SCAR MATURATION IN THE AFRICAN CONTINENTAL ANCESTRY GROUP

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ABSTRACT

The University of Manchester
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Scar maturation in the African Continental Ancestry Group.
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The natural history of scar maturation in humans has been described by Bond et al. (2008b) in a male European Continental Ancestry Group (ECAG). It is important that the natural history of scar maturation in humans is established for all skin types. This study therefore aims to describe clinically and histologically the maturation of scars in male volunteers from the African Continental Ancestry Group (ACAG).

This study was performed as a single centre, methodology trial. Three incisions and a punch biopsy were carried out on each arm. Monthly assessments of the resultant scars included: investigator scar assessments; scar photography; VAS scoring by an Independent External Scar Assessment Panel; and objective measures of colour and scar mechanics. At various time points scars were excised for histology. Sixty male subjects of African Continental Ancestry between the ages of 18-56 years were recruited to take part in the study. The clinical appearance of a scar in the ACAG improves with time. Scar colour mismatch decreases and the mechanical properties of scars improve with time. Scar width increased over the 12 months. With the exception of scar contour and scar redness, a steady state was not achieved. Volunteer skin type was shown to influence the resulting scar appearance and not age. The histology of scar maturation in the ACAG over 12 months was described and scars classified into three groups each displaying a different rate of longitudinal progression of scar maturation. The process of collagen maturation is still ongoing at month 12; many scars demonstrated a prolonged high turnover state of collagen synthesis and degradation, rete ridge restoration and angiogenesis were still ongoing with persistent inflammation identified in scars up to Month 12. There is a strong correlation shown between the Clinical VAS scores and the Histology VAS scores for the papillary dermis which is of better quality than the reticular dermis. There is some evidence that young people (ACAG) and volunteers with darker skin have poorer scar histology. The spectrophotometry data indicated that the Fitzpatrick Skin Type Classification is a useful method of classifying the varying skin colours of this group of volunteers.

In conclusion, scar maturation in the ACAG occurs as a series of defined macroscopic and microscopic stages over the course of 1 year. The process of scar maturation is not complete at 12 months. All scars showed evidence of improvement over the course of the study influenced in part by volunteer skin type and age. Results suggest that scar maturation in this study group occurs at a different rate and is of a different quality, compared to current knowledge of scar maturation in the ECAG.

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THE AUTHOR

I graduated in Medicine from the Queen's University of Belfast in 2001. Following foundation training I commenced surgical training and further specialised in Plastic Surgery. I started this research degree as a Plastic Surgery trainee. As a part-time student I returned to Plastic Surgery training at registrar level. One year later I decided on a career change. I am now in my third year of Anaesthetics training and currently working in a District General Hospital as an Anaesthetics Registrar. During the course of my research I had a nine month break in my career when I had my son Harry who is now 21 months old.

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ABBREVIATIONS

The following abbreviations have been used in this continuation report:

ACAG	African Continental Ancestry Group
ACTH	Adrenocorticotrophic Hormone
ADP	Adenosine Diphosphate
AE	Adverse Event
α-MSH	Alpha-Melanocyte Stimulating Hormone
α-SMA	Alpha- Smooth Muscle Actin
bFGF	basic Fibroblast Growth Factor
CAG	Continental Ancestry Group
cAMP	cyclic Adenosine Monophosphate
CIE	Commission Internationale de Eclairage
CoR	Coefficient of Restitution
CREB	cAMP Response Element-Binding
CRF	Case Report Form
CSF-1	Colony Stimulating Factor-1
DCT	Dopachrome Tautomerase
ECAG	European Continental Ancestry Group
ECM	Extracellular Matrix
EGF	Epidermal Growth Factor
EPC	Endothelial Progenitor Cell
FDA	Food and Drug Administration

FGF	Fibroblast Derived Growth Factor
IDA	Industrial Denatured Alcohol
IESAP	Independent External Scar Assessment Panel
IGF-1	Insulin-like Growth Factor-1
IL-1	Interleukin-1
KGF	Keratinocyte Growth Factor
LFT	Liver Function Test
LREC	Local Research Ethics Committee
MC1R	Melanocortin-1 Receptor
MCP-1	Monocyte Chemotactic Protein-1
MeSH	Medical Subject Headings
MITF	Microphthalmia-Associated Transcription Factor
MMP	Matrix Metalloproteinase
PDGF	Platelet Derived Growth Factor
PKA	Protein Kinase A
POMC	Proopiomelanocortin
PUVA	Psoralen and Ultraviolet A treatment
SAE	Serious Adverse Event
SIA	Spectrophotometric Intracutaneous Analysis
SPT	Skin Phototype
TGF-α	Transforming Growth Factor-alpha
TGF-β	Transforming Growth Factor-beta family

TGF-β1	Transforming Growth Factor-beta 1
TGF-β2	Transforming Growth Factor-beta 2
TGF-β3	Transforming Growth Factor-beta 3
TNF-α	Tumour Necrosis Factor-alpha
TIMP	Tissue Inhibitor of Matrix Metalloproteinase
TRP1	Tyrosinase-Related Protein 1
UK	United Kingdom
uPA	Urokinase-type Plasminogen Activator
US	United States
UV	Ultraviolet
UVA	Ultraviolet A
UVR	Ultraviolet Radiation
VAS	Visual Analogue Scale
VEGF	Vascular Endothelial Growth Factor
VSS	Vancouver Scar Scale

INTRODUCTION AND REVIEW OF THE LITERATURE

1.1 Introduction

In humans, scarring in the skin after trauma or surgery is a major medical problem (Metcalfe and Ferguson, 2005). It often results in adverse aesthetics, loss of function, restriction of tissue movement and/or growth and adverse psychological effects (Ferguson and O'Kane, 2004). A study of the psychology of scarring found that 76% of women and 33% of men in the UK were concerned about a scar on their bodies that a plastic surgeon wound consider acceptable (Carr et al., 2000). ‘In the UK, wounds cost the National Health Service £1 billion a year and, although a large proportion of the cost is due to inappropriate delivery of care, modification of systems of care will provide only part of the answer to this clinical challenge’ (Thomas and Harding 2002). Advances in the management of both chronic and acute wounds will come from a greater understanding of the central mechanisms that control normal wound healing.

Until recently the natural history of scar maturation in humans had not been formally described. Bond et al. (2008b) carried out an observational study of scar maturation from both a clinical and histological standpoint over a 12 month period. One limitation of this study is that it considers a male European Continental Ancestry Group (ECAG) alone. Scar severity is variable and depends on numerous factors such as scar position, age, gender and race (O'Kane, 2002). Anecdotal evidence exists suggesting scarring is more pronounced in darker skin. Keloid scars are a particular type of scarring that

occurs with a specific racial prevalence mainly in Afro-Caribbean and Asian races. This noted it is important that the natural history of scar maturation in humans is established for all skin types. This study will consider the maturation of scars in the African Continental Ancestry Population Group (ACAG).

The following review of published literature will consider ancestral differences in human biology, melanocyte biology, normal wound healing and methods of scar assessment relevant to this study.

1.2 Ancestral Differences in Human Biology

1.21 Introduction

The Oxford Dictionary (2005) defines race as being each of the major divisions of humankind, having distinct physical characteristics; a group of people sharing the same culture, language, etc.; an ethnic group; a group of people or things with a common feature; and in the biological sense as a distinct population within a species. Anthropologists consider classifications based on skin colour should be discarded (Montagna et al., 1993).

Rawlings (2006) in a review of ethnic skin types describes race as being classified genetically and favours the classification system described by Coon (1962). Coon (1962) in a book 'The Origin of Races' uses a self-described conservative and tentative classification of the living peoples of the world into five basically geographical groups:

Caucasoid: Europeans, Arabs, Indians, Pakistanis.

Negroid: Africans, African Americans, African Caribbeans.

Mongoloid: Asians.

Australoid: Australian Aborigines.

Capoid: Kung San tribe of Africa (Coon, 1962).

In 2004 the Medical Subject Headings (MeSH) descriptor Racial Stocks and its four children (Australoid Race, Caucasoid Race, Mongoloid Race, and Negroid Race) were deleted along with Blacks and Whites. MeSH is the National Library of Medicine's controlled vocabulary thesaurus. Persons are now classified using the MeSH sub tree into population groups based on continental ancestry or ethnic groups (Aspinall, 2005).

Persons

- Population Groups
    - Continental Population Groups
        - African Continental Ancestry Group
            - African Americans
        - American Native Continental Ancestry Group
            - Indians, Central American
            - Indians, North American
            - Indians, South American
            - Inuits
        - Asian Continental Ancestry Group
            - Asian Americans
        - European Continental Ancestry Group
        - Oceanic Ancestry Group
- Ethnic Groups
    - African Americans
    - Arabs
    - Gypsies
    - Hispanic Americans
        - Mexican Americans
    - Inuits
    - Jews

This review will be limited mainly to discussion of the two largest population groups. The classification of these population groups are based on Food and Drug Administration (FDA) guidance (2005) in order to maintain a standardised approach to collecting race and ethnicity information in clinical trials. They also reflect the 2004 changes to MeSH headings for race and ethnic groups, with terms such as race, Caucasian and Black no longer being used. The two population groups are the African Continental Ancestry Group (ACAG) and the European Continental Ancestry Group (ECAG). These categories can be traced back to those recommended by the FDA, while fitting with the new MeSH vocabulary.

Definition of Terms

African Continental Ancestry Group (previously Black) – individuals whose ancestral origins are in the continent of Africa. Under the FDA guidance (2005) the black group includes persons having origin in any of the black racial groups of Africa (FDA, 2005). It includes people who indicate their population group as African American, or West Indian.

European Continental Ancestry Group (previously Caucasian) – a person having origins in any of the original peoples of Europe. Under the FDA guidance the white (Caucasian) group also includes the Middle East (Bahrain, Egypt, Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Palestinian Territory, Oman, Qatar, Saudi Arabia, Sudan, Syrian Arab Republic, United Arab Emirates, and Yemen), and North Africa (Algeria, Egypt, Libya, Morocco, Tunisia, Western Sahara) (FDA, 2005).

Another method of classification is the Skin Phototype (SPT) system classically used by dermatologists to categorise individuals (Taylor, 2002). Sun reactive "skin typing" was described in 1975 by Fitzpatrick specifically to classify persons with white skin (types I-IV) in order to select the correct initial doses of ultraviolet A (UVA) in the application of oral methoxsalen photochemotherapy (PUVA) (Fitzpatrick, 1988). A review by Fitzpatrick (1988) highlights that brown- and black-skinned persons (types V and VI) were included in the classification by Pathak et al and Fitzpatrick at a later stage. 'Sun-reactive skin types are based on the verbal response regarding a single unprotected sun exposure in early summer (burning tendency) and the degree of facultative pigmentation after repeated ultraviolet (UV) exposures (tanning ability)' (Rampen et al., 1988). Rampen et al. (1988) studied the self-reported tendencies to burn and tan in a large (790) number of white young adults. Only 41.1% of the cases were classifiable according to Fitzpatrick's original proposals. Rampen et al (1988) concluded that self-reported burning-tanning histories form an unreliable method of skin typing. However, according to Fitzpatrick (1988) a careful interview should allow the clinician to categorise individuals into the following skin types.

Type I	White skin. Always burns, never tans.
Type II	White skin. Always burn, minimal tan.
Type III	White skin. Burns minimally, tans moderately and gradually.
Type IV	Light brown skin. Burns minimally, tans well.
Type V	Brown skin. Rarely burns, tans deeply.
Type VI	Dark brown/black skin. Never burns, tans deeply.

Roberts (2008) reviewed current methods of skin type categorisation. The review recognised the Fitzpatrick scale as an invaluable tool for communication within the dermatological specialty. It is used to determine the skins burning tendency and tanning ability (Fitzpatrick, 1988). However, it fails to evaluate or describe two problems often encountered with darker pigmented skin types: pigmentation alterations and scarring (Roberts, 2008). Roberts (2008) sought to address this issue by proposing a new classification system. The Roberts Skin Type Classification system is a four part system, including the clinician's evaluation of phototype, hyperpigmentation, photoaging and scarring according to a numeric scale. Roberts (2008) deems the scale will positively impact on physician communication and serve as a universal language to classify, evaluate and properly treat varying patient skin types. As yet there is no literature to support this view. A more objective method of skin type classification is certainly required. Meanwhile, the Fitzpatrick scale offers a simple and practical method of classifying a patient's skin phototype.

1.22 Biological Differences

There are many similarities between individuals of different continental ancestry groups; however, there are also some biological differences.

Burt et al (1995) present the results of a US national survey which showed that hypertension is more common and more severe in the ACAG then in the ECAG.

There is variation in tumour biology between ECAG and ACAGs. A review by Bowen (2006) reports a lower incidence of breast cancer in women of ACAG compared to those of ECAG, however, a variation in prognostic features means that the ACAG has a higher age-adjusted breast cancer mortality rate. This poor survival rate from breast cancer is also seen in colorectal (Chien et al., 2005) and prostate cancer (Freedland and Isaacs, 2005). Skin cancer, however, is rare in those in the ACAG (Fleming et al., 1975). Burchard et al (2003) emphasise that socioeconomic status and access to care have to be taken into account when considering ancestral disparities in health.

Bain (1996) carried out a systematic investigation of the total and differential white cell counts and platelet counts in healthy volunteers of various ethnic origins and showed that the ACAG had a lower total white cell, neutrophil and platelet counts than the ECAG.

Ketchum et al (1974) have reviewed several series and on this basis report that individuals of the ECAG are less inclined to form keloid scars than those of African or Asian CAG (Ketchum, Cohen, & Masters 1974). Alhady (1969) also reports that keloid scars are more common in the ACAG with the ACAG:ECAG ratio varying between studies reviewed from 2:1 to 14:1. Alhady (1969) analysed 175 cases of keloid in the multi-racial population of West Malaysia from 1959 to 1967 and found that Chinese individuals were more likely to develop keloid scars than Indian or Malaysian Individuals. There are no epidemiological studies in recent literature to support these findings.

1.23 Skin Morphology Differences

Understanding and quantifying racial differences in skin function are important for skin care and the prevention and treatment of skin diseases (Berardesca and Maibach, 2003). Andersen and Maibach (1979) in a review article report that although colour is the most striking racial skin difference, it is only one piece of a biologic mosaic. They describe skin changes that are more pronounced in black skin:

1. Pigment lability – frequent hyper- and hypopigmentation
2. Follicular responses and follicular disease
3. Mesenchymal responses: fibroplastic and granulomatous.

Montagna and Carlisle (1991) carried out a study comparing the structure and function of facial skin of black and white women 22 to 50 years of age. Listed below are some of the differences they found:

1. Blacks have a significantly higher mean TEWL (transepidermal water loss) values than whites (Berardesca and Maibach, 2003).
2. Black skin has fewer elastic fibres everywhere than does white skin.
3. Black skin has minimal elastosis and the epidermis rarely shows atrophied areas.
4. Black skin appears to have more superficial, sub-epidermal blood vessels than white skin, regardless of age.
5. Black skin contains many large, dilated lymph vessels everywhere, regardless of age.

6. Black skin has a thick compact dermis with the distinction between papillary and reticular layers even less clear than it is in white skin. The collagen fibre bundles in the reticular dermis are smaller than in white skin and arranged almost orthogonally, running mostly parallel to the epidermis.
7. Epidermal melanocytes in black skin are essentially similar to those in white skin, except they are larger, have more branching processes and are found mainly among the basal keratinocytes.
8. Black skin melanosomes differ from white skin in number, size, composition and distribution.

A review by La Ruche & Cesarini (1992) of the histology and physiology of black skin highlights that skin structure in all races is essentially the same under the microscope but morphological differences do exist, particularly within the epidermis. They report the following:

1. The stratum corneum is equal in thickness but more compact in black skin compared to white skin.
2. There is a higher lipid content in black skin.
3. Black skin has a higher electrical resistance, suggesting black epidermis would be less well hydrated than white epidermis.
4. The number of sweat glands and sweating is similar.
5. Vasomotor functions differ between black skin and white skin but skin vascularity is similar.
6. Sebum production is equal in both skin types.

7. Pigmentation is the most outstanding difference between black and white skins.
8. Arrangement of melanin pigment in the epidermis provides a superior mechanism for photoprotection among black skin. The main site of ultraviolet filtration in white skin is the stratum corneum, in black skin it is the malpighian layers.
9. Blacks are less susceptible to acute and chronic actinic damage than whites.
10. Increased skin pigment in black skin reduces the cutaneous production of vitamin D.

Giraedeau et al (2009) carried out a study of the dermal component of Caucasian and African skin types. They analysed skin biopsies obtained from 57 healthy adult women at breast reduction surgery i.e. UV-unexposed skin. Thirty-four specimens were of Caucasian origin and 23 were of African origin. They found that neither epidermal thickness nor superficial dermis thickness was significantly different in African versus Caucasian subjects. The dermal-epidermal junction length in African skin was about threefold that in Caucasian skin and the junction was more convoluted in African skin. No differences were noticed as regards elastic and collagen fibre organisation (Girardeau et al., 2009). MCP-1 is a central chemokine involved in an inflammatory activation cascade and plays an important role in wound healing. The results of this study showed that African skin type fibroblasts express twice as much MCP-1 as Caucasians. Girardeau et al (2009), suggest that in an inflammatory environment, this may contribute to make black skin more prone to pathologies or disorders such as keloids or acne.

This said, other authors have studied the histology of black skin and have agreed with Szabo et al (1969) that there are few major differences other than the greater number and packaging of melanosomes (McDonald, 1988). The biology of human pigmentation will now be considered.

1.3 Biology of Human Pigmentation

1.31 Introduction

A key feature that characterises different continental ancestry groups (races) is skin colour. Skin colour varies dramatically between different continental ancestry groups from dark to light. The biological basis of this variation in human skin colour has been studied by numerous investigators over the years. Westerhof (2006) reviewed the history of human pigmentation. He found the first mention to be of a disorder of human pigmentation, most likely vitiligo, in 2200BC; however it wasn't until the 19th century when the melanocyte was discovered that the true source of human pigmentation was revealed. In the years between many imaginative theories have been proposed. 'Onesiaritus (380-305 BC), one of the writers on the expedition by Alexander the Great to India attributed black skin colour to the influence of hot water falling from the sky, whereas Aristotle (382-322 BC), perhaps based on observations of browning food associated with baking, attributed it to the burning sun and regarded heat as the cause of dark skin hue' (Westerhof, 2006).

Edwards and Duntley (1939) carried out a spectrophotometer study on the pigments of human skin using the Hardy recording spectrophotometer as a basis for the objective measurement of the colour of living human skin. Measurements were made on 20 volunteers and on samples of cadaver skin. The wave-length by wave-length analysis given by the spectrophotometer allowed an identification of the substances which give rise to skin colour. Edwards and Duntley (1939) found that skin colour is the result of

the interplay of four pigments: melanin produced by melanocytes; oxygenated haemoglobin (red) in the dermal capillaries; deoxygenated haemoglobin (bluish) in the dermal venules; and carotene (yellow) found in the dermis and in the keratinocytes of the epidermis. Edwards and Duntley (1939) also found that a further component of skin colour arises from the phenomenon of light scattering. They found that 'were it not for light scattering, which tends to raise the blue end of the reflected spectrum, the skin would be much redder than it is, since all the skin pigments have in common a preponderant absorption in the blue' (Edwards and Duntley, 1939). When the source of human skin colour is considered the main emphasis falls on melanin.

Melanin is the main source of human pigmentation and the variation seen in constitutive skin colour of individuals of different continental ancestry is primarily due to the differences in the amount of melanin within the epidermis (Quevedo et al., 1975). Edwards and Duntley's spectrophotometry studies confirm the histological observation that differences in the quantity of melanin are alone responsible for the variation in the colour of those of different ancestry (Edwards and Duntley, 1939). Melanin pigmentation of human skin is conventionally divided into two components (Quevedo et al., 1972). 'Constitutive skin colour is defined as the level of melanisation generated within the epidermis of an individual through the operation of cellular genetic programs in the absence of influences from ultraviolet light' (Quevedo and Holstein, 2006). Facultative skin colour or "tan" includes the short-lived immediate tanning and the absolute increases in melanin pigmentation or delayed tanning

reactions which are both elicited by direct exposure to ultraviolet radiation (Jimbow et al., 1976, Quevedo et al., 1972).

1.32 Origin of the Melanocyte

Melanocytes originate in the neural crest as precursor cells termed melanoblasts (Goding, 2007). The melanoblasts migrate from the dorsal portion of the closing neural tube to their final locations where they populate the basal layer of the epidermis and the hair follicles as melanocytes (Lin and Fisher, 2007, Slominski et al., 2004). The melanocyte is by definition the only cell capable of melanin synthesis (Morgan et al., 1975). Each melanocyte in the human epidermis forms a relationship with local keratinocytes, which transport and in some cases degrade the melanin received from the melanocytes. The association of the melanocyte with the keratinocytes was described by Fitzpatrick and Breathnach (1963) as the "epidermal-melanin unit" (Yaar and Gilchrest, 2004). A review by Slominski et al (2004) describes this relationship, where each single, well differentiated, melanocyte interacts with a complement of ~36 viable keratinocytes at various stages of progression to the upper cornified layer of the epidermis. In order for the melanocytes to proliferate they need to de-couple from the keratinocytes. The melanocytes then re-couple to the matrix and to keratinocytes after division to form another epidermal melanin unit (Haass et al., 2005).

1.33 Function of the Melanocyte

Melanocytes are located mainly in the epidermis, dermis and hair bulb of the mammalian skin (Hirobe, 2005). The most important and phenotypically obvious function of melanocytes is the manufacture of melanin. Melanin synthesis occurs within the melanocyte in specialised organelles termed melanosomes (Goding, 2007). Melanosomes are 'distinctive sub-cellular particles which differ from mitochondria; are unique in their localisation within the cytoplasm of melanin-forming cells; contain a specialised metabolic pathway in which tyrosine is converted to melanin; and appear to be quite inert sub-cellular particles without biological function except in the formation of melanin' (Seiji et al., 1963). The melanin containing melanosomes are transported within the melanocyte from the perinuclear region to the tips of dendrite extensions of the melanocyte surface (Busca and Ballotti, 2000, Quevedo et al., 1985). The formation of melanocyte dendrites is essential for the successful transfer of melanosomes (Scott, 2006). The process by which melanosomes are transferred from melanocytes via their dendrites to adjacent keratinocytes is poorly characterised. Once in keratinocytes the melanosomes assume a position over the 'sun-exposed' side of nuclei to form cap-like structures, shielding the nuclear material from ultraviolet radiation (UVR) (Lin and Fisher, 2007, Rees, 2003). As the keratinocytes differentiate and move upwards in the epidermis the melanosomes undergo degradation reducing the melanin to fine particles (Quevedo and Holstein, 2006). The melanin continues to exert a photo protective activity on the cells beneath by casting a shadow (Rees, 2003).

It is the varying number, size, composition and distribution of melanosomes that gives rise to the differences in human pigmentation, whereas melanocyte numbers tend to remain constant (Lin and Fisher, 2007).

Melanin Formation

Melanin is the protein-bound pigment synthesised in melanocytes (Morgan et al., 1975). Melanin is not a pure substance but composed of a mixture of pigments. There are two major types of melanin pigments: black or brown eumelanin; and yellow or red phaeomelanin (Montagna et al., 1993). These melanins are synthesised in melanosomes that contain the specific enzymes required for proper melanin production (Busca and Ballotti, 2000). The melanin pigments combine in different proportions which tend to vary with ethnicity and photo exposure (Alaluf et al., 2002). Both melanin types can be synthesised & released by the same melanocyte (Slominski et al., 2004). Although melanosomes can produce either of the melanin pigments and coexist in the same cell, they cannot within the same pathway. Melanosomes commit to either eu- or pheomelanogenesis.

Lin & Fisher (2007) describe both melanin pigments as being derived from a common tyrosinase dependent pathway, with the same precursor tyrosine. Tyrosine and tyrosinase are both found in the cytoplasm of the melanocyte (Morgan et al., 1975). The rate limiting reaction of melanogenesis is the hydroxylation of tyrosine to dopaquinone, catalysed by tyrosinase. The eumelanin and phaeomelanin pathways diverge from this point. Tyrosinase is essential in the synthesis of both melanins,

however, two other well characterised enzymes, tyrosinase-related protein 1 (TRP1) and dopachrome tautomerase (DCT), seem to more crucial in eumelanin synthesis (Busca and Ballotti, 2000).

A review by Morgan, Gilchrest and Goldwyn (1975) describes four stages in the maturation of the melanosomes. Stages I and II involve melanisation through the action of tyrosinase resulting in light coloured melanosomes. Stages III and IV continue without tyrosinase action and the melanoprotein is polymerised and darkened. Melanosomes can be transferred to keratinocytes at varying stages of this process of melanisation thereby influencing skin colour.

'The number of epidermal melanocytes in human skin vary from one region to another, but all human beings, regardless of skin colour, have approximately the same number of them in any specific area of sun-protected skin' (Quevedo et al., 1985). The different racial colours are due to differences in the size, number and distribution of melanosomes (Szabo et al., 1969), rather than the number of cutaneous melanocytes which is essentially constant in individuals of different ancestry.

In considering pigmentation, there are several things we need to consider; the amount of melanin being produced; the chemical composition determined by the relative amounts of either eumelanin or phaeomelanin; the stage of maturation of melanosomes when transferred to keratinocytes; and how the melanin is packaged into

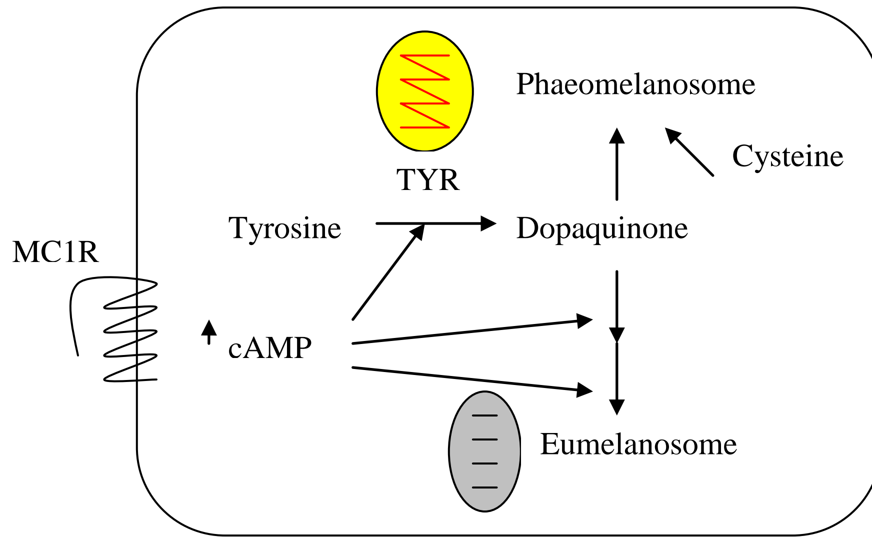
melanosomes. As melanosomes vary in shape and size this alters light scattering and also influences colour (Quevedo and Holstein, 2006).

Control of Melanin Formation

'The variation in skin pigmentation among humans is an obvious trait that is under strict genetic control' (Abdel-Malek et al., 1993). Hormones and sunlight are also important factors in the regulation of skin colour. When considering genetic factors, the melanocortin 1 receptor (MC1R) gene is the only gene that is known to explain physiological variation in human pigmentation (Rees, 2000).

MC1R encodes a seven pass transmembrane G protein coupled receptor that when bound by an agonist induces the production of cyclic adenosine monophosphate (cAMP) (Lin and Fisher, 2007, Rees, 2000). The receptor acts like a pigmentary switch. Proopiomelanocortin (POMC) peptides alpha-melanocyte stimulating hormone (α-MSH) and adrenocorticotrophic hormone (ACTH) are agonists of human MC1R, and cause an increase in production of eumelanin through elevated cAMP levels (Lin and Fisher, 2007). This involves a cascade where protein kinase A (PKA) and then cAMP response element-binding (CREB) transcription factor are activated, which results in an up regulation of microphthalmia associated transcription factor (MITF) (Busca and Ballotti, 2000). Figure 1 provides a simple illustration of the cellular pathway of melanogenesis (Barsh, 2003)

Figure 1 Cellular Pathway of Melanogenesis



MC1R – melanocortin receptor 1
TYR - Tyrosinase

'MITF binds and activates melanogenic gene promoters', for example, the tyrosinase promoter. In the case of tyrosinase, MITF stimulates tyrosinase gene expression, which in turn results in increased melanin production (Busca and Ballotti, 2000). cAMP activates another cascade leading to the phosphorylation of MITF which targets the transcription factor to proteasomes for degradation. Taken together, these complex processes would allow a fine tuning of melanocyte differentiation leading to melanin synthesis (Busca and Ballotti, 2000).

Vance and Goding (2004) describe MITF as being 'at the heart of a regulatory network of transcription factors and signalling pathways that control the survival, proliferation and differentiation of melanoblasts and melanocytes' consequently the MITF gene is essential for the generation of mature melanocytes. This however

represents knowledge to date and is likely to only be one aspect of this finely tuned process.

Loss of function mutations at the MC1R are associated with a switch from eumelanin to phaeomelanin production (Rees, 2000, Lin and Fisher, 2007). There is a great diversity at the human MC1R. A review by Rees (2003) explains how MC1R is a determinant of pigmentary phenotype regulating the relative amounts of eumelanin and phaeomelanin produced. Interestingly, the majority of red haired individuals are compound heterozygotes or homozygotes for up to five loss-of-function mutations at the MC1R (Rees, 2000), providing phenotypic evidence of a switch to phaeomelanin production.

In addition to genetic factors, the regulation by the tissue environment, especially by the keratinocytes is important. Keratinocyte-derived factors, such as α-MSH, proopiomelanocortin (POMC) and nerve growth factor, variously modulate cAMP levels in the melanocyte; increase tyrosinase gene transcription; induce melanocyte proliferation, migration and dendricity; and enhance melanocyte survival (Yaar and Gilchrest, 2004, Hirobe, 2005). Rousseau et al (2007) have shown that POMC, which can be processed to ACTH, is secreted by epidermal keratinocytes and acts through the MC1R to increase melanogenesis and dendricity in melanocytes. Schwann et al (2001) hypothesises that the effect that α-MSH will have on melanocytes is related to the amount of melanin substrate (tyrosine) available. Schwann et al (2001) found that at low concentrations of tyrosine, α-MSH stimulates proliferation of melanocytes not

pigmentation. Conversely, high levels of tyrosine prevented α-MSH induced proliferation and resulted in differentiation of melanocytes and increased melanin synthesis. Tyrosine levels also play a part in this complex melanin synthesis pathway (Schwahn et al., 2001).

Human pigmentation can also be stimulated by ultraviolet radiation. 'UV-induced skin darkening involves an increase in the melanocyte number as well as stimulation of melanin neosynthesis and melanocyte dendricity, a crucial morphological feature required for melanin transfer to keratinocytes (Busca and Ballotti, 2000). Skin phototype is a classification system based on an individual's ability to tan and susceptibility to sunburn, however the validity and reliability of skin type is still in controversy (Kawada, 2000).

1.34 Ancestral Differences in the Morphology of the Epidermal-Melanin Unit

It is easy to recognize racial colour differences at the ultrastructural level, most obviously in the host cells of the melanosomes, that is, the keratinocytes (Szabo et al., 1969). It is the variation in the quantity, packaging and distribution of epidermal melanin within keratinocytes that accounts for skin colour variation between groups of different continental ancestry (Jimbow et al., 1976).

Melanosomes in black skin are mature (stage III-IV) therefore more pigmented, more numerous, single, ovoid and membrane-bound, whereas, those in white skin are less mature (stage I-II) therefore less pigmented, smaller and bound together in groups by a

membrane (Barsh, 2003, Montagna et al., 1993). When the large melanosomes of dark skin are transferred singly to the keratinocytes they remain intact, however, the clumps of melanosomes in a light skin are disrupted by lysosomes leaving a "melanin dust" within the keratinocytes (Morgan et al., 1975). Alaluf (2002) has reported that the variation in melanosomes size is progressive with African skin having the largest melanosomes, European skin the smallest melanosomes and the melanosomes of Indian, Mexican and Chinese skin being intermediate in size (Thong et al., 2003).

1.35 The Role of Human Pigmentation

It has long been a question, why black skin is black and why white skin is white. There are several theories for the role of human pigmentation, such as photo protection and thermoregulation.

It is a physical property of black to absorb heat and of white to reflect it. Montagna (1993) has stated that black skinned people have poor physiological mechanisms for living in cold environments. Conversely, Robins (1991) has reviewed skin colour and thermoregulation and has reported that skin colour is unimportant in heat tolerance.

Daniels & Johnson (1972) support the common view that light skin represents an open melanin shutter to maximize the synthesis of vitamin D and dark skin represents a partially closed melanin shutter to protect the skin from direct ultraviolet radiation. An obvious clue to the link between melanin and photo protection resides in the prevalence of skin cancer (Robins, 1991). Skin cancer is uncommon in those of

African Continental Ancestry (Fleming et al., 1975). Fair-skinned, light haired individuals of European Continental Ancestry who burn easily and tan poorly (for example those of a Celtic background) are more susceptible to skin cancer (Robins, 1991). The ability of an individual to increase in melanin pigmentation influences the amount of damage caused by ultraviolet light. A tan is the result of ultraviolet light injury but it also protects against further injury (Morgan et al., 1975).

Kaidbey et al (1979) evaluated the photoprotective role of melanin by comparing the transmission of ultraviolet (UV) radiation through skin samples of black and white skin. They found that on average, five times as much ultraviolet light (UVB and UVB) reaches the upper dermis of white skin as reaches that of black skin. They report that the superior photoprotection of black epidermis is due not only to increased melanin content but also to other factors related to packaging and distribution of melanosomes (Kaidbey et al., 1979). Large numbers of melanosomes dispersed throughout cells of the epidermis of black skin offer considerable protection from harmful UV radiation. McDonald (1988) believes that melanin pigmentation offers no other advantages or disadvantages to black skin.

1.36 Melanocyte Biology and Wound Healing

There is a lack of discussion in the literature of the behaviour of melanocytes in human wound healing, or the processes involved in hyperpigmentation and hypopigmentation. Animal studies of melanocyte behaviour in full and partial thickness wounds in guinea pigs have shown that melanocyte migration occurs with keratinocytes, from hair

follicles to re-pigment wounds (Pepper, 1954). A review of skin pigmentation by Morgan, Gilchrest & Goldwyn (1975) reports observations of skin healing after dermabrasion and laceration also showing the migration of melanocytes from hair follicles and the healing edge of the wound into the healing skin. These melanocytes are reported to be producing melanin by the eighth to tenth day.

Melanocyte migration into human scar tissue was studied by Dressler et al (2001) in an effort to establish an objective method of estimating scar age. They report that melanocytes migrate from the immediately adjacent epidermis into the replenished epidermal defect and recolonise the epidermis. They found that 'melanocytes migrate into scar tissue as it ages and their number within the epidermal basal layers alters during the maturation of a scar'. Botella-Estrada (1999) carried out one of the few histological studies on scars. They assessed scars following excision of melanotic and non-melanotic skin tumours. They believed that the scarring process was responsible for the scar pigmentation irrespective of the tumour excised, suggesting the 'existence of an induction process of scar tissue acting on melanocytes of the overlying epidermis' (Botella-Estrada et al., 1999). Snell (1963) carried out an experiment on guinea pigs showing that melanocytes gradually migrate into the healing wound as early as day 6-8. Snell also found that the number of melanocytes in the new epithelium rose to greater than the surrounding normal skin. Melanocyte biology of wound healing requires further investigation.

