INTRODUCTION

Scarring is the most important long-term complication of burn injuries or trauma on the skin. Treatment options for managing such scars are limited to surgical correction, dermabrasion or laser resurfacing techniques ***(Majid and Imran, 2015).***

Carbon dioxide (CO_2) laser is an ablative laser device that produces energy in the far-infrared region at a wavelength of 10,600 nm. Resurfacing with CO2 laser is highly effective in treating scars and ageing skin ***(Graber et al., 2008)***. Laser resurfacing is supposed to work by stimulating collagen production in the dermis and by dermal remodeling of collagen fibers ***(Majid and Imran, 2015).*** However, a really long down-time and high incidence of adverse effects has limited its use in routine dermatology practice. Some long term adverse effects can also occur including permanent hypopigmentation, hyperpigmentation and permanent scarring ***(Alster and Hirsch, 2003).***

Since its introduction in 2007, ablative fractional resurfacing (AFR) has gained popularity in the cosmetic improvement of multiple skin conditions including dyschromia, acne, surgical, and burn scars ***(Bowen, 2010)***. Fractional photothermolysis (FP) circumvents many of the above mentioned disadvantages of laser resurfacing ***(Hantash and Mahmood, 2007)***. With FP, only a fraction of the whole skin is treated in a pixilated pattern while the intervening skin remains intact. Treatment with FP leads to formation of longitudinal microthermal zones (MTZs) in the skin which are separated by healthy, untreated skin with an intact epidermis ***(Ozog et al., 2013).*** This allows the treating physicians to go for much deeper treatment than with traditional laser resurfacing with more rapid healing and less down time ***(Majid and Imran, 2014).*** Additionally, the adverse effects encountered with FP are transient and less severe than with full skin resurfacing ***(Anderson et al., 2014).***

Fractional CO2 laser resurfacing has been successfully used in the treatment of atrophic acne scars and for skin rejuvenation. There are also some reports of its usefulness in hypertrophic as well as burn scars ***(Anderson et al., 2014).***

AIM OF THE WORK

The aim of this thesis is to assess the efficacy of fractional ablative CO_2 Laser in the treatment of linear depressed traumatic scars of the face in male patients.

Chapter I

SCARS

Introduction:

Skin, the largest organ in the body, is vital for humans to protect against dehydration, bleeding and environmental microorganisms. Humans have evolved a sophisticated mechanism of wound healing to plug any gap in skin integrity quickly, re - epithelialize over the defect and rapidly replace the lost dermis with new matrix. The final product is not normal skin, for adult human skin does not regenerate, but rather is a repair in the form of a scar, which is visible on the skin surface ***(Wong et al., 2013).***

Scars:

Definition:

Cutaneous scarring is a macroscopic disturbance of the skin architecture manifesting itself as an elevated or depressed area, with an alteration of skin texture (e.g. hard or soft), color (e.g. hyperpigmentation or erythema), vascularity, nerve supply and biomechanical properties (e.g. elasticity). Scar formation is an inevitable consequence of wound healing in which the normal skin is replaced by a fibrous tissue ***(Garg et al., 2014).***

Scars continue to remodel for a long time after wounding, and cannot be considered to be in a steady state condition until at least 2 years post - wounding ***(Eming et al., 2007).***

Scarring of skin is a major clinical problem resulting in adverse cosmesis and loss of function ***(Gilliver et al., 2007).***

In addition to the potential for cosmetic disfigurement ***(Bayat et al., 2003)***, scars may be associated with a number of physical comorbidities including hypertrichosis/ hypotrichosis, dyshidrosis, tenderness/pain, pruritus, dysesthesias, and functional impairments such as contractures, all of which may be compounded by psychosocial factor ***(Krakowski et al., 2016).***

Wound healing:

Wound healing is one of the most complex biological processes where the skin repairs itself after injury ***(You and Han, 2014).*** Skin wound healing is a dynamic and highly regulated process of cellular, humoral and molecular mechanisms which begins directly after wounding and might last for years ***(Reinke and Sorg, 2012).***

Stages of wound healing:

Traditionally wound healing is divided into 4 overlapping phases known as (1) haemostasis, (2) inflammation, (3) cellular proliferation and (4) remodeling ***(Macri et al., 2009).***

1) Coagulation and haemostasis phase (the vascular response):

The healing cascade begins immediately after injury by platelet aggregation and deposition of fibrin clot at the site of injury ***(Velnar et al., 2009).***

