were slightly elongated in methylP + EGF + PDGF and NS + EGF and were flat and elongated in methylP + EGF + PDGF, methylP + NS, and NS + EGF + PDGF. The combination of EGF + PDGF showed superior effect on transformation of fibroblasts to myofibroblasts, suggesting synergic effect at day 7 post-wounding. But delay in the transformation of fibroblast to myofibroblast were observed in NS + NS, NS + PDGF and NS + EGF rabbits. This indicated that, non-steroid treated groups had less transformed fibroblast to myofibroblast and that the use of PDGF enhanced the transformation of fibroblast to myofibroblast.

The scar had similar connective tissue fibre density in group NS + EGF as that in the skin, while the fibres size were similar with that in methylP + EGF, methylP + NS, NS + NS and NS + EGF + PDGF but fused (mated) in group NS + EGF + PDGF. The metylP + NS and methylP + EGF treated group had the best fibre size relative to skin. This finding also showed that the topical administration of EGF enhanced modulation effect on fibre density in the final scar, and the addition of EGF to methylP enhanced fibre size. Thus a balanced may be needed in the use of EGF with methylP to achieve not only good fibre size but also good fibre density. The connective tissue fibres were thin in groups methylP + PDGF, methylP + EGF + PDGF, and NS + EGF. Hence the use of PDGF or EGF + PDGF on methylP treated rabbits results in thin connective tissue fibres. Common in all the groups were the purple colour of the connective tissue fibres in the scar unlike its pinkish colour in the normal skin, this demonstrated that the connective tissue fibres in the wound were immature fibres. In this study the inflammatory cells population on day 3 was observed to be less than that on day 7. This result could be due to the increase in the population of fibroblast into the wound site. Rozman and Bolta (2007) also reported increase cell population between day 7 and 14. Similar increase in the counts was reported by Olczyk*et al.* (2014) that cells of the inflammatory phase neutrophil and platelets usually lasts for 3 days post-wounding and are interchanged during the repair phase of wound healing by influx of lymphocyte, eosinophil, and basophil (Hart, 2002;

Daniel and Mathieu, 2006). Bin *et al*. (2011) also reported that fibroblast transformed into 3 forms: myofibroblast, cells for protein synthesis and cells for collagen synthesis. Hence, the need of high influx for fibroblast at wound stroma may also account for the high cellular wound stroma as compared to that on day 3. However, reduction in cell population was observed between day 3 and 7 in NS +NS, NS + EGF + PDGF and methylP + EGF + PDGF, suggesting that infiltration of inflammatory cells, mainly macrophages, neutrophils and platelets, as reported by MacCornic *et al.* (2014) andOlczyk*et al.* (2014) that the non-cellular wound environment is changed to cellular within 48 hours post-wounding, and this was excessive at 72 hours in all the groups. Scanty fibroblast at day 7 in same groups likely account for the larger scar size. In this study EGF + PDGF had less stimulating effects on fibroblast. Furthermore, mast cell count at the wound milieu diminishes as wound healing progresses (da Silva*et al.*, 2014). Increased myofibroblast was observed at day 7, compared to that recorded on day 3 post-wounding, this was also observed by Calfee and Manning (2002) who stated that at day 7 fibroblast specialise to myofibroblast to reduce wound edges. Vascularity also increased from day 3 to day 7, which was expected in order to maintain optimum of oxygen and nutrient supply at the wound site. Diegelmann (2004), Barrientos*et al.* (2008) and Eming *et al.* (2014) also reported that neovascularization transports oxygen and essential nutrient further into the damage site.

Tensile strength was high in the methylP + PDGF (76.8), moderate in methylP + EGF (71.3), and low in NS + EGF (70.8). This finding signifies that the combination of methylP + PDGF had some synergic relations in tensile strength. However, the EGF with or without methylprednisolone had good tensile strength. This finding implied that, EGF increased tensile strength of rabbits wound and had no influence on methylP. The ultrasound also showed methylP + EGF, NS + EGF followed by methylP + PDGF. This was also consistent with the Vancouver scar scale methylP + EGF, NS + EGF followed by methylP + PDGF in this study. In all cases the NS + NS had the worse rating in tensile strength, ultrasound and the Vancouver scar scale. This showed that, the use of methylprednisolone and cytokines resulted in a better scar compared to the control.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

The effects of a single topical application of EGF and PDGF with or without the parenteral administration of methylprednisolone resulted in varied effects on wound healing process in rabbits:

The use of both cytokines resulted in early epithelialisation as well as early resolution of wound retraction by 2 days, hence may reduce the risk of wound infection and counter the delaying effect of methylprednisolone on epithelialisation in rabbit wound healing process. EGF + PDGF enhanced neovascularisation at day 3. MethylP + EGF + PDGF recorded more vascular bud at day 7, than NS + EGF + PDGF; granulation tissue was fully formed in all treatments by day 10 post-wounding. Methylprednisolone recorded less cellular influx at day 3, that is, methylP + NS compared to NS + NS, similar response was achieved with the use of methylP + EGF compared to NS + EGF, methylP + EGF + PDGF relative to NS + EGF + PDGF. Furthermore, a synergic anti-inflammatory activity was achieved with the use of the methylP + EGF. PDGF demonstrated being a stronger anti-inflammatory cytokines than EGF, and their combination also resolved retraction within 3 days.Methylprednisolone alone at 0.5 mg/kg resulted in early transformation of fibroblasts to myofibroblasts, but the transformation was delayed in groups treated with NS + NS and NS + EGF + PDGF. The use of methylprednisolone alone at 0.5 mg/kg resulted in superior collagen arrangement relative to its use with any of the cytokine, or in groups not treated with the steroid. Collagen was more in NS + EGF +PDGF and more organised in the methylP + EGF treated rabbits. This was also reflective on the tensile strength MethylP + EGF (71.25 N/cm^2). However, for aesthetic purpose, methylP + NS (0.32 cm^2) and NS + PDGF (0.25 cm^2) recorded the least scar size.

Blood cell counts were not influenced in the study except for the undulating pattern which was more in the steroid treated rabbits and the reduced platelet count which was observed in the methylprednisolone treated group. Surgical procedure, wound healing and stress associated with them did not influence circulating cytokines levels. However, the use of methylprednisolone prevented itching which was observed in the normal saline-treated rabbits. Furthermore, hair re-growth was faster (10 days) in the methylprednisolone treated rabbits and slow (14) days in the non-methylprednisolone treated rabbits.

6.2 Conclusions

The use of NS + EGF + PDGF results in early and enhanced epithelialisation, however resulted in larger scar tissue, thus, enhanced epithelialisation may not be desired in this specie for aesthetic purpose.

1. The study showed that the administration of methylprednisolone at 0.5 mg/kg resulted in early transformation of fibroblast to myofibroblast but transformation was delayed in group treated with NS + NS and NS + EGF + PDGF.

2. Reduced period of wound retraction was achieved with the use methylprednisolone, EGF or PDGF, thus, reduced wound surface area for contamination and reduced chances of wound infection.

3. Platelet-derived growth factor is a stronger anti-inflammatory cytokine compared to epidermal growth factor.

4. Smallest scar size was achieved with the use of PDGF and methylP + PDGF, thus, maybe important in cosmetic surgeries.

5. The administration of methylprednisolone at 0.5mg/kg in wound management to enhance collagen fibre arrangements in scar tissue.

6. Tensile strength was best observed in methylP + PDF and methylP + EGF, thus, are relevant in management of wounds in a weight carrying regions like the ventral aspects of the abdomen.

7. The use of ultrasound in tissue harmonic and Doppler mode at the frequency of 5 Hz as a non- invasive, affordable and convenient tool for scar tissue evaluation.

Finally, the administration of methylprednisolone at 0.5 mg/kg in wound healing management was beneficial, and maybe a good therapy for scar quality. Though proper wound dressing had significant influence on wound healing in terms of time and scar quality, but can be enhanced by the administration of cytokines and methylprednisolone.

6.3 Recommendations

In view of the above conclusions we recommend that more work be done on:

1. The synergic effects of EGF and PDGF in wound healing.

2. The concentration of PDGF and EGF at wound site should be carried out to give a based-line idea as to the quantity that may need to be added at wound site which can also be in relation to the wound size.

3. The injection of these cytokines into the wound stroma, rather than topical administration.

4. The use of water-soluble medium as vehicle for the cytokines in wound treatments like honey.

REFERENCES

Abcam manual. (2017). http://www.abcam.com/ps/products/100/ab100504/documents/ab100504-EGF-Human-ELISA-Kit-ab100504-protocol-plain-v2.pdf. Retrieved 16/03/2017.

Abcam manual. (2017). http://www.abcam.com/ps/products/100/ab100623/documents/ab100623-PDGF-Human-ELISA-Kit-ab100623-protocol-plain-v2.pdf. Retrieved 16/03/2017.

Afshari, M., Larijani, B., Fadayee, M., Darvishzadeh, F., Ghahary, A., Pajouhi, M., Bastanhagh, M. H., Baradar-Jalili, R. and Vassigh, A. (2005). Efficacy of topical epidermal growth factor in healing diabetic foot ulcers.*Therapy*, 2(5):759-765.

Ahmed, E. M. A. (2015). Hydrogel: Preparation, characterization, and applications: A review. Journal *of Advanced Research*,6(2):105–121.

Alturkistani, H. A., Tashkandi, F. M. and Mohammedsaleh, M. Z. (2016).Histological Stains: A Literature Review and Case Study. *Global Journal of Health Science*, 8(3): 72–79.

Amendt, C., Mann, A., Schirmacher, P. and Blessing, M. (2002). Resistance of keratinocytes to TGF β-mediated growth restriction and apoptosis induction accelerates re-epithelialization in skin wounds.*Journal of Cell Science*, 115(10):2189-2198.

Anaya, D. A. and Dellinger, E. P. (2006). The obese surgical patient: a susceptible host for infection. *Surgical Infection (Larchmt)*, 7(5):473-480.

Anderson, K. and Hamm, R. L. (2012). Factors that impair wound healing. *Journal of the American College of Clinical Wound Specialists*, 4(4):84-91.

Ani, A. (2010). Receptor behind testosterone activity plays key role. *Wound Healing.* blog.taragana.com/science/2010/01/05/receptor-behi. Retrieved 2014/07/11.

Arnold, M. and Barbul, A. (2006). Nutrition and wound healing. *Plastic and reconstructive surgery*, 117(7 Suppl):42S-58S.

Atiba, A., Marzok, M. and Ghazy, A. (2014). Comparison of *Aloe vera* and silver sulfadiazine in the treatment of deep second-degree burn in dogs. *Global Veterinaria*, 13(5):733-737.

Atiyeh, B. S., Dibo, S. A. and Hayek, S. N. (2009). Wound cleansing, topical antiseptics and wound healing.*International Wound Journal*, 6(6):420-430.

Badiavas, E. V. and Falanga, V. (2003) Treatment of chronic wounds with bone marrow-derived cells. *Archives of Dermatology,* 139(4):510-516.

Bae, I. H., Park, J. W. and Kim, D. Y. (2014). Enhanced regenerative healing efficacy of a highly skin-permeable growth factor nanocomplex in a full-thickness excisional mouse wound model. *International Journal of Nanomedicine*, 9(1):4551—4567.

Barnea, Y., Weiss, J. and Gur, E. (2010). A review of the applications of the hydrofiber dressing with silver (Aquacel Ag®) in wound care. *Journal of Therapeutics and Clinical Risk Management*,6(1):21–27.

Barrientos, S., Brem, H., Stojadinovic, O. and Tomic-Canic, M. (2014). Clinical application of growth factors and cytokines in wound healing. *Wound Repair and Regeneration*, 22(5):569-578.

Barrientos, S., Stojadinovic, O., Golinko, M. S., Brem, H. and Tomic-Canic, M. (2008). Growth factors and cytokines in wound healing. *Wound Repair and Regeneration*, 16(5):585–601.

Bhanot, R. and Mago, J. (2016). Corticosteroids in dentistry. *Indian Journal of Dental Science,* 8(4):252-254.

Bishop, A. (2008). Role of oxygen in wound healing. *Journal of Wound Care,* 17(9):399-402.