Mature scars are noticeable mainly because they appear paler than the surrounding skin. Velangi & Rees (2001) asked the question ‘Why are Scars Pale?’ The original hypothesis was that paleness is due to a decrease in melanocyte number or activity within a scar. However, they believe, although their numbers are small, that this hypothesis is incorrect. They propose an alternative hypothesis that ‘a combination of both vascular and optical factors relating to dermal or epidermal characteristics are more important’ (Velangi and Rees, 2001). ‘The epidermis over a scar will always be slightly paler than the surrounding skin, since the avascular nature of the underlying fibrous tissue reduces the contribution of the blood pigments to normal skin colour’ (Snell, 1963).

Scarring in Pigmented Skin

Scar severity can be influenced by a large number of factors including age, sex, skin thickness and tension, the position of the scar on the body and patient population group. There is extensive literature on the formation of keloid and hypertrophic scars, but there are no systematic comparative studies on skin wound healing in those of African Continental Ancestry. Montagna, Prota & Kenney (1993) in a review of ‘Black Skin’ have concluded that ‘other than colour and the readiness to form hypertrophic scar and keloids in black skin, there are probably no basic differences in the process of wound healing between Whites and Blacks’.

Where differences are observed among population segments, clinical trials focused in these population groups are essential (Taylor and Wright, 2005). Despite the evidence

for differences in disease pattern or outcomes, until recently, clinical trials were generally conducted with populations homogenous with respect to age, gender, and/or ethnicity and the results were then generalised to all populations (Heiat et al., 2002). Evaluation of differences in scarring in sub-segments of the population is essential to understand the biological mechanisms of scarring and to provide therapies to reduce this. Such understanding provides the opportunity to develop strategies for the improvement of wound outcomes for everyone.

Scarring is known to be typically more severe in highly-pigmented skin. It is surprising that no in-depth investigations have been conducted into why coloured skin types scar worse than white skin types, and as a consequence the biological and molecular basis for this difference in the severity of scarring remains unclear. The processes and mechanisms underlying and regulating melanin pigmentation of the skin in mammals, including man, are well documented above and primarily result from the close interaction between epidermal melanocytes (which synthesise melanosomes containing melanin) and keratinocytes (that acquire the melanosomes secondarily and serve in their transport) (Slominski et al., 2004). Differences in skin pigmentation can occur as a result of differences in the quantity, type (phaeomelanin and eumelanin) and distribution of melanin produced by epidermal melanocytes. Scars in all ethnicities can typically appear hypo- or hyper-pigmented compared to the surrounding skin. Whilst the molecular mechanisms involved in regulating melanin production and melanocyte biology have been studied in depth with respect to ethnic variation and photoexposure, this is not the case for scarring. It is therefore currently unclear how and whether

melanocytes contribute to the scarring process, particularly with respect to scar severity and appearance, and what the molecular mechanisms underlying melanocyte biology in this process are. ‘Excessive scarring shows a clear genetic basis with racial tendencies, for example it is commoner in black and oriental people than in white people’ (Ferguson and Leigh, 1998). Additionally, the reason why some individuals scar worse than others and take longer to complete scar maturation are questions yet to be answered, as is whether the age-related effects on healing and scarring that have been documented in white skinned individuals also extend to coloured skinned individuals.

1.4 Wound Healing

1.41 Introduction

Rapid healing after injury is essential for survival, and in the majority of mammalian organs the endpoint is scar formation. It is possible that *Homo sapiens* evolved to close wounds rapidly, at the expense of perfect regeneration (Ferguson and O'Kane, 2004). In layman terms a scar is a mark left on the skin or within body tissue after the healing of a wound or burn (2005, Daniels and Johnson, 1972). Ferguson et al (1996) define cutaneous scarring as 'the macroscopic disturbance of the normal structure and function of the skin architecture, resulting from the end-product of a healed wound'.

A wound is a disruption of normal anatomical structure and more importantly, function of skin (Diegelmann and Evans, 2004). The primary function of skin is to serve as a protective barrier against the environment (Singer and Clark, 1999). Wound healing is a complex process resulting in the restoration of anatomical continuity and function. Knowledge of this process is constantly evolving. Different experimental models have been used to investigate the process of wound healing and most of our knowledge to date is derived from animal studies. Consequently, we are obliged to consider our knowledge of the process in humans as restricted. Although there are recent papers out there that are now beginning to ratify some of those animal model systems with clinical studies in the human (Occleston et al., 2008a, Bond et al., 2008b). A common theme exists in the literature, in that the process is best considered in three stages with the understanding that these various stages in wound healing overlap significantly. The

first phase is that of inflammation including haemostasis and the immune response; followed by a proliferative phase involving re-epithelialisation and neovascularisation; concluding with a phase of maturation and remodelling (Broughton et al., 2006, Ferguson and Leigh, 1998).

Timeline for Wound Healing

Wound healing, as discussed, is traditionally divided into phases. These phases overlap significantly and there is no agreement in the literature on the exact timeline for wound healing. Table 1 gives a summary of the common timelines quoted in the literature (Broughton et al., 2006, Keast and Orsted, 2009).

Table 1 Phases of Wound Healing

Phase of Healing	Days Post Injury
Haemostasis	Immediate
Inflammation	Day 1 – Day 4-6
Proliferative	Day 4 – Day 14-21
Remodelling	Day 8 – Year 1-2

1.42 Inflammatory Phase

The inflammatory response can be considered in two phases, the early phase which leads to haemostasis and the late phase involving recruitment and activity of inflammatory cells.

Haemostasis

Haemostasis is the cessation of blood loss from a damaged vessel; it occurs in minutes and heralds the start of healing. Tissue injury at wounding results in the disruption of blood vessels and extravasation of blood constituents into the surrounding tissues (Singer and Clark, 1999). The exposure of blood to extravascular tissue components, such as, adenosine diphosphate (ADP) released form injured tissue and to collagen, activates platelets and initiates both the intrinsic and extrinsic pathways of the blood coagulation cascade (Hart, 2002, Broughton et al., 2006). Blood loss is reduced by vasoconstriction and vasoconstrictors such as thromboxane A2 and prostaglandin 2-α are released from cell membranes after injury (Broughton et al., 2006). Platelets aggregate and adhere to exposed collagen. Platelets secrete factors which interact with and stimulate the intrinsic clotting cascade through production of thrombin which in turn initiates the formation of fibrin from fibrinogen (Furie and Furie, 1988). The fibrin mesh strengthens the platelet aggregate into a stable haemostatic plug (Keast and Orsted, 2009). The platelet plug not only re-establishes haemostasis it provides a provisional extracellular matrix for cell migration and wound healing ensues (Singer and Clark, 1999). Platelets, in addition to achieving haemostasis, initiate the healing response through release of important cytokines, the two most important being platelet derived growth factor (PDGF) and the transforming growth factor β family (TGF-β) (Diegelmann and Evans, 2004).

Immune Response

Inflammation is a type of non-specific immune response. Blood borne inflammatory cells, such as neutrophils, monocyte-macrophages and T-lymphocytes, migrate to the wound site in response to a variety of cell-attraction factors. Adhesion molecules are expressed by endothelial cells lining wound site capillaries, this is necessary to recruit cells from the circulating blood (Miller and Nanchahal, 2005). Inflammatory cells are responsible for wound debridement and later the orchestration of new-tissue synthesis (Hart, 2002).

Neutrophils are the first of the inflammatory leucocytes to appear in the wound and are the predominant cell type within 24 hours after injury (Hart, 2002, Diegelmann and Evans, 2004). This innate immune cell mediates the first line of defence and heralds the start of the inflammatory response of wound healing (Dovi et al., 2004). The main function of the neutrophil is thought to be the elimination of foreign material, specifically contaminating bacteria (Hart, 2002). Neutrophils are equipped with highly active anti-microbial substances and proteinases to remove dead and devitalised tissue components such as elastin, fibrin, fibronectin, collagen and proteoglycans (Hart, 2002, Eming et al., 2007). Neutrophils complete the task of cleaning up the wound within a few days of injury, recruitment then stops, and the number of neutrophils at the wound site falls.

There is debate in the literature regarding the importance of neutrophils in wound healing. Simpson and Ross (1972) examined the healing response in a neutropenic

animal model. They found that there was no discernible difference between the control and neutropenic wounds in relation to wound debridement or the extent of repair of incisional wounds under sterile conditions (Simpson and Ross, 1972). Dovi et al. (2003) also carried out a similar study in a neutropenic mouse model of normal wound healing to determine if neutrophils affect the repair process. The results were consistent with previous work and extended the findings by showing that neutrophil-depleted mice displayed significantly accelerated wound re-epithelialisation (Dovi et al., 2003). Neutrophils can provide an effective local barrier against bacterial invasion but no other role for neutrophils is supported in normal tissue repair (Simpson and Ross, 1972, Dovi et al., 2004).

As neutrophil numbers decline, another inflammatory leucocyte, the monocyte, starts to enter the wound in increasing numbers (Simpson and Ross, 1972), in response to chemoattractants such as extracellular matrix protein and TGF-β family (Singer and Clark, 1999). Neutrophils and monocytes are both derived from haematopoietic stems cells found in the bone marrow (Diegelmann et al., 1981). There are two main types of stem cells in the bone marrow, haemopoietic stem cells and mesenchymal stem cells. A review by Wu et al (2007), describes how bone marrow stem cells have the capacity to leave the bone marrow, circulate in the blood, and home to injured tissue (Wu et al., 2007). As monocytes extravasate from the circulation they become activated and differentiate into mature macrophages (Eming et al., 2007). They first appear within 48 to 96 hours post-injury and generally reach a peak around the third day post-injury (Metcalfe and Ferguson, 2005). Macrophages are not abundant at the wound site until

24 to 48 hours after the arrival of neutrophils, and cells of the adaptive immune response not until 7 days after injury (Dovi et al., 2004). An important function of macrophages in wound healing is to accelerate the regression of the inflammatory response through the elimination of neutrophils by phagocytosis (Dovi et al., 2004, Hart, 2002). Macrophages bind to specific proteins of the extracellular matrix by their integrin receptors, an action that stimulates phagocytosis of microorganisms and fragments of extracellular matrix by the macrophages, and metamorphosis into inflammatory macrophages (Singer and Clark, 1999). They are thought to play an integral role in the successful outcome of the healing process through the synthesis of numerous potent growth factors, such as, colony stimulating factor 1 (CSF-1), tumour necrosis factor α (TNF-α), transforming growth factor β (TGF-β), transforming growth factor α (TGF-α), basic fibroblast growth factor (bFGF), platelet derived growth factor (PDGF) and insulin like growth factor (IGF-1 (Eming et al., 2007). These growth factors direct the activity of inflammatory cells and of those cells responsible for new tissue synthesis, such as fibroblasts, endothelial cells and keratinocytes (Hart, 2002).

Macrophages are considered essential to healing (Dovi et al., 2004). Studies in 1975 by Leibovich and Ross established the critical role of macrophages. Macrophage depletion using antisera in a guinea pig animal model resulted in significant delay of fibroblast and collagen deposition in the wound site (Eming et al., 2007, Diegelmann et al., 1981). A review by Eming et al (2007) shows that recent studies support these early findings and that delay in wound closure is associated with a significantly

reduced infiltration of neutrophils and macrophages and defective wound repair. Macrophages play a pivotal role in the transition between inflammation and repair (Singer and Clark, 1999). Their presence marks the near end of the inflammatory phase (Diegelmann et al., 1981) and their continued presence after resolution of acute inflammation reflects their ongoing involvement in tissue synthesis and remodelling (Hart, 2002).

In acute wounds T-lymphocyte recruitment occurs after that of neutrophils and monocyte-macrophages. They appear in significant numbers around the fifth day post-injury, with peak numbers occurring about the seventh day after injury (Metcalfe and Ferguson, 2005). A review by Hart (2002) states that based on animal experiments, T-lymphocytes, while not essential for wound repair, play an important regulatory role during the process of wound healing. On the other hand a paper by Metcalfe and Ferguson (2005) reviews previous work by Chettibi and Ferguson (1999) stating that 'in contrast with polymorphonuclear leukocytes, the presence and activation of both macrophages and lymphocytes in the wound is critical to the progress of the normal healing process'.

Mast cells are an additional leucocyte subset present in the skin. These cells release a variety of pro-inflammatory mediators and cytokines that are responsible for the characteristic signs of inflammation around the wound site (Diegelmann and Evans, 2004, Eming et al., 2007). Since ancient times the defining clinical features of inflammation have been known in Latin as rubor (redness), calor (warmth), tumor

(swelling) and dolor (pain) (Tracy, 2006). These hallmarks of inflammation were first described by the Roman physician and medical writer Celsus in the 1st century A.D.

It is interesting to note that early foetal wounds heal without a scar. The most important difference between foetal and adult wound healing is a reduction in the inflammatory response and the levels of TGF-β in its three isoforms. In adult healing wounds there is a high level of TGF-β1 and TGF- β2 whereas in healing foetal wounds the predominant isoform is TGF-β3 (O'Kane, 2002). Shah et al (1995) showed that modulating the healing of incisional rat wounds with addition of anti-TGF-β1 and -β2 antibodies or addition of exogenous TGF-β3 reduced cutaneous scarring. Occleston et al (2008b) have found that scar improvement efficacy demonstrated in preclinical models has translated into humans. They have found in human volunteer clinical trials that recombinant human transforming growth factor β3 (Avotermin) administered at the time of surgery to full thickness incisions results in statistically significant improvements in scar appearance compared with placebo and/or standard care (Occleston et al., 2008b). This result supports the central role of TGF-β family as one of the most important growth factors during wound healing (Hammar, 1993).

In summary the inflammatory response to injury is a collection of vascular and cellular processes that:

- Achieve haemostasis
- Defend the body against alien substances
- Dispose of dead and dying tissue

- Generate an environment conducive to the synthesis of new tissue (Hart, 2002)

As the inflammatory response resolves the repair process begins in a wound now prepared for new tissue deposition.

1.43 Proliferative phase

Keratinocytes are the predominant cell type within the epidermis, and in the dermis, the fibroblast is the predominant cell. Endothelial cells constitute the dermal vasculature providing a network of blood vessels to support both fibroblast and keratinocyte activities (Stephens and Thomas, 2002). The proliferative phase of wound healing involves a shift from an inflammatory to a synthesis-driven granulation tissue (Werner et al., 2007), leading to a new epithelium (re-epithelialisation), dermal architecture (fibroplasia and granulation tissue formation) and vasculature (neovascularisation).

Re-epithelialisation

Re-epithelialisation is widely accepted to be one of the major processes in wound healing that ensures successful repair (Braiman-Wiksman et al., 2007). Epidermal keratinocytes near the wound edge become mobilised to migrate and proliferate over the denuded wound surface, leading to the restoration of an epithelial surface and of the barrier surface of the skin (Dovi et al., 2004). This process actually begins in hours after injury demonstrating how the phases of wound healing overlap (Singer and Clark, 1999). Wound healing studies in animal models by Braiman-Wiksman et al. (2007)

showed that keratinocytes at the wound edge begin to migrate across the wound gap. Keratinocytes are not restricted to migration over newly formed matrix, they can migrate over any wound related matrix including clot-related debris (Braiman-Wiksman et al., 2007). During normal re-epithelialisation, keratinocytes migrate along a path of least resistance, dissecting underneath the scab while remaining superficial to the underlying viable dermis and wound bed (Parks, 1999).

Initially keratinocytes migrate from the edges of the wound and from residual epithelial structures stimulated to do so by numerous factors such as altered calcium levels, loss of contact inhibition, exposure to damaged extracellular matrix, alterations in tension within the epithelium and exposure to growth factors/cytokines (Stephens and Thomas, 2002). The growth factor stimulus for epithelial proliferation and chemotaxis is epidermal growth factor (EGF) and transforming growth factor α (TGF-α) produced by activated platelets and macrophages. Interleukin-1 (IL-1) and TNF-α, inflammatory cytokines, upregulate fibroblasts to produce keratinocyte growth factor (KGF) which is also important in directing this process (Broughton et al., 2006). In order to be able to migrate across the wound keratinocyte cells undergo a number of phenotypic changes. The cellular cytoskeleton is altered, there is loss of tight desmosomal contacts with adjacent keratinocytes, loss of hemidesmosomal binding of the keratinocyte to the underlying dermis and acquisition of a migratory appearance (Stephens and Thomas, 2002).

Migrated cells alone would not be sufficient to replace the damaged epithelium hence cell proliferation is crucial to ongoing wound healing. One or two days after injury, proliferation of keratinocytes at the wound margin is stimulated by local growth factors. Migration over a provisional matrix continues until the wound space is covered completely. The mitotically active basal cells of the epidermis are involved in differentiation and stratification of the epithelium and reformation of the basement membrane to complete the re-epithelialisation process (Stephens and Thomas, 2002).

Neovascularisation

Neovascularisation is the process in which new vessels are formed as a result of vasculogenesis, angiogenesis and arteriogenesis (Stavrou, 2008).

Vasculogenesis is the process of blood vessel formation occurring de novo following recruitment of endothelial progenitor cells (EPCs) from the bone marrow which differentiate into endothelial cells (Stavrou, 2008). This process differs from angiogenesis where new blood vessels are formed from pre-existing blood vessels. Mobilisation of EPCs to the circulation is thought to be modulated by cytokine pathways and VEGF. On reaching the wound area, EPCs differentiate into mature endothelial cells and continue the process of regenerating the vascular network by forming capillaries, arterioles and venules de novo (Stavrou, 2008)

Angiogenesis, the growth of new blood vessels from pre-existing host vasculature, is a vital part of tissue repair (Brown et al., 2002). Ineffective angiogenesis will result in

impaired healing and a chronic wound (Ferguson and Leigh, 1998). A review by Hartlapp (2001) reports that newly formed blood vessels can represent over 50% of the granulation tissue mass found in early wounds. Wound repair itself stimulates a vigorous angiogenic response. In addition to hypoxia and matrix components (Cox, 2003), the main angiogenic stimulators include vascular endothelial growth factor (VEGF) and bFGF, both of which have been identified in human wounds (Nissen et al., 1998). Platelets are also a source of angiogenic factors, such as PDGF, VEGF & TGF-βs, which induce endothelial cell migration and proliferation (Stavrou, 2008). Macrophages produce TNFα, which also stimulates this process marked by endothelial cell migration and capillary formation (Broughton et al., 2006). Angiogenesis is a complex process that relies on the extracellular matrix in the wound bed. Angiogenesis occurs in parallel to fibroplasia and granulation tissue formation, commencing at day two or three post injury (Cox, 2003). In order for the migration of endothelial cells into the wound area to occur, vascular permeability, loosening of the peri-endothelial support and disruption of the basement membrane occurs under the action of proteolytic enzymes such as uPA and MMPs (Stavrou, 2008). The fragmentation of the basement membrane facilitates capillary sprouting and migration into the injured site in response to FGF, VEGF and other angiogenic factors (Tonnesen et al., 2000). As degeneration of the basement membrane is occurring the surrounding matrix of fibrin clot and immature collagen is also degraded to allow newly formed capillaries to fill the wound (Cox, 2003). Endothelial cell proliferation produces a continual supply of endothelial cells for capillary extension, with capillary sprouts branching and joining to form arcades through which blood flow begins (Tonnesen et al., 2000). By

this method, angiogenic capillary sprouts invade the wound clot and within a few days organise into a microvascular network throughout the granulation tissue. Formation of new blood vessels is necessary to supply the required oxygen, nutrients and inflammatory cells to the damaged tissue in addition to facilitating removal of debris and the deposition of granulation tissue for wound closure (Singer and Clark, 1999, Brown et al., 2002). Eventually there is growth factor down regulation as the new wound vasculature is capable of maintaining the viability of the tissue, angiogenesis subsides and is suppressed by an apoptotic pathway where blood vessels that are no longer necessary are removed (Stavrou, 2008, Ferguson and Leigh, 1998). As collagen density increases during formation of a scar the density of blood vessels decreases (Tonnesen et al., 2000). The majority of new vessels eventually regress as mature scars are known to be relatively avascular (Arnold and West, 1991).

Fibroplasia and Granulation Tissue Formation

Fibroblasts, which are of mesenchymal origin, are the predominant cell type of the proliferative phase of wound healing (Diegelmann and Evans, 2004). They migrate into the wound site from the surrounding tissue and are responsible for the synthesis, deposition and remodelling of the extracellular matrix (Singer and Clark, 1999). Ross and Benditt (1961) report they first appear at 24 hours in a healing guinea pig skin wound. The provisional matrix of the clot of fibrin, fibronectin and vitronectin provide a scaffold or conduit for initial fibroblast migration (Singer and Clark, 1999). Cell migration is stimulated by PDGF, EGF and TGF-β family plus extracellular matrix

molecules such as hyaluronic acid and fragments of damaged matrix (Stephens and Thomas, 2002).

Migration into the wound is facilitated by the induction of proteolytic activity involving matrix metalloproteinases (MMPs), tissue inhibitors of MMP (TIMPs) and the urokinase type plasminogen activator (uPA)/plasmin/plasminogen activator inhibitor pathway (Parks, 1999). These proteinases play a major role in degradation and turnover of the extracellular matrix (Stephens and Thomas, 2002).

Cell migration would be insufficient to provide enough fibroblasts to produce the nascent dermal tissues. Resident fibroblasts undergo cellular proliferation in response to growth factors PDGF, TGFβ-1, EGF and FGF resulting in fibroblasts replacing macrophages as the predominant cell type within the wound. They synthesise non-collagenous proteins, such as fibronectin and hyaluronic acid, as well as collagen type I and III (Miller and Nanchahal, 2005). Production of the new extracellular matrix (ECM) termed granulation tissue commences. The new wound connective tissue appears macroscopically as pink and granular, hence the term granulation tissue. It consists of new blood vessels, fibroblasts, inflammatory cells, endothelial cells, myofibroblasts, and the components of a new, provisional ECM. This provisional ECM differs in composition from normal ECM facilitating greater cell migration. Later this provisional matrix is replaced with an ECM that more closely resembles that found in non-injured tissue.

Fibroblasts deposit ECM molecules. ECM is composed of several different proteins providing multiple functions. Some proteins, such as elastin and collagen confer strength and resiliency to tissues. Several different matrix glycoproteins, such as laminins, fibronectin, thrombospondins, and many others, form aggregates of varying degrees of complexity, providing a substratum for cell adhesion, and are important for numerous protein–protein interactions. Proteoglycans, in which the mass of carbohydrate exceeds the mass of protein, function in tissue hydration (Parks, 1999).

Fibroblast production of collagen in response to PDGF is important to wound healing (Broughton et al., 2006). It modulates the nature of the cellular infiltrate and provides tensile strength to the mature wound. There are 19 known types of collagen. Type III is initially rapidly synthesised providing an early wound matrix and scaffold for fibroblast migration whereas type I collagen is synthesised 72 hours after injury providing long-term strength to the wound (Stephens and Thomas, 2002).

Fibroblasts are also involved in wound contraction. As granulation tissue is laid down a proportion of activated fibroblasts gradually transform into myofibroblasts, under the stimulation of TGF-β family (Quan et al., 2004) These specialised cells have morphological and biochemical properties intermediate between those of a fibroblast and a smooth muscle cell (Spyrou and Naylor, 2002). Myofibroblasts transiently express a marker of smooth muscle differentiation α-smooth muscle actin (α-SMA) (Spyrou and Naylor, 2002). They have contractile ability causing contraction of granulation tissue and pulling the wound edges together below the epithelium by a

mechanism similar to that in smooth muscle cells (Miller and Nanchahal, 2005, Desmouliere et al., 2005). 'The high contractile force generated by myofibroblasts is beneficial for physiological tissue remodelling but detrimental for tissue function when it becomes excessive such as in hypertrophic scars' (Hinz, 2007).

Once an abundant collagen matrix has been deposited in the wound, fibroblasts gradually decrease production of collagen. Homeostasis occurs with production and degradation of collagen becoming equal. Newly laid down collagen becomes cross-linked into thicker bundles, this is the formation of a scar and the remodelling phase of wound healing commences (O'Kane, 2002). Of interest, historically, we have been lead to believe that cellular proliferation ceases during this phase of wound healing. Bond et al (2008b) in a human wound healing study found persistent fibroblastic density consistent with the existence of a high turnover state similar to the proliferative phase at Month 4 during scar maturation.

1.44 Remodelling Phase

Tissue remodelling is the final process to occur following injury and also the longest, taking up to 2 years to complete (O'Kane, 2002). During this phase wounded skin regains its strength and elasticity through reorganisation of collagen and elastic fibres for final reconstruction of the dermis. As dermal remodelling occurs scar formation takes place (Braiman-Wiksman et al., 2007). Macroscopically dermal scars typically have a colour mismatch to surrounding skin, are harder to the touch than surrounding skin and have very little elasticity. Histologically, dermal architecture is altered with

thinner collagen bundles which are more densely packed than that of normal skin. Newly formed fine collagen fibres tend to align with the epidermis and have not been arranged in the ‘basket weave’ pattern of normal dermis. Elastin content is decreased (O'Kane, 2002) and the dermal-epidermal junction is flat due to the failure of rete ridge restoration (Miller and Nanchahal, 2005).

Collagen remodelling during the transition from granulation tissue to scar involves both synthesis and catabolism of collagen at a low rate (Singer and Clark, 1999). During remodelling hyaluronic acid is replaced by sulphated proteoglycans which are produced by mature scar fibroblasts contributing to improved tissue resilience (Miller and Nanchahal, 2005). Degradation of collagen and turnover of the extracellular matrix is controlled by proteolytic enzymes termed proteinases. These enzymes cleave peptide bonds in the central regions of polypeptides. The two most important groups in wound healing are serine- and metallo-proteinases. The serine proteinase family includes plasmin, leucocyte elastase and cathepsin G, which play a part in extracellular matrix remodelling and removal of damaged matrix components (Barrick et al., 1999). The matrix metalloproteinases (MMP) are a family of endopeptidases which can metabolise most of the macromolecular components of the extracellular matrix (Stephens and Thomas, 2002). They are not present normally in skin and are produced by macrophages, endothelial cells, keratinocytes and fibroblasts (Singer and Clark, 1999). They are required for successful migration of cells into the wound and remodelling of the extracellular matrix (Parks, 1999). A fine balance is necessary, activated MMPs are susceptible to inhibition by tissue inhibitors of MMPs (TIMPs)

and the balance between MMPs and TIMPs facilitates successful wound repair (Stephens and Thomas, 2002).

Remodelling and scar maturation results in dermal tissues with greater strength. Collagen remodelling with the formation of larger bundles and an increase in the number of inter-molecular cross-links maximises the scar strength but only up to 70% of that of normal skin (Singer and Clark, 1999). Brown et al (2002) describe wound healing as terminating leaving a acellular and avascular scar.

1.45 Clinical Studies of Wound Healing

There are few studies in wound healing in humans. However, Occleston et al (2008a) in the development of TGF-β3 as a scar prevention and reduction therapeutic have shown that data generated in pre-clinical models of scarring (rats) are predictive of effects in humans. Bond et al (2008b) found on reviewing the literature that histological studies have not been performed in scars over a longitudinal time course in humans and that animal studies have rarely observed the process beyond the first three months after wounding. Consequently they carried out a study of maturation of the human scar, aiming to provide a description of scar maturation until 1 year post injury. They were able to provide a macroscopic and histological description of scar maturation including a representative description and a description of those individuals referred to as either ‘poor outliers’ or ‘excellent’ outliers. The poor subset invariably contained individuals younger than 30 years of age whereas the opposite was true for the excellent subset comprising individuals older than 55 years of age. The most

surprising finding was that the rate of scar maturation varied within the study group, almost according to age. Older subjects displayed accelerated maturation and younger subjects had a retarded rate of maturation characterised by a prolonged high turnover rate (Bond et al., 2008b). Persistent fibroblastic density at Month 4, indicating a high turn-over state similar to the proliferative phase, would appear to contradict the commonly held view of wound healing, whereby cellular proliferation is thought to cease early during the granulation phase (Bond et al., 2008b, Singer and Clark, 1999). This prolonged high turn-over phase has not been shown in previous animal models of healing, and consequently there may be more about the process of human wound healing that can only be fully characterised through human wound healing studies such as this.

Another study by Bond et al (2008a) aimed to describe the natural history of scar redness in humans as this has not formally been described for humans (or animals) in the literature. Bond et al (2008a) found that scar redness fades on average at Month 7 and is influenced by wound type and position. Some individuals still had a red scar at Month 12 following wounding and this correlated with an increased size and number of scar blood vessels. The authors attribute persistent redness not to ongoing inflammation but to the numbers of blood vessels within the scar. Bond et al (2008a) propose the use of the term “rubor perseverans” rather than erythema to describe the physiologic redness of a normal scar as it matures beyond the first month, a process that does not involve inflammation.

Table 2 Summary of the Clinical and Histological findings of Bond (2009) and Bond et al (2008a, 2008b) in Studies of Maturation of the Human Scar in Subjects of European Continental Ancestry

	Clinical and Histological Findings
1.	The clinical appearance of a scar correlates with its histological appearance.
2.	Human scars improve with time as they mature but in 12 months there is no evidence of a steady state being reached.
3.	Three distinct groups, each displaying a different rate of longitudinal progression of scar maturation were identified from within the study group. Most subjects belonged to a "representative" subset but distinct "poor" and "excellent" subsets were also identified.
4.	Healthy older people produce scars of better clinical and histological quality than young people.
5.	Healthy older people (>55 years) produce scars which mature more quickly than scars of young people.
6.	Younger subjects (<30 years) displayed a prolonged high turnover state and a retarded rate of maturation.
7.	Clinical scoring of scars was influenced by scar type, position on the arm, subject age and subject BMI.
8	Clinical scoring of scars was influenced mainly by scar characteristics of scar colour and scar distortion.
9.	Scar redness faded at an average of seven months following wounding and was influenced by wound type and position.
10.	Scar mechanical properties improved with time (assessed with a Torsional Ballistometer) but did not return to those of normal skin in 12 months.
11.	Absence of any ongoing inflammatory processes in all scars at month 12 post wounding.

1.46 Alterations of Wound Healing

There can be alterations in the normal healing response (Occleston et al., 2008a). Thomas and Harding (2002) describe a clinical spectrum of human wound healing. At one end is ‘excessive healing’, with fibrosis and the deposition of excessive scar tissue, for example keloid scars. At the other end of the spectrum are wounds that fail to heal such as chronic skin ulcers (Thomas and Harding, 2002).

Excessive Healing

This occurs in fibrotic lesions where wound healing has become locked in the proliferative phase and remodelling has been reduced. Fibrosis can be defined as ‘the replacement of the normal structural elements of the tissue by distorted, non-functional and excessive accumulation of scar tissue’ for example keloid and hypertrophic scars in the skin, tendon adhesions, liver cirrhosis, scleroderma, Crohn’s disease (Diegelmann and Evans, 2004). A detailed discussion of the many conditions associated with abnormal wound healing is beyond the scope of this review. Keloid scars provide an interesting example of excessive scarring given the increased incidence in the African Continental Ancestry Group.

Keloid disease is a dermal fibroproliferative tumour of unknown aetiology, which represents an abnormal pathological response to cutaneous wound healing (Bayat et al., 2004b). Keloid scars occur exclusively in humans and represent the extreme end of the spectrum of scar formation (Bayat et al., 2005). The molecular biology is still not clear. Histopathologically, keloid is characterised by the presence of whorls and

nodules of thick, hyalinized collagen bundles or keloidal collagen with mucinous ground substance and relatively few fibroblasts (Lee et al., 2004). Clinically, keloid is characterised by a painful or pruritic raised scar that grows beyond the boundary of the original injury and does not regress with time (Bayat et al., 2004a). Keloid is a common disease in Black and Asian populations with an incidence ranging from 4.5 to 16% (Lee et al., 2004).

Failure to Heal

In conditions such as non-healing ulcers, the orderly process of wound healing is lost. The ulcerated skin is locked into a state of chronic inflammation characterised by abundant neutrophil infiltration with associated reactive oxygen species and destructive enzymes (Diegelmann and Evans, 2004) and often a local microbial environment that proves difficult to treat. Healing can only proceed when inflammation is controlled.

1.5 Scar Assessment

1.51 Introduction

There are an increasing number of subjective and objective tools available to the researcher for scar evaluation. There is no general agreement on the most appropriate tool or tools (van Zuijlen et al., 2002). No study can use all available tools, reviewed below are the scar evaluation tools used in this study.

1.52 Scar Assessment Scales

When carrying out research in scarring we need to be able to follow and quantify the progress of a scar over time. There are several commonly used scar assessment scales. The Vancouver scar scale (VSS) (Sullivan et al., 1990) is widely used in clinical practice and research, (Baryza and Baryza, 1995), however it is best used in burn scar assessment (Roques and Teot, 2007). Quinn et al (1995) were the first to report the use of a visual analogue scale (VAS) to measure the cosmetic outcomes of wounds. They found it a reproducible and valid measure of aesthetic outcome. This approach was borrowed from the realm of pain research and was logical because, like pain, aesthetics are quite subjective (Singer et al., 2007). A more recent study by Duncan et al (2006) confirms that the VAS meets the required standards of consistency, reliability, validity, and feasibility. Most importantly it has been shown to have a high level of sensitivity in the type of scar which this study will be assessing. However, although reliable it does not provide a reason why a scar is bad or good. Beausang et al (1998) devised a scale that is appropriate for assessment of a wide range of scars. It involves clinical

assessment using the Clinical (Manchester) Scar Assessment Scale (Appendix 4) and an overall assessment made on a VAS (score of '0' indicated normal skin and a score of '100' a very poor scar). This is more appropriate for the assessment of linear scars and with the VAS is more comprehensive than the VAS alone. This is the main method of clinical scar assessment in this study.

1.53 Spectrophotometry

It is difficult to find a reliable objective measure of scar pigmentation. Historically, the measurement of skin colour has been subjective e.g. black, white, olive. Now we are able to use quantitative methods in the form of reflectance spectrophotometry. Dermatologists tend to use the tristimulus systems whereby colour is represented according to one of a number of CIE indexes such as the LAB score, where colour is defined on three axes, light/dark, red/green and yellow blue (Shriver and Parra, 2000). Skin darkness is measured by the spectrophotometer and it also allows us to distinguish between the darkening caused by the reddening in inflammation and that caused by increased melanin (Alaluf et al., 2002). Conversely, Rees (2003) is cautious when considering the individual aspects of the index as representative of biological qualities, such as blood or melanin, believing instead that it is questionable whether differences in erythema and melanin can be separated as the two spectra are likely to overlap. Li-Tsang et al (2003) carried out a study to assess the validity of the spectrocolorimeter as an instrument to quantify scar pigmentation. Results showed good inter-rater reliability (intraclass correlation coefficient 0.50-0.90) and test-retest reliability (intraclass correlation coefficient 0.95-0.99) amongst three assessors when

the spectrocolorimeter was used to assess a variety of scars. This device will be used in this study as an objective measurement of scar colour.

1.55 Ballistometry

It is important to be able to assess objectively the mechanical properties of a scar; however there is no consensus on the methodology for doing so. Jemec et al (2001) carried out one of the few studies on scar mechanics assessment and found the Ballistometer to provide reproducible data on skin mechanics. The Torsional Ballistometer used in this study provides us with an objective means by which to measure scar mechanics. Ballistometry involves measurements of the skin surface after it has been struck by a known mass with a known force (Jemec et al., 2001). The Ballistometer essentially provides a short tap on the skin, registering the subsequent oscillations and describing them in terms of frequency and amplitude.

There are four parameters:

- Indentation - *Indentation* is the peak penetration depth of the probe tip beneath the skin surface level, and reflects softness.
- Alpha - *Alpha* is the rate of energy damping, and large values indicate non-elastic damping materials.
- Coefficient of restitution - The *coefficient of restitution (CoR)* is the bounce height relative to the start height, and large values indicate high elasticity of the sample.
- And area - *Area* describes the area between the bounce profile and the skin zero datum (i.e., the sum of the area under the curve described by the probe vs. the resting

level of the surface of the skin) and is therefore correlated to both softness and elasticity.

1.6 Aim & Objectives of the Project

1.61 Aim of the Project

This study aims to describe clinically and histologically the maturation of scars in male volunteers from the African Continental Ancestry Group.