The blood clot and platelets trapped within it are not only important for haemostasis, as the clot also provides a provisional matrix as a scaffold structure for cell migration (such as leukocytes, keratinocytes, fibroblasts and endothelial cells) and as a reservoir of growth factors in the subsequent phases of the haemostatic and inflammatory phases. The cytoplasm of platelets contains α-granules filled with growth factors and cytokines, such as platelet derived growth factor (PDGF), transforming growth factor-β (TGF-β), epidermal growth factor and insulin-like growth factors ***(Reinke and Sorg, 2012).***

2) Inflammatory phase (the cellular response):

The humoral and cellular inflammatory phase follows next, with the aim of establishing an immune barrier against invading micro-organisms and removal of damaged tissue (fig.1). It is divided into two separate phases, an early inflammatory phase and a late inflammatory phase ***(Hart, 2002).***

a) Early inflammatory phase

Starting during the late phase of coagulation and shortly thereafter, the early inflammatory response has many functions. It activates the complement cascade and initiates molecular events, leading to infiltration of the wound site by neutrophils, whose main function is to prevent infection ***(Broughton et al., 2006).*** The neutrophils start with the critical task of phagocytosis to destroy and remove bacteria, foreign particles and damaged tissue ***(Velnar et al., 2009).*** Furthermore, they act as chemoattractants for other cells that are involved in the inflammatory phase ***(Reinke and Sorg, 2012).***

b) Late inflammatory phase

As a part of the late inflammatory phase, 48-72 hours (approximately 2-3 days) after injury, macrophages appear in the wound and continue the process of phagocytosis ***(Profyris et al., 2012)*** as well as secretion of growth factors, chemokines and cytokines which activate the next phase of wound healing (proliferative phase) ***(Gurtner et al., 2008).***

Macrophages have many functions including host defense, the promotion and resolution of inflammation, the removal of apoptotic cells and the support of cell proliferation and tissue restoration following injury ***(Koh and DiPietro, 2011).*** Macrophages also have a role in the synthesis of numerous potent growth factors such as TGF-β, TGF-α, basic FGF, PDGF and VEGF, which promote cell proliferation and the synthesis of extracellular matrix (ECM) molecules by resident skin cells ***(Reinke and Sorg, 2012).***

The last cells to enter the wound site in the late inflammatory phase are lymphocytes, attracted 72 h after injury by the action of interleukin-1 (IL-1), complement components and immunoglobulin G (IgG) breakdown products. The IL-1 plays an important role in collagenase regulation, which is later needed for collagen remodelling, production of extracellular matrix components and their degradation ***(Velnar et al., 2009).***

There is evidence that the amount of inflammation determines the extent of scar formation ***(Reinke and Sorg, 2012).***

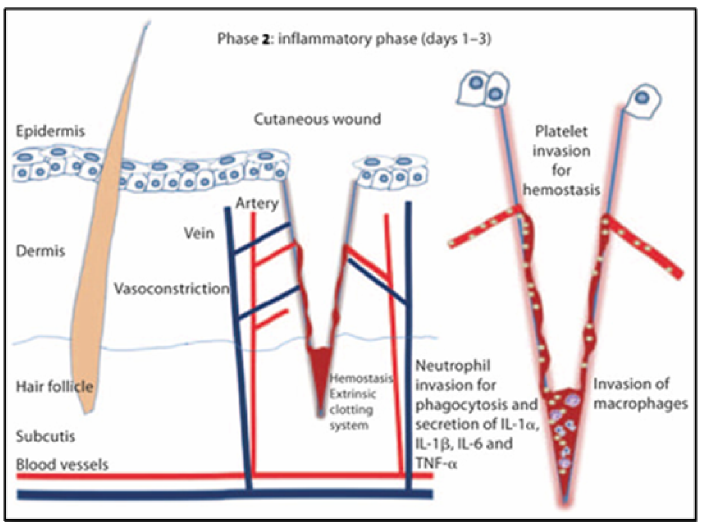

Fig. (1): Inflammatory phase after a cutaneous cut hemostasis and invasion of inflammatory cells ***(Reinke and Sorg, 2012).***

3) Proliferative Phase:

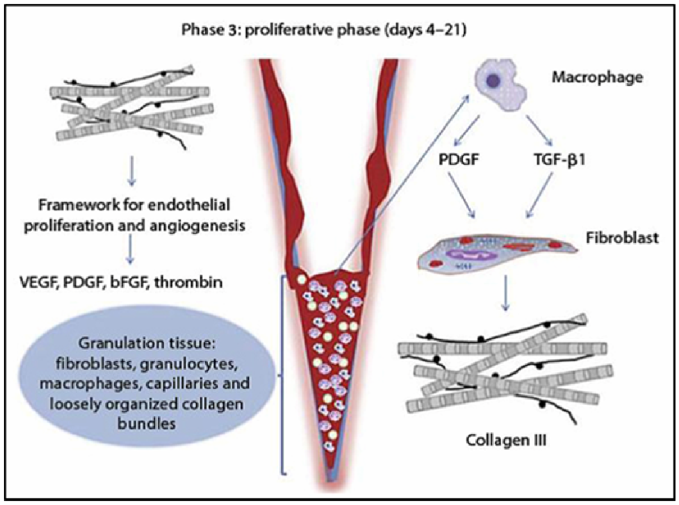

Fig. (2): Proliferative phase; organization of the thrombus, secretion of growth factors synthesis of collagen III and the beginning of angiogenesis ***(Reinke and Sorg, 2012).***

The proliferative phase starts on the third day after wounding and lasts for about two weeks thereafter. It is characterized by fibroblast migration and deposition of newly synthesized extracellular matrix and collagen **(fig. 2)** acting as a replacement for the provisional network composed of fibrin and fibronectin. At the macroscopic level, this phase of wound healing can be seen as an abundant formation of granulation tissue ***(Velnar et al., 2009).***

This step can be roughly divided into the following phases:

a) Fibroblast migration

Following injury, fibroblasts and myofibroblasts in the surrounding tissue are stimulated to proliferate for the first 3 days they then migrate into the wound, being attracted by factors such as TGF-β and PDGF, that are released by inflammatory cells and platelets. Once in the wound, they proliferate profusely and produce the matrix proteins hyaluronan, fibronectin, proteoglycans and type 1 and type III procollagen ***(Müller et al., 2012).***

By the end of the first week, abundant extracellular matrix accumulates which further supports cell migration and is essential for the repair process. Now, fibroblasts change to their myofibroblast phenotype. At this stage, they contain thick actin bundles below the plasma membrane and actively extend pseudopodia. Wound contraction, that helps to approximate the wound edges, then takes place as these cell extensions retract. Having accomplished this task, redundant fibroblasts are eliminated by apoptosis ***(Li et al., 2007)***.

b) Re-epithelialization

The re-epithelialization process is ensured by local keratinocytes at the wound edges and by epithelial stem cells from hair follicles or sweat glands ***(Lau et al., 2009).*** After the enzymatic loosening of the intercellular desmosomes via collagenase and elastase, activated keratinocytes can migrate along the preformed fibrin blood clot in the higher layers of the granulation tissue ***(Bauer et al., 2005)***. This process is called the 'shuffling' of keratinocytes and describes the ability of these cells to migrate competitively along a chemotactic gradient established by mediators such as IL-1, and over a fibronectin rich matrix into the center of the wound ***(Reinke and Sorg, 2012).***

c) Collagen synthesis

Collagens are an important component in all phases of wound healing. Synthesized by fibroblasts, they impart integrity and strength to all tissues and play a key role, especially in the proliferative and remodelling phases of repair. Unwounded dermis contains 25% type III collagen, whereas wound granulation tissue expresses 40% type III collagen ***(Velnar et al., 2009).***

d) Angiogenesis

The first step in new vessel formation is the binding of growth factors to their receptors on the endothelial cells of existing vessels, thereby activating intracellular signaling cascades ***(Hong et al., 2014).*** The activated endothelial cells secrete proteolytic enzymes which dissolve the basal lamina. Thus, the endothelial cells are now able to proliferate and migrate into the wound, a process also known as 'sprouting' ***(Wong et al., 2013).*** The newly built sprouts form small tubular canals which interconnect to others forming a vessel loop.

Finally, the initial blood flow completes the angiogenic process ***(Reinke and Sorg, 2012).***

e) Granulation tissue formation

The last step in the proliferation phase is the development of the acute granulation tissue ***(Clark, 2001)***. As a transitional tissue it replaces the fibrin-/fibronectin-based provisional wound matrix and might produce a scar by maturation ***(Nauta et al., 2011).*** Furthermore, it is characterized by a high density of fibroblasts, granulocytes, macrophages, capillaries and loosely organized collagen bundles ***(Reinke and Sorg, 2012).***

4) Remodelling Phase:

As the final phase of wound healing, the remodelling phase is responsible for the development of new epithelium and final scar tissue formation **(Fig. 3).** This phase starts from day 21 ***(Reinke and Sorg, 2012)*** and may last up to 1 or 2 years ***(Ramasastry, 2005).*** The tensile strength of the wound increases progressively in parallel with collagen collection. Collagen fibres may regain approximately 80% of the original strength compared with unwounded tissue ***(Velnar et al., 2009).***