Bjarnsholt, T., Kirketerp-Møller, K., Jensen, Ø. P., Madsen, K. G.,Phipps, R., Krogfelt, K., Høiby, N. and Givskov, M. (2008). Why chronic wounds won't heal: a novel hypothesis. *Wound Repair and Regeneration*, 16(1):2-10.

Bjerregaard, L. S., Jensen, P. F., Bigler, D. R., Petersen, R. H., Møller-Sørensen, H., Gefke, K., Hansen, H. J. and Kehlet, H. (2017). High-dose methylprednisolone in video-assisted thoracoscopic surgery lobectomy: a randomized controlled trial. *European Journal of Cardiothoracic Surgery*, doi: 10.1093/ejcts/ezx248.

Boateng, J. S., Matthews, K. H., Stevens, H. N. and Eccleston, G. M. (2008). Wound healing dressings and drug delivery systems: A review. *Journal of Pharmaceutical Sciences*, 97:2892–2923.

Borena, B. M., Martens, A., Broeckx, S. Y., Meyer, E., Chiers, K., Duchateau, L. and Spaas, J. H. (2015). Regenerative skin wound healing in mammals: state-of-the-art on growth factor and stem cell based treatments. *Cellular Physiology and Biochemistry*, 36:1-23.

Bosanquet, D. C., Rangaraj, A., Richards, A. J., Riddell, A., Saravolac, V. M. and Harding, K. G. (2013). Topical steroids for chronic wounds displaying abnormal inflammation. *Annalas of Royal Colledge of Surgeons of England*, 95(4):291-296.

Boyapati, L. and Wang, H. L. (2007). The role of stress in periodontal disease and wound healing. *Journal of Periodontology 2000*,44(1):195-210.

Boykin, J. V. Jr. and Baylis, C. (2007). Hyperbaric oxygen therapy mediates increased nitric oxide production associated with wound healing: A preliminary study.*Advances in Skin and Wound Care*, 20(7):382-388.

Broughto, G. 2nd., Janis, J. E. and Attinger, C. E. (2006). The basic science of wound healing. *Plastic and Reconstructive Surgery*, *117(Suppl*):12S-34S.

Bryden, J. S. (2015). Delayed wound healing: what to do?http://c.ymcdn.com/sites/www.michvma.org/resource/resmgr/mvc_proceedings_2015/stanley_02.pdf. Retrieved on 2016/05/19.

Bugada, D., De Gregori, M., Compagnone, C., Muscoli, C., Raimondi, F., Bettinelli, S., Avanzini, M. A., Cobianchi, L., Peloso, A., Baciarello, M., Dagostino, C., Giancotti, L. A., Ilari, S., Lauro, F., Grimaldi, S., Tasciotti, E., Fini, M., Jotti, G. M. R. S., Meschi, T., Fanelli, G. and Allegri, M. (2015). Continuous wound infusion of local anesthetic and steroid after major abdominal surgery: study protocol for a randomized controlled trial. *Trials*, 16(1):357.

Burgess, C. (2008). Topical vitamins. *Journal of Drugs in Dermatology,* 7(1):S2-S6.

Busti, A. J., Hooper, J. S., Amaya, C. J. and Kazi, S. (2005). Effects of perioperative anti-inflammatory and immunomodulating therapy on surgical wound healing. *The Journal of Human Pharmacologyand Drug Theraphy*, 25(11):1566-1591.

Calfee, T. and Manning, T. O. (2002). Non-healing subcutaneous wounds in the cat and proposed surgical management techniques. *Clinical Techniques in Small Animal Practice*, 17(4):162-167.

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

Carvalho, B., Clark, B., Yeomans, B. and Angst, M. S. (2012). Collecting and measuring wound exudate Biochemical mediators in surgical wounds. *Journal of Visualized Experiments,* 68(1). doi: 10.3791/50133.

Casacó, A., Fuente, D., Ledón, N., Fernández, A. and Crombe, T. (2012). Anti-epidermal growth factor/epidermal growth factor receptor therapeutic anti-cancer drugs and the wound healing Process. *Journal of Cancer Science and Therapy*, 4(10):324-329.

Charmandari, E., Kino, T. and Chrousos, G. P. (2004). Glucocorticoids and their actions: an introduction. *Annals of New the York Academic of Sciences,* 1024; pp. 1-8.

Chamanga, E. (2015). Will providing care closer to home result in more complex wound care. *Journal of Community Nursing,* 29(5):32-38.

Chappi, D. M., Suresh, K. V., Patil, M. R., Desai, R., Tauro, D. P., Bharani, K. N. S. S., Parkar, M. I. and Babaji, H. V. (2015). Comparison of clinical efficacy of methylprednisolone and serratiopeptidase for reduction of postoperative sequelae after lower third molar surgery. *Journal of Clinical and Experimental Dentistry*, 7(2):197-202.

Chaurand-Lara, J. and Facio-Umaña, J. A. (2013). Methylprednisolone injection following the surgical extraction of impacted lower third molars: A split-mouth study. *OpenJournal of Stomatology,* 3(2): 192-196.

Chen, J., Yu, L., Chen, L., Wu, X., Tang, P., Yin, J., Jiang, T., Yin, G. and Fan, J. (2017). Surgical trauma and low-dose methylprednisolone modulate the severity of the acute-phase response induced by zoledronic acid infusion. *Experimental and Therapeputic Medicine,* 14 (2):1802-1808.

Chen, Q., Chen, H., Zhu, L. and Zheng, J. (2015). Fundamentals of double network hydrogels.*Journal of Materials Chemistry B,*3(18):3654-3676.

Chikuda, H., Yasunaga, H. and Takeshita, K. (2014) "Mortality and morbidity after high-dose methylprednisolone treatment in patients with acute cervical spinal cord injury: a propensity-matched analysis using a nationwide administrative database." *Emergency Medicine Journal*, 31(3):201–206.

Chugh, A., Singh, S. and Chugh, V. (2017). Submucosal injection of dexamethasone and methylprednisolone for the control of postoperative sequelae after third molar surgery: randomized controlled trial. *International Journal of Oral and Maxillofacial Surgery,*doi: http://dx.doi.org/10.1016/j.ijom.2017.07.009.

Colin, F. R., Leif, S., Richard, W., Jared, O. and Alistair, R. (2017). Impact of Methylprednisolone on Postoperative Quality of Recovery and Delirium in the Steroids in Cardiac Surgery Trial: A Randomized, Double-blind, Placebo-controlled Substudy. *Anesthesiology*, 126(2):223-233.

Contini, S. and Scarpignato, C. (2013). Caustic injury of the upper gastrointestinal tract: A comprehensive review. *World Journal of Gastroenterology*, 19(25):3918–3930.

Dabiri, G., Damstetter, E. and Phillips, T. (2016). Choosing a wound dressing based on common wound characteristics. *Advances in Wound Care* (New Rochelle), 5(1):32–41.

da Costa, M. A., Campos, A. C., Coelho, J. C., de Barros, A. M. and Matsumoto, H. M. (2003). Oral glutamine and the healing of colonic anastomoses in rats. *Journal of Parenteral and Enteral Nutrition,* 27(3):182-185.

Daigle, J. C., Kerwin, S., Foil, C. S. and Merchant, S. R. (2001). Draining tracts and nodules in dogs and cats. *Topics in Companion Animal Medicine*, 16(4):214-218.

da Silva, E. Z. M., Jamur, M. C. and Oliver, C. (2014). Mast cell function: A new vision of an old cell. *Journal of Histochemistry and Cytochemistry*, 62(10):698–738.

Daniel, D. S. and Mathieu, M. G. (2006). Clinical Exposures: A dog with a non-healing flank wound. http://veterinarymedicine.dvm360.com/clinical-exposures-dog-with-nonhealing-flank-wound. Retrieved 2016/08/21.

Daunton, C., Kothari, S., Smith, L. and Steele, D. (2012). A history of materials and practices for wound management. *Wound Practice and Research,* 20(4):174-184.

Davidson, J. R. (2015). Current Concepts in Wound Management and Wound Healing Products. *Veterinary Clinics of North America: Small Animal Practice*, 45(3): 537-564.

de Mello, V. D., Kolehmainen, M., Schwab, U., Mager, U., Laaksonen, D. E., Pulkkinen, L., Niskanen, L., Gylling, H., Atalay, M., Rauramaa, R. and Uusitupa, J. I. M. (2008). Effect of weight loss on cytokine messenger RNA expression in peripheral blood mononuclear cells of obese subjects with the metabolic syndrome. *Metabolism,* 57(2):192-199.

Demidova-Rice, T. N., Hamblin, M. R. and Herman, I. M. (2012). Acute and impaired wound healing: pathophysiology and current methods for drug delivery, part 1: normal and chronic wounds: biology, causes, and approaches to care. *Advance Skin Wound Care*, 25(7):304-314.

Deodhar, A. K. and Rana, R. E. (1997). Surgical physiology of wound healing: a review. *Journal of Postgraduate Medicine*, 43(2):52-56.

Deuel, T. F., Kawahara, R. S., Mustoe, T. A. and Pierce, A. F. (1991). Growth factors and wound healing: Platelet-derived growth factor as a model cytokine. *Annual Review of Medicine,* 42(1):567-584.

Deshmukh, C. T. (2007). Minimizing side effects of systemic corticosteroids in children. *Indian Journal of Dermatology*, 73(4):218-221.

de Souza M. V. (2016). Osteoarthritis in horses - Part 2: a review of the intra-articular use of corticosteroids as a method of treatment. *Brazilian Archives of Biology and Technology*, 59: http://dx.doi.org/10.1590/1678-4324-2016150025. Assessed 26/10/2017

Dhall, S., Do, D. C., Garcia, M., Kim, J., Mirebrahim, S. H., Lyubovitsky, J., Lonardi, S., Nothnaqel, E. A., Schiller, N. and Martins-Green, M. (2014). Generating and reversing chronic wounds in diabetic mice by manipulating wound redox parameters. *Journal of Diabetes Research*, 2014: 562625. doi:10.1155/2014/562625.

Dhivya, S., Padma, V. V. and Santhini, E. (2015). Wound dressings – a review. *Biomedicine*, 5(4):24-28.

Diegelmann, R. F, and Evans, M. C. (2004). Wound healing: an overview of acute, fibrotic and delayed healing. *Frontier in Bioscience*, 9(1):283 – 289.

Dogan, S., Demirer, S., Erkek, B., Kiziltay, A., Hasirci, N., Muftuoglu, S., Nazikoglu, A., Renda, N., Dincer, U. D., Elhan, A. and Kuterdem, E. (2009). Epidermal growth factor-containing wound closure enhances wound healing in non-diabetic and diabetic rats. *International Wound Journal*, 2(1):107–115.

Driver, V. R., Yao, M., Kantarci, A., Gu, G., Park, N. and Hasturk, H. (2013). A prospective, randomized clinical study evaluating the effect of transdermal continuous oxygen therapy on biological processes and foot ulcer healing in persons with diabetes mellitus. *Ostomy Wound Management*, 59(11):19-26.

Dudas, M., Wysocki, A., Gelpi, B. and Tuan, T. L. (2008). Memory coded throughout our bodies: molecular and cellular bases of tissue regeneration. *Pediatric Research*,63(1):502-512.

Durmus, M., Karaaslan. E., Ozturk, E., Gulec, M., Iraz, M., Edali, N. and Ersoy, M. O. (2003). The effects of single-dose dexamethasone on wound healing in rats. *Anaesthesia and Analgesia*, 97(5):1377-1380.

Edmonds, M., Bates, M., Doxford, M., Gough, A. and Foster, A. (2000). New treatments in ulcer healing and wound infection. *Diabetes and Metabolic Research Reviews*, 16(suppl):S51-S54.

Edwards, R. and Harding, K. G. (2004). Bacteria and wound healing. *Journal of Current Opinion in Infectious Disease*, 17(2):91-96. PMID:15021046.

Efron, P. A. and Moldawer, L. L. (2004). Cytokines and wound healing: the role of cytokine and anticytokine therapy in the repair response. *Journal of Burn Care and Rehabilitation*; 25(2):149-160.