1.62 Objectives of the Project

1. To describe the natural history of scarring and maturation in volunteers of African Continental Ancestry through analysis of clinical assessments.
2. To understand more about pigmentation of scars in volunteers of African Continental Ancestry with the non-invasive measures of spectrophotometry.
3. To understand more about the mechanics of scars in volunteers of African Continental Ancestry with the non-invasive measures of Ballistometry.
4. To describe the natural history of scarring and maturation in volunteers of African Continental Ancestry through descriptive histology.

2 MATERIALS AND METHODS

2.1 Study Design

2.11 Protocol

Clinical trial protocol RNO-0065 for this study (and associated patient information sheets and consent forms) was reviewed and approved by Manchester Regional Research Ethics Committee on the 20th Nov 2006 (LREC Ref: 06/Q1407/217). It describes a single site open, parallel group, uncontrolled methodology trial, to compare the process of scarring in different population groups of healthy volunteers. This thesis will consider the process of scarring in the African Continental Ancestry Population Group.

Subjects were selected for screening from a database of volunteers who had previously contacted Renovo to register their interest in participating in clinical trials. Advertisements were also posted in order to recruit 60 volunteers from the African Continental Ancestry Population Group. Volunteers had to self-ascribe themselves to this Population Ancestry Group. For completeness in data collection record was made if volunteers had any blood relatives belonging to a different Continental Ancestry Group (CAG) from their own.

All volunteers provided their written, informed consent to participate in this study, with consent obtained before any study-specific procedures were undertaken.

Volunteers were given at least 24 hours to consider the subject-information literature before attending a screening visit. All of the trial information received by volunteers was approved by Manchester Regional Research Ethics Committee.

Three incisions and a punch biopsy where carried out on each arm. The tissue from the punch biopsy was used for baseline genomics and histology and was not analysed in this study. The incision scars and punch biopsy scar were assessed clinically on a monthly basis for 12 months. At various time points scars were excised for histology providing information at each month of scar development. Following scar excision the resultant excisional scar continued to be assessed every month. One of the three incisions was not excised but assessed monthly for twelve months.

Measures to Avoid Bias

Subjects were assigned to different groups, according to when they entered the trial. An attempt was made, to include within each trial group subjects of varying ages. 10 patients were recruited to each group. An attempt was made to place 2-4 from the category 18-30 years, 2-4 from the category 31-54 years, 2-4 from the category 55-85 years in each group in order to achieve comparable age demographics.

2.2 Subject Population

2.21 Number

Sixty male subjects of African Continental Ancestry between the ages of 18-85 years who had given written informed consent were recruited to take part in this study. Volunteers had to fulfil certain inclusion/exclusion criteria.

2.22 Inclusion Criteria

1. African Continental Ancestry Group males aged 18-85 years who have given written informed consent.
2. Subjects with a body mass index within the permitted range for their height using Quetelet's index-weight (kg)/height^2 (m). The permitted index is 15-35 kg/m^2.
3. Subjects who have, clinically acceptable results for the laboratory tests specified in the trial protocol. All laboratory tests having been performed within 28 days of the subject’s first surgery date.

2.23 Exclusion Criteria

The following subjects were excluded from the trial:

1. Subjects who on direct questioning and physical examination had a personal or family history or evidence of keloidal scarring.
2. Subjects with tattoos or previous scars within 3cm of the area to be incised during the trial.

3. Subjects who had surgery in the area to be incised within one year of the first day of trial participation.
4. Subjects with a history of a bleeding disorder or who were receiving anti-coagulant or anti-platelet therapy.
5. Subjects, who on direct questioning and physical examination, had evidence of any past or present clinically significant disease that may affect the endpoints of the trial. For example: Coagulation disorders, diabetes, immuno-mediated conditions and clinically significant skin diseases or allergies.
6. Subjects with a clinically significant skin disorder that was chronic or currently active and which the Investigator considered would adversely affect the healing of the acute wounds or involved the areas to be examined in this trial.
7. Subjects with any clinically significant medical condition or history that would impair wound healing including:
    a. Significant rheumatoid arthritis.
    b. Chronic renal impairment significant for their age.
    c. Significant hepatic impairment (Liver Function Tests (LFTs) >3 times upper limit of normal).
    d. Congestive heart failure.
    e. Current active malignancy or history of malignancy in the last 5 years.
    f. Immunosuppression or chemotherapy within the last 12 months.
    g. A history of radiotherapy at the areas to be studied.
    h. Uncontrolled diabetes mellitus.

8. Subjects with a history of hypersensitivity to any of the dressings used in this trial.
9. Subjects who were taking regular, continuous, oral corticosteroid therapy.
10. Subjects undergoing investigations or changes in management for an existing medical condition.
11. Subjects with a history of drug abuse, or with a positive drug of abuse test for cocaine, amphetamines, methamphetamines, opiates or benzodiazepines during the screening period. Subjects with a positive drug of abuse test which in the opinion of the Investigator is due to a permitted prescription or over the counter medication were not excluded from the trial.
12. Subjects who, in the opinion of the Investigator, were unlikely to complete the trial for whatever reason.
13. Subjects who had any clinically significant neurological impairment or disease.
14. Subjects who had an active infection.

2.24 Withdrawal / Discontinuation Criteria

Subjects were free to leave the trial by withdrawing their consent to participate at any time for any reason. The reason for leaving the trial was documented in the Case Report Form (CRF).

2.3 Treatment of Subjects

2.31 Trial Schedule

Each volunteer was assigned to 1 of 6 specific groups (G1 to G6). Volunteers were then given a schedule for further visits based on the group assignment, including when they should re-attend for scar excision. The time points for all surgical procedures are illustrated in Table 3 below. After initial surgery at Day 0 all subjects returned at two selected time points as outlined for excision of two scars (proximal and distal incision scars) from the first then the second trial arm. Excision of the punch biopsy scars from both arms occurred at the time of last wound excision visit.

Time Points	G1	G2	G3	G4	G5	G6
Day 0	PB/I	PB/I	PB/I	PB/I	PB/I	PB/I
Mth 1	1st Exc Visit					
Mth 2		1st Exc Visit				
Mth 3			1st Exc Visit			
Mth 4				1st Exc Visit		
Mth 5					1st Exc Visit	
Mth 6						1st Exc Visit
Mth 7	2nd Exc Visit					
Mth 8		2nd Exc Visit				
Mth 9			2nd Exc Visit			
Mth 10				2nd Exc Visit		
Mth 11					2nd Exc Visit	
Mth 12						2nd Exc Visit

PB/I – Punch Biopsy and incisions made on both arms.
1st Exc Visit – Excision of proximal and distal healing incisions on 1st arm.
2nd Exc Visit – Excision of proximal and distal healing incisions on 2nd arm and excision of healing punch biopsy wound on both arms.

Table 3 Time Points for All Surgical Procedures.

2.32 Allocation to Groups

An attempt was made to include within each trial group subjects of varying ages. This proved difficult due to the lack of older volunteers.

Recruitment within each population group section was prioritized to Group 6 then Group 5 and so on in order to achieve good time management.

2.33 Concomitant Medication

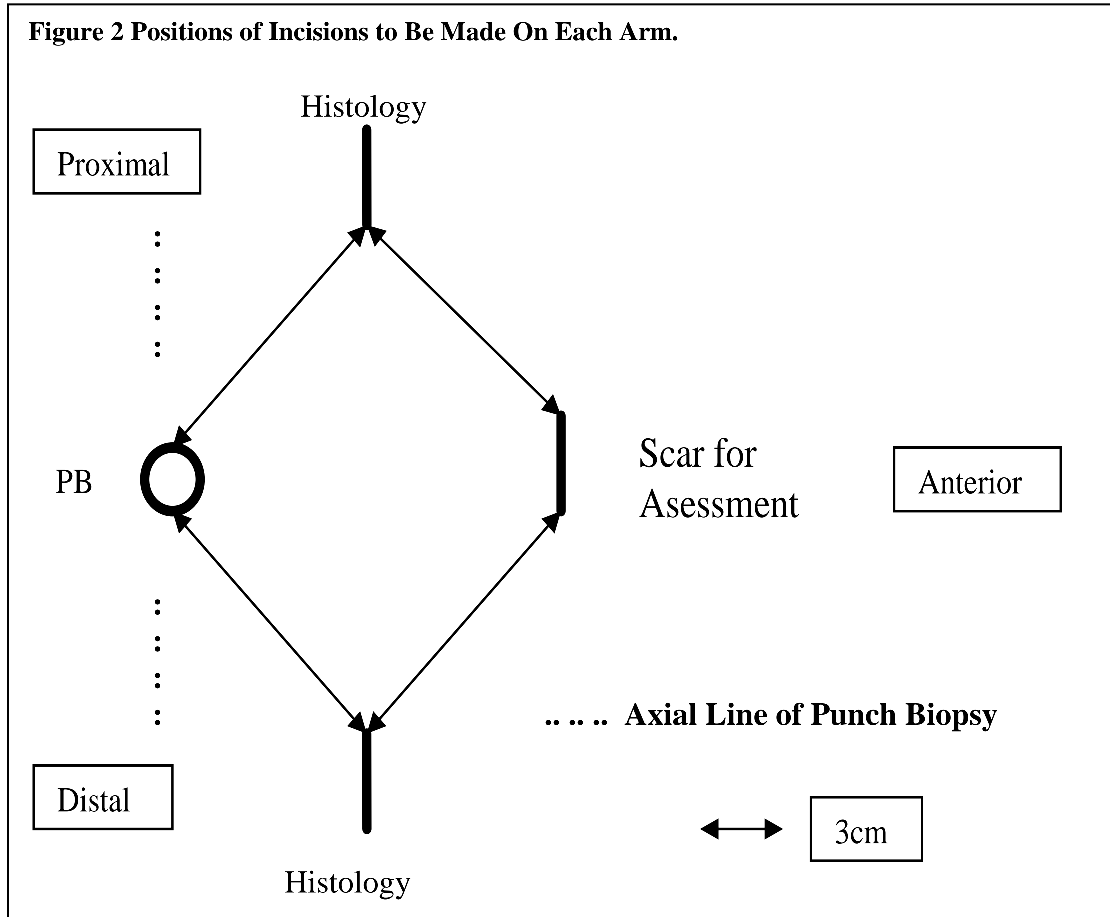
All subjects were administered 1% lignocaine with adrenaline 1:200 000 around each site before incision. All other concomitant medications taken during the trial period were recorded in the individual subject's CRF.

2.4 Clinical Procedures

2.41 Site Preparation Procedure

The sites of the four incisions on each arm were marked on the skin using a pen and template.

Figure 2 Positions of Incisions to Be Made On Each Arm.



Marks were placed on the inner aspect of the upper arm and at least 3 cm apart in a diamond position, Distal (scar to be excised for histology), Proximal (scar to be excised for histology), Anterior (scar for long term assessment) and Posterior (punch biopsy) as in Figure 2. An axial reference line was drawn through the punch biopsy

site, using the provided template. The areas for incision were anaesthetised with 1% lignocaine with adrenaline 1:200,000, by injection around the incision sites.

2.42 Site Incision, Closure and Dressing

Full thickness 1cm skin incisions and a 5mm punch biopsy were made at the marked sites on Day 0 (see Figure 2). The width of the wound from each 1cm incision was measured using sterile callipers. Each incision was closed with sterile closure strips and mastisol, and dressed with a standardised padded waterproof dressing. The posterior punch biopsy site was dressed alone. No sutures were used at this stage to ensure comparability with Trial RNO-888-1001 which provides data on scarring in European Continental Ancestry Group (Caucasian).

2.43 Excision Visits

All subjects returned at two selected time points as outlined by Table 2 for excision of two healing incisions (proximal and distal scars), from the first and then from the second trial arm, using an ellipse. Excision of the punch biopsy wounds from both arms occurred at the time of the 2nd excision visit.

1st Excision Visit

The sites of the two incisions to be excised on the 1st trial arm were marked as ellipses on the skin using a pen and template. The areas for excision were anaesthetised with 1% lignocaine with adrenaline 1:200,000, by injection

around the excision sites. As soon as the area to be excised was fully anaesthetised, the whole healing incisional wound was excised cosmetically using an ellipse. The samples were placed in collection tubes and appropriately labelled. The excised area was sutured to achieve a cosmetically acceptable result. Each wound was closed with subcuticular sutures using 5/0 Prolene. Additional sterile closure strips and mastisol were applied to the wounds and then dressed with a standardised padded waterproof dressing.

2nd Excision Visit

The sites of the two incisional scars to be excised were marked on the skin of the 2nd trial arm using a pen and template. The punch biopsy scar for excision on each arm was marked as an ellipse in the orientation considered to produce the best cosmetic result following wound closure i.e. in the orientation of scar distortion. All areas for excision were anaesthetised with 1% lignocaine with adrenaline, by injection around the excision sites. As soon as the area to be excised was fully anaesthetised, each healing wound was excised cosmetically using an ellipse. The samples were placed in collection tubes and appropriately labelled. Each wound was closed with subcuticular sutures using 5/0 Prolene. Additional sterile closure strips and mastisol were applied to the wounds and then dressed with standardised padded waterproof dressing.

2.44 Biopsy Handling for Histology

Histology specimens were placed onto a labelled specimen cassette, the lid closed and the whole cassette placed into the fixative (10% buffered formal saline) at room temperature. As soon as was practically possible the sample(s) stored in 10% buffered formal saline were transported to Renovo Research Laboratories, following fixation, the samples were transferred into 50% IDA (Industrial denatured alcohol). The samples were kept in this reagent until ready to be further processed and examined to determine measures of wound scarring and melanocyte biology.

Tissue processing is the process where the samples are dehydrated in increasing concentrations of IDA. Following dehydration, a clearing reagent (Xylene) is introduced to the samples. Xylene is miscible with IDA and also the eventual paraplast wax. Following the Xylene, paraplast wax is pumped into the samples under vacuum. The ultimate aim of tissue processing is to replace the water in the tissue with paraffin wax (providing stability to the tissue).

The samples were embedded in Paraplast wax blocks which were then cooled and cut on a microtome. Sections were taken at 5 microns thickness and mounted on Superfrost Plus charged slides. Once dried, the slides were ready for staining in the Autostainer XL. Further details of the staining protocols are found in Appendix 2. Following staining, the slides were cover slipped using pertex mountant on the CV3500 Cover slipper. Slides were viewed on LEICA DFC420 FX Microscope and photographed using Leica QWin V3 software.

2.5 Scar Assessments

2.51 Histological Scar Assessments

Specimens were fixed, wax embedded and sectioned. All specimens were stained with Masson's Trichrome and assessed by 2 trained histologists according to a modified version of the microscopic VAS of Beausang et al (1998). The specimens were scored according to the following criteria and following a standard operating procedure:

- Epidermal Restitution
- Angiogenesis and Inflammation
- Collagen Organisation
- Visual Analogue Scale Assessment of Collagen Organisation
- Other Scar Features

Epidermal Restitution

The restoration of rete ridges and epithelial thickness at the scar area was assessed by comparing these to the surrounding normal skin.

Rete ridge restoration	Epithelial Thickness
0 = normal	0 = normal
1 = some restoration	1 = thinner than normal skin
2 = no restoration	2 = thicker than normal skin

Angiogenesis and Inflammation

The number and size of blood vessels and the level of inflammation in the scar were assessed by comparing these to the surrounding normal skin.

Number of blood vessels
0 = similar to normal dermis
1 = less than normal dermis
2 = higher than normal dermis

Size of Blood Vessels
0 = similar to normal dermis
1 = less than normal dermis
2 = higher than normal dermis

Level of inflammation
0 = similar to normal dermis
1 = less than normal dermis
2 = higher than normal dermis

Collagen Organisation

The orientation, density and thickness of collagen fibres in the papillary and reticular regions of the scar were assessed compared to the surrounding normal dermis. The following scores were carried out for the papillary and reticular dermis separately.

Collagen fibre orientation
0 = normal basket weave pattern
≤ 10% abnormal
1 = 11 – 25% abnormal
2 = 26 – 50% abnormal
3 = 51 – 75% abnormal
4 = 76 - 100% abnormal
5 = Keloid-like fibre orientation

Collagen fibre density
0 = normal fibre density
≤ 10% abnormal
1 = 11 – 25% abnormal
2 = 26 – 50% abnormal
3 = 51 – 75% abnormal
4 = 76 - 100% abnormal
5 = Keloid-like fibre density

Collagen fibre thickness
0 = normal fibre thickness
≤ 10% abnormal
1 = 11 – 25% abnormal
2 = 26 – 50% abnormal
3 = 51 – 75% abnormal
4 = 76 - 100% abnormal
5 = Keloid-like fibre thickness

Visual Analogue Scale Assessment of Collagen Organisation

The overall quality of the collagen fibre organisation within the scar area compared to the surrounding normal dermis was scored on a Visual Analogue Scale.

Other Scar Features

Scar Elevation was also assessed:

Scar elevation
0 = Normal (in line with normal dermis)
1 = Raised (above normal dermis)
2 = Depressed (below normal dermis)
3 = Not interpretable

2.52 Clinical Scar Assessments

Various assessments were carried out at monthly follow-up visits (see Appendix 1).

Photography

Digital photographs were taken at each monthly follow-up visit. Photography conditions and camera settings were standardised (see Appendix 3). The images were given a unique descriptor, which correlates with the patient identification number, site number and visit number.

Investigator Scar Assessment

The following scar assessments were performed by the Investigator:

- VAS: A 100mm visual analogue scale (VAS) was used to record assessment of appearance of all scars. A score of ‘0’ indicated normal skin and a score of ‘100’ a very poor scar.

- Redness: The presence of redness in each of the scars was assessed.

- Clinical Scar Assessment Scale (see Appendix 4).

- Investigator Global Assessment Scale (see Appendix 5): The Investigator graded each scar using a categorical scar assessment scale.

Spectrophotometry

The colour of each scar was assessed using an X-rite™ SP62 Spectrophotometer with a 4mm aperture. The spectrophotometer reading was taken from the centre of each scar. The spectrophotometer measures lightness (L*) redness/greenness (a*), yellowness/blueness (b*), chroma (C*) and hue (h) in accordance with the Commission Internationale de Eclairage (CIE) international standards on colour perception. The colour of a control area of normal skin was also assessed at the same time as the scar colour assessment.

Ballistometer

The mechanical properties of each scar were assessed using a Torsional Ballistometer manufactured by Dia-Stron Limited (Andover, UK). A control area of normal skin was assessed in addition to the central portion of the scar.

2.6 Safety Assessments

2.61 Local Tolerability at Wound Site

The Investigator assessed itch, burn and pain at each site. These was assessed as none (0), or present (1) and qualified as (a) localised to the area of the incision, or (b) diffuse around the area of the incision. Only if the symptom was clinically worse than that expected for normal wounds at the specific time of assessment was it recorded as an adverse event. These assessments were carried out on Day 0 and at post-operative Day 7.

The Investigator assessed the wound and the skin around the wound, under good lighting conditions for the presence of erythema, oedema and wound exudate. Skin responses were graded according to the following scale illustrated in Table 4:

Severity	Erythema	Oedema	Exudate
None	0	0	0
Mild	2	2	2
Moderate	4	4	4
Severe	6	6	6
Very severe	8	8	8

Table 4 Local Wound Tolerability Scale

2.62 Laboratory Safety Screen

Blood and urine samples were collected for the following laboratory safety tests at screening only:

- Haematology
- Clinical Chemistry
- Drugs of Abuse
- Urinalysis

2.63 Adverse Events

An adverse event is any untoward medical occurrence in a volunteer or clinical subject involved in a clinical trial and which does not necessarily have a causal relationship with the trial related intervention (i.e. surgical incision/excision/biopsy/dressings).

All AEs occurring between Day 0 and the Month 12 visit were recorded. Adverse event severity was classified according to the following definitions:

Mild: Discomfort noticed but does not interfere with the subject's daily routines.

Moderate: Discomfort sufficient to interfere with some aspect of the subject's daily routines

Severe: Discomfort so severe it prevents subject continuing daily routines.

Deciding the relationship of an adverse event to trial related interventions is the responsibility of the investigating physician and is based on clinical judgment. The following guidelines were used, which are based on the Karch-Lasagna classification (Karch and Lasagna, 1977):

ALMOST DEFINITE

- Distinct temporal relationship with trial related intervention.
- Event cannot be explained by subject's clinical state or other factors.

PROBABLE

- Reasonable temporal relationship with trial related intervention.
- Event cannot easily be explained by subject's clinical state or other factors.

POSSIBLE

- Reasonable temporal relationship with trial related intervention.
- Event could be explained by subject's clinical state or other factors.

UNLIKELY

- Poor temporal relationship with trial related intervention and/or
- Event easily explained by subject's clinical state or other factors.

UNRELATED

- Event occurred before Day 0 or
- Event or intercurrent illness due wholly to factors other than the trial related intervention.

A Serious Adverse Event is any untoward medical occurrence that:

- Results in death.
- Is life threatening.
- Requires hospitalisation or prolongation of existing hospitalisation.
- Results in persistent or significant disability / incapacity.
- Results in a congenital anomaly / birth defect.

This was not a drug trial; however serious adverse events were recorded and reported to the regulatory affairs department at Renovo.

2.64 Medical Examination

A physical examination was conducted by the physician.

Blood pressure and pulse were measured using a digital blood pressure monitor after the subject had been supine for five minutes.

A 12-lead ECG was recorded using the QRS Diagnostic Pocket View ECG system or similar.

2.7 Other Assessments

2.71 Demographic Data

The following demographic data were recorded:

- Date of birth
- Age
- Gender
- Population Group.
- Section to record relatives that belong to a different Population Group
- Height
- Weight
- Body Mass Index
- Hand Dominance
- Fitzpatrick Skin Type (see Appendix 6)

2.8 Statistical Design

2.81 Sample Size Calculation

The trial was not formally powered but a sample size of 60 volunteers was selected. This sample size has been chosen to fall in line with previous study RNO-888-1001 which considered wound healing in The European Continental Ancestry Group.

2.82 Data Handling

All assessments and data relating to each volunteer were recorded in a CRF. These were stored in a secure storage room. Data was assimilated by the author and a data management team.

2.82 Scar Analysis

Statistical analysis for clinical data was achieved using Statistical Analysis Software (SAS). Most of the statistical information is descriptive. However, multiple regression analysis was used to assess which scar features influenced Clinical VAS scores. One-way ANOVA testing was used when considering the relationship between, VAS scores and age, and scar width and age. When analysing variance of VAS scores by Fitzpatrick Skin Types p values were calculated from least squares mean estimates.

Statistical analysis of histology data was achieved using Microsoft Excel and is mostly descriptive. In Chapter 5 ANOVA testing is used to analyse a potential relationship between the spectrophotometer data and Fitzpatrick Skin Type.

3 SCAR MATURATION – CLINICAL PERSPECTIVE

3.1 Introduction

Until recently the natural history of scar maturation in humans had not been formally described. Bond et al. (2008b) carried out an observational study of scar maturation from both a clinical and histological standpoint over a 12 month period. One limitation of this study is that it considers a male European Continental Ancestry Group (ECAG) alone. It is important that the natural history of scar maturation in humans is established for all skin types. This Chapter will present the clinical results over a study period of 12 months in relation to maturation of scars in the African Continental Population Group (ACAG).

3.2 Materials and Methods

A large amount of clinical data was collected utilising the study design outlined in Chapter 2. It was recorded in a Case Report Form (CRF) for each volunteer.

The following clinical data were collected for each scar at each monthly visit attended:

1. Investigator scar assessments
    - Visual analogue scoring and clinical scar assessment according to the Clinical Scar Assessment Scale
    - Global assessment scoring
2. Scar photographs
    - Visual analogue scoring of projected images by an Independent External Scar Assessment Panel (IESAP)
3. Spectrophotometry readings
4. Ballistometry

There were 5 investigators involved in carrying out scar assessments. Investigators were tested on the Renovo Scoring System, which is an electronic data capture system that allows for the rapid data basing of scores generated from assessment of projected images of scars by assessors. This is the system that is used in the screening of lay panel members. All investigators demonstrated consistency in both VAS and ranking methods.

For the purpose of presenting the results the data collected from the anterior scar of the dominant arm of each volunteer has been used in the analyses. This provides 12 months of clinical data. The other scars have been excised at different time points according to Table 2 in Chapter 2; consequently clinical data is not available for these scars for a full 12 month period. The anterior scar of the dominant arm is used in order to be consistent in the choice of which anterior scar data is used. This aspect of the study design also provides a scar which can be clinically assessed beyond the 12 month period.

3.3 Study Subjects - Results

3.31 Time period of the study

Sixty patients were successfully recruited between February 2006 and January 2007.

3.32 Disposition of Subjects

The disposition of subjects is provided in Table 5.

Table 5 Subject Disposition

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Overall
Enrolled	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Discontinued	4 (40%)	1 (10%)	2 (20%)	3 (30%)	2 (20%)	2 (20%)	14 (23%)
Completed	6 (60%)	9 (90%)	8 (80%)	7 (70%)	8 (80%)	8 (80%)	46 (77%)
Reason for Discontinuation:							
Adverse Event	0	0	0	0	0	0	0
Lost to Follow-up	4 (40%)	0	2 (20%)	3 (30%)	2 (20%)	2 (20%)	13 (22%)
Withdrew Consent	0	0	0	0	0	0	0
Protocol Violation	0	0	0	0	0	0	0
Other	0	1 (10%)	0	0	0	0	1 (10%)

3.33 Demographic and Other Baseline Characteristics

All subjects in the study were male and made up from the planned study population. The racial background was such that 100% of the population self-ascribed to the African CAG, with 33.3% having a close relative of alternative ancestry (28.3% were 50% European Continental Ancestry; 1.7% were 25% European Continental Ancestry;

1.7% were 12.5% European Continental Ancestry; 1.7 % were 25 % Asian Continental Ancestry. Volunteer ages ranged between 18 and 56 years with a mean age of 33 years. Mean BMI was 25.1 kg/m² and ranged between 19.5 kg/m² and 31.6 kg/m². Demographic data for all volunteers is summarised in Table 6 and group demographics are summarised in Table 7.

Demographics	**Number (%)**
Race: no. (%)	
African Continental Ancestry Group	60 (100%)
Alternate Ancestry/ Mixed Race:	
No alternate ancestry	40 (66.6%)
$^1/_8$ European CAG	1 (1.7%)
¼ European CAG	1 (1.7%)
½ European CAG	17 (28.3%)
¼ Asian CAG	1 (1.7%)
Age (years)	
Median	32
Mean	33
Range	18 – 56
Sex: no. (%)	
Male	60 (100%)
Body-mass index (kg/m²)	
Mean	25.1
Standard deviation	2.88
Range	19.5-31.6
Smoking history: no. (%)	
Non-smoker	31 (52%)
$\leq$10 cigarettes per day	15 (25%)
>10 – $\leq$15 cigarettes per day	12 (20%)
>15 – $\leq$20 cigarettes per day	2 (3%)
>20 cigarettes per day	0
Alcohol consumption: no. (%)	
$\leq$10 units per week	51 (85%)
>10 – $\leq$14 units per week	6 (10%)
>14 units per week	3 (5%)
Fitzpatrick Skin Type:	
Group VI	22 (35.7%)
Group V	24 (40%)
Group IV	14 (23.3%)

Table 6 Volunteer Demographics

Table 7 Group Demographics

Group Demographics	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Race: no. (%)						
Mixed Race	2 (20%)	3 (30%)	5 (50%)	6 (60%)	2 (20%)	2 (20%)
No Alternate Ancestry	8 (80%)	7 (70%)	5 (50%)	4 (40%)	8 (80%)	8 (80%)
Age (years)						
Median	34	30	34	30	32	36
Mean	36.2	30.7	31.6	31.5	32.5	33.6
Range	26-56	19-43	18-42	25-45	20-47	19-53
Sex: no. (%)						
Male	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Body-mass index (kg/m²)						
Mean	22.8	26.6	24.7	24.5	26.5	25.3
Smoking history: no. (%)						
Non-smoker	3 (30%)	8 (80%)	5 (50%)	3 (30%)	7 (70%)	5 (50%)
≤10 cigarettes per day	4 (40%)	2 (20%)	5 (50%)	7 (70%)	2 (20%)	4 (40%)
>10 – ≤15 cigarettes per day	2 (20%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)
>15 – ≤20 cigarettes per day	1 (10%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)	0 (0%)
>20 cigarettes per day	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Alcohol consumption: no. (%)						
≤10 units per week	8 (80%)	10 (100%)	7 (70%)	8 (80%)	8 (80%)	10 (100%)
>10 – ≤14 units per week	2 (20%)	0 (0%)	1 (10%)	1 (10%)	2 (20%)	0 (0%)
>14 units per week	0 (0%)	0 (0%)	2 (20%)	1 (10%)	0 (0%)	0 (0%)
Fitzpatrick Skin Type:						
Group VI	5 (50%)	4 (40%)	2 (20%)	1 (10%)	6 (60%)	4 (40%)
Group V	5 (50%)	2 (20%)	5 (50%)	4 (40%)	3 (30%)	5 (50%)
Group IV	0 (0%)	4 (40%)	3 (30%)	5 (50%)	1 (10%)	1 (10%)

3.4 Scar Clinical Assessments – Results

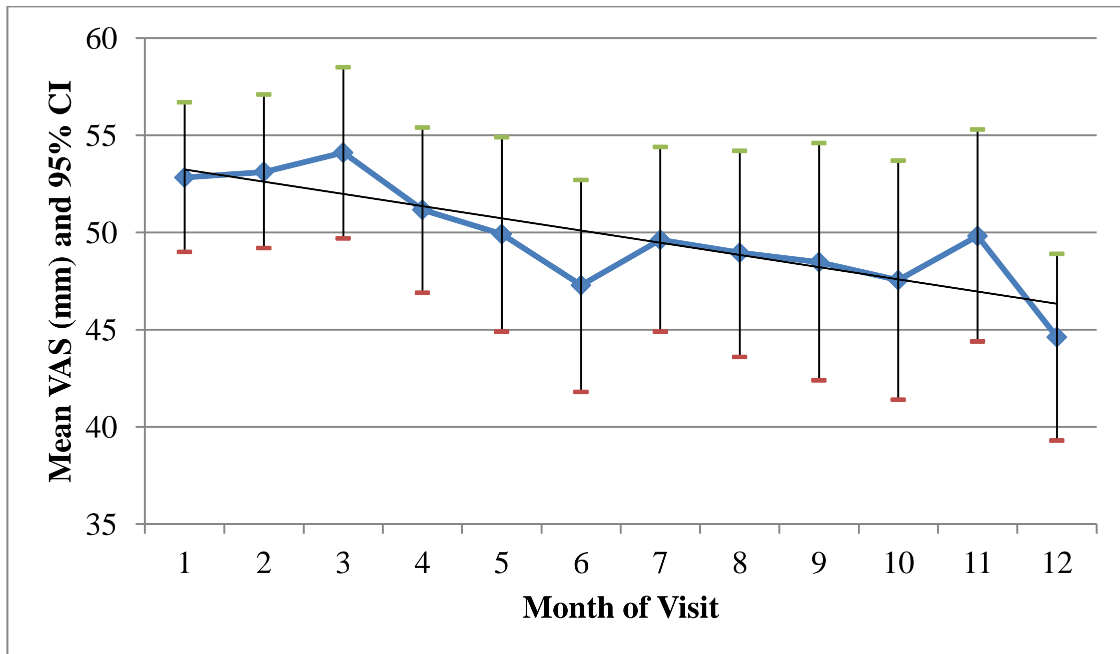
3.41 Investigator Visual Analogue Scale (VAS) Scores

The mean VAS (where 0mm = normal skin and 100mm = worst hypertrophic scar) scored by the Investigator showed a decrease over the course of 12 months indicative of an overall improvement in scar appearance with time. Table 8 shows the mean Clinical VAS for an incisional scar at each month of the study for the entire population. In the initial 3 months the mean Clinical VAS showed no improvement. However from Month 3 onwards a small improvement was seen at each month except for Month 11. Figure 3 shows no steady state was reached, with the mean Clinical VAS continuing to improve up to Month 12.

Table 8 Clinical VAS Scores by Visit

Month	N	Mean VAS (mm)	SD	Min	Median	Max	95% CI Mean
1	59	52.83	14.87	18.0	53.0	98.0	(49.0, 56.7)
2	57	53.12	15.00	16.0	55.0	79.0	(49.2, 57.1)
3	57	54.11	16.56	17.0	52.0	83.0	(49.7, 58.5)
4	54	51.17	15.67	18.0	51.0	80.0	(46.9, 55.4)
5	53	49.92	18.08	18.0	53.0	81.0	(44.9, 54.9)
6	52	47.29	19.55	8.0	49.5	84.0	(41.8, 52.7)
7	52	49.63	17.11	16.0	50.0	86.0	(44.9, 54.4)
8	51	48.96	18.48	7.0	50.0	84.0	(43.6, 54.2)
9	50	48.48	21.56	8.0	45.0	88.0	(42.4, 54.6)
10	49	47.55	21.27	8.0	52.0	78.0	(41.4, 53.7)
11	50	49.82	19.14	13.0	48.5	88.0	(44.4, 55.3)
12	53	44.62	19.14	10.0	47.0	82.0	(39.3, 48.9)

Figure 3 Mean Clinical VAS scores by visit



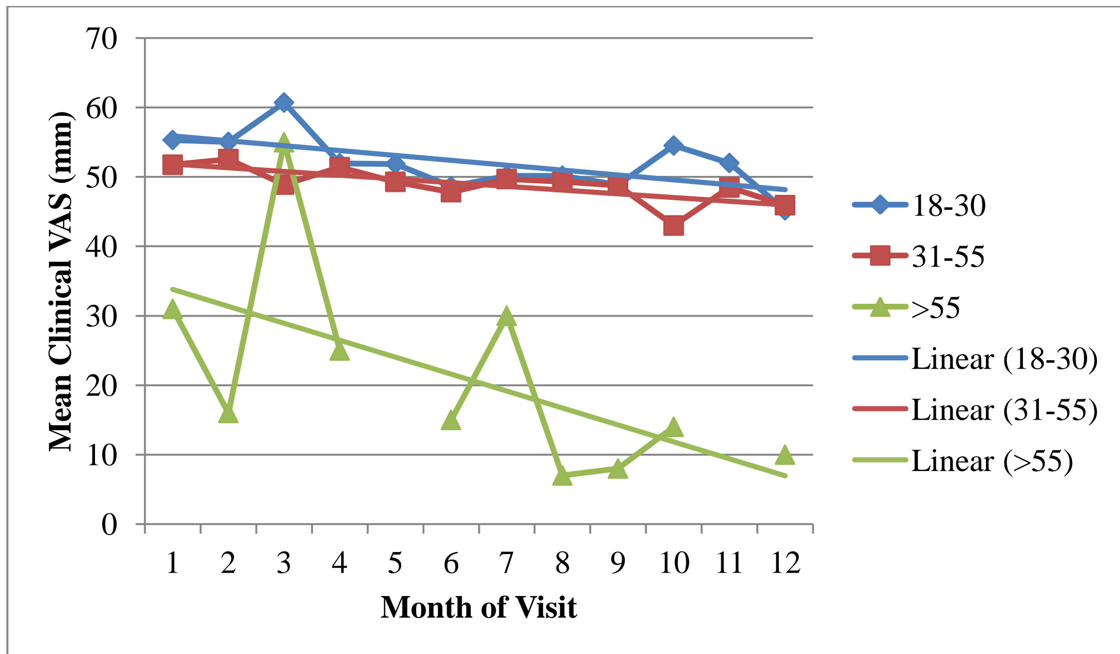
Subjects were subdivided into 3 subgroups based on their age, such that mean VAS could be assessed in a young middle-aged and older population of individuals. An assessment of the improvement in the African populations by age group showed few observable differences between subjects aged 18-30 years and 31-54 years, with the exception of Month 3 in favour of older subjects (p=0.0081) and a trend at Month 10 (p=0.0623) (Figure 4 & Table 9). No conclusions can be made regarding the eldest (55-85 years) age group due to the recruitment of a single volunteer in the African population in this group.