Synthesis and breakdown of collagen as well as extracellular matrix remodelling take place continuously and both tend to equilibrate to a steady state after injury ***(Gurtner et al., 2008).*** Matrix metalloproteinase (MMP) enzymes, produced by neutrophils, macrophages and fibroblasts in the wound, are responsible for the degradation of collagen. Their activity is tightly regulated and synchronized by inhibitory factors. Gradually, the activity of tissue inhibitors of metalloproteinases (TIMP) increases, culminating in a drop in activity of metalloproteinase enzymes, thereby promoting new matrix accumulation ***(Gilliver et al., 2006).***

In this stage there is decrease in the relative levels of fibronectin, proteoglycans and type III collagen and an increase in the levels of predominantly type I collage. The degree of collagenous cross‑linking also varies with time after wounding: the early wound has fewer and more immature cross‑links compared with the later wound, which has extensive mature cross‑links, resulting in a more insoluble collagenous matrix ***(Driskell et al., 2013).***

The end result is a fully matured scar with a decreased number of cells and blood vessels and a high tensile strength ***(Velnar et al., 2009).***

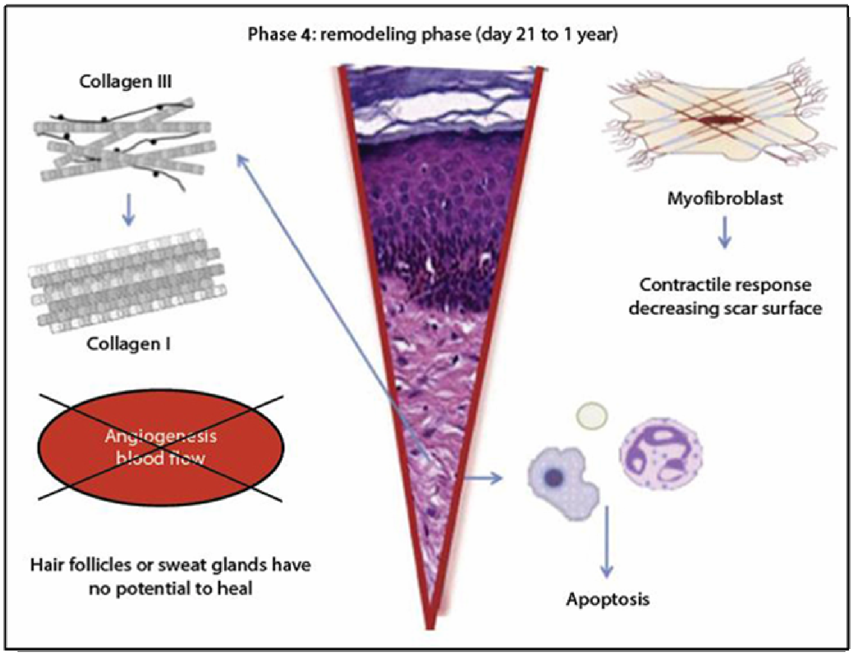

Fig. (3): Remodeling phase; regenerative processes fade and are followed by reorganization of the connective tissue and contractile response ***(Reinke and Sorg, 2012).***

Factors affecting wound healing:

The wound healing depends upon many factors. These factors can be divided as wound factors, surgeon factors and patient factors ***(Sharma and Wakure, 2013).***

<u>(A) Wound factors</u>

a) Size

- Wide wounds that are primarily closed give a poorer scar due to tension on the suture line.

b) Cause

- Traumatic and excisional wounds heal poorer than surgical incisional wounds ***(Lin et al., 2014).***

c) Anatomical

d) Location on trunk and extremities gives poorer scar than the head and neck region ***(Sharma and Wakure, 2013).***

e) Oxygenation

- Oxygen is important for cell metabolism, especially energy production by means of Adenosine Triphosphate (ATP), and is critical for nearly all wound healing processes. It prevents wounds from infection, induces angiogenesis, increases keratinocyte differentiation, migration, and re-epithelialization, enhances fibroblast proliferation and collagen synthesis, and promotes wound contraction ***(Greaves et al., 2013).***

f) Infection

- Once skin is injured, micro-organisms that are normally sequestered at the skin surface gain access to the underlying tissues ***(Kahn et al., 2011)***. *P.aeruginosa* and *Staphylococcus* appear to play an important role in bacterial infection in wounds. Many chronic ulcers probably do not heal because of the presence of biofilms containing *P. aeruginosa*, thus shielding the bacteria from the phagocytic