El-Okdi, N., Smaili, S., Raju. V., Shidyak, A., Gupta, S., Fedorova, L., Elkareh, J., Periyasamy, S., Shapiro, A. P., Kahaleh, M. B., Malhotra, D., Xie, Z., Chin, K. V. and Shapiro, J. I. (2008). Effects of cardiotonic steroids on dermal collagen synthesis and wound healing. *Journal of Applied Physiology,*pp. 105(1): 30-36.

Emery, C. F., Kiecolt-Glaser, J. K., Glaser, R., Malarkey, W. B. and Frid, D. J. (2005). Exercise accelerates wound healing among healthy older adults: a preliminary investigation. *The Journal of Gerontology. Series A: Biological Science and Medical Science*, 60(11):1432-1436.

Eming, S. A., Martin, P. and Tomic-Canic, M. (2014). Wound repair and regeneration: mechanisms, signaling, and translation. *Science Translation Medicine,* 3(6):265.

Enoch, S. and Price, P. (2004). Cellular, molecular and biochemical differences in the pathophysiology of healing between acute wounds, chronic wounds and wounds in the aged. www.worldwidewounds.com/2004/august/enoch/pathophy. Retrieved on 2014/07/11.

Etim, N. N., Enyenihi, G. E., Akpabio, U. and Offiong, E. E. (2014). Effects of nutrition on haematology of rabbits: a review. *European Scientific Journal*, 10(3):413-424.

Falanga, V. (2005). Wound healing and its impairment in the diabetic foot. *TheLancet*; 366(9498):1736–1743.

Falanga, V. (2004) Advanced treatments for non-healing chronic wounds. *European Wound Association Journal*, 4(2): 11- 13.

Fan, W.-P. L., Rashid, M. and Enoch, S. (2010). Current advances in modern wound healing. *Wounds UK*, 6(3):22-36.

Fernandez, R. S., Griffiths, R. D. and Ussia, C. (2001). Wound cleansing: which solution, what technique? *Primary Intention*, 9(2):51-58.

Fife, C. E. and Carter, M. J. (2012). Wound care outcomes and associated cost among patients treated in US outpatient wound centers: data from the US wound registry. *A Compendium of Clinical Research and Practice*, 24(1):10–17.

Fitoz, S., Atasoy, C., Yagmurlu, A. and Akyars, S. (2001). Psoas abscess secondary to tuberculous lymphadenopathy: case report. *Journal of Abdominal Imaging*, 26(3):323-324.

Fleischmajer, R. (1961). The lack of systemic hydrocortisone effects after massive and prolonged external applications. www.nature.com/jid/journal/v36/n1/abs/jid196 15a.ht. Retrieved 14/07/2016

Foey, A., Green, P., Foxwell, B., Feldmann, M. and Brennan, F. (2001). Cytokine-stimulated T cells induce macrophage IL-10 production dependent on phosphatidylinositol 3-kinase and p70S6K: Implications for rheumatoid arthritis. *Arthritis Research and Therapy*, 4(1):64-71.

Fong, J. and Wood, F. (2006). Nanocrystalline silver dressings in wound management: a review. *International Journal of Nanomedicine*, 1(4): 441–449.

Fong, J., Wood, F. and Fowler, B. (2005). A silver coated dressing reduces the incidence of early burn wound cellulitis and associated costs of inpatient treatment: Comparative patient care audits. *Burns*, 31(5):562–567.

Fontana, L., Eagon, J. C, Colonna, M. and Klein, S. (2007). Impaired mononuclear cell immune function in extreme obesity is corrected by weight loss. *RejuvenationResearch*, 10(1):41-46.

Franz, M. G., Steed, D. L. and Robson, M. C. (2007). Optimizing healing of the acute wound by minimizing complications. *Current Problems in Surgery*, 44(11):691-763.

Gabriel, A., Rosenberg, L. Z. and de la Torre, J. I. (2009). Wound healing and growth factors. *The Journal of Plastic Reconstructive Surgery*. The University school of medicine.http://emedicine, medscape.com/article/1298196.

Gardner, S. E., Frantz, R. A. and Doebbeling, B. N. (2001). The validity of the clinical signs and symptoms used to identify localized chronic wound infection. *Wound Repair and Regeneration*, 9(3):178-86.

Geethalakshmi, R., Sakravarthi, C., Kritika, T., Arul Kirubakaran, M. and Sarada, D. V. L. (2013). Evaluation of antioxidant and wound healing potentials of *Sphaeranthus amaranthoides* burm.f. *BioMed Research International*, 109(2):607 – 611.

Gillitzer, R. and Goebeler, M. (2001). Chemokines in cutaneous wound healing. *Journal of Leukocyte Biology*, 69(4):513-521.

Gilliver, S. C. and Ashcroft, G. S. (2007). Sex steroids and cutaneous wound healing: The contrasting influences of estrogens and androgens. *Climacteric : The Journal of the International Menopause Society*, 10 (4):276-288.

Godbout, J. P. and Glaser, R. (2006). Stress-induced immune dysregulation: implications for wound healing, infectious disease and cancer. *Journal of Neuroimmune Pharmacology*, 1(4):421-427.

Gohel, M. S., Windhaber, R. A., Tarlton, J. F., Whyman, M. R. and Poskitt, K. R. (2008). The relationship between cytokine concentrations and wound healing in chronic venous ulceration.*Journal of Vascular Surgery*, 48(5):1272-1277.

Goldman, R. (2004). Growth factors and chronic wound healing: past, present and future. *Advances in Skin and Wound Care,* 17(1):24 – 35.

Gonzalez, A. C. D. O., Costa, T. F., Andrade,Z. D. A. and Medrado, A. R. A. P. (2016). Wound healing - A literature review. *Anais Brasileiros de Dermatologia*, 91(5):614–620.

Gosain, A. and DiPietro, L. A. (2004). Aging and wound healing. *World Journal of Surgery*, 28(3):321-326.

Gower, S. B., Weisse, C. W. and Brown, D. C. (2009). Major abdominal evisceration injuries in dogs and cats: 12 cases (1998–2008). *Journal of American Veterinary Medical Association*, 234(12):1566–1572.

Grazul-Bilska, A. T., Johnson, M. L., Bilski, J. J., Redmer, D. A., Reynolds, L. P., Abdullah, A. and Abdullah, K. M. (2003). Wound healing: the role of growth factors. *Drugs of Today*, 39(10): 787-800.

Greco, J. A 3rd., Castaldo, E. T., Nanney, L. B., Wendel, J. J., Summitt, J. B., Kelly, K. J., Braun, S.A., Hagan, K. F. and Shack, R. B. (2008). The effect of weight loss surgery and body mass index on wound complications after abdominal contouring operations. *Annals of Plastic Surgery*, 61(3):235-242.

Grover, V. K., Babu, R. and Bedi, S. P. S. (2007). Steroid therapy-current indications in practice. *IndianJournalofAnaesthesia*, 51(5):389-393.

Guo, S. and DiPietro, L. A. (2010). Factors affecting wound healing. In. *Journal of Dental Research*, 89(3):219-29.

Gupta, A, and Kumar, P. (2015). Assessment of the histological state of the healing wound. *Plastic and Aesthetic Research,* 2(5):239-42.

Gurtner, G. C., Werner, S., Barrandon, Y. and Longaker, M. T. (2008). Wound repair and regeneration. *Nature*, 453(7193):314-322.

Halim, A. S., Khoo, T. L. and Saad, A. Z. M. (2012). Wound bed preparation from a clinical perspective.*Indian Journal of Plastic Surgery,* 45(2):193-202.

Han, G. and Ceilley, R. (2017). Chronic wound healing: A review of current management and treatments. *Advances in Therapy*, 34(3):599-610.

Han, S. (2015). Clinical pharmacology review for primary health care providers: II. Steroids. *Translational and Clinical Pharmacology*, 23(1):15-20.

Harding, K. G., Morris, H. L. and Patel G. K. (2002). Science, medicine and the future: healing chronic wounds. *British Medical Journal*, 324(7330):160-163.

Hart, J. (2002). Inflammation: Its role in the healing of acute wounds. *Journal of Wound Care,* 11(6): 205 – 209.

Heyman, H., Van De Looverbosch, D. E. J., Meijer, E. P. and Schols, J. M. G. A. (2008). Benefits of an oral nutritional supplement on pressure ulcer healing in long-term care residents. *Journal of Wound Care,* 17(11):476-480.

Hodde, J. (2006). Extracellular matrix as a bioactive material for soft tissue reconstruction. *Australian and New Zealand Journal of Surgery,* 76(12):1096 – 1100.

Hofman, D., Moore, K., Cooper, R., Eagle, M. and Cooper, S. (2007). Use of topical corticosteroids on chronic leg ulcers. *Journal of Wound Care*, 16(5):227-230.

Holt, D. R., Kirk, S. J., Regan, M. C., Hurson, M., Lindblad, W. J. and Barbul, A. (1992). Effect of age on wound healing in healthy human beings. *Surgery;* 112 (2):293-298.

Hong, J. P. and Park, S. W. (2014). The combined effect of recombinant human epidermal growth factor and erythropoietin on full-thickness wound healing in diabetic rat model. *International Wound Journal*, 11(4):373–378.

Hunt, T. K., Hopf, H. and Hussain, Z. (2000). Physiology of wound healing. *Advances in Skin and Wound Care,* 13: 6.

Hurlbert, R. J., Hadley, M. N., and Walters B. C. (2013). “Pharmacological therapy for acute spinal cord injury.” *Neurosurgery*, 72(suppl 2):93–105.

Jaiswal, S. S., Gambhir, R. P. S., Agrawal, A. and Harish, S. (2010). Efficacy of topical recombinant human platelet derived growth factor on wound healing in patients with chronic diabetic lower limb ulcers. *TheIndian Journal of Surgery,* 72(1):27-31.

Jee, C. H., Eom, N. Y., Jang, H. M., Jung, H. W., Choi, E. S., Won, J. H., Hong, I. H., Kang, B. T., Jeong, D. W. and Jung, D. I. (2016). Effect of autologous platelet-rich plasma application on cutaneous wound healing in dogs. *Journal of Veterinary Science*, 17(1):79-87.

Jian-dong, L. J. and Chen, A. F. (2005). Nitric oxide: a newly discovered function on wound healing. *Acta Pharmacologica Sinica*, 26(3): 259–264.

Johnson, S. (2015). Five steps to successful wound healing in the community. *Journal of Community Nursing*, 29(4):30-39.

Juge-Aubry, C. E, Henrichot, E. and Meier, C. A. (2005). Adipose tissue: a regulator of inflammation. *Best Practice Research Clinical Endocrinology and Metabolism*, 19(4):547-566.

Keylock, K. T., Vieira, V. J., Wallig, M. A., DiPietro, L. A., Schrementi, M. and Woods, J. A. (2008). Exercise accelerates cutaneous wound healing and decreases wound inflammation in aged mice. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 294(1):R179-R184.

Khoosal, D. and Goldman, R. D. (2006). Vitamin E for treating children's scars. *Canadian Family Physician*, 52(7):855-856.

Kim, C. J., Kato, K., Yoshiki, T. Okada, Y. and Tani, T. (2003). Intractable duodeno-cutaneous fistula after nephrectomy for stone pyonephrosis: report of a case. *ActaUrologicaJaponica*, 49(9):547-550.

Kim, M. H. and Kang, Y. J.(2000). The effect of anesthetic technique on white blood cell response to surgery in patients who underwent laparoscopic cholecystectomy and cesarian section. *Korean Journal of Anesthesiology*, 39(3): 374-379.

Knighton, D. R., Ciresi, K. F., Fiegel, V. D., Austin, L. L. and Butler, E. L. (1986). Classification and treatment of chronic nonhealing wounds. Successful treatment with autologous platelet-derived wound healing factors (PDWHF). *Annals of Surgery*, 204(3):322 – 330.

Kreindler, J. L., Watkins, M. L., Lettis, S., Tal-Singer, R. and Nicholas, L. (2016). Effect of inhaled corticosteroids on blood eosinophil count in steroid-naïve patients with COPD. *British Medical Journal of Open Respiratory and Research*, 3(1): e000151.

Krischak, G. D., Augat, P., Claes, L., Kinzl, L. and Beck, A. (2007). The effects of non-steroidal anti-inflammatory drug application on incisional wound healing in rats. *Journal of Wound Care*, 16(2): 76-78.