Table 9 Mean Clinical VAS Scores by Age and Visit

Visit	Age (years)	N	Mean	SD	95% CI mean	P-value #
Month 1	18-30	25	55.32	16.446	(48.5, 62.1)	0.3714
	31-54	32	51.75	13.467	(46.9, 56.6)	
	55-85	1	31.00			
Month 2	18-30	25	55.04	14.310	(49.1, 60.9)	0.5272
	31-54	30	52.57	14.383	(47.2, 57.9)	
	55-85	1	16.00			
Month 3	18-30	25	60.72	15.507	(54.3, 67.1)	0.0081
	31-54	30	48.90	16.162	(42.9, 54.9)	
	55-85	1	55.00			
Month 4	18-30	24	51.96	18.758	(44.0, 59.9)	0.8975
	31-54	28	51.39	12.503	(46.5, 56.2)	
	55-85	1	25.00			
Month 5	18-30	24	51.88	18.367	(44.1, 59.6)	0.6057
	31-54	28	49.29	17.529	(42.5, 56.1)	
	55-85	0				
Month 6	18-30	23	48.65	19.869	(40.1, 57.2)	0.8802
	31-54	27	47.81	19.151	(40.2, 55.4)	
	55-85	1	15.00			
Month 7	18-30	23	50.17	19.345	(41.8, 58.5)	0.9245
	31-54	27	49.70	15.546	(43.6, 55.9)	
	55-85	1	30.00			
Month 8	18-30	23	50.17	18.280	(42.3, 58.1)	0.8673
	31-54	26	49.31	17.774	(42.1, 56.5)	
	55-85	1	7.00			
Month 9	18-30	22	48.86	22.790	(38.8, 59.0)	0.9878
	31-54	26	48.77	19.623	(40.8, 56.7)	
	55-85	1	8.00			
Month 10	18-30	21	54.52	22.234	(44.4, 64.6)	0.0623
	31-54	26	43.00	19.083	(35.3, 50.7)	
	55-85	1	14.00			
Month 11	18-30	22	52.00	19.305	(43.4, 60.6)	0.5303
	31-54	27	48.48	19.439	(40.8, 56.2)	
	55-85					
Month 12	18-30	22	45.18	18.636	(36.9, 53.4)	0.8892
	31-54	29	45.93	19.131	(38.7, 53.2)	
	55-85	1	10.00			

P-value from one-way ANOVAs (equivalent to a t-test). Age group 55-85 is excluded from these analyses due to low n.

Figure 4 Clinical VAS by Age at Baseline and Visit



Mean Clinical VAS was assessed in relation to each subject's Fitzpatrick Skin Type. An analysis of Clinical VAS based on Fitzpatrick Skin Type Classification of the subjects, showed that those subjects with Fitzpatrick Skin Type IV had scars which scored consistently better at every month of assessment on the Clinical VAS in comparison to those subjects with Fitzpatrick Skin Type V and Type VI (Figure 5). This difference increases as the scars mature. At Month 12 the mean Clinical VAS for subjects with Fitzpatrick Skin Type IV was 33.5 in comparison to 48.8 and 48.0 for those with Fitzpatrick Skin Type V and VI respectively indicating the degree of difference. Those subjects with Fitzpatrick Skin Type V and Type VI consistently showed minimal difference in Clinical VAS at every month of assessment.

Figure 5 Clinical VAS by Fitzpatrick Scores and Visit

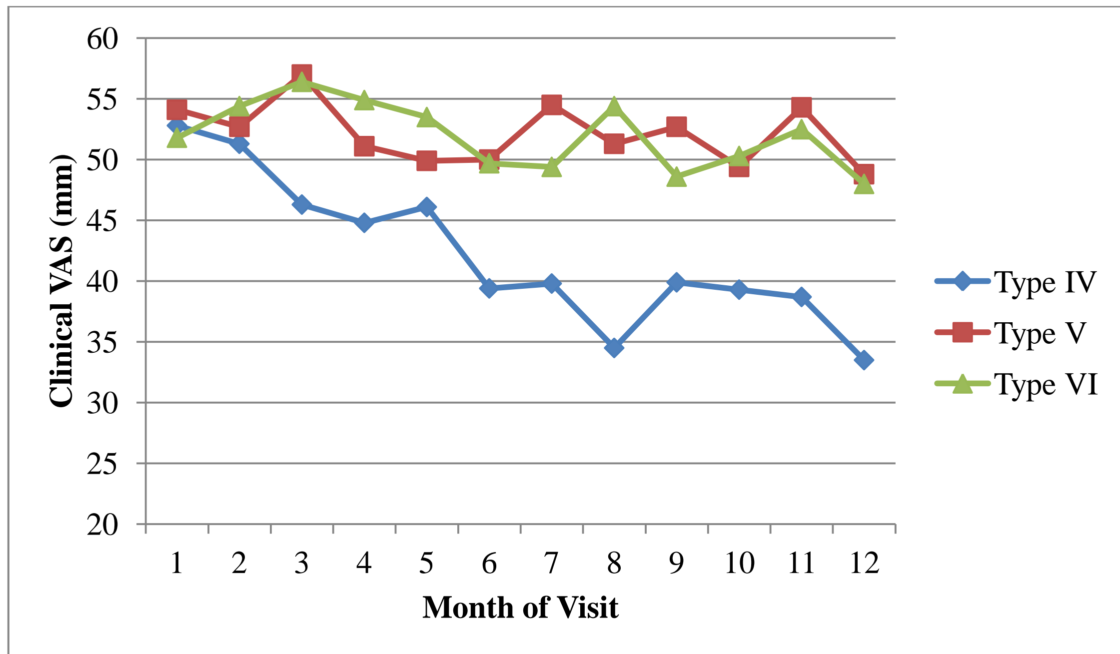


Table 10 Differences in Clinical VAS by Fitzpatrick Score and Visit

Visit	Type IV – Type V P-value	Type IV – Type VI P-value	Type V – Type VI P-value
Month 1	0.7960	0.3531	0.6152
Month 2	0.7911	0.5702	0.7235
Month 3	0.0683	0.0852	0.9164
Month 4	0.2819	0.0828	0.4399
Month 5	0.5664	0.2564	0.5250
Month 6	0.1559	0.1677	0.9615
Month 7	0.0280	0.1412	0.3374
Month 8	0.0138	0.0037	0.5805
Month 9	0.1160	0.2742	0.5685
Month 10	0.2219	0.1832	0.9082
Month 11	0.0342	0.0548	0.7702
Month 12	0.0295	0.0357	0.8869

Table 10 presents p-values calculated from least squares mean estimates from an analysis of variance of Clinical VAS scores by Fitzpatrick scores. This data shows that there is a statistically significant difference between the VAS scores of volunteers of Fitzpatrick Skin Type IV and those of volunteers with darker skin types at some time points. However, there is no statistical difference in the VAS scores for Fitzpatrick Skin Type V and VI at any time point.

3.42 IESAP (Lay) Visual Analogue Scale Scores

The IESAP (Lay) using standardised photographs was undertaken for the 12 months of the study. Images were obtained and standardised using the photographic and processing techniques outlined in Chapter 2 and Appendix 3. To assess the scar images an external panel of independent volunteers (lay people) were recruited. Assessment of these images was performed using the Renovo Scoring System, which is an electronic data capture system that allows for the rapid databasing of scores generated from assessment of projected images of scars by assessors. Panel members were fully trained in the use of this system and issued with new usernames and passwords prior to them scoring any of the images for the trial. All panel members (prior to selection for this panel) were screened and had demonstrated consistency in both VAS and ranking methods. During screening they scored 160 VAS images and 120 ranking images (selected from previous Renovo trials). The 160 VAS images consisted of 70 images shown once and a further 10 images that were shown 9 times each. The data from these repeat images were used to calculate a value for the ICC for each subject and a score of ≥ 0.81 on this test indicated a subject had scored consistently and could be

used in a VAS panel. The 120 ranking images consisted of 40 pairs of images that were each repeated 3 times. These ranking data were analysed for percentage agreement and a score of ≥ 70% indicated a subject was consistent enough to take part in a panel assessing ranking images.

A single panel comprising 5 female and 3 male volunteers was used to assess all of the scar images originating from the study. 8 sessions were required to complete scoring. In each sitting, 10% of the images that were to be scored were repeated for consistency checking. For example, if a sitting had 30 images to be scored, then 3 images from that 30 were selected as repeats giving a total of 33 images to be scored in that sitting. Consistency checks on the scores for the repeat images from the VAS sittings were performed on the combined Month 1 to Month 12 data using a t-test. A value of <0.05 was used to indicated that a panel member had failed to score consistently and that their data should be excluded from the analysis of the trial. Consequently, 1 female volunteer was excluded from the analysis due to failure of the consistency checks and 1 male volunteer was excluded as he failed to complete all scoring sessions.

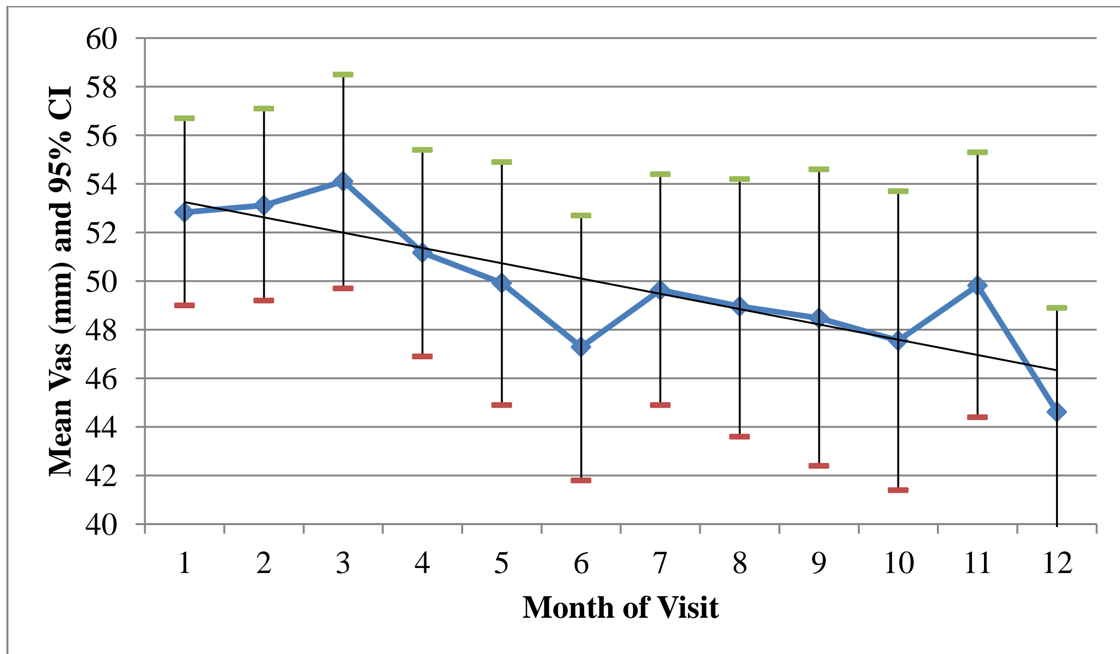
This assessment by the IESAP (lay panel), as with the investigator assessments, showed a small decrease in VAS scores indicating a small but observable improvement in scar appearance over time (Figure 6 & Table 11). In the initial 3 months the mean IESAP VAS showed no improvement which is another similar and supportive finding to that seen with the mean Clinical VAS. At Month 11 the mean lay panel VAS rose again. The lay panel scores tended to be worse than the clinical scores

of the investigators, with mean VAS scores of 52.83 and 44.62 for Month 1 and Month 12 respectively by the investigators and 59.24 and 54.87 for Month 1 and Month 12 respectively by the lay panel. However, there was a similar trend with both sets of data.

Table 11 IESAP (Lay) VAS Scores by Visit

Month	N	Mean VAS (mm)	SD	Min	Median	Max	95% CI Mean
1	58	59.24	15.68	11.7	61.9	87.2	(55.1, 63.4)
2	56	62.32	13.53	31.0	62.1	87.3	(58.7, 65.9)
3	55	58.49	17.80	16.5	61.8	87.6	(53.7, 63.3)
4	53	54.77	19.77	17.6	56.1	91.3	(49.3, 60.2)
5	52	54.39	19.73	14.5	58.8	93.9	(48.9, 59.9)
6	51	51.64	20.01	16.9	55.3	88.3	(46.0, 57.3)
7	51	51.90	19.72	14.9	53.9	90.7	(46.3, 57.4)
8	50	53.21	19.04	23.9	56.4	93.3	(47.8, 58.6)
9	49	50.38	18.34	17.6	49.8	94.7	(45.1, 55.7)
10	48	51.05	16.89	19.6	50.9	91.1	(46.2, 56.0)
11	49	56.72	17.19	23.4	56.1	88.0	(51.8, 61.7)
12	52	54.87	19.86	18.2	54.7	94.8	(49.3, 60.4)

Figure 6 Mean IESAP VAS scores by visit



Mean IESAP VAS was assessed in relation to each subject's Fitzpatrick Skin Type. An analysis of IESAP VAS based on Fitzpatrick Skin Type Classification of the subjects, showed that the subjects with Fitzpatrick Skin Type IV had scars which scored better on the IESAP VAS when compared to subjects with Fitzpatrick Skin Type V and Type VI (Figure 7). This is comparable to the finding using mean Clinical VAS. As seen with the Clinical VAS the difference between Type IV and Type V and VI increases as the scars mature. Similarly, those subjects with Fitzpatrick Skin Type V and Type VI consistently showed minimal difference in IESAP VAS at every month of assessment.

Figure 7 IESAP (Lay) VAS by Fitzpatrick Scores and Visit

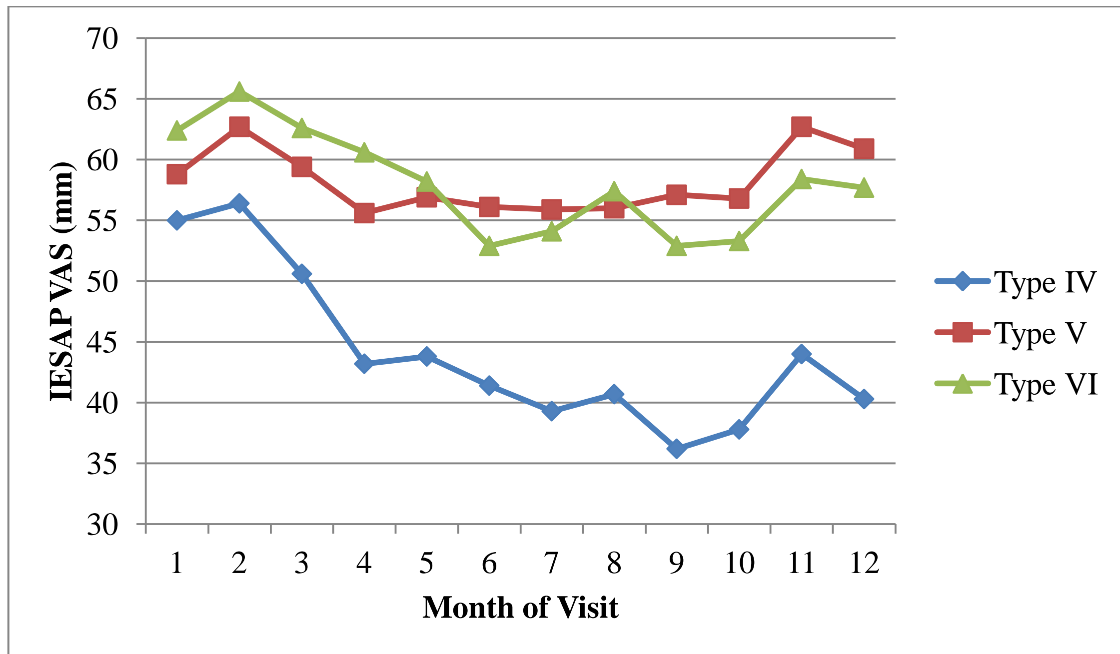


Table 12 Differences in IESAP (lay) VAS by Fitzpatrick Score and Visit

Visit	Type IV – Type V P-value	Type IV – Type VI P-value	Type V – Type VI P-value
Month 1	0.4788	0.1764	0.4599
Month 2	0.1880	0.0566	0.4713
Month 3	0.1578	0.0607	0.5587
Month 4	0.0782	0.0140	0.4014
Month 5	0.0691	0.0428	0.8329
Month 6	0.0515	0.1244	0.6091
Month 7	0.0288	0.0479	0.7668
Month 8	0.0314	0.0181	0.8071
Month 9	0.0016	0.0095	0.4438
Month 10	0.0024	0.0111	0.4890
Month 11	0.0036	0.0201	0.4118
Month 12	0.0039	0.0118	0.5896

Table 12 presents p-values calculated from least squares mean estimates from an analysis of variance of IESAP (lay) VAS scores by Fitzpatrick scores. This data shows that there is a statistically significant difference between the VAS scores of volunteers of Fitzpatrick Skin Type IV and those of volunteers with darker skin types at most time points. The p-value is consistently <0.05 from Month 7 onwards when considering Type IV against Type V and Type IV against Type VI. However, there is no statistical difference in the VAS scores between Fitzpatrick Skin Type V and VI at any time point.

3.43 Clinical Scar Assessment (Manchester) Scale

3.431 Scar Width

An assessment of scar width, as determined by the Investigator, showed there to be a trend towards an increase in the width of the scar over the course of 12 months (Figure 8 & Table 13). An analysis of scar width based on age of the subjects, as with the Clinical VAS, showed that the youngest group of subjects (i.e. 18-30 years) had the wider scars when compared to subjects aged 31-55 years (Figure 9). Table 14 shows that, with the exception of Months 7 and 12, the difference in scar width seen at each month between these two age groups is significant ($p<0.05$). The difference in scar width measurements between these two age groups is much more marked than the difference in Clinical VAS measurements. No conclusions can be made regarding the eldest (55-85 years) age group due to the recruitment of a single volunteer in the African population in this age group.

Figure 8 Mean Scar Width (mm) by Visit

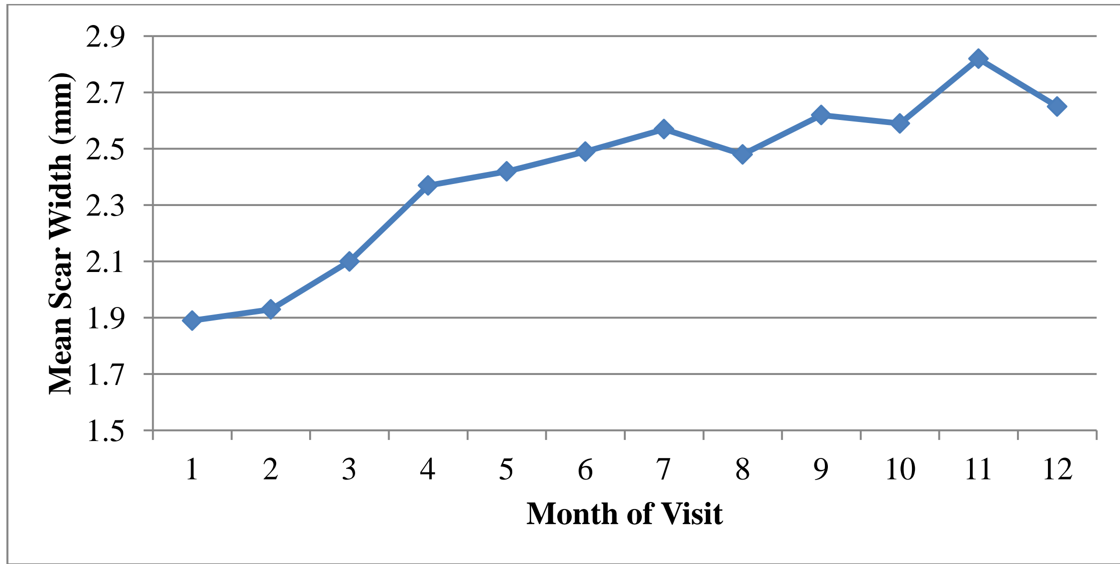


Table 13 Mean Scar Width (mm) by Visit

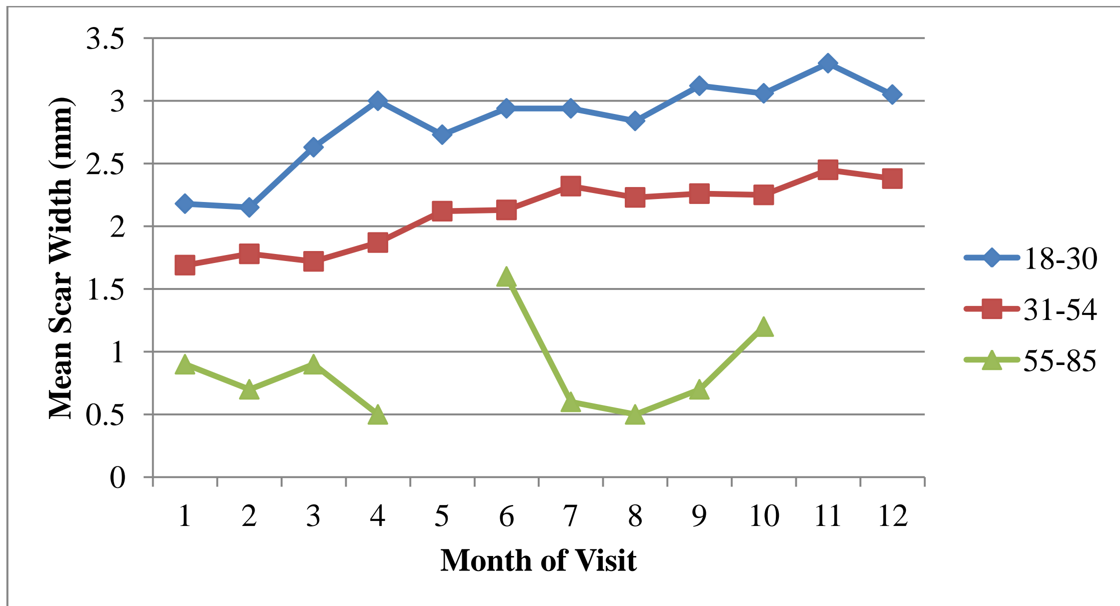
Month	N	Mean Scar Width (mm)	SD	Min	Median	Max	95% CI Mean
1	58	1.89	0.771	0.4	1.7	3.9	(1.7, 2.1)
2	56	1.92	0.690	0.5	1.9	3.7	(1.7, 2.1)
3	56	2.11	0.829	0.8	2.0	3.9	(1.9, 2.3)
4	53	2.36	1.139	0.5	2.3	6.3	(2.0, 2.7)
5	52	2.42	0.952	1.0	2.2	4.8	(2.2, 2.7)
6	51	2.49	1.049	0.5	2.3	5.4	(2.2, 2.8)
7	51	2.57	1.172	0.6	2.5	5.9	(2.2, 2.9)
8	50	2.47	1.105	0.5	2.3	5.5	(2.2, 2.8)
9	49	2.61	1.185	0.7	2.5	6.2	(2.3, 3.0)
10	48	2.59	1.102	0.8	2.4	5.4	(2.3, 2.9)
11	49	2.83	1.216	0.9	2.6	5.7	(2.5, 3.2)
12	52	2.64	1.226	0.7	2.5	6.4	(1.8, 3.3)

Table 14 Mean Scar Width (mm) by Age and Visit

Visit	Age (years)	N	Mean	SD	95% CI mean	P-value #
Month 1	18-30	25	2.18	0.879	(1.8, 2.5)	0.0169
	31-54	32	1.69	0.597	(1.5, 1.9)	
	55-85	1	0.90			
Month 2	18-30	25	2.15	0.665	(1.9, 2.4)	0.0412
	31-54	30	1.78	0.647	(1.5, 2.0)	
	55-85	1	0.7			
Month 3	18-30	25	2.63	0.784	(2.3, 3.0)	0.0000
	31-54	30	1.72	0.595	(1.5, 1.9)	
	55-85	1	0.90			
Month 4	18-30	24	3.00	1.112	(2.5, 3.5)	0.0001
	31-54	28	1.87	0.836	(1.5, 2.2)	
	55-85	1	0.50			
Month 5	18-30	24	2.73	0.991	(2.3, 3.1)	0.0262
	31-54	28	2.15	0.844	(1.8, 2.5)	
	55-85	0				
Month 6	18-30	23	2.94	1.029	(2.5, 3.4)	0.0052
	31-54	27	2.13	0.933	(1.8, 2.5)	
	55-85	1	1.60			
Month 7	18-30	23	2.94	1.246	(2.4, 3.5)	0.0560
	31-54	27	2.32	0.997	(1.9, 2.7)	
	55-85	1	0.60			
Month 8	18-30	23	2.84	1.169	(2.3, 3.3)	0.0462
	31-54	26	2.23	0.921	(1.9, 2.6)	
	55-85	1	0.50			
Month 9	18-30	22	3.12	1.216	(2.6, 3.7)	0.0095
	31-54	26	2.26	0.978	(1.9, 2.7)	
	55-85	1	0.70			
Month 10	18-30	21	3.06	1.145	(2.5, 3.6)	0.0102
	31-54	26	2.25	0.922	(1.9, 2.6)	
	55-85	1	1.20			
Month 11	18-30	22	3.30	1.311	(2.7, 3.9)	0.0131
	31-54	27	2.45	1.001	(2.1, 2.8)	
	55-85					
Month 12	18-30	22	3.05	1.305	(2.5, 3.6)	0.0507
	31-54	29	2.38	1.072	(2.0, 2.8)	
	55-85	1	0.90			

P-value from one-way ANOVAs. Age group 55-85 is excluded from these analyses due to low n.

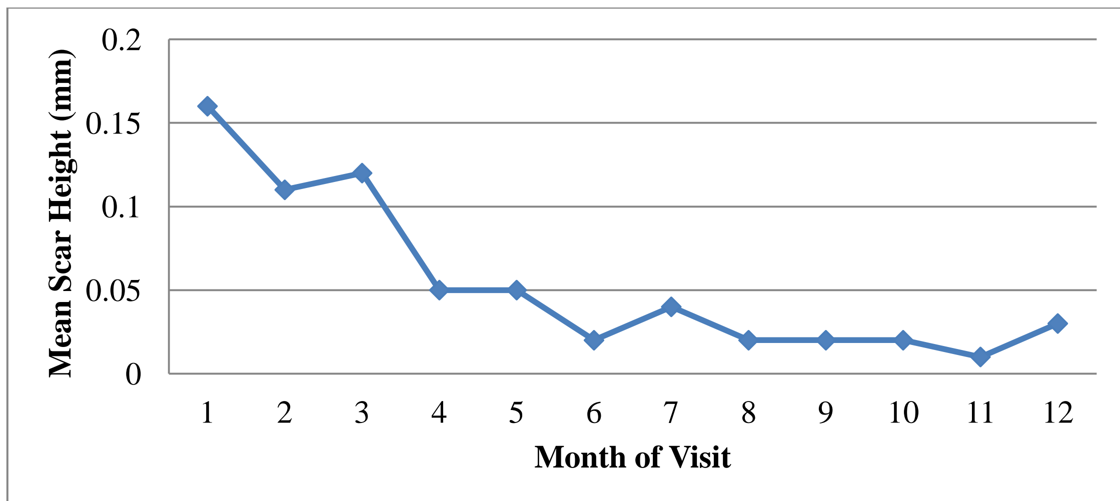
Figure 9 Comparisons of Scar Width (mm) by Age of the Population



3.432 Scar Height

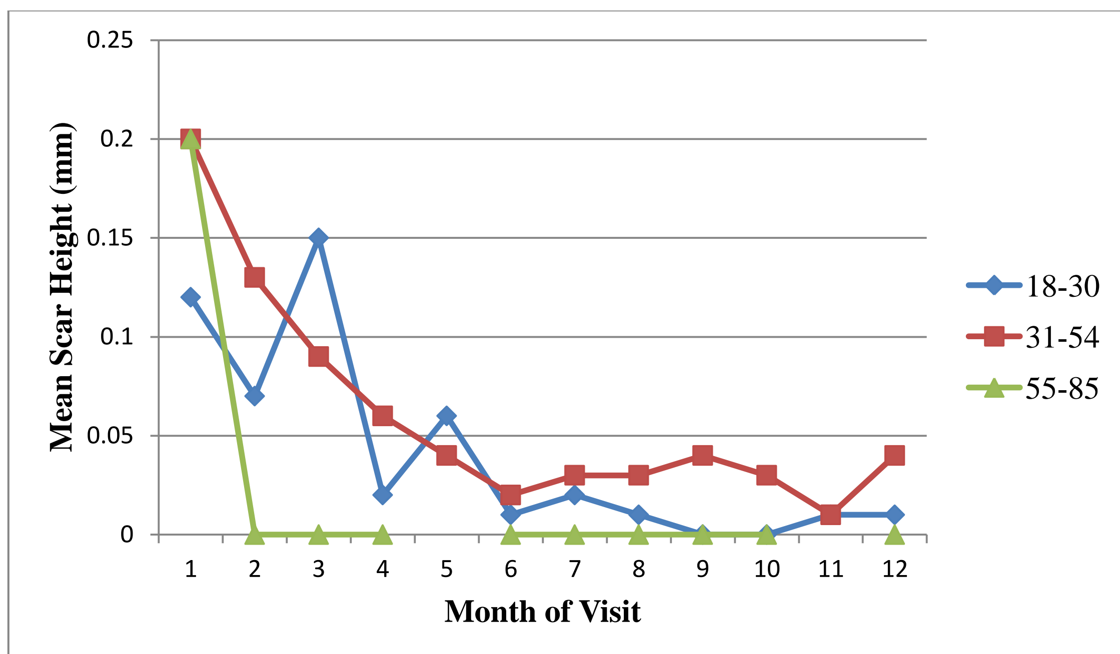
An assessment of scar height as determined by the Investigator showed there to be a trend towards a reduction in scar height over the course of 12 months, suggesting a flattening of the scar over time (Figure 10).

Figure 10 Scar Height (mm) by Visit



When scar height is considered according to the age group of the subjects (Figure 11), no clear trend is seen. In fact the youngest group of subjects, aged 18-30 years, tended to have scars which scored lower for height when compared to subjects aged 31-54 years. No conclusions can be made regarding the eldest (55-85 years) age group due to the recruitment of a single volunteer in this age group.

Figure 11 Comparisons of Scar Height (mm) by Age of the Population

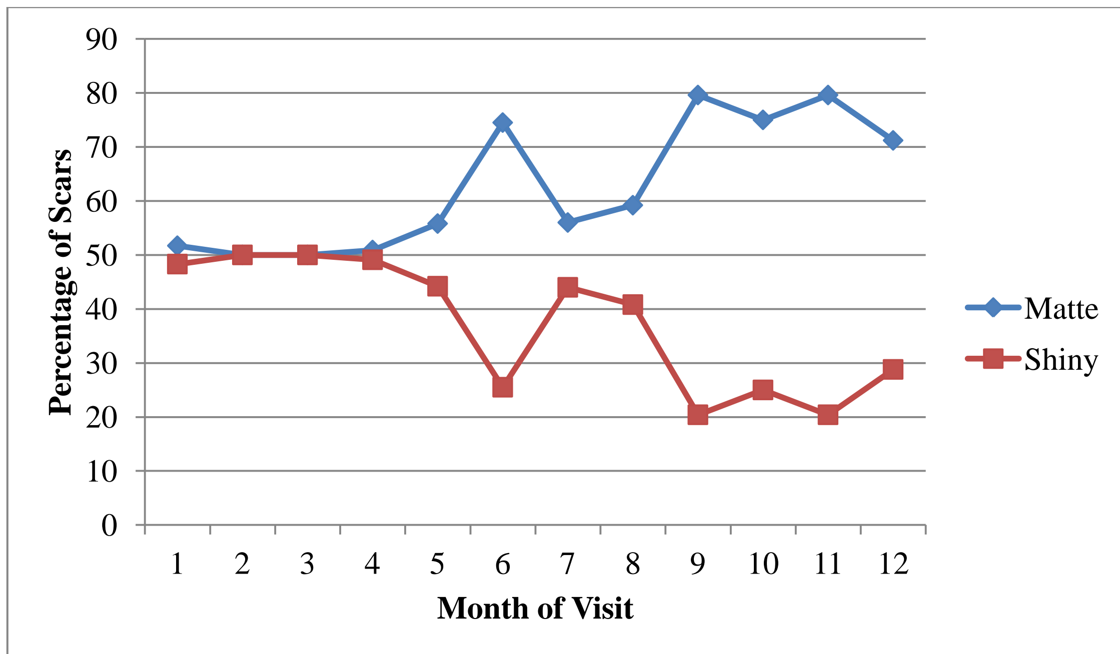


3.433 Scar Appearance

During each monthly visit, the investigator undertook an assessment of the scars and determined on a dichotomous scale, whether it was more “Matte” or “Shiny”. There was a clear trend towards an increase in the number of matte scars as time progressed (Figure 12). Since shiny scars are generally more noticeable, due to their ability to reflect light, this apparent trend agrees with the improvement in scar appearance noted

by both the clinician (VAS) and IESAP (lay) VAS. It may also influence any perceived colour mismatch making this assessment inaccurate.

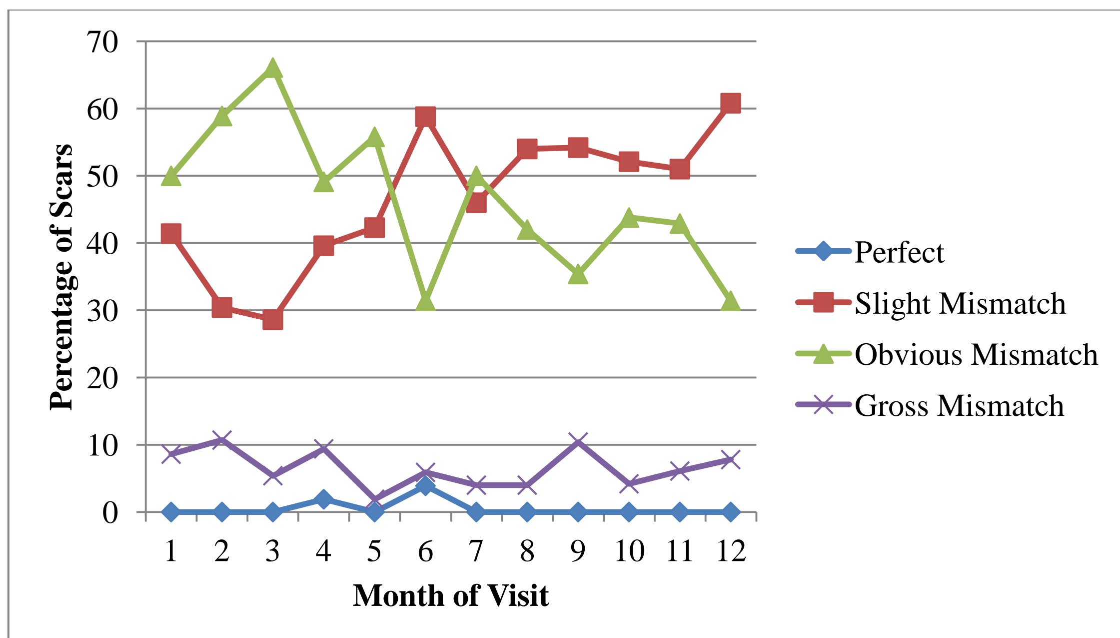
Figure 12 Percentage of Scars Rated as "Matte" or "Shiny" by the Investigator



3.434 Scar Colour

During each monthly visit, the investigator undertook an assessment of the colour of the scars in comparison to the "normal" surrounding skin, with a view to determining whether the scar matched "perfectly", whether there was a "slight mismatch", an "obvious mismatch or a "gross mismatch". In the case of a mismatch, the investigator determined whether the scar was "darker" or "lighter". By Month 12, the majority of scars showed a "slight mismatch" with the surrounding skin (Figure 13). Interestingly, up to and including Month 11, scars were generally "darker" compared to the surrounding skin. These findings are subjective and will be considered later with the objective measures of colour obtained with the use of the spectrophotometer.