Kumar, R., Jindal, A., Kaur, A. and Gupta, S. (2015). Therapeutic plasma exchange—a new dawn in the treatment of pemphigus vulgaris. *Indian Journal of Dermatology*, 60(4):419.

Kupper, T. S. and Fuhlbrigge, R. C. (2004). Immune surveillance in the skin: mechanisms and clinical consequences. *Nature Reviews. Immunology*, 4(3):211-222.

Labler, L., Mica, L., Härter, L., Trentz, O. and Keel, M. (2006). Influence of V. A. C.-therapy on cytokines and growth factors in traumatic wounds. *Zentralblatt Fur Chirurgie*, 31(suppl 1):S62 – S67.

Landén, N. X., Li, D. and Ståhle, M. (2016). Transition from inflammation to proliferation: a critical step during wound healing. *Cellular and Molecullar life Sciences*, 73(20):3861–3885.

Langer, V. and Rajagopalan, S. (2012). Evaluation of recombinant human platelet-derived growth factor as an agent for wound bed preparation in traumatic wounds. *Indian Journal of Plastic Surgery*, 45(2):203-208.

Larson, B. J. B.S., Longaker, M. T. and Lorenz, H. P. (2010). Scarless fetal wound healing: a basic science review. *Plastic and Reconstructive Surgery*, 126(4):1172-1180.

Lashkarizadeh, M, R., Garshasbi, M., Dabiri, S., Lashkarizadeh, M. and Shabani, M. (2015). Evaluation of wound healing and post-operative intra-abdominal adhesions in opium addicted rats. *Physiology and Pharmacology*, 19(3): 193-199.

Lauterbach, S., Kostev, K. and Kohlmann, T. (2010). Prevalence of diabetic foot syndrome and its risk factors in the UK. *Journal of Wound Care*, 19(8):333–337.

Lazarus, G., Valle, M. F., Malas, M., Qazi, U., Maruthur, N. M., Doggett, D., Fawole, O. A., Bass, E. B. and Zenilman, J. (2014). Chronic venous leg ulcer treatment: future research needs. *Wound Repair and Regeneration*, 22(1):34-42.

Lee, D. E., Ayoub N. and Agrawal, D. K. (2016). Mesenchymal stem cells and cutaneous wound healing: novel methods to increase cell delivery and therapeutic efficacy. *Stem Cell Research and Therapy*, 7(1):37.

Lemmens, L., Claes, V. and Uzzell, M. (2008). Managing patients with metastatic colorectal cancer on bevacizumab. *British Journal of Nursing*, 17(5):944-949.

Leung, A., Crombleholme, T. M. and Keswani, S. G. (2012). Fetal wound healing: implications for minimal scar formation. *CurrentOpinioninPediatrics*, 24(3):371-378.

Lew, M., Lew, S., Drazek, M. and Pomianowski, A. (2015). Penetrating eye injury in a dog: a case report. *Veterinarni Medicina*, 60(4):213-221.

Lin, B. and Wang, J. H.-C. (2011). Fibroblast and myofibroblast in wound healing: Force generation and measurement. *Journal of Tissue Viability*, 20(4):108-120.

Lin, F., Baldessari, F. and Gyenge, C. C. (2008). Lymphocyte electrotaxis in vitro and in vivo. *TheJournal of Immunology*, 181(1):2465 – 2471.

Linehan, J. D., Kolios, G., Valatas, V., Robertson, D. A. F. and Westwick, J. (2005). Effect of corticosteroids on nitric oxide production in inflammatory bowel disease: are leukocytes the site of action? *AmericanJournal of Physiology, Gastrointestinal and Liver Physiology*, 288(2):261-267.

Liu, D., Ahmet, A., Ward, L., Krishnamoorthy, P., Mandelcorn, E. D., Leigh, R., Brown, J. P., Cohen, A. and Kim, H. (2013). A practical guide to the monitoring and management of the complications of systemic corticosteroid therapy. *Allergy, Asthma and Clinical Immunology*, 9**(**1):30-54.

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

Longaker, M. T., Chiu, E. S., Adzick, N. S., Stern, M., Harrison, M. R. and Stern, R**.** (1991). Studies in fetal wound healing. V. A prolonged presence of hyaluronic acid characterizes fetal wound fluid. *Annals of Surgery*. 213(4):292-296.

Longui, C. A. (2007). Glucocorticoid therapy: minimizing side effects. *Jornal de Pediatria*, 83(5):S163-S171.

Loning, T., Schmiegelow, P. and Vom Dahl, M. (1983). Wound repair of the oral mucosa. *Virchows Archive,* 398(3):237-246.

Mayet, N., Choonara, Y. E., Kumar, P., Tomar, L. K., Tyagi, C., Du Toit, L. C. and Pillay, V**.** (2014). A comprehensive review of advanced biopolymeric wound healing systems. *JournalofPharmaceuticalSciences.* 103(8):2211–2230.

McCarty, S. M. and Percival, S. L. (2013). Proteases and delayed wound healing. *Advances in Wound Care*, 2(8):438–447.

MacCornick, S., de Noronha, S. A. A. C., Chominski, V., de Noronha, S. M. R., Ferreira, L. M. and Gragnani, A. (2014). Clinical use of growth factors in the improvement of skin wound healing. *Open Journal of Clinical Diagnostics*, 4(4):227-236.

MacLeod, A. S. and Mansbridge, J. N. (2016). The innate immune system in acute and chronic wounds. *Advances in Wound Care*, 5(2):65-78.

Martin, E. A. (2003). *Concise Medical Dictionary*. Sixth edition. Oxford University Press New York . pp. 746.

McDaniel, J. C., Belury, M., Ahijevych, K. and Blakely, W. (2008). Omega-3 fatty acids effect on wound healing. *Wound Repair and Regeneration*, 16(3):337-45.

McDonnell, G. and Russell, D. A. (1999). Antiseptics and disinfectants: activity, action, and resistance. *Clinical Microbiology Reviews,* 12(1):147–179.

Micó-Llorens, J. M., Satorres-Nieto, M., Gargallo-Albiol, J., Arnabat-Domínguez, J., Berini-Aytés, L. and Gay-Escoda, C. (2006). Efficacy of methylprednisolone in controlling complications after impacted lower third molar surgical extraction. *European Journal of Clinical Pharmacology*, 62(9):693-698.

Milne, C. T. (2008). Wound and skin care center; *American Association of Nurses*. www.peace health.org/kbase/cam/hn-1056000.htm Retrieved on 2015/11/28.

Minka, N. S. and Ayo, J. O. (2011). Modulating role of Vitamins C and E against transport-induced stress in pullets during the hot-dry conditions. *ISRN Veterinary Science,* 497138.doi: 10.5402/2011/497138.

Misdorp, W. (2004). Mast cells and canine mast cell tumours. A review. *Veterinary Quarterly,* 26(4):156-169.

Mohammad, S., Abdallah, M. S., Madi, A. F. and Rana, M. A. (2015). The best use of systemic corticosteroids in the intensive care units, Review. *Journal of Steroids and Hormonal Science*, 6(1):149.

Momeni, A., Heier, M., Bannasch, H. and Stark, G. B. (2009). Complications in abdominoplasty: a risk factor analysis. *Journal of Plastic, Reconstructive and Aesthetic Surgery*, 62(10):1250 - 1254.

Morgan, T. (2015). Are your wound management choices costing you money? *Journal of Community Nursing*, 29(4):17-20.

Mustoe, T. A., Cooter, R., Gold, M. H., Hobbs, F. D., Ramelet, A. A., Shakespeare, P. G., Stella, M., Téot, L., Wood, F. M. and Ziegler, U. E. (2002). International clinical recommendation on scar management. *Journal of Plastic and Reconstructive Surgery,* 110(2):560–572.

Nalliappan G. N., Venkataraman, S. S., Daniel, R., Aravind, R. J. and Kumarakrishnan, V. B. (2012). Molecular biology of wound healing. *Journal of Pharmacy and Bioallied Sciences*, 4(Suppl 2):S334–S337.

Nandini, V. V., Venkatesh, K. V. and Nair, K. C. (2008). Alginate impressions: A practical perspective. *Journal of conservative dentistry,* 11(1): 37–41.

Natarajan, S., Williamson, D., Stiltz, A. J. and Harding, K. (2000). Advances in wound care and healing technology.*American Journal of Clinical Dermatology,* 1(5):269-75.

Nath, C. and Gulati S. C. (1998). Role of cytokines in healing chronic skin wounds. *Acta Haematologica,* 99(1):175-179.

Nikitina, S. A., Habibrakhmanova, V. R. and Sysoeva, M. A. (2016). Composition and biological activity of triterpenes and steroids of chaga mushroom. *Biomedit□s□inskai□a□ Khimii□a□*, 62(4): 363-375.

Olczyk, P., Mencner, L. and Komosinska-Vassev, K. (2014). The role of the extracellular matrix components in cutaneous wound healing. *BioMed Research International*. http://dx.doi.org/10.1155/2014/747584.

Ousey, K., Cook, L., Young, T. and Fower, A. (2016). Hydrocolloids in practice: Pressure ulcer, skin integrity. *Wounds*, 13(4):11-18.

Pavletic, M. M. and Trout, N. J. (2006). Bullet, bite and burn wounds in dogs and cats. *The* Veterinary Clinics of North America. *Small Animal Practice,* 36(4):873-893.

.

Pastar, I., Stojadinovic, O., Yin, N. C., Ramirez, H., Nusbaum, A. G., Sawaya, A., Patel, S. B., Khalid, L., Isseroff, R. R. and Tomic-Canic, M. (2014). Epithelisation in wound healing: a comprehensive review. *Advances in Wound Care*, 3(7): 445-464.

Peng, Y., Li, P., Zhao, Z. A., Chen, L., Zhao, X. G., Chen, X., Zhao, Y., Xiong, R. P., Ning, Y. L., Yang, N., Ye, J. and Zhou, Y. G. (2016). Comparative evaluation of the wound-healing potency of recombinant bFGF and ski gene therapy in rats. *Growth Factors* 34(3-4):119-127.

Percival, N. J. (2002). Classification of Wounds and their Management. *Surgery* (Oxford), 20(5):114-117.

Percival, K. R. and Radi, Z. A. (2016). A modified Verhoeff's elastin histochemical stain to enable pulmonary arterial hypertension model characterization. *European Journal of Histochemistry*, 60(1):2588.

Petrie, N .C., Yao F. and Eriksson, E. (2003). Gene therapy in wound healing. *Surgical Clinics of North American,* 83 (3):597-616.

Price, P., Fogh, K., Glynn, C., Krasner, D. L., Osterbrink, J. and Sibbald, R.G. (2007). Why combine a foam dressing with ibuprofen for wound pain and moist wound healing?. *International Wound Journal*, 4(Suppl 1):1–3.

Póvoa, P., Salluh, J. I.., Martinez, M. L., Guillamat-Prats, R., Gallup, D., Al-Khalidi, H. R., Thompson, B. T., Ranieri, V. M. and Artigas, A. (2015). Clinical impact of stress dose steroids in patients with septic shock: insights from the PROWESS-Shock trial. *Critical Care*, 19(1):193-202.

Quesenberry, P. J., Colvin, G. A., Lambert, J. F., Frimberger, A. E., Dooner, M. S., Mcauliffe, C. I., Miller, C., Becker, P., Badiavas, E., Falanga, V. J., ElfenbeinG.

and Lum, L. G. (2002). The new stem cell biology. *Transactions of the American Clinical and Climatological Association*, 113(1):182-207.

Quist, E. M., Tanabe, M., Mansell, J. E. and Edwards, J. L., (2012). A case series of thermal scald injuries in dogs exposed to hot water from garden hoses (garden hose scalding syndrome). *VeterinaryDermatology*, 23(1):162-166.

Rabess, C. (2015). Understanding the link between wound care and nutrition. *Journal of Community Nursing*, 29(4):60-65.

Ramlet, A. A., Hirt-burri, N., Raffoul, W., Scaletta, C., Pioletti, D. P., Offord, E., Mansourian, R. and Applegate, L. A. (2009). Chronic wound healing by fetal cell therapy may be explained by differential gene profiling observed in fetal versus old skin cells. *Experimental Gerontology*, 44(1):208 – 218.

Rana, M. S., Hashem, M. A., Sakib, M. N. and Kumar, A. (2014). Effect of heat stress on blood parameters in indigenous sheep. *Journal of Bangladesh Agricultural University*, 12(1): 91–94.

Raut, G., Vanmali, H., Pimpliskar, M. and Rahul, J. (2015). Efficacy of some antiseptics and disinfectants. A review. International Journal of Pharmacy and Pharmaceutical research. *Human Journals*, 4(4):182-197.

Reinke, J. M. and Sorg, H. (2012). Wound repair and regeneration. *European Surgical Research*, 49(1):35–43.

Rivera, A. E. and Spencer, J. M. (2007). Clinical aspects of full thickness wound healing. *Clinics inDermatology,* 25(1):39 – 48.

Robson, M. C., Steed, D. L. and Franz, M. G. (2001). Wound healing: biologic features and approaches to maximize healing trajectories. *Current Problems in Surgery*, 38(2): 72 – 140.

Rodriguez, P. G., Felix, F. N., Woodley, D. T. and Shim, E. K. (2008). The role of oxygen in wound healing: a review of the literature. *Dermatologic Surgery*. 34(9):1159-1169.

Rodriguez-Menocal, L., Salgado, M., Ford, D. and Van Badiavas, E. (2012). Stimulation of skin and wound fibroblast migration by mesenchymal stem cells derived from normal donors and chronic wound patients. *Stem Cells Translational Medicine,* 1(3):221–229.

Rolfe, K. J. and Grobbelaar, A. O. (2012). A review of fetal scarless healing. *International Scholarly Research Notices*, *Dermatology*. ID 698034. doi: 10.5402/2012/698034.

Rowan, M. P., Cancio, L. C., Elster, E. A., Burmeister, D. M., Rose, L. F., Natesan, S., Chan, R. K., Christy, R. J. and Chung, K. K. (2015). Burn wound healing and treatment: review and advancements. *Critical Care* 19(1): 243.

Rozman, P. and Bolta, Z. (2007). Use of platelet growth factors in treating. wounds and soft-tissue injuries. *Acta Dermatovenerologica Alpina, Pannonica, et Adriatica*,16(4):156-165.

Sabolinski, M. L., Alvarez, O., Auletta, M., Mulder, G. and Parenteau, N. L. (1996). Cultured skin as a 'smart material' for healing wounds: experience in venous ulcers. *Biomaterials*, 17(3):311-320.

Salcido, R. (1999). Anabolic steroids and wound healing. *Advances in Skin and wound care*, 12(8):391-395.

Salmerón-Sánchez, M. and Dalby, M. J. (2016). Synergistic growth factor micro-environments *Chemical Communication*,52(1):13327-13336.

Sardari, K., Dehgan, M. M., Mohri, M., Emami, M. R., Mirshahi, A., Maleki, M., Barjasteh, M. N. and Aslani M. R. (2006). Macroscopic aspect of wound healing (contraction and epithelisation) after topical application of allicin in dogs. *Comparative Clinical Pathology in Dogs*. 15(4):231-235.

Sauerland, S., Nagelschmidt, M., Mallmann, P. and Neugebauer, E. A. (2000). Risks and benefits of preoperative high dose methylprednisolone in surgical patients: a systematic review. *Drug Safety*, 23(5):449-461.

Scappaticci, F. A., Fehrenbacher, L., Cartwright, T., Hainsworth, J. D., Heim, W., Berlin, J., Kabbinavar, F., Novotny, W., Sarkar, S. and Hurwitz, H. (2005). Surgical wound healing complications in metastatic colorectal cancer patients treated with bevacizumab. *Journal of Surgical Oncology*. 91(3):173-180.

Schreml, S., Szeimies, R. M., Prantl, L., Karrer, S., Landthaler, M. and Babilas, P. (2010). Oxygen in acute and chronic wound healing. *British Journal of Dermatology*, 163(2):257–268.

Schultz, G. S., Sibbald, R. G., Falanga, V., Ayello, E. A., Dowsett, C., Harding, K., Romanelli, M., Stacey, M C., Teot, L. and Vanscheidt, W. (2003). Wound bed preparation: a systematic approach to wound management. *Wound Repair and Regeneration*, 11(1):S1–S28.

Scott, L. J. (2007). Bevacizumab: in first-line treatment of metastatic breast cancer. *Drugs*. 67(12):1793-1799.

Sevuk, L., Vayisoğlu, Y., Korlu, S., Çömelekoğlu, Ü., Arpacı, R. B., Aktaş, S., Helvacı, I., Ayaz, L., Dağtekin, A., Göçer, P., Aladağ, Z. C., Karataş, M. A. and Ümit

Talat, D. U. (2014). The effects of methylprednisolone and vitamin A on the healing of traumatic peripheral nerve paralysis. In. *The Journal of International Advanced Otology*, 10(3):275-280.

Sevuk, U., Baysal, E., Altindag, R., Yaylak, B., Adiyaman, M. S., Ay, N., Beyazit, U. and Alp, V. (2016). Role of methylprednisolone in the prevention of postpericardiotomy syndrome after cardiac surgery. *European Reviews, for Medical and Pharmacological Sciences*, 20(3):514-519.

Shah, P., Keppler, L, and Rutkowski, J. (2014). A review of platelet derived growth factor playing pivotal role in bone regeneration. *Journal of Oral Implantology*, 40(3):330-340.

Shaikh, S.,Verma, H., Yadav, N., Jauhari, M. and Bullangowda, J. (2012). Applications of steroid in clinical practice: *A Review International Scholarly Research Notices: Anesthesiology,* 11(1):1-11.

Shepherd, A. A. (2003). Nutrition for optimum wound healing. *Nursing Standard*, 18(6):55-58.

Shimada, H., Ochiai, T., Okazumi, S., Matsubara, H., Nabeya, Y., Miyazawa, Y., Arima, M., Funami, Y., Hayashi, H., Takeda, A., Gunji, Y., Suzuki, T. and Kobayashi, S. (2000). Clinical benefit of steroid therapy on surgical stress in patients with esophageal cancer. *Surgery*, 128(5):791-798.

Shingel, K. I., Faure, M. P., Azoulay, L., Roberge, C. and Deckelbaum, R. J. (2008). Solid emulsion gel as a vehicle for delivery of polyunsaturated fatty acids: implications for tissue repair, dermal angiogenesis and wound healing. *Journal of Tissue Engeneering and Regenerative Medicine*, 2(7):383–393.

Shivanna, S., Priye, S., Singh, D., Jagannath, S., Mudassar, S. and Reddy D. P. (2016). Efficacy of methylprednisolone and lignocaine on propofol injection pain: A randomised, double-blind, prospective study in adult cardiac surgical patients. *Indian Journal of Anaesthesia*, 60(11):848-851.

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

Slavkovsky, R., Kohlerova, R., Jiroutova, A., Hajzlerova, M., Sobotka, L., Cermakova, E. and Kanta, J. (2010). Effects of hyaluronan and iodine on wound contraction and granulation tissue formation in rat skin wounds. *Clinical and experimental Dermatology*, 35(4):373-379.

Soo, C., Beanes, S. R., Hu, F. Y., Zhang, X., Dang, C., Chang, G., Wang, Y., Nishimura, I., Freymiler, E., Lonagker, M. T., Lorenz, H. P. and Ting, K. (2003). Ontogenic transition in fetal wound transforming growth factor-beta regulation correlates with collagen organization. *TheAmerican Journal of Pathology*,163(6):2459-2476.

Sood, A., Granick, M. S. and Tomaselli, N. L. (2014). Wound dressings and comparative effectiveness data. *Advances inWoundCare*, 3(8):511-529.

Sosa, I. J., Reyes, O. and Kuffler, D. P. (2008). Elimination of a pressure ulcer with electrical stimulation – a case study. *Puerto Rico Health Sciences Journal,* 27(2):175–179.

Spiriev, T., Laleva, L. and Enchev, Y. (2014). Surgical decompression and methylprednisolone in the treatment of traumatic spinal cord injury - What are the Evidences? Review of the literature.*Journal of Neurological Surgery*, 75(1):35.

Stanley, A. and Osler, T. (2001). Senescence and the healing rates of venous ulcers. *Journal of Vascular Surgery,* 33(6):1206–1211.

Staudte, K. L., Hopper, B. J. and Gibson, N. R. (2004). Use of ultrasonography to facilitate surgical removal of non-enteric foreign bodies in 17 dogs. *TheJournal of Small Animal Practice*, 45(8):395-400.

Steenfos, H. H. (1994). Growth factors and wound healing. *Scandinavian Journal of Plastic and Reconstructive Surgery,* 28(2):95-105.

Sumano, H., Goiz, G. and Clifford, V. (2002). Use of electrical stimulation for wound healing in dog. *Israel Journal of Veterinary Medicine*, 57(2):1–9.

Sun, B. K., Siprashvili, Z. and Khavari, P. A. (2014). Advances in skin grafting and treatment of cutaneous wounds. *Science*, 346(6212):941–945.

Tajiri, T., Yoshida, S., Ishibashi, N., Tanaka, K., Muraoka, T. and Shirouzu, K. (2002). Effect of prophylactic intracerebroventricular injection of methylprednisolone on nitrogen and catecholamine excretion in the urine after laparotomy in rats. *Journal of Surgical Research*, 103(2):272–278.

Tandon, R., Verma, K., Chawla, B., Sharma, N., Titiyal, J. S., Kalaivani, M. and Vajpayee, R. B. (2009). Intravenous dexamethasone vs methylprednisolone pulse therapy in the treatment of acute endothelial graft rejection. *Eye (London, England),* 23(3):635-639.

Telgenhoff, D. and Shroot, B. (2005). Cellular senescence mechanisms in chronic wound healing. *Cell Death and Differenciation*, 12(7):695–698.

Theoret, C. L. and Wilmink, J. M. (2013). Aberrant wound healing in the horse: Naturally occurring conditions reminiscent of those observed in man. *Wound Repair and Regeneration,* 21(3):365-371.

Tiaka, E. K., Papanas, N., Manolakis, A. C. and Georgiadis, G. S. (2012) Epidermal growth factor in the treatment of diabetic foot ulcers. *Perspective in Vascular Surgery and Endovascular Therapy,*24(1):37-44.

Tiigimae-Saar, J., Leibur, E. and Tamme, T. (2010). The effect of prednisolone on reduction of complaints after impacted third molar removal. *Stomatologija,* 12(1):17-22.

Tiwari, S., Chauhan, M., Shahapurkar, V. V., Akhtar, Md. J., Grover, A., Eshan, S. P. and Nerkar, E. (2014). Importance of southampton wound grading system in surgical site infection. *Journal of Evolution of Medical and Dental Sciences,* 3(20):5491-5495.

Tong, B. C. and Barbul, A. (2004). Cellular and physiological effects of arginine. *Mini Reviews in Medicinal Chemistry*, 4(8):823-832.

Toy, L. W. (2005). Matrix metalloproteinases: their function in tissue repair. *Journal of Wound Care*, 14(1):20 – 22.

Traversa, B. and Sussman, G. (2001). The role of growth factors, cytokines and proteases in wound management, primary intention. *The Australian Journal of Wound Management*, 9(4):161-167.

Tricco, A. C., Cogo, E., Isaranuwatchai, W., Khan, P. A., Sanmugalingham, G., Antony, J., Hoch, J. S. and Straus, S. E. (2015). A systematic review of cost-effectiveness analyses of complex wound interventions reveals optimal treatments for specific wound types. Open Peer Review. *BMC Medicine*, 13(1):90.

Tsourdi, E., Barthel, A., Rietzsch, H., Reichel, A. and Bornstein, S. R. (2013). Current aspects in the pathophysiology and treatment of chronic wounds in diabetes mellitus. *Biomed Research International*, 2013(2013); 385641. doi: 10.1155/2013/ 385641.

Tunçcan, T., Yalçın, S., Demir, C. F., Akın, M. M., Karlıdağ, T., Keleş, E. and Kaygusuz, I. (2016). Efficacy of cortexin and methylprednisolone on traumatic facial nerve paralysis. *Journal of International Advanced Otology*, 12(1): 303-309.