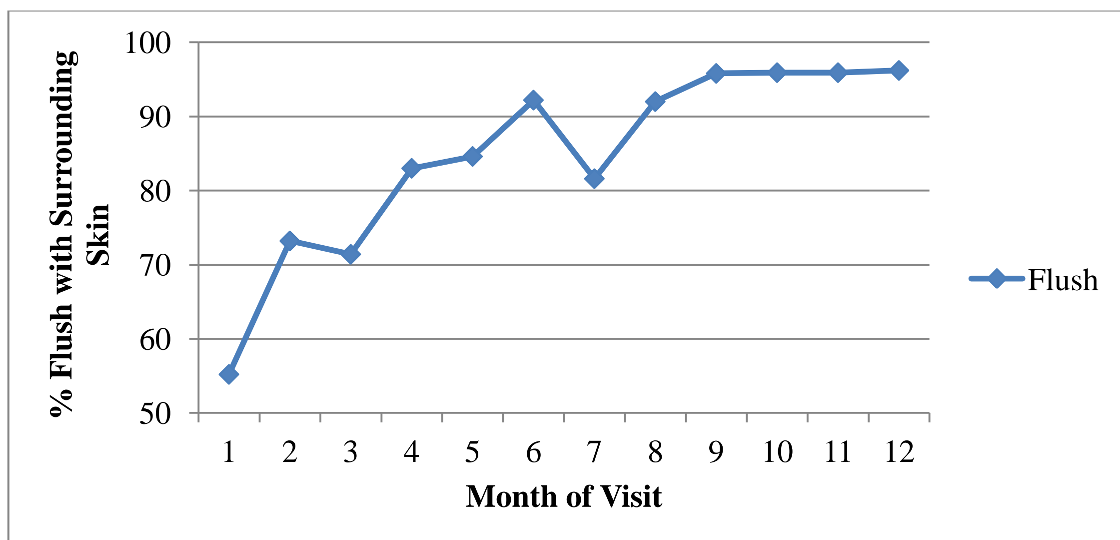
Figure 13 Colour of Scars by Visit



3.435 Scar Contour

At each assessment visit, the investigator undertook an assessment of the contour of the scar and categorised it according to whether it was "flush with the surrounding skin", "slightly proud", "hypertrophic" or "keloid". No keloid scars were observed throughout the study and a hypertrophic scar was noted in only 1 subject on 2 occasions (Month 11 and Month 12). At Month 12 over 95% of scars were rated as flush with the surrounding skin. As shown in Figure 14 a steady state has been reached. In fact the percentage of scars deemed flush with the surrounding skin remains relatively unchanged from Month 9 onwards. This finding is consistent with the measures of scar height illustrated in Figure 10, indicating that the process of scar maturation that allows a scar to become flush with surrounding skin has completed within the study period.

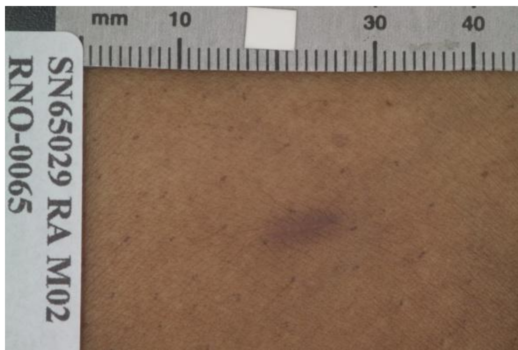
Figure 14 Percentage of Scars Rated as "Flush with the Surrounding Skin"



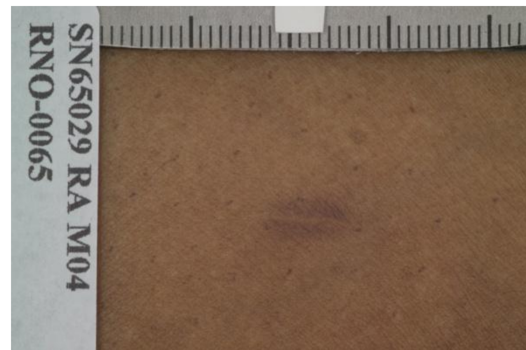
3.436 Scar Distortion

At each assessment visit, the investigator undertook an assessment of the “distortion” of the scar and categorised it as “none”, “mild”, “moderate” or “severe”. Distortion refers to any alteration in the linear shape of the scar or puckering of the surrounding skin. Several scars and the score they received for distortion are illustrated in Figure 15.

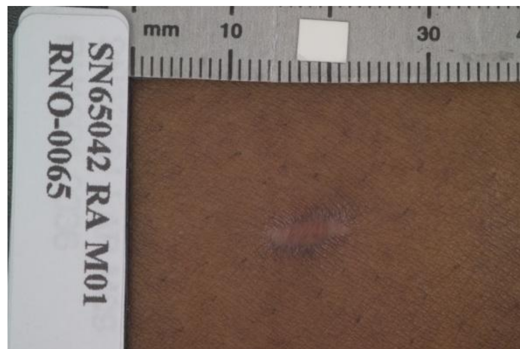
Figure 15 Photographs of Scars Illustrating "Distortion" Scores



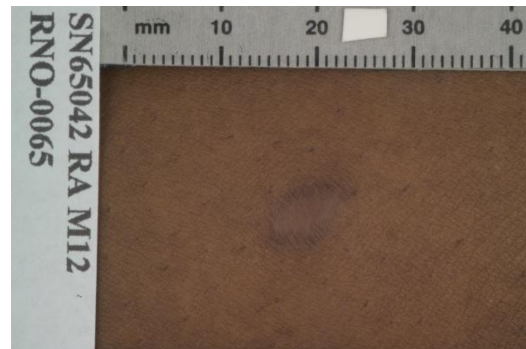
None



Mild



Moderate



Severe

Table 15 Scar "Distortion" by Visit

Month	Scar "Distortion" (%)			
	None	Mild	Moderate	Severe
1	15.8	63.2	21.1	0
2	8.9	66.1	25.0	0
3	8.9	64.3	25.0	1.8
4	9.4	41.5	41.5	7.5
5	5.9	56.9	29.4	7.8
6	16.0	42.0	34.0	8.0
7	16.0	50.0	20.0	14.0
8	14.0	48.0	28.0	10.0
9	25.0	27.1	27.1	20.8
10	22.9	35.4	29.2	12.5
11	26.5	36.7	24.5	12.2
12	30.8	36.5	25.0	7.7

At most time points the majority of scars were rated as "mild" (Table 15).

3.437 Scar Texture

Over the 12 months the investigator undertook an assessment of the "texture" of the scar and categorised it as "normal", "just palpable", "firm" or "hard. By Month 12, the majority of scars were rated as "normal" (Table 16).

Table 16 Scar "Texture" by Visit

Month	Scar "Texture" (%)			
	Normal	Just Palpable	Firm	Hard
1	3.5	66.7	28.1	1.8
2	14.3	62.5	23.2	0
3	14.3	67.9	17.9	0
4	26.4	56.6	17.0	0
5	34.6	61.5	3.8	0
6	52.9	45.1	2.0	0
7	71.4	24.5	4.1	0
8	76.0	18.0	6.0	0
9	81.6	14.3	4.1	0
10	78.7	19.1	2.1	0
11	77.1	18.8	2.1	2.1
12	78.8	19.2	1.9	0

3.438 How Scar Features Influence Clinical VAS Scores

Five scar features have been considered, scar appearance, colour, contour, distortion and texture. Table 17 presents multiple regression analysis of the data at Month 12 to identify which scar features influence the scar clinical VAS score at this time point. Colour is the most statistically relevant feature, followed by distortion. The other features were not statistically relevant, although it should be noted that the distribution across categories of 'contour' wasn't really sufficient for a proper analysis.

Table 17 Clinical VAS Scores at Month 12 vs. Scar Features - Multiple Regression Analysis

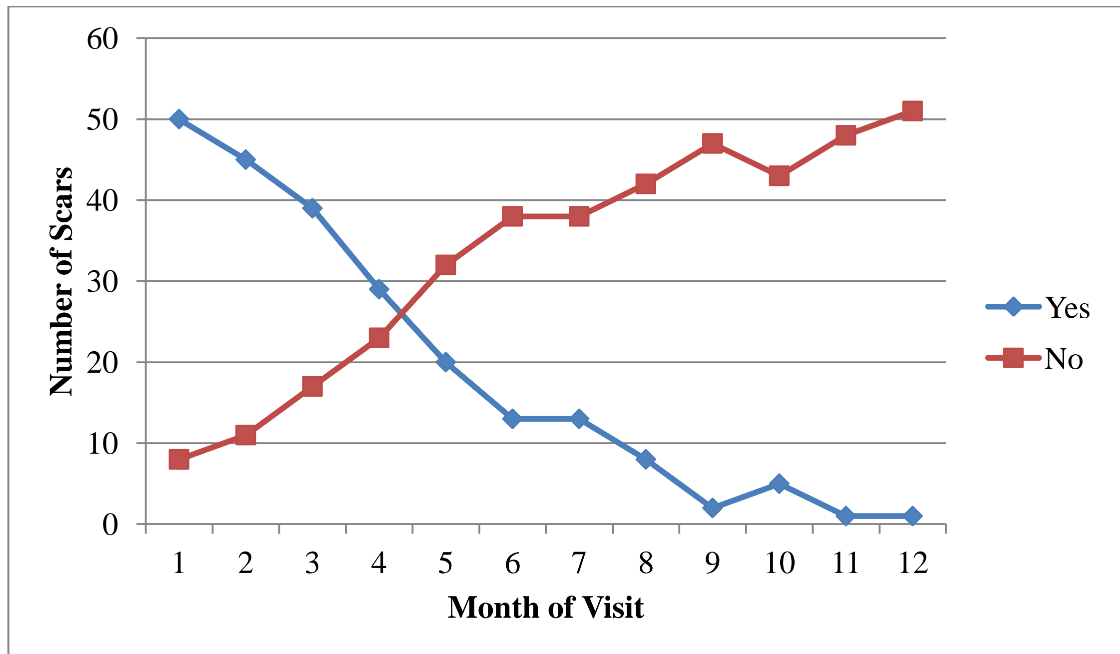
Scar Feature	Class	N	Mean	SD	P-value #
Appearance	Matte	37	42.59	18.329	0.2459
	Shiny	14	49.00	20.415	
Colour	Gross/Obvious Mismatch	20	58.75	10.047	0.0010
	Slight Mismatch	31	35.06	17.504	
Contour	Flush	49	43.33	18.602	0.5837
	Hypertrophic/ slightly proud	2	69.50	2.121	
Distortion	Moderate/Severe	17	57.53	11.875	0.0156
	None/Mild	34	37.76	18.461	
Texture	Firm/ Just Palpable	10	50.60	16.761	0.2439
	Normal	41	42.83	19.310	

p-value taken from an ANCOVA with colour, appearance, contour, distortion and texture at month 12 as covariates

3.44 Presence of Redness

At each assessment time point, the investigator undertook a subjective assessment as to the presence of redness at the scar. During the course of the clinical trial, the prevalence of redness reduced (Figure 16). By Month 9 almost all scar redness had faded. Unusually there was a slight increase at Month 10 in the number of scars reported as red. However, by Month 12 only 1 volunteer (2%) was considered to have persistent scar redness. In the darker skin types it was difficult to be accurate with this assessment. In section 3.463 spectrophotometric assessments of the scars is presented as an objective assessment method of scar redness and supports the clinical assessment carried out.

Figure 16 'Is Redness Present'?



3.45 Investigator Global Assessment Scale

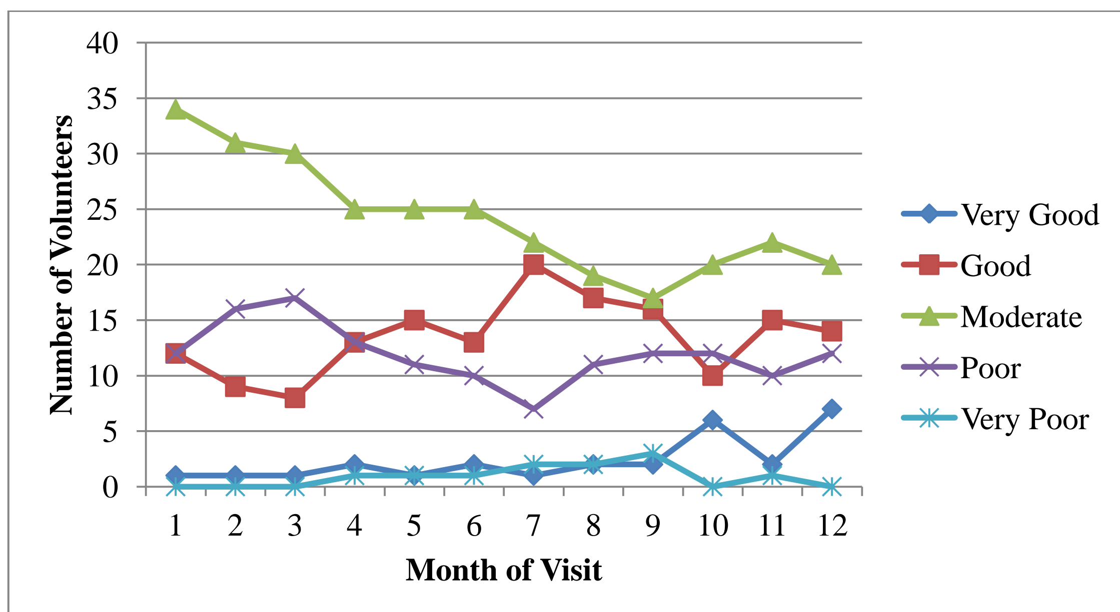
At each of the assessment visits, the Investigator undertook a subjective assessment of the scars according to a categorical scale, in which the scars were classified as "Very Good", "Good", "Moderate", "Poor" or "Very Poor". At Month 12, the majority of scars in the African population were rated as "Moderate" (Table 18). Although from Month 1 to Month 12 the numbers decreased from 58% to 38%. The number of "Very Good" scars increased from 1% to 13% from Month 1 to Month 12. No scars were rated as "Very Poor" at Month 1 or Month 12 but a few were in the interim, with 6% rated "Very Poor" at Month 9. The "Good" and "Poor" scars started at Month 1 similar at 20%, numbers then fluctuated up and down over the twelve months and ended up similar at Month 12 at 26% and 23% respectively.

Table 18 Investigator Global Assessment Scale

Month	N	Very Good	Good	Moderate	Poor	Very Poor
1	59	1 (1%)	12 (20%)	34 (58%)	12 (20%)	0
2	57	1(2%)	9 (16%)	31 (54%)	16 (28%)	0
3	57	1(2%)*	8 (14%)*	30 (54%)*	17 (30%)*	0
4	54	2 (4%)	13 (24%)	25 (46%)	13 (24%)	1 (2%)
5	54	1 (2%)*	15 (28%)*	25 (47%)*	11 (21%)*	1 (2%)*
6	52	2 (4%)*	13 (26%)*	25 (49%)*	10 (20%)*	1 (2%)*
7	52	1 (2%)	20 (39%)	22 (42%)	7 (14%)	2 (4%)
8	51	2 (4%)	17 (33%)	19 (37%)	11 (22%)	2 (4%)
9	51	2 (4%)*	16 (32%)*	17 (34%)*	12 (24%)*	3 (6%)
10	49	6 (12%)	10 (20%)	20 (41%)	12 (27%)	0
11	50	2 (4%)	15 (30%)	22 (44%)	10 (20%)	1 (2%)
12	53	7 (13%)	14 (26%)	20 (38%)	12 (23%)	0

***Percentages calculated excluding missing data point(s)**

Figure 17 Investigator Global Assessment Scale by Month



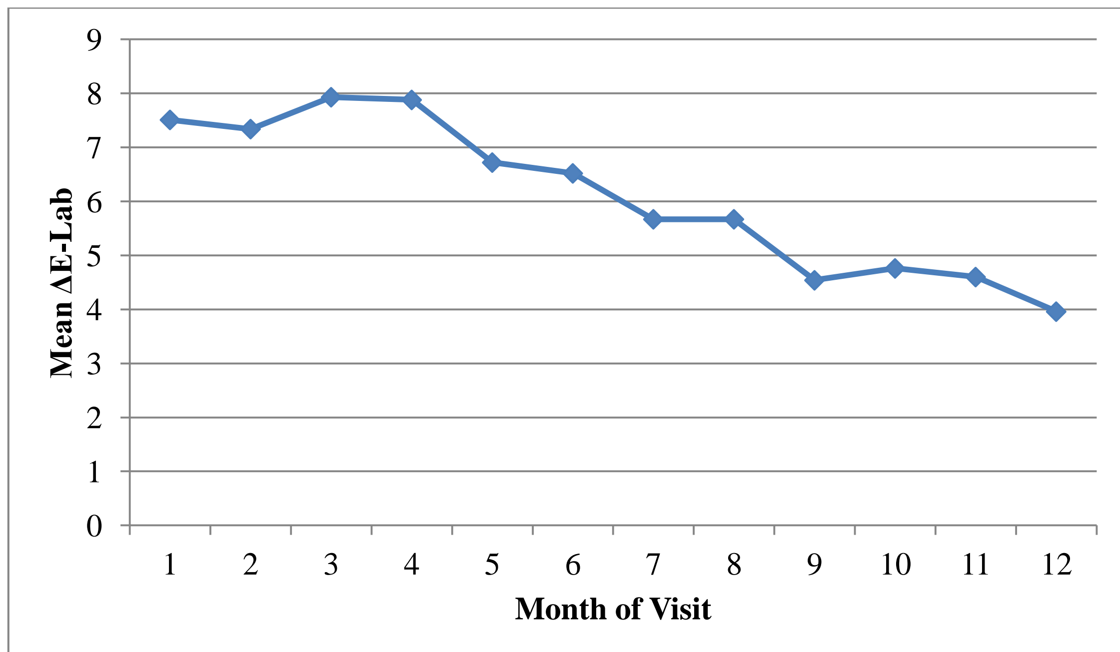
3.46 Spectrophotometry

A spectrophotometer was used at each of the assessment time points to determine the colour of both the scar and the surrounding skin. The difference between the measurements of the scar and normal skin were used to calculate four measurements; ΔE-Lab (the overall colour difference); Δl (the difference in lightness and darkness); Δa (the difference in the red/green axis); Δb (the difference in the yellow/blue axis).

3.461 ΔE-Lab

Over the course of the clinical study, it was noted that the ΔE-Lab calculated from the spectrophotometry data reduced, consistent with a better overall blending of the scar with the surrounding skin (Figure 18).

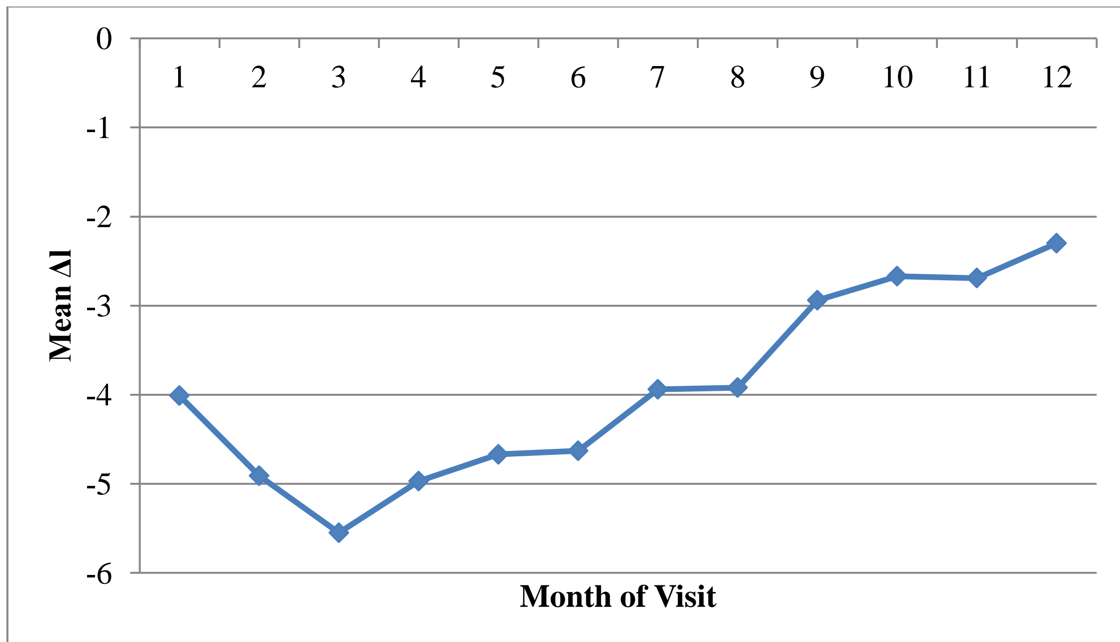
Figure 18 Changes in the Mean ΔEab



3.462 Δl

Δl measures the difference in darkness / lightness between the scar and the surrounding skin; the more negative a value, the lighter the scar is compared to the surrounding skin; the more positive a number, the darker the scar is compared to the surrounding skin. In the first three months the mean Δl value became more negative indicating a scar lighter than normal skin. From Month 3 onward the mean Δl value steadily increased. As shown in Figure 19, the scars starting from Month 3 become less light with time and begin to normalise with the surrounding skin. The mean Δl value does not return to zero during the period of the study and does not reach a steady state indicating that the maturation process is still ongoing at Month 12 as regards pigmentation changes.

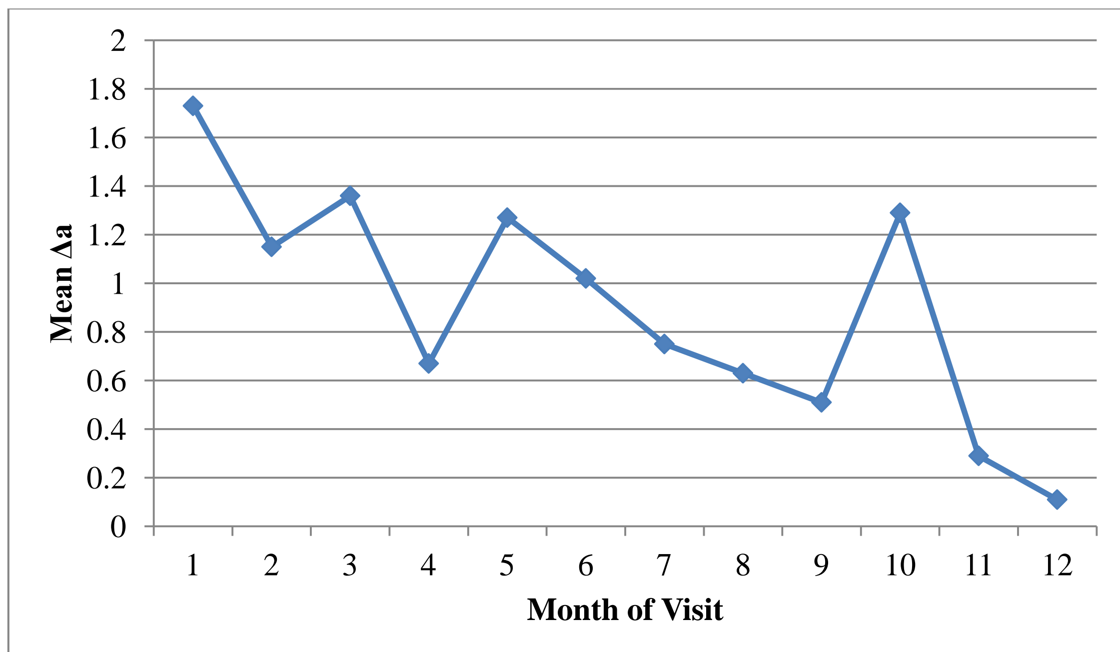
Figure 19 Changes in the Mean Δl



3.463 Δa

Δa measures the difference in the red/green axis between the scar and the surrounding skin. In this case, a negative value means the scar is less red than the surrounding skin, whereas a positive value indicates a scar which is redder than the surrounding skin. Consistent with the subjective assessment of redness (Figure 16), the Δa value derived from the spectrophotometry was shown to reduce over time, indicating that the scar became less red with time; normalising the appearance of the scar compared to the surrounding skin (Figure 20). A more detailed look at the values shows a peak is seen at Month 10. This finding is also consistent with the subjective assessment of redness (Figure 16), as is the sharp decrease that follows from Month 10 to Month 12 with a mean Δa of 0.11 at the end of the study period.

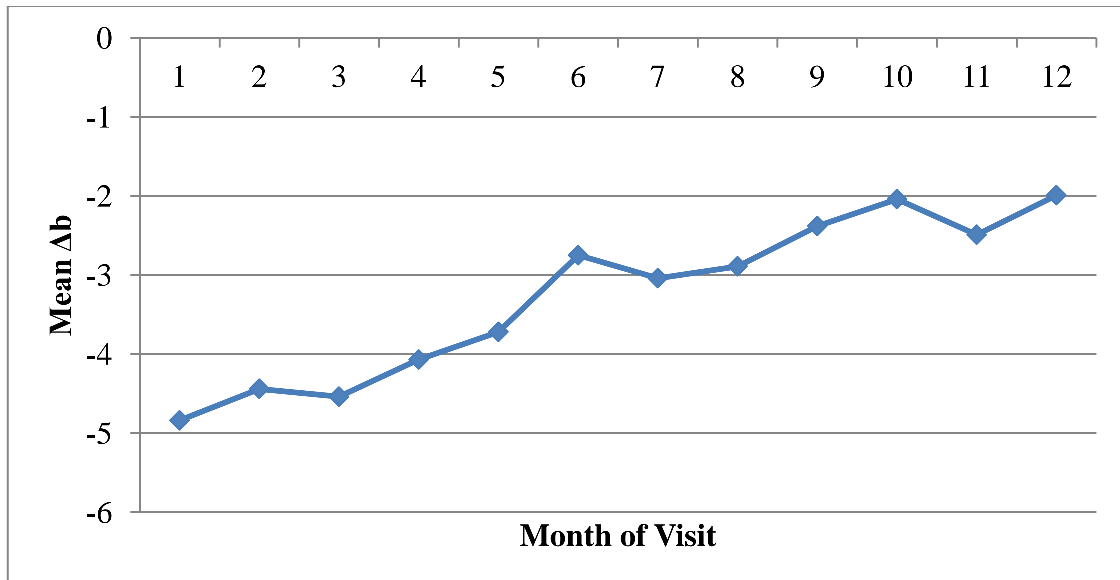
Figure 20 Changes in the Mean Δa



3.464 Δb

Δb measures the difference in the yellow/blue axis between the scar and the surrounding skin. In this case, a negative value indicates a scar that is less yellow than the surrounding skin, whereas a positive value indicates a scar which is more yellow than the surrounding skin. Over the course of the study, it was observed that there was an increase from a negative number (i.e. less yellow scar), consistent with the scar colour normalising with the surrounding skin (Figure 21). This is seen as a steady increase over the twelve month study period. It continues to improve up to Month 12 with no steady state reached indicating that this particular aspect of scar maturation in relation to scar colour is still ongoing at Month 12.

Figure 21 Changes in the Mean Δb



3.47 Ballistometer

From Month 3 onwards, Ballistometry [Dia-Stron Torsional Ballistometer (Dia-Stron Limited, Andover, UK)] was used to determine the elasticity of the scar compared to the surrounding skin. As discussed in Chapter 1 Ballistometry involves measurements of the skin surface after it has been struck by a known mass with a known force (Jemec et al., 2001). The Ballistometer essentially provides a short tap on the skin, registering the subsequent oscillations and describing them in terms of frequency and amplitude.

A range of measures was collected. There are four parameters:

- Indentation (mm) –the peak indentation depth of the probe tip below the skin surface level. Softer skin will have a larger indentation.
- Alpha – This measures the rate of energy damping. A lower value shows more elastic skin, i.e. it shows that the skin returns to normal more quickly.
- Coefficient of Restitution – This is the bounce height relative to the start height. A value of 0= no elasticity and a value of 1=good elasticity.
- Area - *Area* describes the area between the bounce profile and the skin zero datum (i.e., the sum of the area under the curve described by the probe vs. the resting level of the surface of the skin) and is therefore correlated to both softness and elasticity.

Normal skin tends to be softer and more elastic then scarred skin and this is reflected in ballistometry readings as:

- Higher values than a scar for “indentation” because it is softer. Softer skin will have a larger indentation.
- Lower values than a scar for “alpha” because the lower the value the more elastic the skin.
- Values closer to 1 than a scar for “coefficient of restitution” because it is more elastic. A value of 1 indicates good elasticity.
- Higher values than a scar for “area” because it correlates positively with softness and elasticity.

Scars tend to be harder and less elastic than normal skin and this is reflected in ballistometry readings as:

- Lower values than normal skin for “indentation” demonstrating the increased hardness/firmness of a scar.
- Higher values than normal skin for “alpha” demonstrating the decreased elasticity of scarred skin.
- Lower values than normal skin for “coefficient of restitution” demonstrating the decreased elasticity of scarred skin with a value of 0 indicating no elasticity.
- Lower values than normal skin for “area” indicating a scar is harder and less elastic.

Table 19 Ballistometer Readings - Indentation (mm) by Visit

Month	N	Mean Indentation (mm)	SD	Min	Median	Max	95% CI Mean
3	53	0.458	0.1021	0.19	0.43	0.70	(0.43, 0.49)
4	52	0.459	0.0978	0.23	0.46	0.69	(0.43, 0.49)
5	50	0.496	0.1092	0.31	0.47	0.79	(0.46, 0.53)
6	49	0.510	0.1142	0.21	0.52	0.75	(0.48, 0.54)
7	48	0.521	0.1001	0.34	0.52	0.74	(0.49, 0.55)
8	49	0.514	0.1150	0.17	0.52	0.87	(0.48, 0.55)
9	45	0.519	0.1011	0.28	0.51	0.81	(0.49, 0.55)
10	43	0.501	0.1043	0.24	0.50	0.74	(0.47, 0.53)
11	48	0.518	0.1360	0.33	0.49	1.03	(0.48, 0.56)
12	51	0.474	0.1103	0.05	0.49	0.71	(0.44, 0.51)

Figure 22 Ballistometer - Mean Indentation by Visit

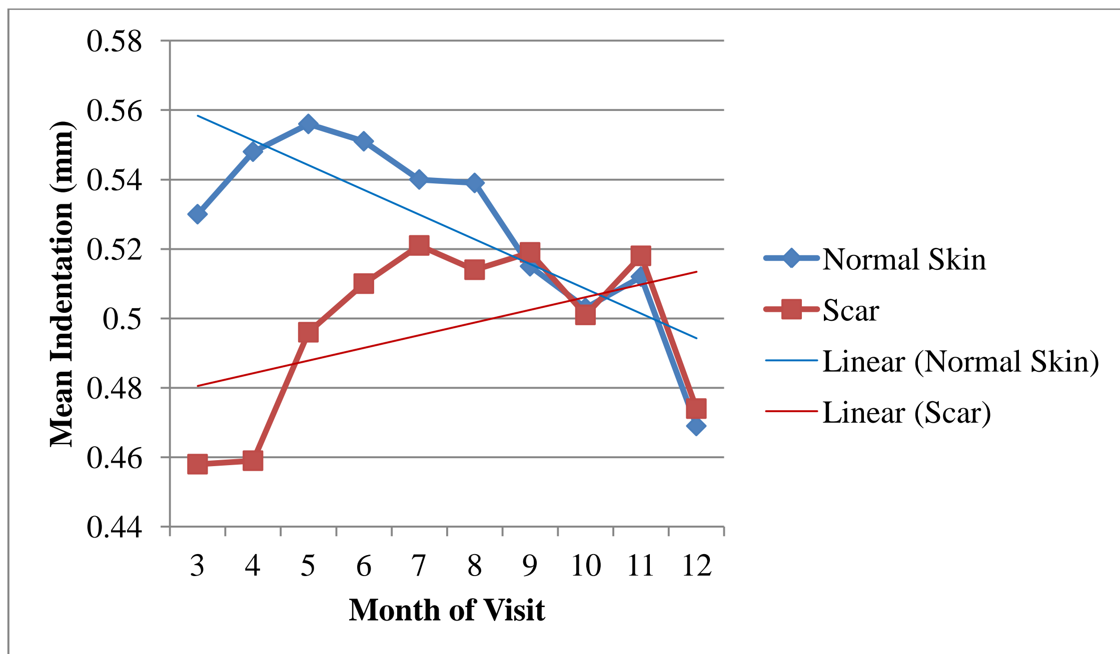


Table 20 Ballistometer Readings - Alpha by Visit

Month	N	Mean Alpha	SD	Min	Median	Max	95% CI Mean
3	53	0.0354	0.00655	0.023	0.035	0.060	(0.034, 0.037)
4	52	0.0356	0.00680	0.025	0.034	0.055	(0.034, 0.038)
5	50	0.0339	0.00828	0.018	0.034	0.069	(0.032, 0.036)
6	49	0.0335	0.00633	0.022	0.033	0.051	(0.032, 0.035)
7	48	0.0307	0.00588	0.019	0.031	0.044	(0.029, 0.032)
8	49	0.0320	0.00823	0.018	0.031	0.058	(0.030, 0.034)
9	45	0.0295	0.00702	0.017	0.029	0.053	(0.027, 0.032)
10	43	0.0298	0.00766	0.017	0.029	0.047	(0.027, 0.032)
11	48	0.0263	0.00872	0.014	0.025	0.050	(0.024, 0.029)
12	51	0.0260	0.00859	0.015	0.024	0.048	(0.024, 0.028)

Figure 23 Ballistometer - Mean Alpha by Visit

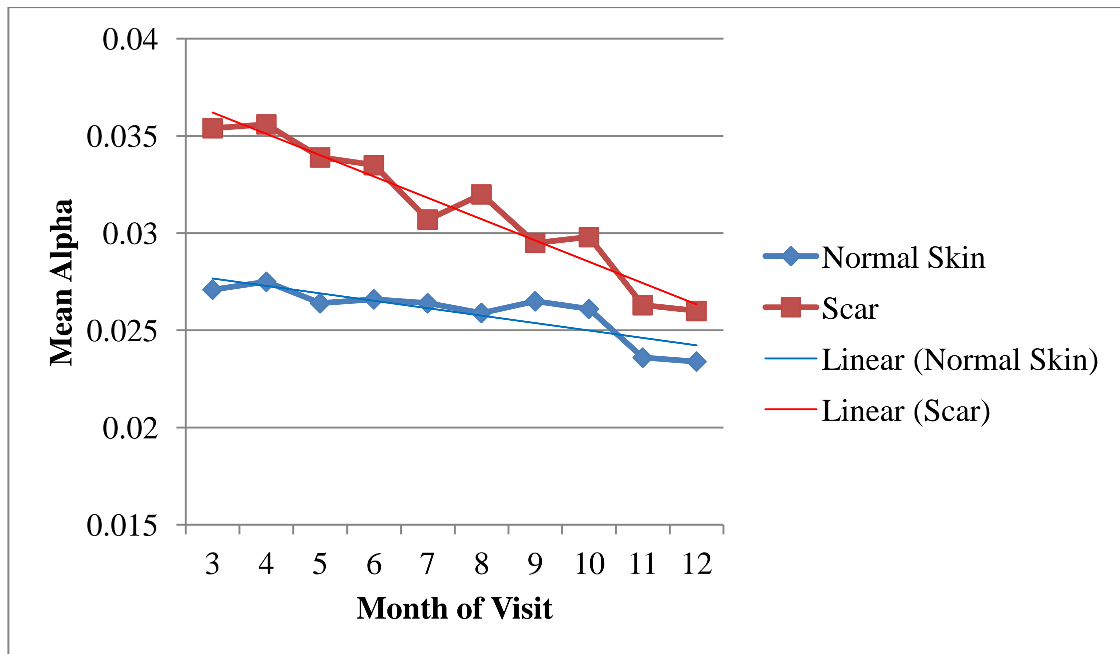


Table 21 Ballistometer Readings - Coefficient of Restitution by Visit

Month	N	Mean Coefficient of Restitution	SD	Min	Median	Max	95% CI Mean
3	53	0.610	0.0371	0.50	0.61	0.70	(0.60, 0.62)
4	52	0.599	0.0859	0.06	0.61	0.73	(0.58, 0.62)
5	50	0.623	0.0468	0.52	0.63	0.73	(0.61, 0.64)
6	49	0.619	0.0414	0.50	0.62	0.76	(0.61, 0.63)
7	48	0.648	0.0560	0.53	0.64	0.78	(0.63, 0.66)
8	49	0.645	0.0636	0.48	0.64	0.80	(0.63, 0.66)
9	45	0.668	0.0630	0.57	0.64	0.79	(0.65, 0.69)
10	43	0.673	0.0643	0.54	0366	0.79	(0.65, 0.69)
11	48	0.706	0.0736	0.51	0.72	0.82	(0.68, 0.73)
12	51	0.719	0.0701	0.56	0.74	0.81	(0.70, 0.74)