Ueno, C., Hunt, T. K. and Hopf, H. W. (2006). Using physiology to improve surgical wound outcomes. *Plastic and Reconstructive Surgery,* 117(7):59S-71S.

Velnar, T., Bailey, T. and Smrkolj, V. (2009). The wound healing process: an overview of the cellular and molecular mechanisms. *The Journal of International Medical Research,* 37(5):1528 – 1542.

Veves, A., Falanga, V., Armstrong, D. G. and Sabolinski, M. L. (2001). Graftskin, a human skin equivalent, is effective in the management of noninfected neuropathic diabetic foot ulcers: a prospective randomized multicenter clinical trial. *Diabetes Care,* 24(2):290-295.

Wagner, A. E., Huck, G., Stiehl, D. P., Jelkmann, W. and Hellwig-Bürgel, T. (2008). Dexamethasone impairs hypoxia-inducible factor-1 function. *Biochemical and Biophysical Research Communications,* 372(2):336-340.

Wall, I. B., Moseley, R. , Baird, D. M., Kipling, D., Giles, P., Laffafian, I., Price, P. E., Thomas, D. W. and Stephens, P. (2008). Fibroblast dysfunction is a key factor in the non-healing of chronic venous leg ulcers. *TheJournal of Investigative Dermatology*, 128(10):2526–2540.

Wang, A. S., Armstrong, E. J. and Armstrong, A. P. (2013). Corticosteroids and wound healing: clinical considerations in the perioperative period. *The American Journal of Surgery*, 206(3):410-417.

Weller,C. and Sussman, G. (2006).Wound dressings update. *Journal of Pharmacy Practice and Research,* 36(4):318-324.

Werner, S. andGrose, R. (2003). Regulation of wound healing by growth factors and cytokines. *Physiological Reviews*, 83(3):835-870.

Westgate, S. J., Percival, S. L., Knottenbelt, D. C., Clegg, P. D. and Cochrane, C. A. (2010). Chronic equine wounds: what is the role of infection and biofilms? *Wounds*, 22(6):12-17.

Wicke, C., Halliday, B., Allen, D., Roche, N. S., Scheuenstt, H., Spencer, M. M., Roberts, A. B. and Hunt, T. K. (2000). Effects of steroids and retinoids on wound healing. *Achives of Surgery*, 135(11): 1265-1270.

Wild, T., Rahbarnia, A., Kellner, M., Sobotka, L. and Eberlein, T. (2010). Basics in nutrition and wound healing. *Nutrition*, 26(9): 862-866.

Wilmink, J. M. and van Weeren, P. R. (2005). Second-intention repair in the horse and pony and management of exuberant granulation tissue. *The Veterinary Clinics of North America: Equine Practice*, 21(1):15-32.

Wilson, J. A. and Clark, J. J. (2004). Obesity: impediment to postsurgical wound healing. *Advances in Skin and Wound Care,* 17(8):426-435.

Witte, M. B. and Barbul, A. (2002). Role of nitric oxide in wound repair. *The American Journal of Surgery,* 183(1):406–412.

Wojtowicz, A. M., Oliveira, S., Carlson, M. W., Zawadzka, A., Rousseau, C. F. and Baksh, D. (2014). The importance of both fibroblasts and keratinocytes in a bilayered living cellular construct used in wound healing. *Wound Repair and Regeneration*, 22(2):246-255.

Wong, V. W., Sorkin, M., Glotzbath, J. P., Longaker, M. T. and Gurtner, G. C. (2010). Surgical approaches to creat murine models of human wound healing. *Journal of Biomedicine and Biotechnology,* 2011(2011); ID. 969618.

Woodward, M., Sussman, G., Rice, J., Ellis, T. and Fazio, V. (2010). Nutrition and wound healing.www.worldofwounds.com/.../Expert%20Guide%20Nutrition%20Wound%20Healing_final_lr.pdf. Retrieved 2014/07/11.

Wozniak, S. E., Gee, L. L., Wachtel, M. S. and Frezza, E. E. (2009). Adipose tissue: the new endocrine organ? A review article. *Digestive Diseases and Sciences,* 54(9):1847-1856.

Yagi, L. H.,Watanuki, L. M., Isaac, C., Gemperli, R., Nakamura, Y. M. and Ladeira, P. R. S. (2016). Human fetal wound healing: a review of molecular and cellular aspects. *European Journal of Plastic surgery,* 39(4):239-246.

Yakubu, A. S., Abubakar A. A., Salihu, M. D., Jibril, A. and Isah, I. (2010). Comparative analysis of chlorhexidine gluconate, povidone iodine and chloroxylenol as scrubbing solution. *British Journal of Pharmacology and Toxicology,* 1(2):93-95.

Yang, W., Sherman, V. R., Gludovatz, B., Schaible, E., Stewart, P., Ritchie, R. O. and Meyers, M. A. (2015). On the tear resistance of skin. *Nature Communications* 6(6649). doi:10.1038/ncomms7649.

Yates, C. C., Hebda, P. and 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.

Yokoi, K., Matsuguma, H., Nakahara, R., Kondo, T., Kamiyama, Y., Mori, K. and Miyazawa, N. (2007). Multidisciplinary treatment for advanced invasive thymoma with cisplatin, doxorubicin, and methylprednisolone. *Journal of Thoracic Oncology*, 2(1):73-78.

Young, A. and Mc Naught, C. -E. (2011). The physiology of wound healing. *Surgery*, 29(10): 475-479.

Young, S. R. and Dyson, M. (1990). Effects of therapeutic ultrasound on the healing of full thickness excised wound lesions. *Ultrasonics,* 28;(3): 178-180.

Zulkowski, K. and Albrecht, D. (2003). How nutrition and aging affect wound healing. *Nursing*, 33(8):70–71.

APPENDICES

Appendix 1

Wound Dimensions in Centimetre square (cm^2)

	MethylP + EGF	**NS + EGF**	**MethylP + PDGF**	**NS + PDGF**	**MethylP + NS**	**NS + NS**	**MethylP + EGF + PDGF**	**NS + EGF + PDGF**
Day	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**
0	2±0	2 ±0	2 ±0	2 ±0	2 ±0	2 ±0	2 ±0	2 ±0
3	2.28 ±0.29	2.67 ±0.092	2.54 ±0.12	3.13 ±0.14	2.57 ±0.09	2.61 ±0.17	2.59 ±0.12	2.56 ±0.31
5	2.29 ±0.16	3.01 ±0.267	2.30 ±0.15	2.77 ±0.11	2.4 ±0.13	3.11 ±0.23	2.19 ±0.08	2.43 ±0.38
7	1.81 ±0.08	2.11 ±0.08	1.43 ±0.23	2.21 ±0.129	1.86 ±0.07	2.3 ±0.18	1.91 ±0.03	1.82 ±0.23
10	1.29 ±0.15	1.52 ±0.26	1.05 ±0.22	1.14 ±0.15	1.35 ±0.13	0.93 ±0.11	1.25 ±0.12	1.23 ±0.17
12	0.87 ±0.15	0.81 ±0.15	0.57 ±0.14	0.71 ±0.13	0.82 ±0.07	0.51 ±0.08	0.62 ±0.08	0.87 ±0.21
14	0.61 ±0.12	0.53 ±0.17	0.33 ±0.08	0.63 ±0.14	0.57 ±0.11	0.39 ±0.07	0.49 ±0.04	0.64 ±0.08
16	0.39 ±0.09		0.25 ±0.05	0.58 ±0.09	0.49 ±0.12		0.42 ±0.02	0.54 ±0
18	0.32 ±0			0.56 ± 0				

KEY:- MethylP = Methylprednisolone; EGF = Epidermal growth factor; NS = Normal saline; PDGF = Platelet-derived growth factor

Appendix 2

Wound Epithelisation in Centimetre square (cm^2)

	MethylP + EGF	NS + EGF	MethylP + PDGF	NS + PDGF	MethylP + NS	NS + NS	MethylP + EGF + PDGF	NS + EGF + PDGF
Day	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
0								
3							0.15 ± 0.01	0.13 ± 0.01
5				0.10 ± 0.00	0.1 ± 0.0		0.19 ± 0.01	0.2 ± 0.02
7	0.12 ± 0.01	0.18 ± 0.01	0.16 ± 0.01	0.19 ± 0.01	0.12 ± 01	0.17 ± 0.01	0.27 ± 0.02	0.29 ± 0.02
10	0.15 ± 0.02	0.26 ± 0.02	0.19 ± 0.01	0.37 ± 0.01	0.17 ± 0.01	0.26 ± 0.02	0.36 ± 0.01	0.33 ± 0.03
12	0.24 ± 0.02	0.33 ± 0.02	0.23 ± 0.01	0.37 ± 0.01	0.36 ± 0.01	0.35 ± 0.02	0.41 ± 0.03	0.41 ± 0.07
14	0.27 ± 0.01	0.53 ± 0.03	0.24 ± 0.01	0.49 ± 0.02	0.40 ± 0.02	0.39 ± 0.03	0.42 ± 0.02	0.48 ± 0.02
16	0.33 ± 0.03		0.25 ± 0.02	0.53 ± 0.01	0.49 ± 0.03			0.54 ± 0.03
18	0.32 ± 0.02			0.56 ± 0.02				

KEY:- MethylP = Methylprednisolone; EGF = Epidermal growth factor; NS = Normal saline; PDGF = Platelet-derived growth factor

Appendix 3

PCV (%)

	MethylP + EGF	NS + EGF	MethylP + PDGF	NS + PDGF	MethylP + NS	NS + NS	MethylP + EGF + PDGF	NS + EGF + PDGF
Day	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
0	29.67 ± 1.45	28.67 ± 2.19	29.33 ± 0.67	28 ± 1.53	26.33 ± 1.76	28 ± 1.73	29.67±0.33	29.67 ± 0.88
3	28.33 ± 1.45	30.67 ± 2.03	27.67 ± 2.33	32.33 ± 2.91	25 ± 2.51	33 ± 2.52	33 ± 2	34.67 ± 2.97
5	24 ± 4.04	29 ± 1.7	30 ± 2.08	29.67 ± 2.03	26.33 ± 1.67	26.67 ± 4.33	31 ± 3.22	26.67 ± 3.67
7	27.67 ± 1.33	33.33 ± 3.33	28 ± 1.73	30.67 ± 2.33	27.33 ± 1.45	32.67 ± 5.24	31.67 ± 2.40	33.33 ± 1.33
10	24.67 ± 2.19	26.67 ± 3.18	29.6 ± 1.76	30 ± 2.65	29 ± 3.06	28.67 ± 3.28	26.67 ± 2.91	21 ± 0
12	26.33 ± 2.33	29.67 ± 3.33	30.33 ± 1.86	29.33 ± 2.19	28.33 ± 3.84	27.67 ± 2.40	30 ± 1.73	29 ± 2.52

KEY: PCV = Packed cell volume; MethylP = Methylprednisolone; EGF = Epidermal growth factor; NS = Normal saline; PDGF = Platelet-derived growth factor

Appendix 4

PLT (%)

	MethylP + EGF	NS + EGF	MethylP + PDGF	NS + PDGF	MethylP + NS	NS + NS	MethylP + EGF + PDGF	NS + EGF + PDGF
Day	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
0	467.67 ± 42.34	402.67 ± 28.84	405.33 ± 97.02	281.67 ± 37.47	291 ± 14.42	287.67 ± 16.18	374.67 ± 39.89	349.67 ± 37.20
3	357.33 ± 46.93	393.67 ± 118.18	410.33 ± 117.53	413.33 ± 75.68	275.33 ± 39.03	341.67 ± 83.22	460.33 ± 51.15	331.33 ± 75.84
5	330 ± 81.98	303.67 ± 26.97	342.67 ± 75.30	328 ± 71.04	328.67 ± 67.51	277 ± 68.15	418.67 ± 97.03	347.33 ± 141.87
7	394.33 ± 105.67	284.33 ± 80.34	359.33 ± 33.18	347.33 ± 58.34	308.33 ± 19.68	237.33 ± 46.37	342 ± 65.96	390.67 ±117.03
10	475 ± 16.20	307 ± 67.09	453.67 ± 70.97	284.33 ± 69.85	273.67 ± 35.00	370 ± 78.79	421 ± 51.68	462.67 ± 166.56
12	346.67 ± 64.81	266.33 ± 25.62	418 ± 35.16	298.33 ± 46.77	305.67 ± 56.83	341.67 ± 45.18	420 ± 79.19	363.67 ± 140.72

KEY: PLT= Platelets count; MethylP = Methylprednisolone; EGF = Epidermal growth factor; NS = Normal saline; PDGF = Platelet-derived growth factor

Appendix 5

N (%)

	MethylP + EGF	NS + EGF	MethylP + PDGF	NS + PDGF	MethylP + NS	NS + NS	MethylP + EGF + PDGF	NS + EGF + PDGF
	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
0	38.33 ± 9.17	25.67 ± 3.18	30 ± 10.02	29 ± 3.79	33.67 ± 7.67	32.67 ± 4.81	44 ± 8.02	28.67 ± 9.82
3	41.00 ± 10	23.67 ± 4.26	32.33 ± 8.45	23.33 ± 2.40	33 ± 9.07	33.33 ± 7.69	37.67 ± 7.17	23 ± 1.53
5	35.67 ± 7.54	25.67 ± 2.73	37 ± 8.72	38.33 ± 10.87	34.33 ± 11.35	26.33 ± 7.75	35.67 ± 7.42	24.33 ± 9.49
7	34.67 ± 5.61	24.67 ± 5.24	35 ± 10.54	31.33 ± 7.13	45 ± 6.43	28 ± 7.23	39.33 ± 7.51	22 ± 7.02
10	33.67 ± 8.29	20.33 ± 4.63	33.33 ± 8.69	31 ± 4.58	31.67 ± 9.74	30 ± 1.73	34.67 ± 5.55	29.33 ± 8.25
12	34.67 ± 6.7	24.67 ± 2.60	27 ± 5.57	30.33 ± 4.33	32.33 ± 9.35	35 ± 2.00	40.67 ± 6.36	29 ± 12.58

KEY: N= Neutrophiles; MethylP = Methylprednisolone; EGF = Epidermal growth factor; NS = Normal saline; PDGF = Platelet-derived growth factor

Appendix 6

M (%)

	MethylP + EGF	NS + EGF	MethylP + PDGF	NS + PDGF	MethylP + NS	NS + NS	MethylP + EGF + PDGF	NS + EGF + PDGF
Day	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
0	27 ± 1.00	24 ± 2.08	19.33 ± 2.03	21 ± 0.58	26.67 ± 0.88	24 ± 2.52	26.67 ± 0.88	21.67 ± 1.86
3	27 ± 1.53	23.33 ± 2.91	25.67 ± 5.24	27.33 ± 3.76	28 ± 1.16	19 ± 5.51	28 ± 1.16	25.67 ± 3.76
5	27.33 ± 0.88	23.67 ± 2.73	29 ± 6.43	24.67 ± 3.71	28 ± 3.06	21.67 ± 1.67	28 ± 3.06	21.67 ± 3.38
7	27.67 ± 1.45	21.33 ± 0.33	26.67 ± 3.18	28.67 ± 2.19	29.67 ± 2.91	24 ± 3.46	29.67 ± 2.91	21.67 ± 2.33
10	25.33 ± 0.33	24.67 ± 1.67	26 ± 1.53	24.67 ± 2.73	29.67 ± 3.28	31.33 ± 4.81	29.67 ± 3.28	27.33 ± 4.1
12	24.67 ± 3.18	25 ± 2.89	24 ± 1.16	27 ± 1.16	29.67 ± 5.24	24 ± 3.61	29.67 ± 5.24	22 ± 0.58

KEY: M= Monocytes; MethylP = Methylprednisolone; EGF = Epidermal growth factor; NS = Normal saline; PDGF = Platelet-derived growth factor

Appendix 7

Hb (%)

	MethylP + EGF	NS + EGF	MethylP + PDGF	NS + PDGF	MethylP + NS	NS + NS	MethylP + EGF + PDGF	NS + EGF + PDGF
Day	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
0	29.67 ± 1.46	28.67 ± 2.19	29.33 ± 0.67	28 ± 1.53	26.33 ± 1.76	10.43 ± 0.39	29.67 ± 0.33	29.67 ± 0.88
3	28.33 ± 1.46	30.67 ± 2.03	27.67 ± 2.33	32.33 ± 2.91	25 ± 2.52	9.4 ± 0.87	33 ± 2.00	34.67 ± 2.96
5	24 ± 4.04	29 ± 1.73	30 ± 2.08	29.67 ± 2.03	26.33 ± 1.67	8.83 ± 1.82	31 ± 3.22	26.67 ± 3.67
7	27.67 ± 1.33	33.33 ± 3.33	28 ± 1.73	30.67 ± 2.33	27.33 ± 1.46	10.8 ± 1.91	31.67 ± 2.40	33.33 ± 1.33
10	24.67 ± 2.19	26.67 ± 3.18	29.67 ± 1.76	30 ± 2.65	29 ± 3.06	9.87 ± 1.02	26.67 ± 2.91	21 ± 0.00
12	26.33 ± 2.33	29.67 ± 3.33	30.33 ± 1.86	29.33 ± 2.19	28.33 ± 3.84	9.9 ± 1.18	30 ± 1.73	29 ± 2.52

KEY: Hb = Hemoglobin concentration; MethylP = Methylprednisolone; EGF = Epidermal growth factor; NS = Normal saline; PDGF = Platelet-derived growth factor

Appendix 8

WBC (%)

Day	MethylP + EGF	NS + EGF	MethylP + PDGF	NS + PDGF	MethylP + NS	NS + NS	MethylP + EGF + PDGF	NS + EGF + PDGF
	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
0	29.67 ± 1.45	28.67 ± 2.19	29.33 ± 0.67	28 ± 1.53	26.33 ± 1.76	10.43 ± 0.39	29.67 ± 0.33	29.67 ± 0.88
3	28.33 ± 1.45	30.67 ± 2.03	27.67 ± 2.33	32.33 ± 2.91	25 ± 2.52	9.4 ± 0.87	33 ± 2.00	34.67 ± 2.96
5	24 ± 4.04	29 ± 1.73	30 ± 2.08	29.67 ± 2.03	26.33 ± 1.67	8.83 ± 1.82	31 ± 3.22	26.67 ± 3.67
7	27.67 ± 1.33	33.33 ± 3.33	28 ± 1.73	30.67 ± 2.33	27.33 ± 1.45	10.8 ± 1.91	31.67 ± 2.40	33.33 ± 1.33
10	24.67 ± 2.19	26.67 ± 3.18	29.67 ± 1.76	30 ± 2.65	29 ± 3.06	9.87 ± 1.02	26.67 ± 2.91	21 ± 0.00
12	26.33 ± 2.33	29.67 ± 3.33	30.33 ± 1.86	29.33 ± 2.19	28.33 ± 3.84	9.9 ± 1.18	30 ± 1.73	29 ± 2.52

KEY: WBC= Total Leucocytes Count; MethylP = Methylprednisolone; EGF = Epidermal growth factor; NS = Normal saline; PDGF = Platelet-derived growth factor

Appendix 9

L (%)

Day	MethylP + EGF Mean	NS + EGF Mean	MethylP + PDGF Mean	NS + PDGF Mean	MethylP + NS Mean	NS + NS Mean	MethylP + EGF + PDGF Mean	NS + EGF + PDGF Mean
0	46 ± 8.51	52.67 ± 0.88	53.67 ± 0.67	48.33 ± 6.01	52.67 ± 0.33	45.33 ± 4.49	56.33 ± 1.76	50 ± 6.81
3	35.33 ± 7.31	56.33 ± 3.93	40.33 ± 6.98	43.33 ± 6.23	41 ± 6.51	47.67 ± 3.48	41 ± 5.86	51.33 ± 2.73
5	46.67 ± 6.89	58.67 ± 5.55	40.33 ± 6.96	57 ± 13.45	36.67 ± 6.07	52.67 ± 6.12	40.33 ± 7.43	50.67 ± 8.95
7	40.33 ± 8.45	60.67 ± 2.33	47 ± 3.06	46 ± 8.08	42 ± 10.54	50.67 ± 6.74	48 ± 7.23	53 ± 12.58
10	47 ± 8.54	60.33 ± 4.18	55.67 ± 1.86	44.67 ± 13.69	55.67 ± 1.45	38 ± 5.86	48.67 ± 6.07	46.67 ± 7.22
12	44.33 ± 11.80	52.33 ± 2.33	48 ± 2.08	48.67 ± 3.48	43.67 ± 10.4	45.33 ± 6.01	47 ± 8.19	48.33 ± 12.25

KEY: L= Lymphocytes; MethylP = Methylprednisolone; EGF = Epidermal growth factor; NS = Normal saline; PDGF = Platelet-derived growth factor

Appendix 10

Normal hemogram of Rabbit

Hb	7.02±1.21 – 9.92±0.58
PCV	21±3.53 – 30±1.84
RBC($*10^6/mm^3$)	3.53±.57 – 5.05±.31
WBC($*10^6/mm^3$)	4.68±.36 – 5.9±.69
N%	32.17±1.82 – 34.5±1.8
L	52.17±1.71 – 59.3±1.28
M	0.67±0.21 – 1.5±0.34
Eosinophile	0
Basophile	0
PLT	112-716

Etim et al., 2014

KEY: PCV = Packed cell volume; RBC = Red blood cell count; WBC = White blood cell count; N = Neutrophil count; L = Lymphocyte count; M = Monocyte count; PLT = Platelet count

Appendix 11

EGF Optical density

Day	MethylP + EGF	NS + EGF	MethylP + PDGF	NS + PDGF	MethylP + NS	NS + NS	MethylP + EGF + PDGF	NS + EGF + PDGF
	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
0	0.07 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.1 ± 0.00	0.05 ± 0.00	0.19 ± 0.06	0.32 ± 0.07
3	0.08 ± 0.00	0.06 ± 0.0	0.06 ± 0.00	0.07 ± 0.0	0.18 ± 0.0	0.05 ± 0.00	0.24 ± 0.09	0.14 ± 0.04
5	0.06 ± 0.00	0.07 ± 0.0	0.06 ± 0.00	0.05 ± 0.00	0.07 ± 0.00	0.05 ± 0.00	0.1 ± 0.01	0.06 ± 0.00
7	0.06 ± 0.00	0.05 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.37 ± 0.06	0.05 ± 0.00	0.09 ± 0.01	0.18 ± 0.06
10	0.08 ± 0.00	0.05 ± 0.00	0.07 ± 0.01	0.06 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.08 ± 0.01	0.06 ± 0.00
12	0.24 ± 0.06	0.06 ± 4.41	0.07 ± 0.00	0.06 ± 0.00	0.14 ± 0.04	0.06 ± 0.00	0.44 ± 0.11	0.13 ± 0.04

KEY: MethylP = Methylprednisolone; EGF = Epidermal growth factor; NS = Normal saline; PDGF = Platelet-derived growth factor; S = Standard

Appendix 12

PDGF Optical density

	MethylP + EGF	NS + EGF	MethylP + PDGF	NS + PDGF	MethylP + NS	NS + NS	MethylP + EGF + PDGF	NS + EGF + PDGF
Day	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
0	0.09 ± 0.01	0.06 ± 0.00	0.06 ± 0.00	0.1 ± 0.00	0.06 ± 0.00	0.09 ± 0.01	0.08 ± 0.00	0.06 ± 0.00
3	0.07 ± 0.00	0.06 ± 0.00	0.07 ± 0.00	0.08 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.07 ± 0.00	0.07 ± 0.00
5	0.06 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.08 ± 0.00
7	0.06 ± 0.00	0.08 ± 0.00	0.06 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.07 ± 0.00
10	0.19 ± 0.08	0.06 ± 3.64	0.07 ± 0.00	0.08 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.07 ± 0.00	0.08 ± 0.00
12	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.01	0.08 ± 0.00	0.06 ± 0.00	0.27 ± 0.12	0.08 ± 0.00

KEY: MethylP = Methylprednisolone; EGF = Epidermal growth factor; NS = Normal saline; PDGF = Platelet-derived growth factor; S = Standard