Figure 24 Ballistometer - Mean Coefficient of Restitution by Visit

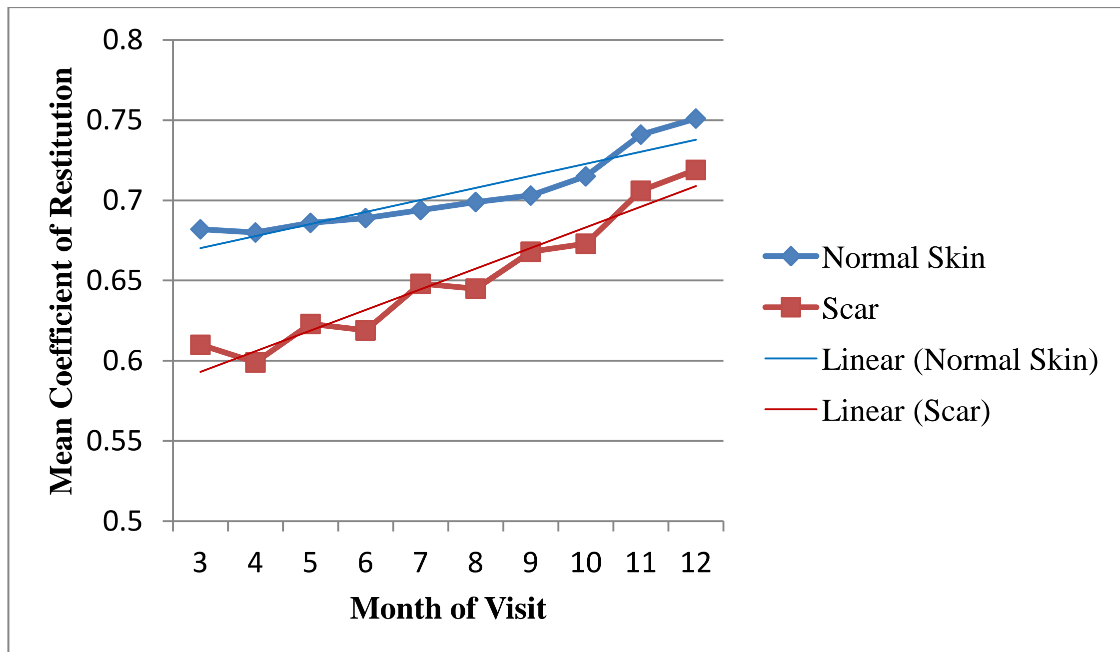
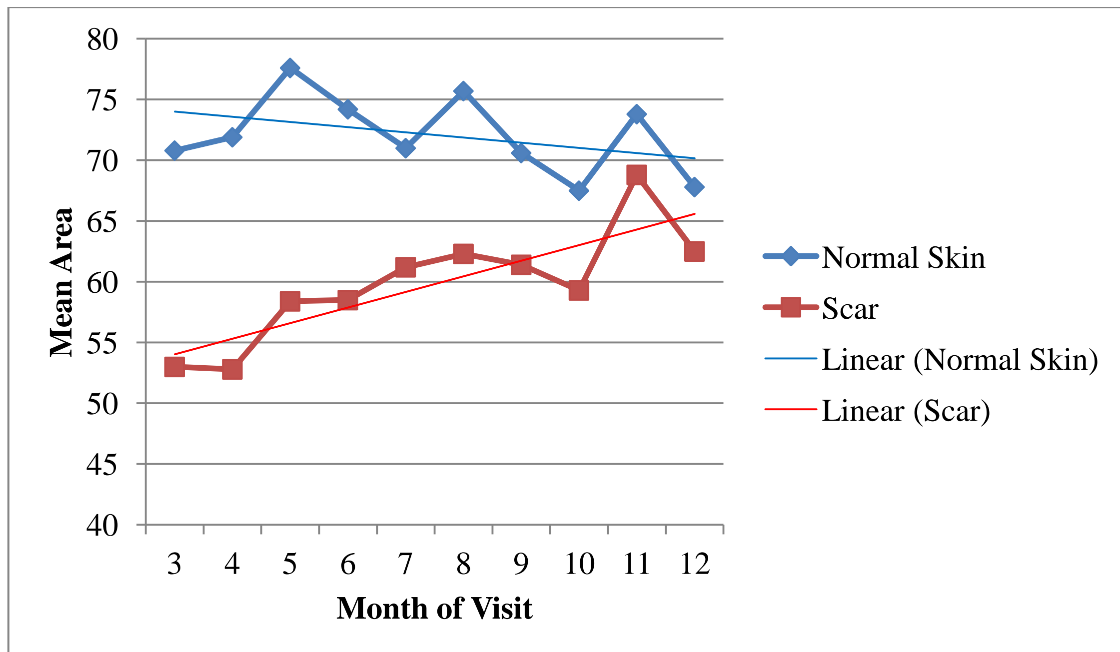


Table 22 Ballistometer Readings - Area by Visit

Month	N	Mean Area	SD	Min	Median	Max	95% CI Mean
3	53	53.0	13.61	24	50.0	86	(49.2, 56.7)
4	52	52.8	15.32	25	50.5	89	(48.5, 57.0
5	50	58.4	18.94	28	54.0	127	(53.1, 63.8)
6	49	58.5	16.79	22	58.0	95	(53.7, 63.3)
7	48	61.2	15.32	32	60.0	96	(56.8, 65.7)
8	49	62.3	20.32	29	62.0	136	(56.4, 68.1)
9	45	61.4	17.47	22	60.0	127	(56.2, 66.7)
10	43	59.3	16.89	31	58.0	95	(54.1, 64.5)
11	48	68.8	32.64	28	61.0	187	(59.3, 78.2)
12	51	62.5	17.90	27	63.0	94	(57.4, 67.5)

Figure 25 Ballistometer – Mean Area by Visit



The results displayed are those taken at each monthly visit commencing at Month 3. There is a nice convergence in the Ballistometer data between the scars and the normal skin as the scars mature. More specifically, the mean indentation of the scar displayed a gradual increase up until Month 12. Graph trend lines (Figure 22) illustrate values for scar and normal skin converge between Month 10 and 11 with the value at Month 12 for the scar appearing to exceed that of normal skin. This is indicative of a rise in pliability over the study period. Mean alpha gradually decreases (Figure 23) up to Month 12 and coefficient of restitution increases (Figure 24). They both failed to regain the values obtained for normal skin with no steady state achieved but indicating an increasing scar elasticity and reduced rate of energy dampening. Mean area rose to near levels for normal skin (Figure 25) correlating to increasing softness and elasticity of the scar. Values for the scar did not reach those values obtained for normal skin.

3.5 Discussion

This chapter describes the clinical process of scar maturation in an African Continental Ancestry Group of volunteers over a 12 month period. Many factors are at play influencing the scar appearance. The length of the process varies for different clinical aspects of scar maturation.

The results presented follow the maturation of the anterior scar of the dominant arm of each volunteer. This ensured that we continued to assess the same scar throughout and did not lose data when scars started to be excised. This aspect of the study design provides a scar which can be assessed beyond the 12 month period. These scars have been followed up at Month 18 and Month 24 but will not be reported in this thesis.

The main method of clinical scar assessment in this study involves a scale devised by Beausang et al (1998) that is appropriate for assessment of a wide range of scars. It involves clinical assessment using the Clinical (Manchester) Scar Assessment Scale (Appendix 4) and an overall assessment made on a VAS (score of '0' indicated normal skin and a score of '100' a very poor scar). Quinn et al (1995) and more recently Duncan et al (2006) have shown that the VAS meets the required standards of consistency, reliability, validity, and feasibility. The Clinical Scar Assessment Scale is shown to have a high level of sensitivity in the assessment of linear scars and with the VAS is more comprehensive than the VAS alone.

The mean Clinical VAS scored by the Investigator showed a gradual decrease over the course of 12 months indicative of an overall improvement in scar appearance over time (Figure 3). However, when the 12 months are considered in more detail several important points should be made. The mean Clinical VAS actually showed no improvement rather a slight deterioration in the initial 3 months. From Month 3 onwards a small improvement was seen at each month except for Month 11. Figure 3 shows no steady state was reached, with the mean Clinical VAS continuing to improve up to Month 12. This suggests that the process of scar maturation is continuing at Month 12. In the literature, the maturation phase of wound healing is often quoted as ongoing in humans up to 1 year (Gurtner, 2007). Occleston et al (2010) in a study looking at pre-clinical models of wound healing and human wound healing found that 'scars are stable and mature at ≥ 70 days postwounding in mice/rats and ≥ 6 months in pigs compared to 6-12 months in man'.

The results from the lay panel, as with the investigator assessments, showed a small decrease in VAS scores indicating a small but observable improvement in scar appearance over time (Figure 6). Consistency was seen with the investigator assessments in that in the initial 3 months the mean lay panel VAS showed no improvement and at Month 11 the mean lay panel VAS rose again. While the trends in both sets of data were similar the lay panel tended to score scars poorer than the investigators and give a smaller range of scores. Mean VAS scores of 52.83 and 44.62 for Month 1 and Month 12 respectively by the investigators and 59.24 and 54.87 for Month 1 and Month 12 respectively by the lay panel illustrate this point. There are

several reasons to explain these findings. The panel members assessed magnified projected images. The panel members had one aspect, the image, on which to base the scar assessment. Clinicians assessed the scar on the volunteer's arm with no magnification and will have considered other aspects such as scar elevation and texture when making the scar assessment.

During the recruitment process we aimed to recruit to different age groups in order to be able to illustrate clinical differences in scar maturation between the young, middle-aged and older population of volunteers. Unfortunately, only 1 volunteer was recruited to the over 55 age group limiting this aspect of the analyses. An assessment of the improvement in the African populations by age group showed few observable differences between subjects aged 18-30 years and 31-54 years, with the exception of Month 3 in favour of older subjects (p=0.0081) and a trend at Month 10 (p=0.0623). No conclusions can be made regarding the eldest (55-85 years) age group for reasons already stated, with the 1 volunteer scoring much better at every visit than his younger counterparts. These findings are in contrast to that of Bond et al (2008b) who carried out a study of maturation of the human scar in a population of European Continental Ancestry, aiming to provide a description of scar maturation until 1 year post injury. They were able to provide a macroscopic and histological description of scar maturation including a 'representative' description and a description of those referred to as either 'poor' outliers or 'excellent' outliers. The poor subset invariably contained individuals younger than 30 years of age whereas the opposite was true for the excellent subset comprising individuals older than 55 years of age. The rate of scar

maturation varied within the study group, almost according to age with older subjects displaying an accelerated rate of maturation and younger subjects a retarded rate of maturation. Ashcroft et al (1997) also found that in aged mice the macroscopic and microscopic appearance of scars were superior to those of younger animals with dermal organisation similar to that of normal dermal "basket-weave" collagen. They observed that 'the rate of healing in young animals appears to be increased at the expense of the scar quality' (Ashcroft et al., 1997).

The racial background was such that 100% of the population self-ascribed to the African CAG, with 33.3% having a close relative of alternative ancestry. However, a more objective method of skin type classification is certainly required. In the absence of a gold standard the Fitzpatrick scale offers a simple and practical method of classifying a patient's skin phototype. It was first described in 1975 by Fitzpatrick and reviewed by Pathak et al and Fitzpatrick at a later stage (Fitzpatrick, 1988) to give a total of 6 skin types. It is used to determine the skins burning tendency and tanning ability (Fitzpatrick, 1988) and is recognised as an invaluable tool for communication within the dermatological specialty (Roberts, 2008). A careful interview should allow the clinician to categorise individuals into one of the six skin types (Table 23).

Table 23 Fitzpatrick Skin Type Classification

Skin Type	Reaction to UVA	Reaction to Sun
Type I	Very Sensitive	Always burns easily, never tans; very fair skin tone
Type II	Very Sensitive	Usually burns easily, tans with difficulty; fair skin tone
Type III	Sensitive	Burns moderately, tans gradually; fair to medium skin tone
Type IV	Moderately Sensitive	Rarely burns, always tans well; medium skin tone
Type V	Minimally Sensitive	Very rarely burns, tans very easily; olive or dark skin tone
Type VI	Least Sensitive	Never burns, deeply pigmented; very dark skin tone

Mean Clinical VAS was shown to be influenced by a subject's Fitzpatrick Skin Type. An analysis of Clinical VAS based on Fitzpatrick Skin Type Classification of the subjects, showed that those subjects with Fitzpatrick Skin Type IV (medium skin tone) had scars which scored consistently better at every month of assessment on the Clinical VAS in comparison to those subjects with Fitzpatrick Skin Type V (dark/olive skin tone) and Type VI (very dark skin tone) (Figure 5). This difference increases as the scars mature. Those subjects with Fitzpatrick Skin Type V and Type VI consistently showed minimal difference in Clinical VAS at every month of assessment. These findings show a real drop in Clinical VAS scar scores between subjects with a medium skin tone and those with dark or very dark skin tone. The lay

panel VAS scores were consistent with the investigators findings. Scar severity can be influenced by a large number of factors including age, sex, skin thickness and tension, the position of the scar on the body and patient population group. Montagna, Prota & Kenney (1993) in a review of 'Black Skin' have concluded that 'other than colour and the readiness to form hypertrophic scar and keloids in black skin, there are probably no basic differences in the process of wound healing between Whites and Blacks'. From these data it is possible to see that scar maturation, without hypertrophic or keloidal activity, is different for subjects with different skin types. It is not as simple as stating that populations with darker skin are more likely to get a keloid scar or a hypertrophic scar. It is also possible age-related differences in scar quality have not been demonstrated in this population group due to the greater influence of volunteer skin type on the scar quality than volunteer age.

Assessment of scar width forms part of the Clinical Scar Assessment Scale. Scar width of the scars increased over the 12 month study period (Figure 8). An analysis of scar width based on age of the subjects, as with the Clinical VAS, showed that the youngest group of subjects (i.e. 18-30 years) had significantly wider scars when compared to subjects aged 31-55 years (Figure 9). The difference in scar width measurements between these two age groups is much more marked than the difference in Clinical VAS measurements. It can be said then that the scar width is unlikely to be a significant factor when investigators score scars using the Clinical VAS. As before, no conclusions can be made regarding the eldest (55-85 years) age group due to the recruitment of a single volunteer in this demographic. The mechanisms by which a

scar increases in width are not completely understood. The next Chapter will consider this in further detail and consider what is going on histologically in a wider scar. Sommerlad and Creasey (1978) in a clinical wound healing study found that scar width was influenced by the method of wound closure. In general, this study demonstrated that scars stretch at a constant rate. The study was carried out over 12 months. The scars almost doubled their width between 3 weeks and 3 months. Between 3 and 6 months scars increased in width by a further 50% but little increase in width was seen thereafter. Figure 8 demonstrated that the most significant increase in scar width occurs in the first 6 months for the ACAG but the scar width does increase up to 12 months post wounding. It is important to point out that the wound closure in this study was identical to that in the ECAG study by Bond et al (2008).

Assessment of scar height forms part of the Clinical Scar Assessment Scale. As expected, scar height steadily decreased over the 12 month study period suggesting a flattening of the scar over time (Figure 10). The Clinical Scar Assessment Scale includes other assessments including assessment of scar appearance, colour mismatch, scar distortion, scar contour and scar texture. The assessment of scar contour is relevant in relation to scar height. Indeed the findings are consistent, in that the percentage of scars deemed flush with the surrounding skin remains relatively unchanged from Month 9 onwards. At Month 12 over 95% of scars were rated as flush with the surrounding skin. As shown in Figure 14 a steady state has been reached. These findings are consistent with the measures of scar height illustrated in Figure 10, indicating that the process of scar maturation that allows a scar to become flush with

surrounding skin has completed within the study period. It is interesting to note, that many of the other assessments of the maturing scar have not reached a steady state by Month 12. It was also unexpected to find that the scars of the older age group tended to be more elevated. This contrasts with the animal study by Marcus et al (2000) which demonstrated that scar prominence/height was significantly less in older animals.

Assessment of scar appearance and scar colour forms part of the Clinical Scar Assessment Scale. There was a clear trend towards an increase in the number of matte scars as time progressed (Figure 12). As regards scar colour, at month 12, the majority of scars showed a "slight mismatch" with the surrounding skin (Figure 13). Interestingly, up to and including Month 11, scars were generally "darker" compared to the surrounding skin. These findings are subjective and should be considered with the objective measures of colour obtained with the use of the spectrophotometer. Since shiny scars are generally more noticeable, due to their ability to reflect light the assessment of scar appearance may influence any perceived colour mismatch making this assessment less accurate. Statistical analysis of the data at Month 12 was used to identify which scar features influence the scar clinical VAS score. Colour is the most statistically relevant feature, followed by distortion with the other features not statistically relevant to clinical VAS scores.

As described in the results section there are several measurements which can be derived from the spectrophotometer data. ΔEab calculated from the spectrophotometry data reduced, consistent with a better overall blending of the scar with the surrounding

skin (Figure 18). As a measure of overall colour difference it shows that at Month 12 the scar has still not colour matched with surrounding skin, consistent with the clinical scar assessment of colour were the majority of scars still show a “slight colour mismatch” at Month 12. Another derived spectrophotometric measurement is Δl which measures the difference in darkness / lightness between the scar and the surrounding skin. In the first three months the mean Δl value became more negative indicating a scar lighter than normal skin. From Month 3 onward the mean Δl value steadily increased. This is in contrast to the subjective assessment of scar colour as part of the Clinical Scar Assessment Scale where scars were generally assessed as “darker” compared to the surrounding skin. The mean Δl value does not return to zero during the period of the study and does not reach a steady state indicating that the maturation process is still ongoing at Month 12 as regards pigmentation changes. The same can be said of Δb which measures the difference in the yellow/blue axis between the scar and the surrounding skin. Over the course of the study, it was observed that there was a steady increase continuing to improve up to Month 12 with no steady state reached indicating that this particular aspect of scar maturation in relation to scar colour is still ongoing at Month 12. The spectrophotometric data highlights that at Month 12 there is still an ongoing active process in scar maturation.

Scar redness was assessed at each month in order to find out when scar redness fades. By Month 9 almost all scar redness had faded. In subjects with very dark skin it is possible that the subjective assessment of redness was not accurate. Interestingly the objective derived measurement of Δa from the spectrophotometer data which measures

redness was consistent with the subjective assessment of redness. Both forms of assessment reveal an increase in scar redness values at Month 10 followed by a sharp decrease from Month 10 to Month 12 with a mean Δa of 0.11 at the end of the study period. McGregor and McGregor (2000) point out that the degree of redness of a scar and the time taken for it to diminish is extremely variable. Dierickx et al. (1995) observed that the time taken for redness to fade can take up to one year. Bond et al (2008a) were the first to describe the natural history of scar redness in humans, with the time point at which scar redness fades previously unknown. They found that on average scar redness fades at Month 7. Of note these findings are in relation to a population of European Continental Ancestry. Our findings for a population of African Continental Ancestry would suggest that the process in scar maturation that leads to scar redness fading is slower in this population.

Mechanical properties are difficult to assess objectively. The longitudinal change in scar mechanics was measured using the Torsional Ballistometer which provides four parameters to consider. Indentation is a direct measure of hardness. The higher the indentation values the softer the substance being assessed. Mean indentation of scars increased over time indicating that as scars mature they become softer. Alpha represents the rate of energy dampening and is an indirect measure of elasticity. Alpha values are inversely related to elasticity. The fall in values for alpha over time demonstrated an improvement in scar elasticity over time. On the other hand, the coefficient of restitution is a direct measure of elasticity. Monthly improvement in this value supports the finding that scar elasticity improves over time. The values generated

for mean area indicated yet again improvement in the softness and elasticity of the scar over time. All of the data generated by the use of Ballistometry supports the pattern of increasing scar softness and elasticity as a scar matures. Once again this process appears to be ongoing and incomplete at Month 12.

3.6 Summary

This Chapter describes how the appearance of a scar improves with time. The process of scar maturation appears to be ongoing at the end of the 12 month time period of this study with the exception of scar contour and scar redness, no steady state has been achieved. The results suggest that the main volunteer demographic to influence the resulting scar appearance is skin type with age not shown to exert significant influence. The scar feature with the most influence on clinical scar scoring was scar colour followed by scar distortion with the other features not statistically relevant to clinical scoring. The results demonstrate that scar colour mismatch decreases over time, but with no steady state achieved, further indicating an ongoing scar maturation process. Scar width was shown to increase over the 12 months of the study, with younger volunteers having a greater propensity to develop wide scars. We have demonstrated how the mechanical properties of scars as measured by the Torsional Ballistometer improve with time. The scar data was not shown to plateau or equilibrate with normal unwounded skin which would suggest that scar maturation is still ongoing.

4 SCAR MATURATION – HISTOLOGICAL PERSPECTIVE

4.1 Introduction

Until recently the natural history of scar maturation in humans had not been formally described. Bond et al. (2008b) carried out an observational study of scar maturation from both a clinical and histologic standpoint over a 12 month period. One limitation of this study is that it considers a male European Continental Ancestry Group (ECAG) alone. It is important however that the natural history of scar maturation in humans is established for all skin types.

Bond et al (2008b) described two distinct groups of outliers from a normal course when studying scar maturation in the ECAG, described either as “poor” or “excellent” based on their appearance in comparison with a “representative” group. In this study I aim to describe scar maturation in volunteers in the ACAG and assess if the same or a different pattern occurs. Three groups of scar type were identified. They were named Type 3, Type 4 and Type 5. These scars at their best were as good as a poor scar in the ECAG. Considering the groups described by Bond et al (2008b) as Type 1 (excellent outlier), Type 2 (representative) and Type 3 (poor outlier), then the findings of this study can be typed to follow on from them.

4.2 Materials and Methods

A large number of histological samples were collected utilising the study design outlined in Chapter 2. Each volunteer in the study had two scars excised at two different time points. 206 samples for histological assessment were collected.

The specimens were fixed, wax embedded and sectioned. All specimens were stained with Masson's Trichrome and assessed by 2 trained histologists according to a modified version of the microscopic VAS of Beausang et al (1998). The histologic assessment of the scars involved semi-quantification of the major architectural abnormalities that are known to occur in scars (Beausang et al., 1998). The scale is modified to include a score for epithelial thickness, scar elevation, angiogenesis and inflammation, and an overall visual analogue scale assessment of collagen organisation. The following data were collected on all specimens:

- Epidermal Restitution Score
- Angiogenesis and Inflammation Score
- Collagen Organisation Score
- Visual Analogue Scale Assessment of Collagen Organisation
- Other Scar Features – scar elevation

4.3 Study Subjects - Results

4.31 Time period of the study

Sixty patients were successfully recruited between February 2006 and January 2007.

4.32 Disposition of Subjects

6 volunteers did not undergo excision of scars at one time point and 5 volunteers did not undergo excision at both time points. Of those 6 volunteers missing one excisional surgery 5 were lost to follow-up and 1 due to ill-health did not undergo surgery (adverse event). Of those 5 volunteers missing both excisional surgery visits one volunteer withdrew consent for excision but continued with follow-up, 1 was withdrawn from excisional surgery by the investigator and the other three were lost to follow-up. This equates to a loss of 32 (13%) samples from a potential 240. Another 21 specimens were not assessed due to problems during processing. This results in 187 specimens available for assessment.

4.33 Demographic and Other Baseline Characteristics

As documented in Chapter 3.

4.4 Scar Histology Assessments – Results

4.41 The Histological Appearance of Scar Specimens

The specimen slides stained with Masson's Trichrome were assessed by this author and two trained histologists. Scar specimens were viewed in order of month of excision on a LEICA DFC420 FX microscope under x10 magnification. The histological appearance of each scar was described providing a description of scar maturation in this population group over the course of 12 months.

The histological slides were described considering the following major architectural features of a scar:

- Epidermal restitution, in terms of rete ridge restoration and maturity of the epithelium;
- The presence of pigment in the epidermis;
- The width of the scar;
- The presence of inflammatory cells;
- The maturity of the new dermis in terms of cellularity and maturity and organisation of the new collagen;
- And a description of the wound margins as many scars showed evidence of undermining of the wound margins with the neo-dermis being wider than the neo-epidermis.

In the process of describing the scar histology, specimens were found to fall into three groups. The three subgroups have been entitled Type 3, Type 4 and Type 5 defined by the histological findings. Scars in the ACAG were found to be poorer than those in the ECAG. A spectrum of wound healing is suggested in the discussion and summary for this chapter; with the best scars (Type 1) occurring in elderly ECAG individuals with optimal wound healing conditions through to scarring in young individuals of the ACAG (Type 5).

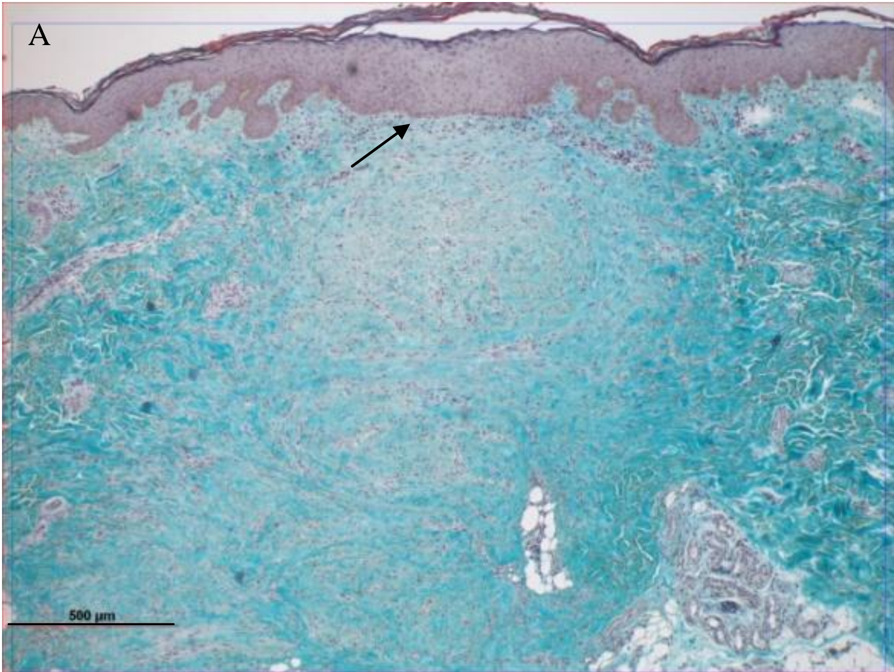
4.411 Summary of Histological Findings at Each Month

To follow are the main histological results presented as descriptions of the three different scar types at each month of scar maturation up to Month 12. Presented along side are images captured of the histological specimens representing interesting features noted during the assessment process. Following this the histological findings are summarised by month and scar type in Table 35, Table 36 and Table 37.

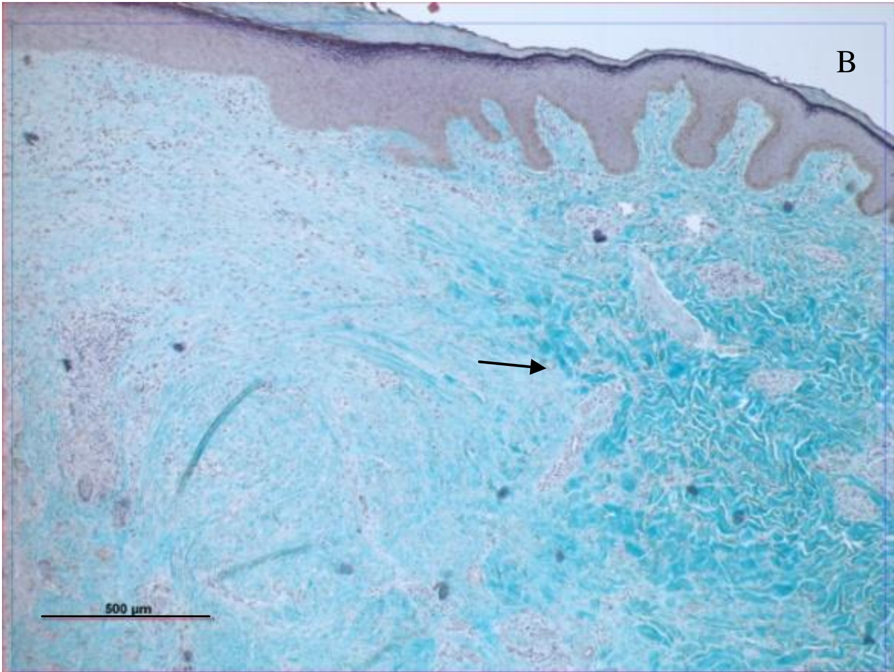
Table 24 Summary of Histological Findings - Month 1

	Month 1
Type 3	➢ No rete ridge reformation; flat epidermal dermal junction ➢ Immature thickened epithelium ➢ No pigment in the basal layer of the epidermis ➢ Narrow scar ➢ Minimal inflammatory cell infiltrate ➢ Cellular dermis with immature wispy collagen ➢ Minimal undermining of the wound margins
Type 4	➢ No rete ridge reformation; flat epidermal dermal junction ➢ Immature thickened epithelium ➢ No pigment in the basal layer of the epidermis ➢ Lots of mixed types of inflammatory cells especially surrounding blood vessels from which they appear to have originated ➢ Highly cellular dermis with immature wispy collagen ➢ Some undermining of the wound margins with the neo-dermis wider than the neo-epidermis
Type 5	➢ No rete ridge reformation; flat epidermal dermal junction ➢ Immature thickened epithelium ➢ No pigment in the basal layer of the epidermis but pigment seen in supra basal parts ➢ Obvious wide scar boundary with wound elevation ➢ Massive inflammatory cell infiltrate ➢ Fine wispy collagen ➢ Significant undermining of the wound margins with the neo-dermis significantly wider than the neo-epidermis

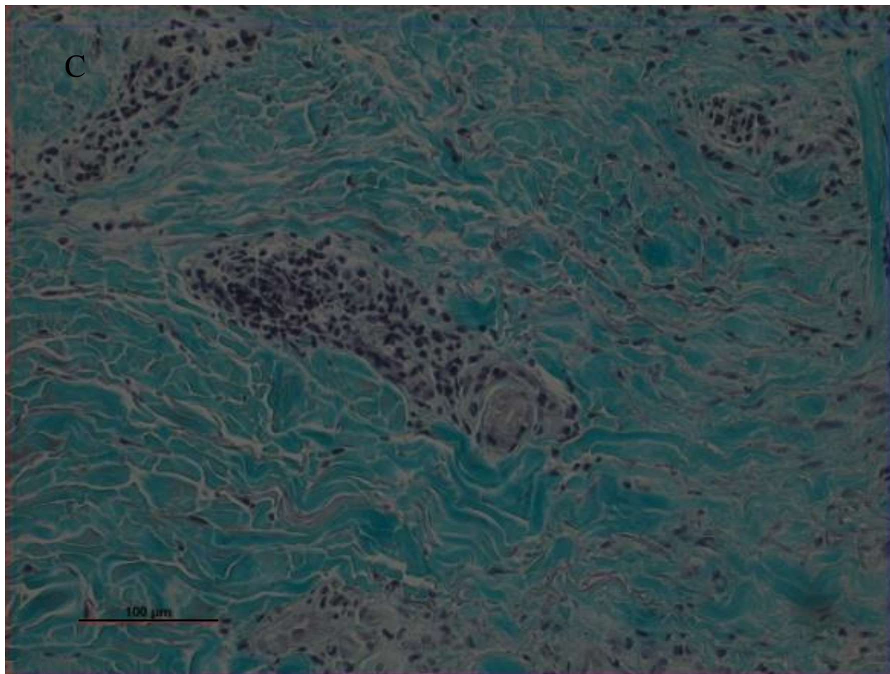
Figure 26 Histology Sections Stained with Masson's Trichrome at Month 1



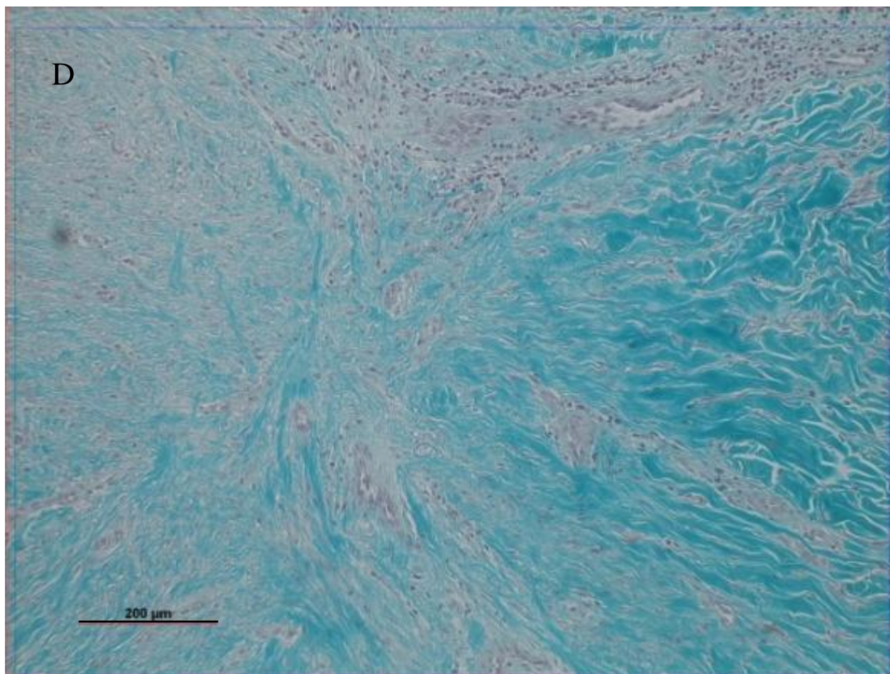
A Illustrating a flat epidermal dermal junction (Scale bar = 500µm)



B Illustrating undermining of the wound margin (Scale bar = 500 µm)



C Illustrating the presence of inflammatory cells (Scale bar = 100µm)