Appendix 13

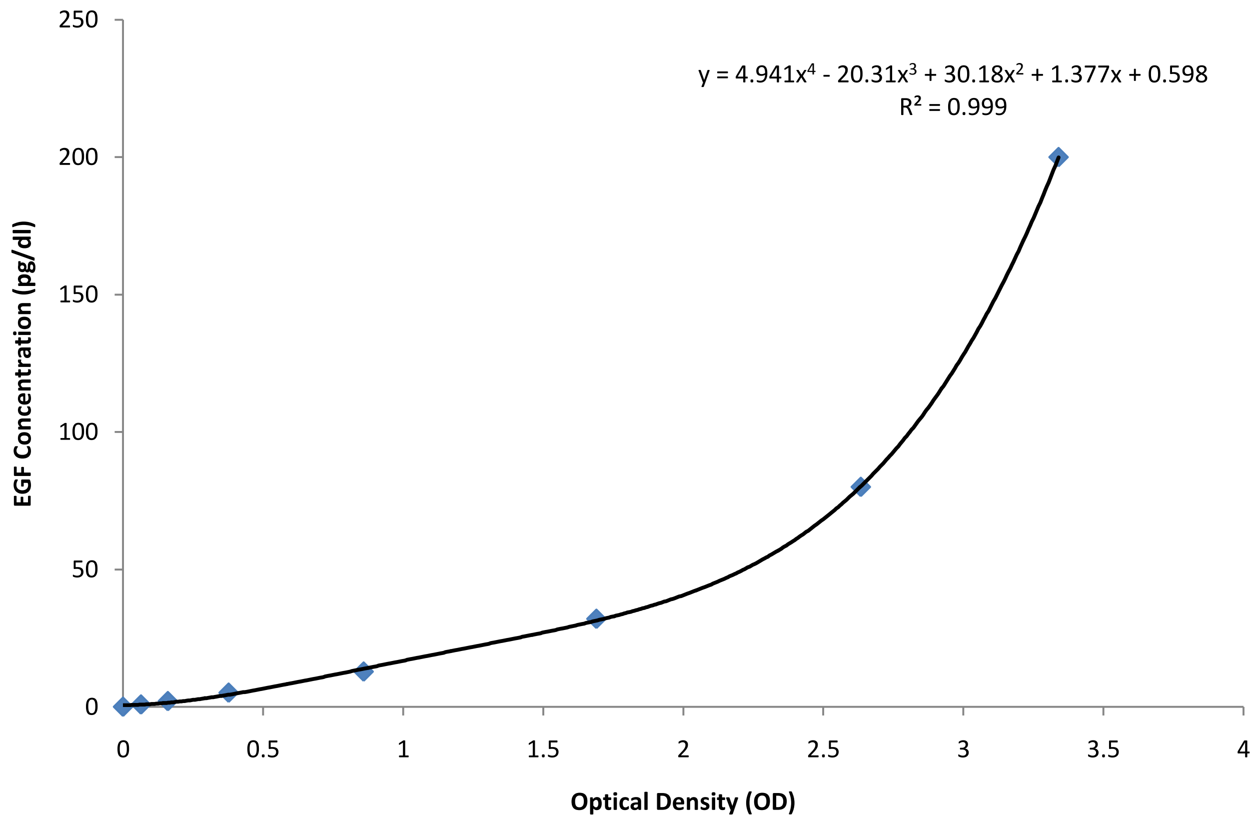
EGF Concentration (pg/dl)

Day	MethylP + EGF	NS + EGF	MethylP + PDGF	NS + PDGF	MethylP + NS	NS + NS	MethylP + EGF + PDGF	NS + EGF + PDGF
0	0.836322	0.785824	12.34871	0.785824	1.018646	0.740654	1.817484	3.517088
3	0.892033	0.785824	13.39881	0.836322	1.711621	0.740654	2.403878	1.329542
5	0.785824	0.836322	11.41193	0.740654	0.836322	0.740654	1.018646	0.785824
7	0.785824	0.740654	12.34871	0.785824	4.30491	0.740654	0.952845	1.711621
10	0.892033	0.740654	12.34871	0.785824	0.836322	0.785824	0.892033	0.785824
12	2.403878	0.785824	12.34871	0.785824	1.329542	0.785824	5.504077	1.244881

KEY: MethylP = Methylprednisolone; EGF = Epidermal growth factor; NS = Normal saline; PDGF = Platelet-derived growth factor

Appendix 14

Standard curve EGF Concentration and Optical density



KEY: EGF = Epidermal growth factor

Appendix 15

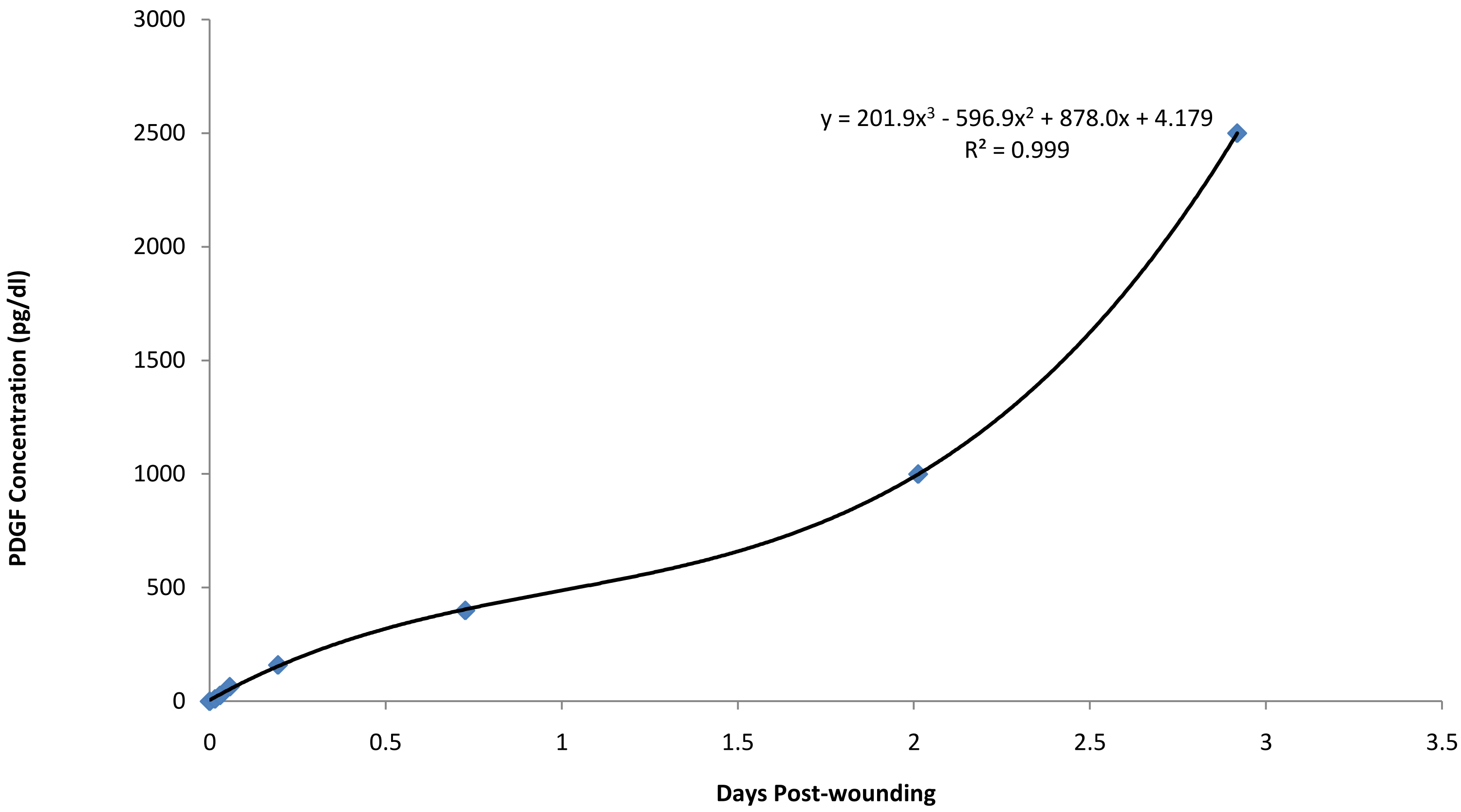
EGF Concentration (pg/dl)

Day	MethylP + EGF	NS + EGF	MethylP + PDGF	NS + PDGF	MethylP + NS	NS + NS	MethylP + EGF + PDGF	NS + EGF + PDGF
0	0.836322	0.785824	12.34871	0.785824	1.018646	0.740654	1.817484	3.517088
3	0.892033	0.785824	13.39881	0.836322	1.711621	0.740654	2.403878	1.329542
5	0.785824	0.836322	11.41193	0.740654	0.836322	0.740654	1.018646	0.785824
7	0.785824	0.740654	12.34871	0.785824	4.30491	0.740654	0.952845	1.711621
10	0.892033	0.740654	12.34871	0.785824	0.836322	0.785824	0.892033	0.785824
12	2.403878	0.785824	12.34871	0.785824	1.329542	0.785824	5.504077	1.244881

KEY: MethylP = Methylprednisolone; EGF = Epidermal growth factor; NS = Normal saline; PDGF = Platelet-derived growth factor

Appendix 16

Standard curve PDGF Concentration and Optical density



KEY:PDGF = Platelet-derived growth factor

Appendix 17:

Ultrasound of scar post-wounding

	MethylP +EGF	NS + EGF	MethylP + PDGF	NS + PDGF	NS + NS	MethylP + NS	MethylP + EGF + PDGF	NS + EGF + PDGF
	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
Scar Size Relative To Scar Line	2.00 ± 0.00	2.00 ± 0.00	2.50 ± 0.50	3.50 ± 0.50	4.00 ± 0.00	3.00 ± 0.00	2.50 ± 0.50	3.00 ± 0.00
Isoechogenicity	1.00 ± 0.00	1.00 ± 0.00	1.00± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
Vascularity	7.00 ± 0.00	7.50 ± 0.50	7.50 ± 0. 50	9.50 ± 1.50	3.50 ± 0.50	6.50 ± 0.50	4.50 ± 1.50	4.50 ± 1.50

KEY: MethylP = Methylprednisolone; EGF = Epidermal growth factor; NS = Normal saline; PDGF = Platelet-derived growth factor

Appendix18:

Wound breaking strenght and tensile strength in N/cm^2

	MethylP +EGF	NS + EGF	MethylP + PDGF	NS + PDGF	NS + NS	MethylP + NS	MethylP + EGF + PDGF	NS + EGF + PDGF
	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
Scar size at day 28 post-wounding	0.32 ± 0.02	0.53 ± 0.01	0.25 ± 0.03	0.54 ± 0.02	0.39 ± 0.03	0.42 ± 0.02	0.43 ± 0.01	0.56 ± 0.02
Wound breaking strenght	22.8 ± 1.2	37.5 ± 0.40	19.2 ± 0.90	36.1 ± 0.4	22.2 ± 0.90	29.7 ± 0.8	30.3 ± 1.3	38.1 ± 1.1
Tensile strenght	71.25 ± 1.25	70.8 ± 1.00	76.8 ± 0.20	66.9 ± 2.10	56.9 ± 1.2	70.75 ± 0.55	70.5 ± 1.60	68 ± 01.77

KEY: MethylP = Methylprednisolone; EGF = Epidermal growth factor; NS = Normal saline; PDGF = Platelet-derived growth factor

Appendix19

Vancouver scar scale score card

	Vascularity		Pigmentation		Pliability		Height		Vancouver scar scale
WOUND (W)	W1	W2	W1	W2	W1	W2	W1	W2	
MethylP + EGF	1	1	1	1	1	1	0	0	3
NS + EGF	1	1	1	1	1	2	0	2	3.5
MethylP + PDGF	1	1	1	1	1	1	0	0	3.5
NS + PDGF	1	1	2	2	2	3	1	0	6.5
MethylP + NS	1	1	2	1	2	2	0	0	4.5
NS + NS	1	1	2	2	3	3	2	2	9
MethylP + EGF +PDGF	1	1	2	2	2	2	0	0	5
NS + EGF + PDGF	1	1	2	1	2	2	0	1	5

KEY: MethylP = Methylprednisolone; EGF = Epidermal growth factor; NS = Normal saline; PDGF = Platelet-derived growth factor