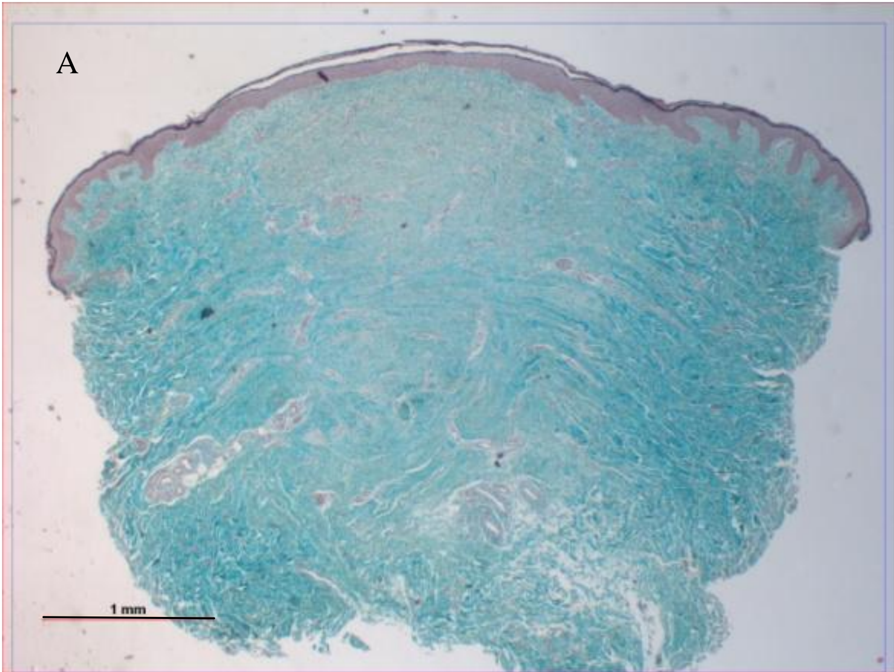


D Illustrating collagen dissolution (Scale bar = 200µm)

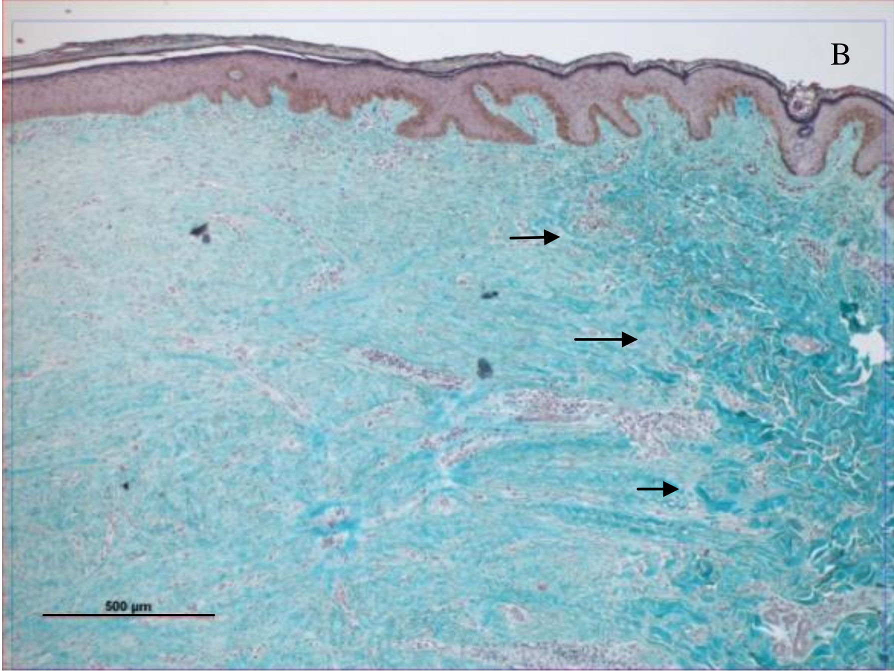
Table 25 Summary of Histological Findings - Month 2

	Month 2
Type 3	➢ Some rete ridge reformation ➢ Maturing epithelium ➢ No pigment in the basal layer of the epidermis but increased pigment at wound margins ➢ Minimal inflammatory cell infiltrate ➢ Cellular dermis with wispy parallel bundles of collagen beginning to mature ➢ Minimal undermining of the wound margins
Type 4	➢ Some rete ridge reformation ➢ Maturing epithelium ➢ No pigment in the basal layer of the epidermis but increased pigment at wound margins ➢ Inflammatory cells present but significantly less than Month 1 ➢ Cellular dermis with immature parallel bands of collagen ➢ Some undermining of the wound margins
Type 5	➢ No rete ridge reformation; flat epidermal dermal junction ➢ Maturing epithelium ➢ No pigment in the basal layer of the epidermis but increased pigment at wound margins ➢ Wide scar ➢ Massive inflammatory cell infiltrate but less than Month 1 ➢ Fine wispy collagen but more mature than Month 1 ➢ Significant undermining of the wound margins with the neo-dermis significantly wider than the neo-epidermis

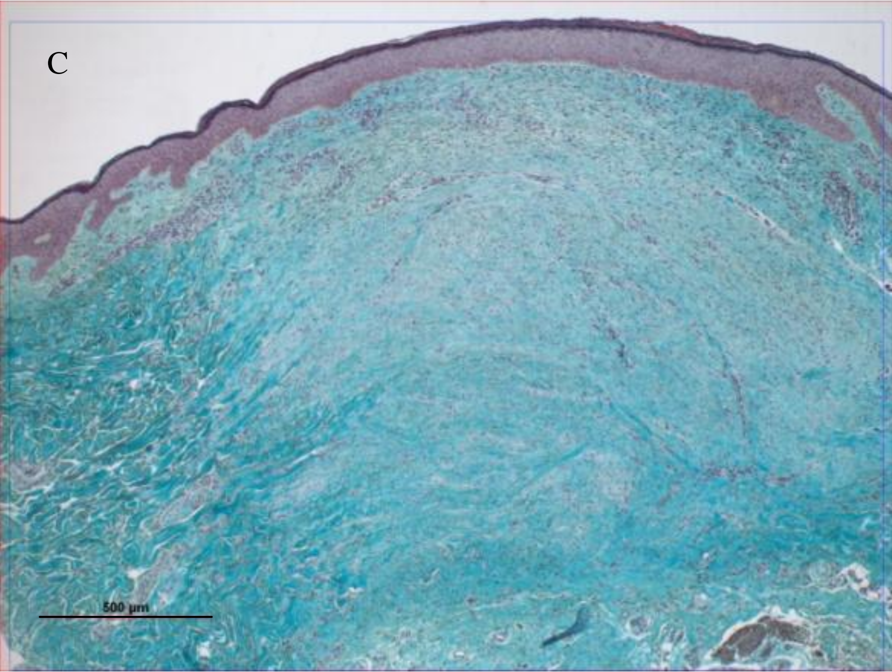
Figure 27 Histology Sections Stained with Masson's Trichrome at Month 2



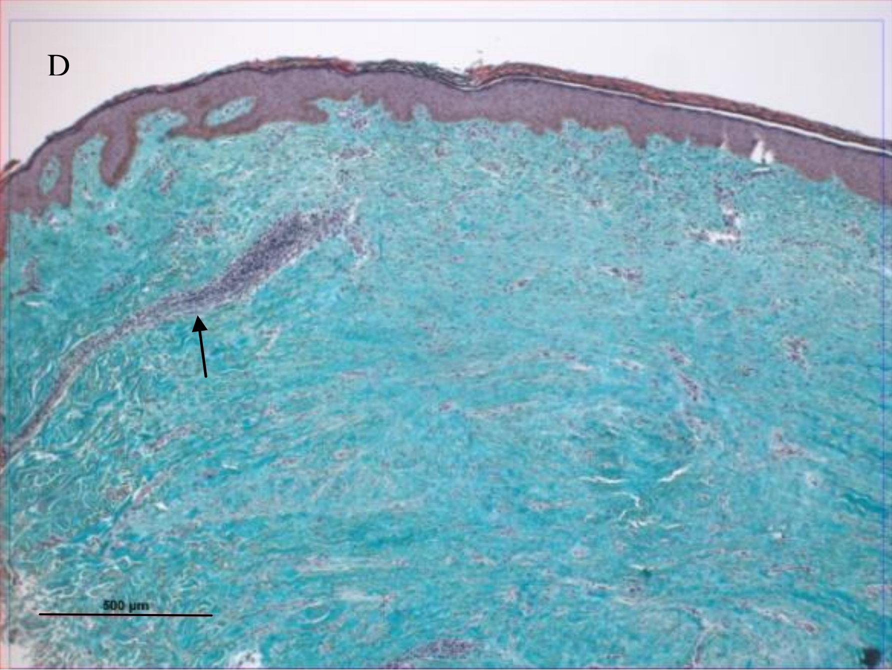
A Illustrating a Type 3 scar with minimal undermining of the wound margin (Scale bar = 1mm)



B Illustrating a Type 4 scar with undermining at the wound margin (Scale bar = 500µm)



C Illustrating invasion of the wound margins in a Type 5 scar (Scale bar = 500µm)

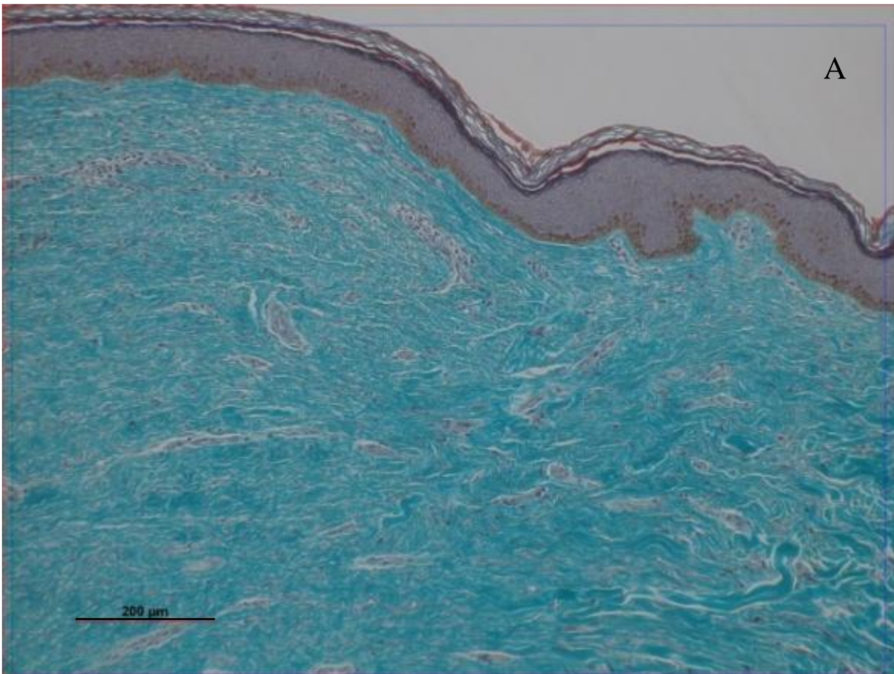


D Illustrating a strand of inflammatory cells in a Type 3 scar (Scale bar = 500µm)

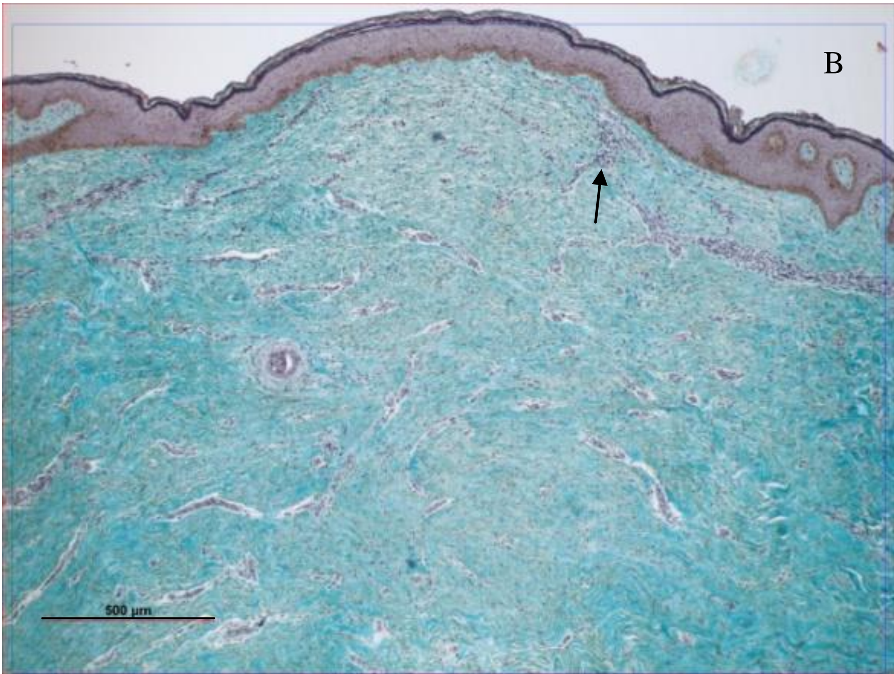
Table 26 Summary of Histological Findings - Month 3

	Month 3
Type 3	➢ Early rete ridge reformation ➢ Maturing epithelium ➢ Pigment in the basal layer of the epidermis and increased pigment at wound margins ➢ Very few inflammatory cells present ➢ Bundles of collagen maturing ➢ No undermining of the wound margin
Type 4	➢ Some rete ridge reformation ➢ Maturing epithelium ➢ Some pigment in the basal layer of the epidermis ➢ Persistent inflammatory cells decreasing ➢ Immature parallel bands of collagen with early signs of remodelling ➢ Minimal undermining of the wound margins
Type 5	➢ No rete ridge reformation; flat epidermal dermal junction ➢ Maturing epithelium ➢ Wide scar ➢ Some pigment in the basal layer of the epidermis and increased melanin at the wound margins ➢ Persistently high numbers of inflammatory cells including giant cells ➢ Immature collagen in parallel bands ➢ Significant undermining of the wound margins with the neo-dermis significantly wider than the neo-epidermis

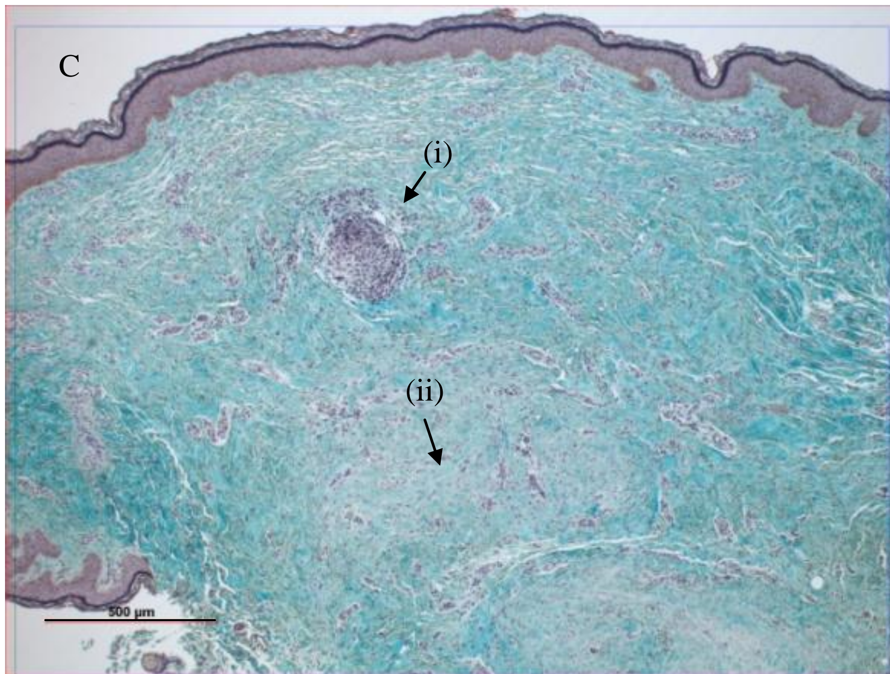
Figure 28 Histology Sections Stained with Masson's Trichrome at Month 3



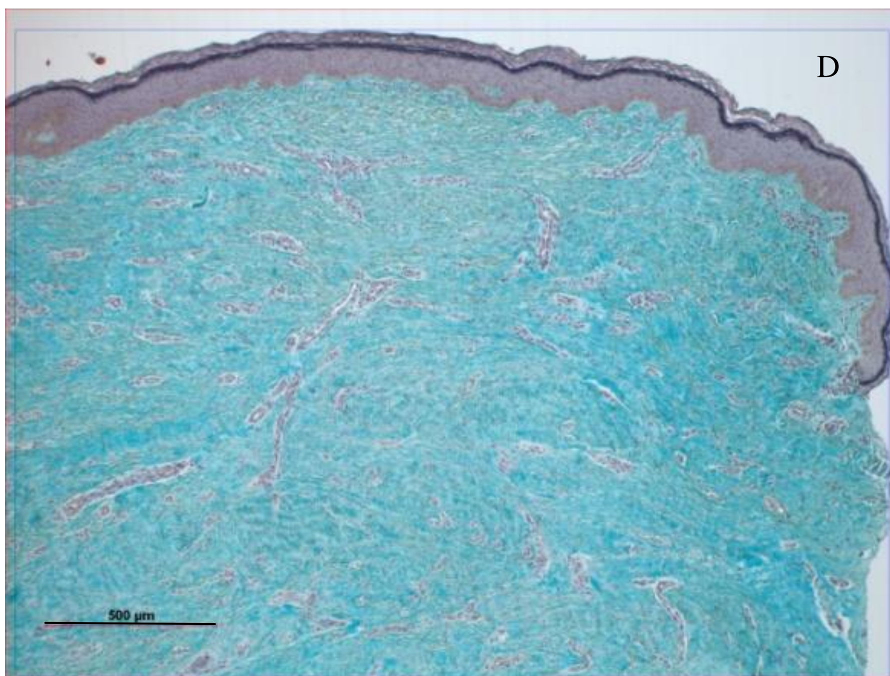
A AType 3 scar with new collagen present (Scale bar = 200µm)



B Illustrating persistent inflammation in a Type 4 scar (Scale bar = 500µm)



C AType 5 scar with (i) persistent inflammation and (ii) high turnover. (Scale bar = 500µm)

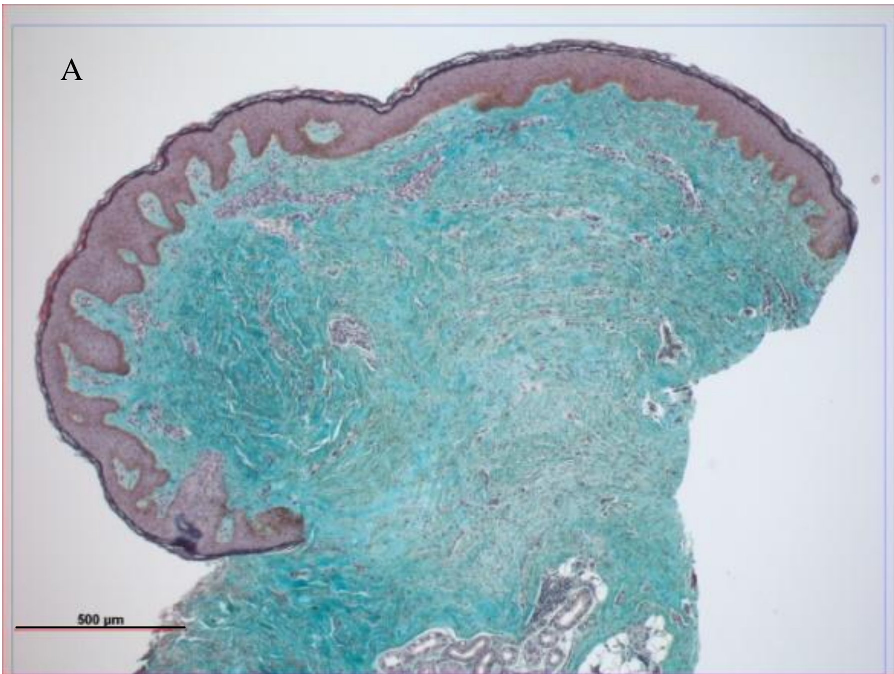


D Type 3 scar with minimal inflammation present (Scale bar = 500µm)

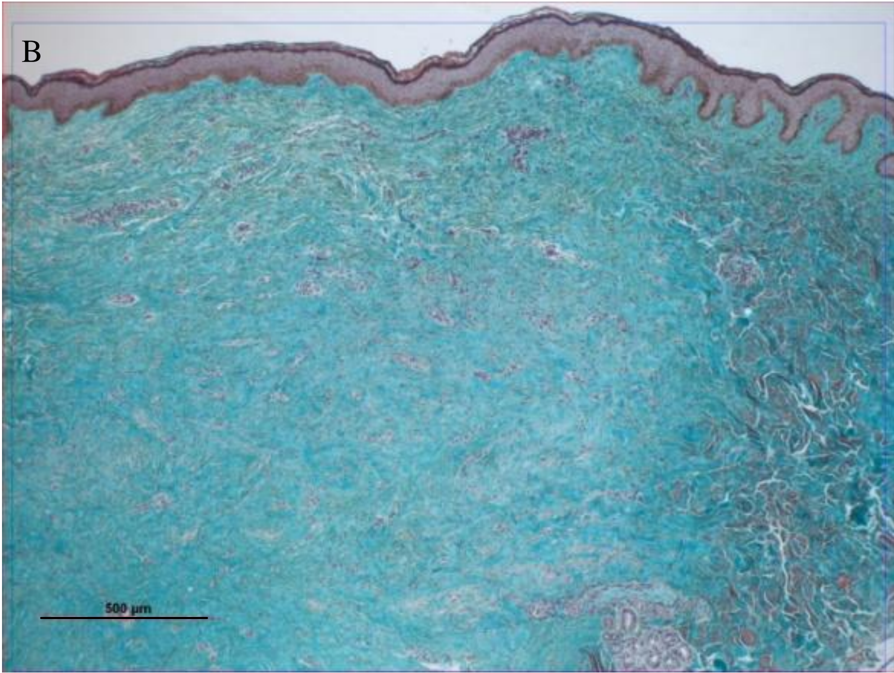
Table 27 Summary of Histological Findings - Month 4

	Month 4
Type 3	➢ Partial rete ridge reformation ➢ Maturing epithelium ➢ Pigment in the basal layer of the epidermis ➢ Pockets of inflammatory cells persist ➢ Some restoration of the papillary dermis and dermal collagen maturing ➢ No undermining of the wound margins
Type 4	➢ Some rete ridge reformation ➢ Maturing epithelium ➢ Some pigment in the basal layer of the epidermis ➢ Persistent inflammatory cells decreasing ➢ Immature parallel bands of collagen with early signs of remodelling and early restoration of papillary dermis ➢ Minimal undermining at the wound margins
Type 5	➢ Early rete ridge reformation ➢ Maturing thickened epithelium ➢ Some pigment in the basal layer of the epidermis with increased pigment at the wound margins ➢ Wide scar with tendency to be raised ➢ Persistently high numbers of inflammatory cells including giant cells slowly resolving ➢ Remnants of degraded old collagen in scar; immature collagen in parallel bands and early restoration of papillary dermis ➢ Significant undermining of the wound margins with the neo-dermis significantly wider than the neo-epidermis

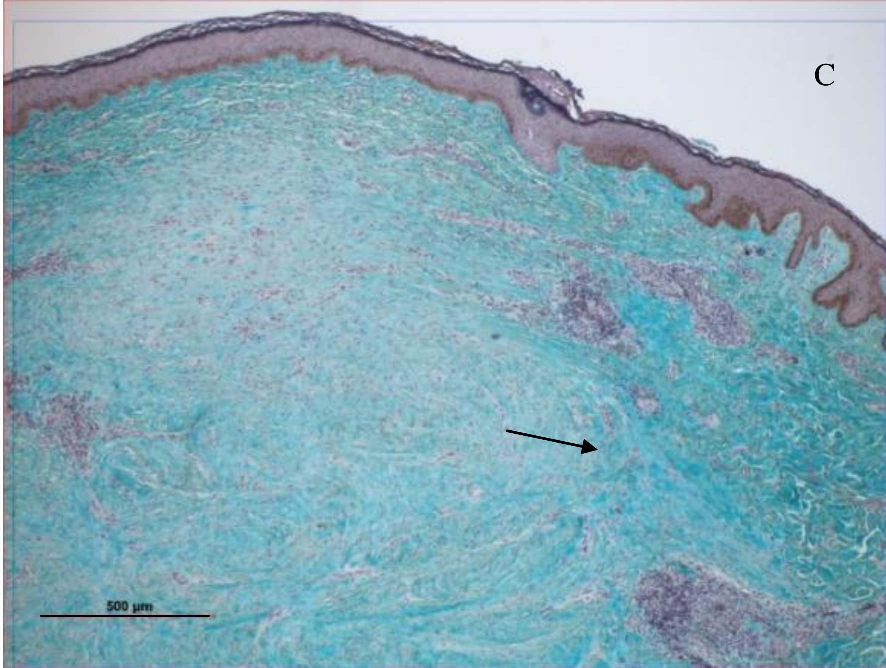
Figure 29 Histology Sections Stained with Masson's Trichrome at Month 4



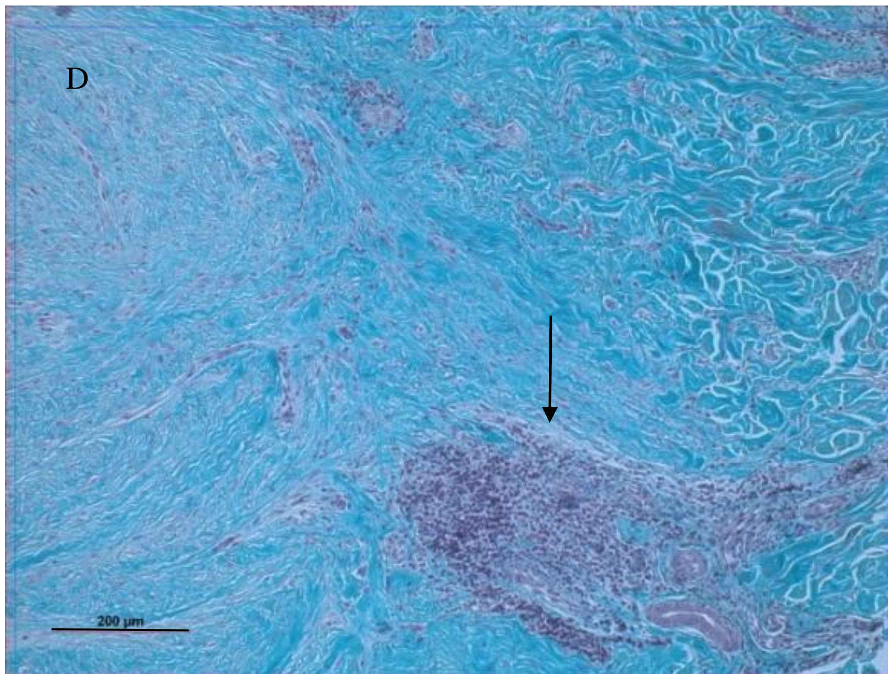
A Illustrating a Type 3 scar (Scale bar = 500µm)



B Illustrating new collagen in a Type 4 scar (Scale bar = 500µm)



C Illustrating undermining of the wound margin in a Type 5 scar (Scale bar = 500µm)

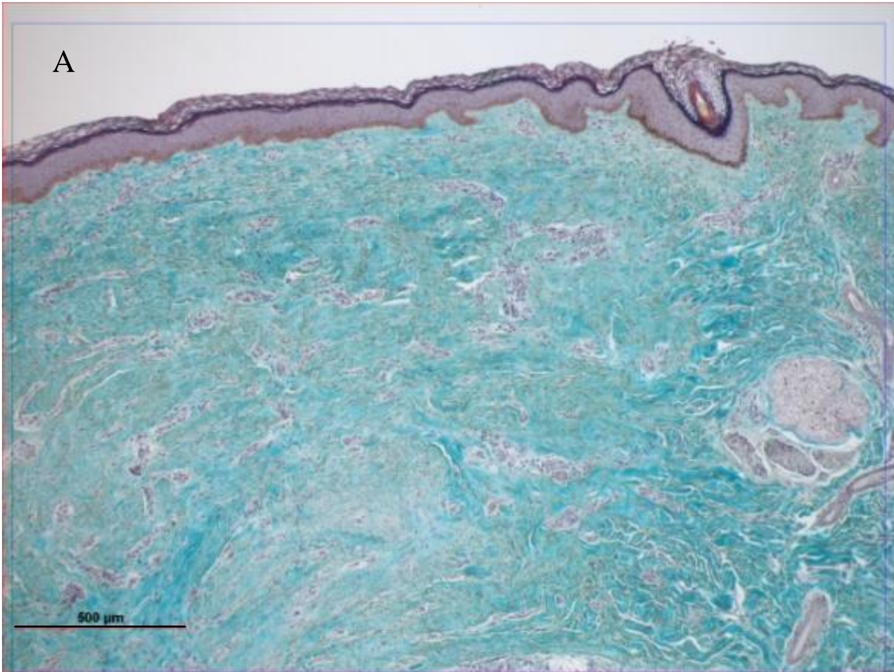


D Illustrating large numbers of inflammatory cells in a Type 5 scar (Scale bar = 200µm)

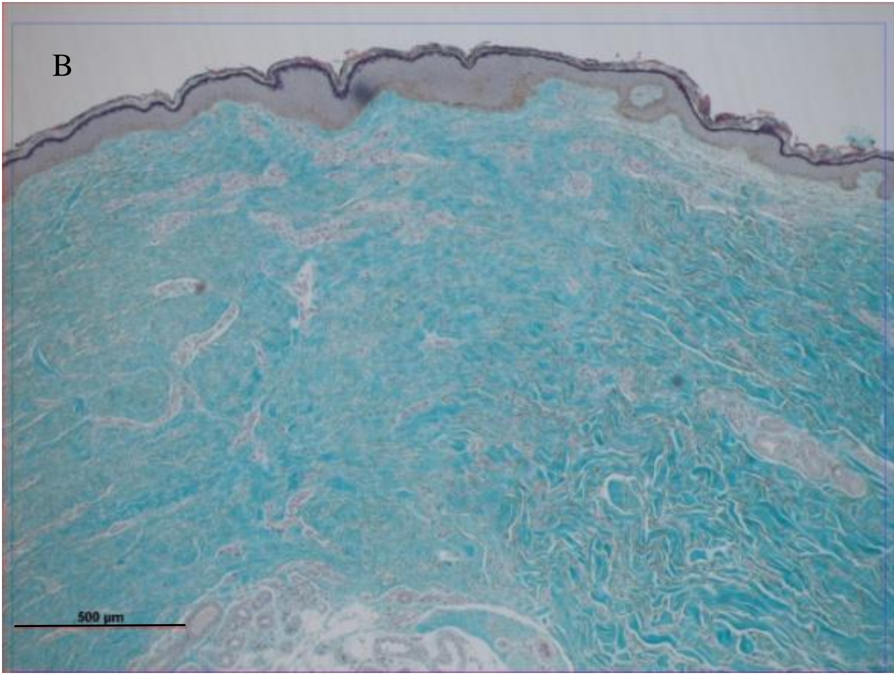
Figure 30 Histology Sections Stained with Masson's Trichrome at Month 5

	Month 5
Type 3	➢ Partial rete ridge reformation ➢ Maturing epithelium ➢ Pigment in the basal layer of the epidermis as in normal skin ➢ No inflammatory cells present ➢ Some restoration of the papillary dermis and remodelling of dermal collagen ➢ No undermining of the wound margins
Type 4	➢ Some rete ridge reformation ➢ Maturing epithelium ➢ Pigment in the basal layer of the epidermis. Occasionally more pigment than in adjacent normal skin ➢ Wide scar ➢ Very few inflammatory cells present ➢ Mostly immature parallel bands of collagen with early signs of remodelling and good restoration of papillary dermis ➢ Minimal undermining of the wound margins
Type 5	➢ Early rete ridge reformation ➢ Maturing thickened epithelium ➢ Wide scar ➢ Pigment in the basal layer of the epidermis and increased pigment at the wound margins ➢ Reduced numbers of inflammatory cells ➢ Remnants of degraded collagen arranged in degradation nodules; immature collagen in parallel bands surrounding nodules of degraded collagen and early restoration of papillary dermis ➢ Significant undermining of the wound margins with the neo-dermis significantly wider than the neo-epidermis

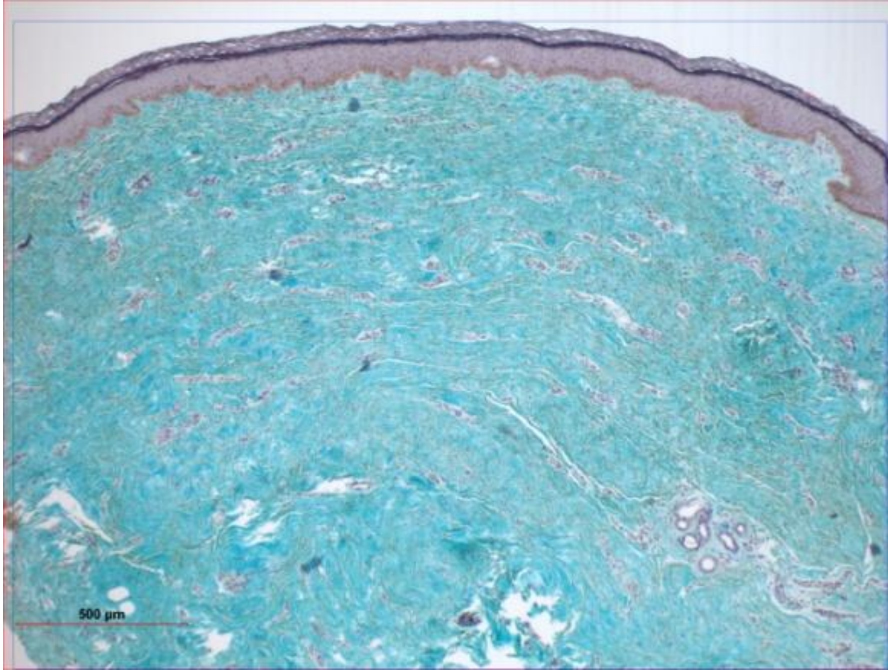
Figure 31 Histology Sections Stained with Masson's Trichrome at Month 5



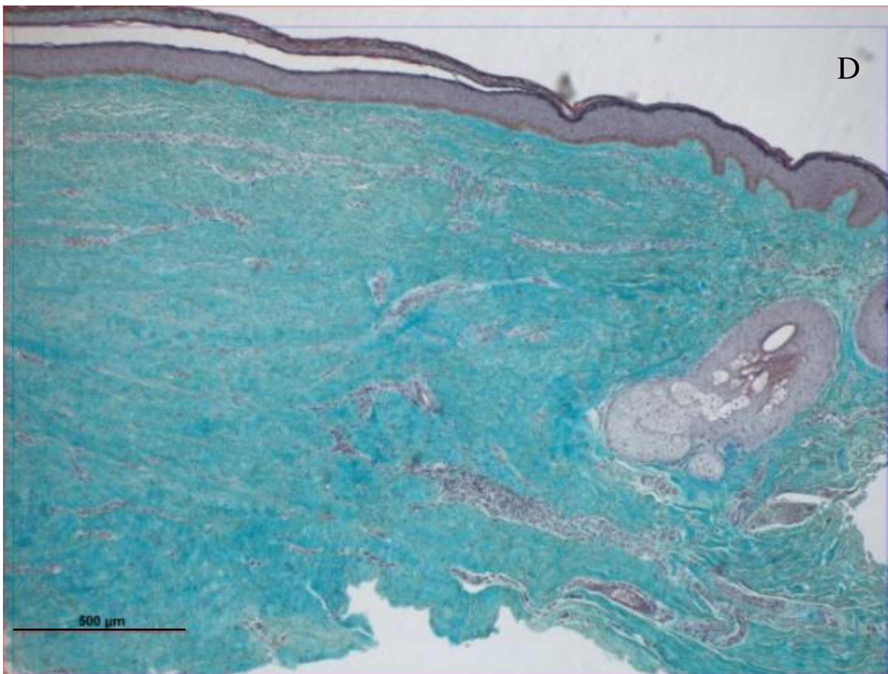
A Illustrating a Type 3 scar (Scale bar = 500µm)



B Illustrating a Type 4 scar (Scale bar = 500µm)



C Illustrating minimal inflammation in a Type 4 scar (Scale bar = 500µm)

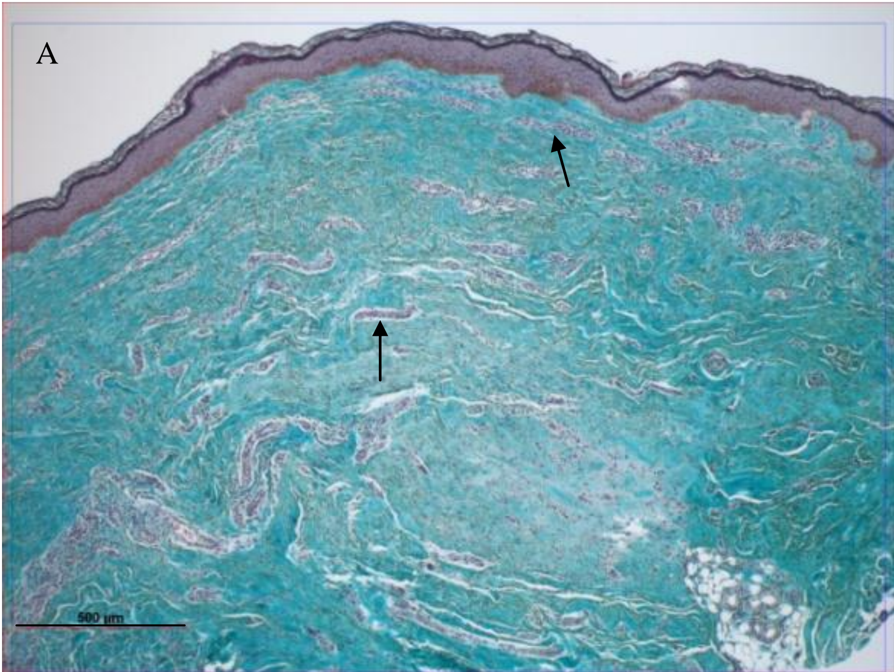


D Illustrating a Type 5 scar (Scale bar = 500µm)

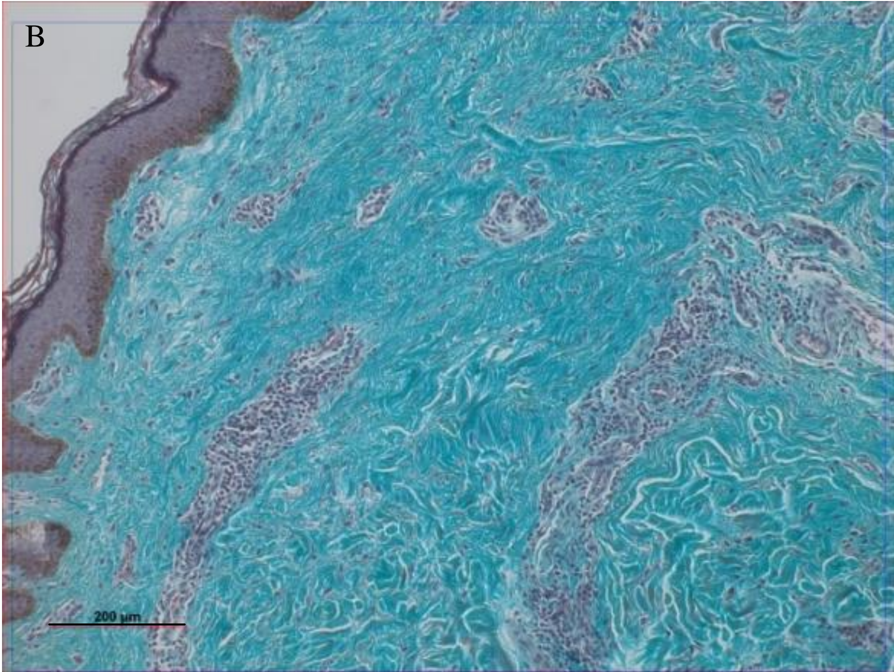
Table 28 Summary of Histological Findings - Month 6

	Month 6
Type 3	➢ Partial rete ridge reformation ➢ Normal epithelial thickness ➢ Pigment in the basal layer of the epidermis as in normal skin ➢ Narrow scar ➢ No inflammatory cells present ➢ Some restoration of the papillary dermis and maturation of dermal collagen ➢ No undermining of the wound margins
Type 4	➢ Some rete ridge reformation ➢ Maturing epithelium ➢ Pigment in the basal layer of the epidermis. Occasionally more pigment than in adjacent normal skin ➢ Wide scar ➢ Inflammatory cells still present especially near blood vessels ➢ Mostly immature parallel bands of collagen with early signs of remodelling and good restoration of papillary dermis ➢ Minimal undermining of the wound margins
Type 5	➢ Some rete ridge reformation ➢ Maturing thickened epithelium ➢ Wide scar ➢ Persistent inflammatory cell presence with giant cells present ➢ Remnants of degraded collagen arranged in degradation nodules; immature collagen in parallel bands surrounding nodules of degraded collagen and early restoration of papillary dermis ➢ Significant undermining of the wound margins with the neo-dermis significantly wider than the neo-epidermis

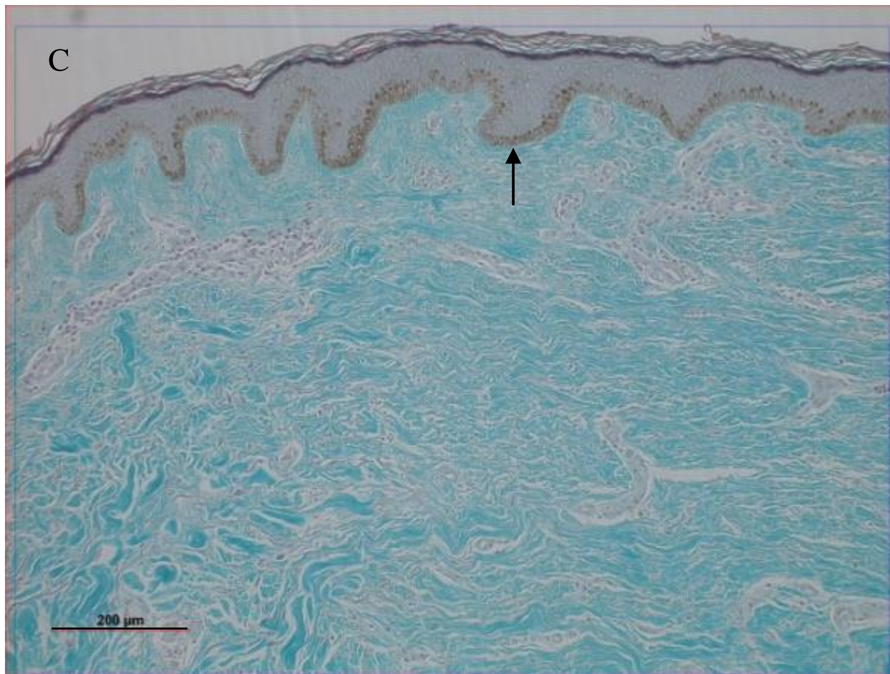
Figure 32 Histology Sections Stained with Masson's Trichrome at Month 6



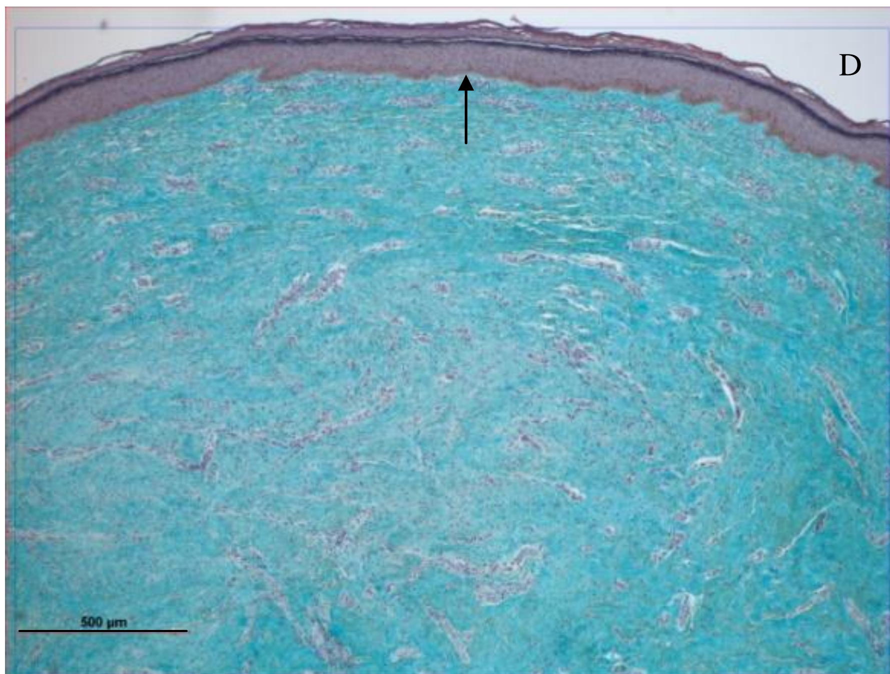
A Illustrating persistent inflammation in a Type 4 scar (Scale bar = 500µm)



B Illustrating a Type 4 scar (Scale bar = 200µm)



C Illustrating melanin staining across a Type 4 scar (Scale bar = 200µm)

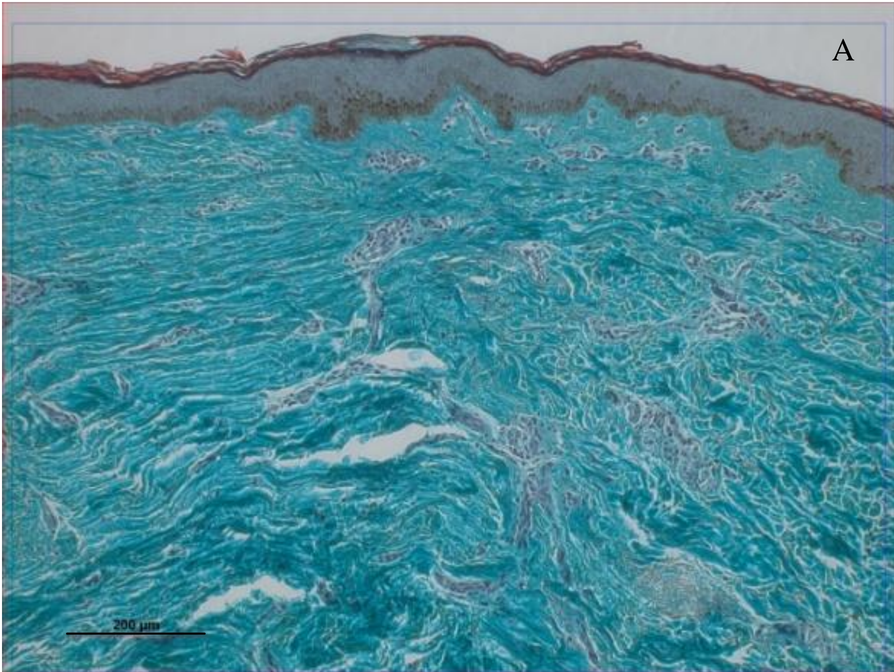


D Illustrating melanin staining across a Type 5 scar (Scale bar = 500µm)

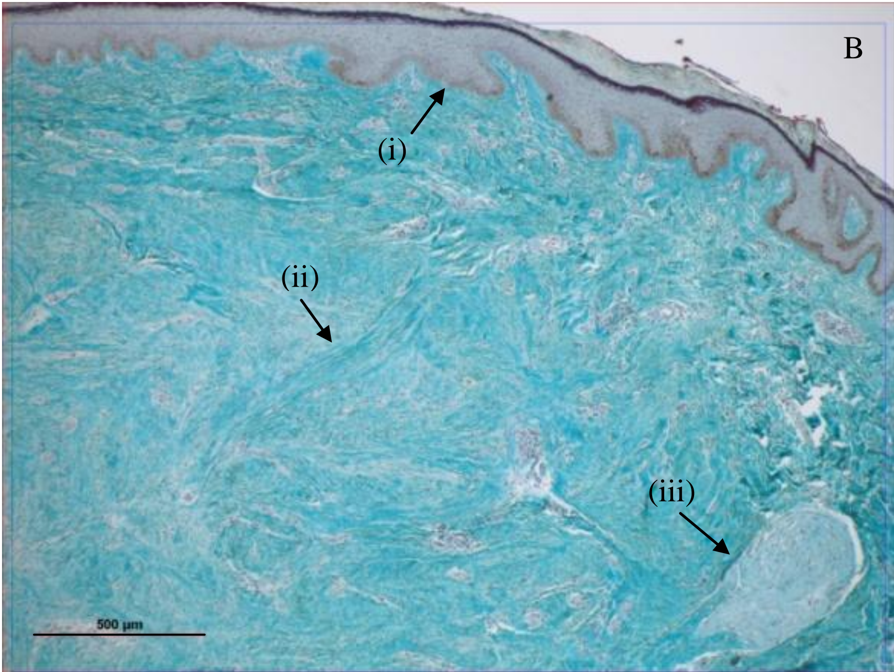
Table 29 Summary of Histological Findings - Month 7

	Month 7
Type 3	➢ Partial rete ridge reformation ➢ Normal epithelial thickness ➢ Pigment in the basal layer of the epidermis as in normal skin ➢ Narrow scar ➢ No inflammatory cells present ➢ Mature papillary dermis and mature reticular dermal collagen with some remodelling ➢ No undermining of the wound margins
Type 4	➢ Some rete ridge reformation ➢ Maturing epithelium ➢ Pigment in the basal layer of the epidermis and in supra basal layers with increased pigment at wound edges ➢ Wide scar ➢ Very few inflammatory cells present ➢ Parallel bands of collagen maturing with early signs of remodelling. Good restoration and maturity of papillary dermis ➢ Minimal undermining of the wound margins
Type 5	➢ Some rete ridge reformation ➢ Maturing thickened epithelium ➢ Pigment in the basal layer of the epidermis and in supra basal layers with increased pigment at wound edges ➢ Wide scar and can be elevated ➢ Minimal inflammatory cell infiltrate ➢ Collagen arranged in parallel bands beginning to mature surrounding nodules of degraded collagen and restoration of papillary dermis ➢ Significant undermining of the wound margins

Figure 33 Histology Sections Stained with Masson's Trichrome at Month 7



A Illustrating a Type 3 scar (Scale bar = 200µm)

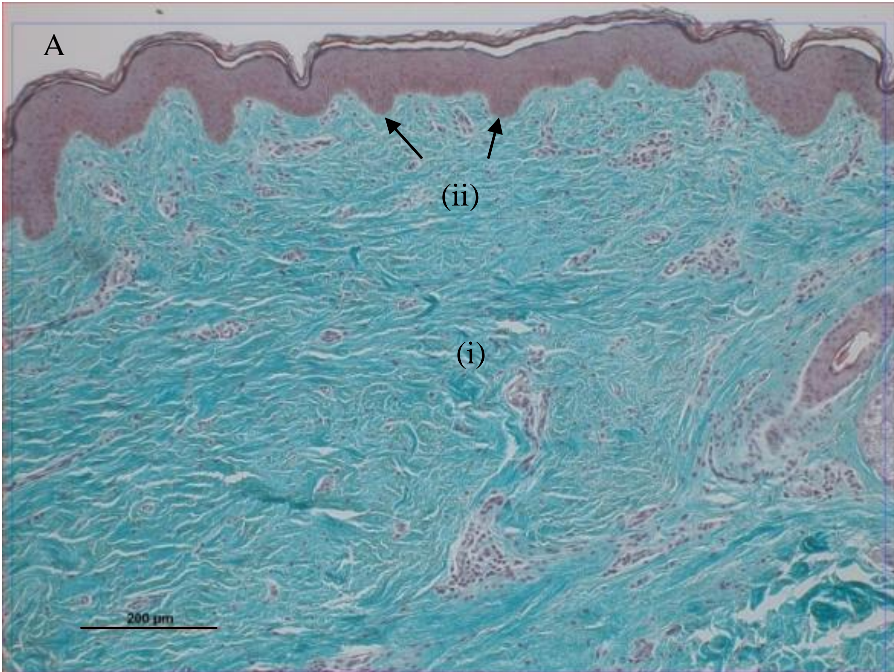


B Illustrating a Type 5 scar with (i) some epidermal restitution, (ii) degradation nodules and swirls of immature collagen, and (iii) a giant cell (Scale bar = 500µm)

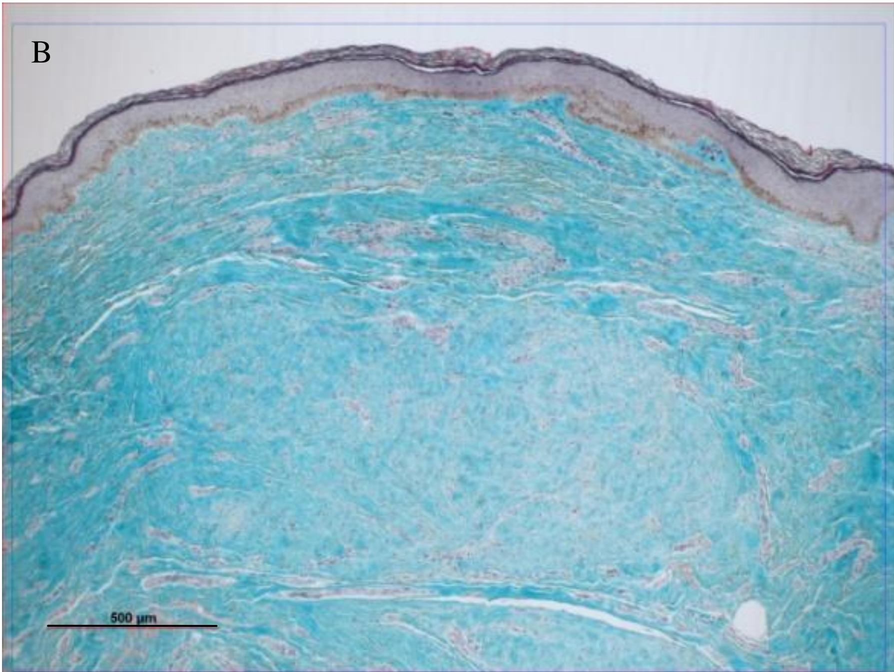
Table 30 Summary of Histological Findings - Month 8

	Month 8
Type 3	➢ Partial rete ridge reformation ➢ Pigment in the basal layer of the epidermis as in normal skin ➢ No inflammatory cells present ➢ Mature papillary dermis with good reformation and mature reticular dermal collagen with some basket weave remodelling ➢ No undermining of the wound margins
Type 4	➢ Some rete ridge reformation ➢ Maturing epithelium ➢ Pigment in the basal layer of the epidermis and in supra basal layers with increased pigment at wound edges ➢ Wide scar ➢ A few inflammatory cells present ➢ Parallel bands of collagen maturing with signs of remodelling; good restoration and maturity of papillary dermis nearing normal ➢ Minimal undermining of the wound margins
Type 5	➢ Good rete ridge reformation ➢ Maturing thickened epithelium ➢ Wide scar and can be elevated ➢ Pigment in the basal layer of the epidermis and in supra basal layers with increased pigment at wound edges ➢ Persistent significant numbers of inflammatory cells and giant cells present ➢ Remnants of degraded collagen arranged in degradation nodules; collagen arranged in parallel bands beginning to mature surrounding nodules of degraded collagen. Restoration of the papillary dermis ➢ Significant undermining of the wound margins

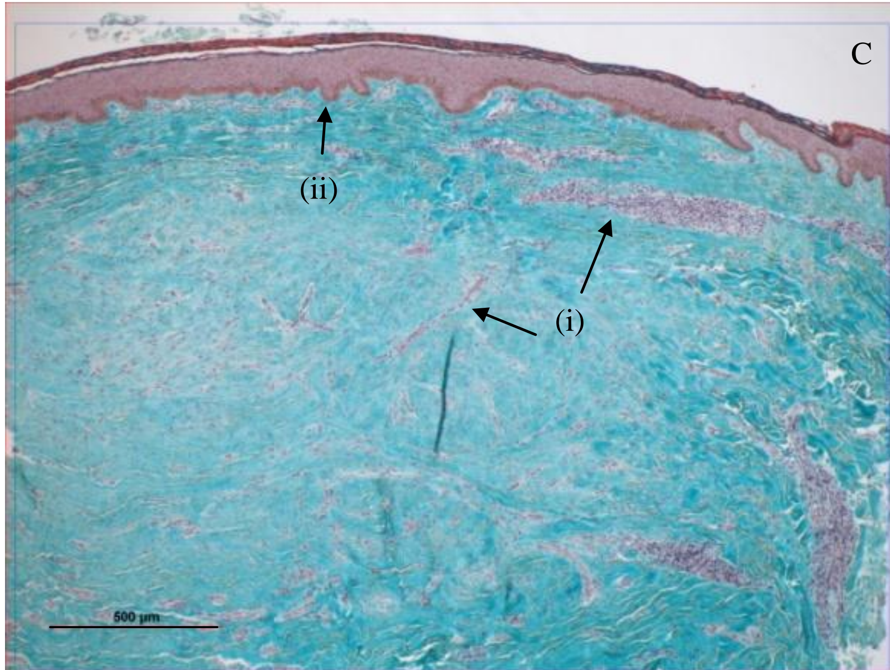
Figure 34 Histology Sections Stained with Masson's Trichrome of Month 8



A Illustrating a Type 3 scar with (i) early collagen basket weaving and (ii) rete ridge reformation (Scale bar = 200µm)



B Illustrating a Type 4 scar with degradation and resynthesis nodules (Scale bar = 500µm)

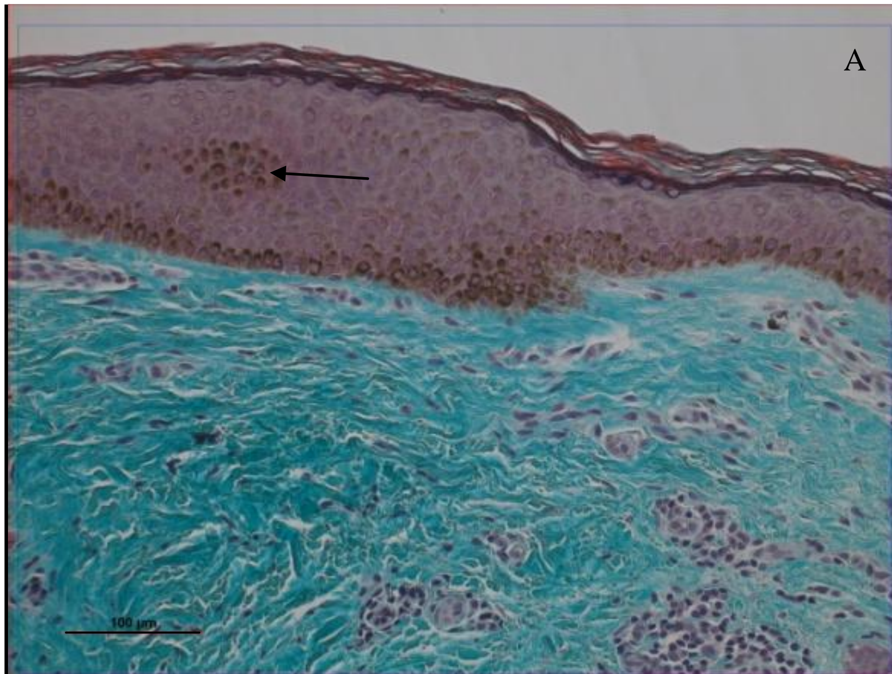


C Illustrating a Type 5 scar with (i) persistent inflammation and (ii) some rete ridge reformation (Scale bar = 500µm)

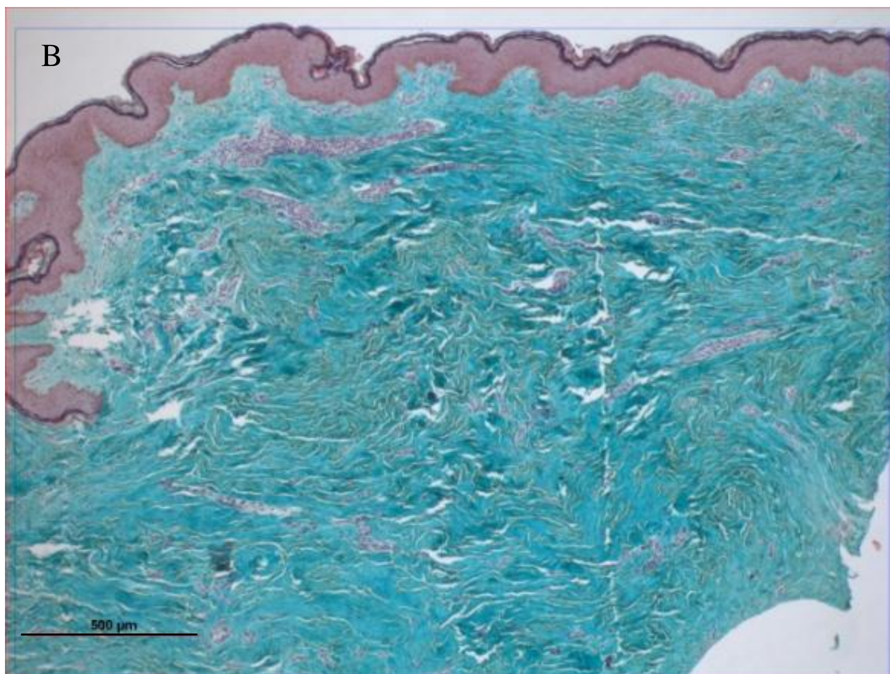
Table 31 Summary of Histological Findings - Month 9

	Month 9
Type 3	➢ Partial rete ridge reformation ➢ Pigment in the basal layer of the epidermis as in normal skin ➢ Narrow scar ➢ Minimal inflammatory cells present ➢ Mature papillary dermis with good reformation and mature reticular dermal collagen (up to 20% basket weave remodelling)
Type 4	➢ Some rete ridge reformation ➢ Maturing epithelium ➢ Pigment in the basal layer of the epidermis and in supra basal layers with increased pigment at wound edges ➢ Wide scar ➢ A few inflammatory cells present ➢ Parallel bands of mature collagen with signs of remodelling and early basket weave formation and good restoration and maturity of papillary dermis nearing normal ➢ Minimal undermining of the wound margin
Type 5	➢ Occasional good rete ridge reformation ➢ Maturing thickened epithelium ➢ Pigment in the basal layer of the epidermis and in supra basal layers with increased pigment at wound edges ➢ Wide scar and can be elevated ➢ Persistent significant number of inflammatory cells with giant cells present ➢ Collagen arranged in parallel bands beginning to mature and restoration of papillary dermis ➢ Significant undermining of the wound margin

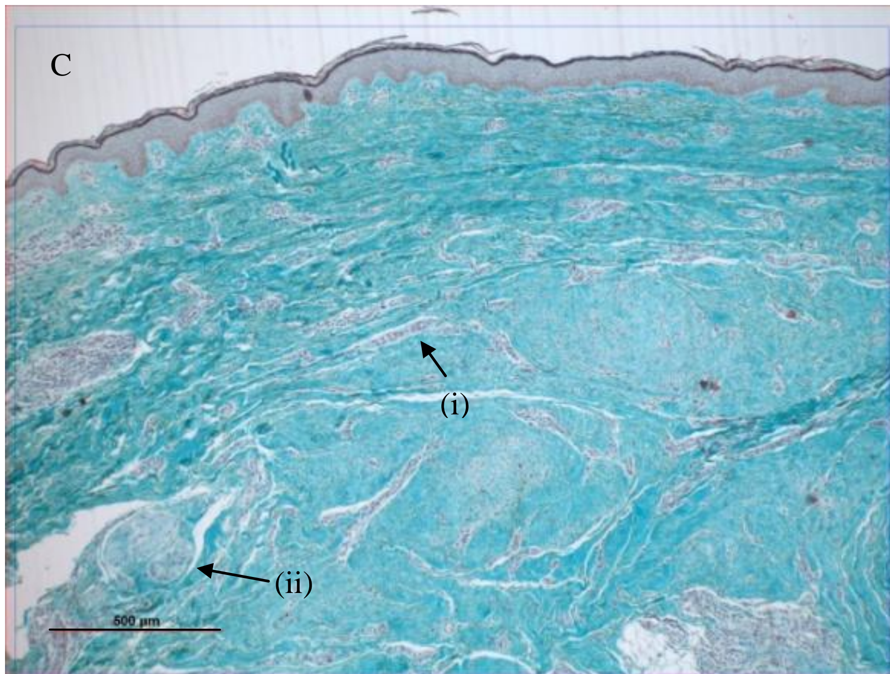
Figure 35 Histology Sections Stained with Masson's Trichrome at Month 9



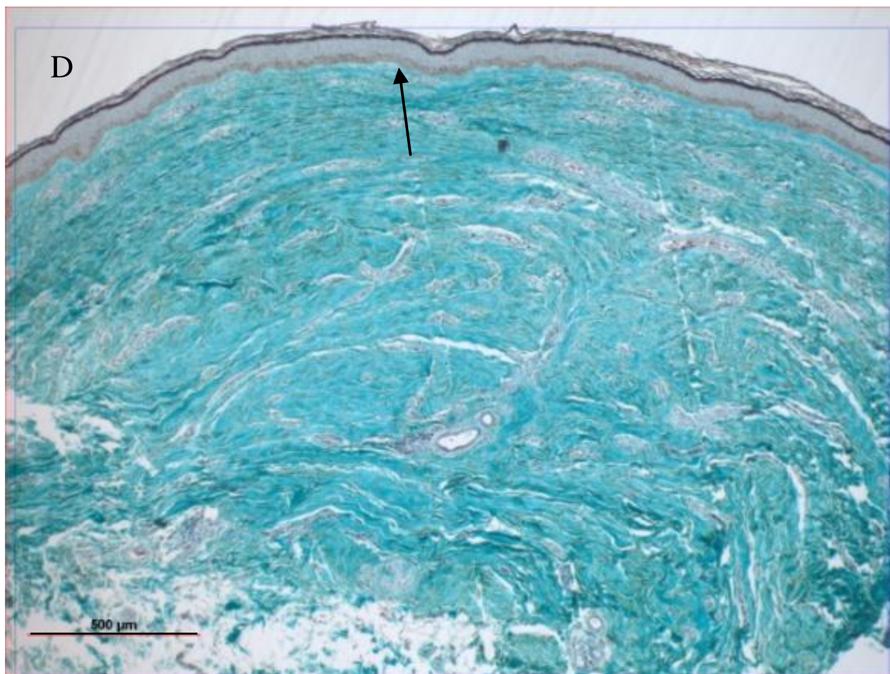
A Illustrating a Type 3 scar with melanin in the suprabasal layers of the epidermis. (Scale bar = 100µm)



B Illustrating a Type 4 scar with mature collagen and some basket weaving (Scale bar = 500µm)



C Illustrating a Type 5 scar with (i) persistent inflammation and (ii) a giant cell. (Scale bar = 500μm)

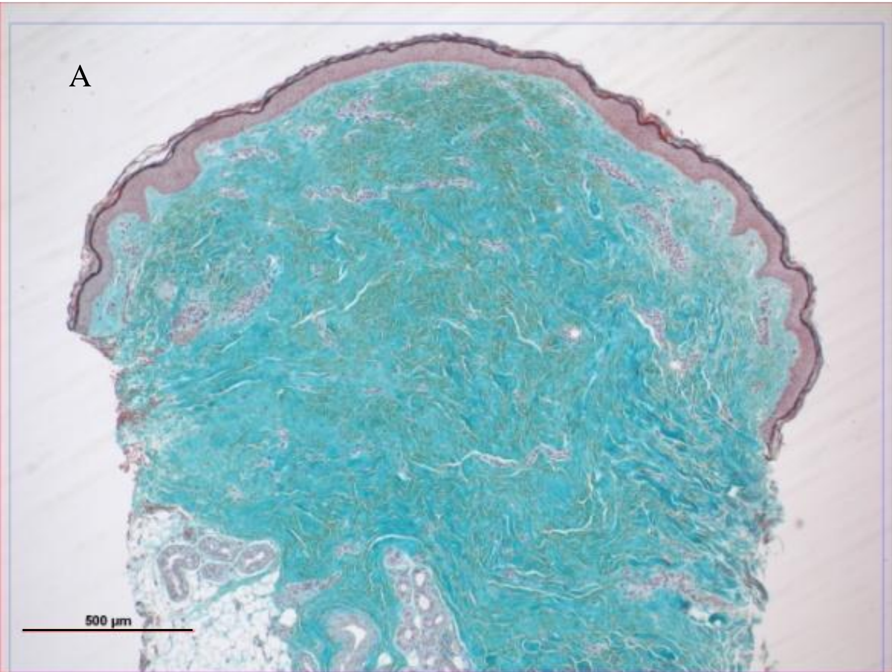


D Illustrating a Type 5 scar with poor reformation of rete ridges (Scale bar = 500μm)

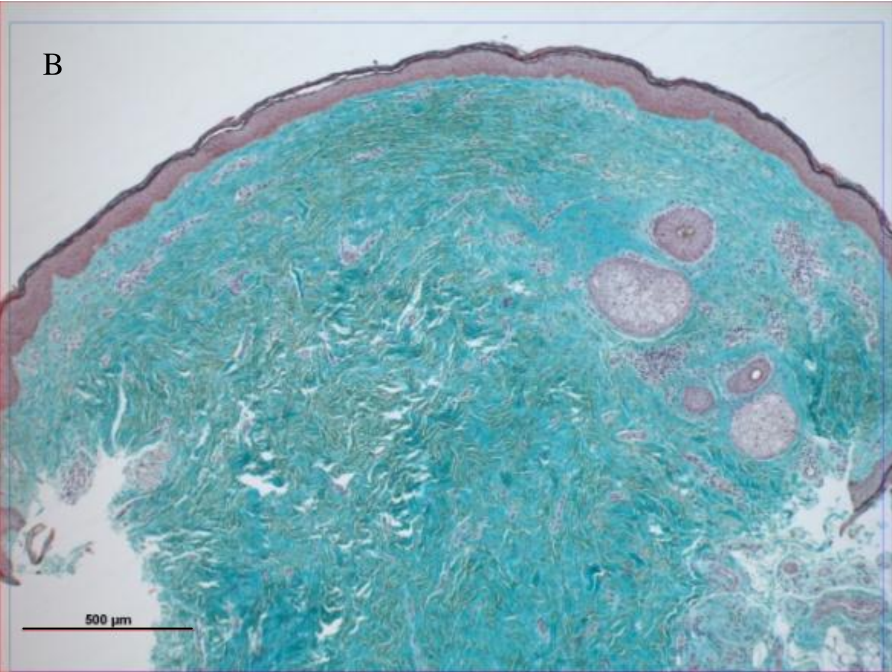
Table 32 Summary of Histological Findings - Month 10

	Month 10
Type 3	➢ Partial rete ridge reformation ➢ Pigment in the basal layer of the epidermis as in normal skin ➢ Minimal inflammatory cells present ➢ Mature papillary dermis with good reformation and mature reticular dermal collagen with up to 20% basket weave remodelling
Type 4	➢ Some rete ridge reformation ➢ Mature epithelium ➢ Pigment in the basal layer of the epidermis as in normal skin with increased pigment at wound edges ➢ Wide scar ➢ A few inflammatory cells present ➢ Parallel bands of mature collagen with signs of remodelling and early basket weave formation and good restoration and maturity of papillary dermis nearing normal ➢ Minimal undermining at the wound margin
Type 5	➢ Good rete ridge reformation in some scars ➢ Normal epithelial thickness ➢ Pigment in the basal layer of the epidermis and in supra basal layers with increased pigment at wound edges ➢ Wide scar and can be elevated ➢ Persistently significant amount of inflammatory cells ➢ Good restoration of papillary dermis seen and collagen arranged in parallel bands slow to mature ➢ Significant undermining of the wound margins

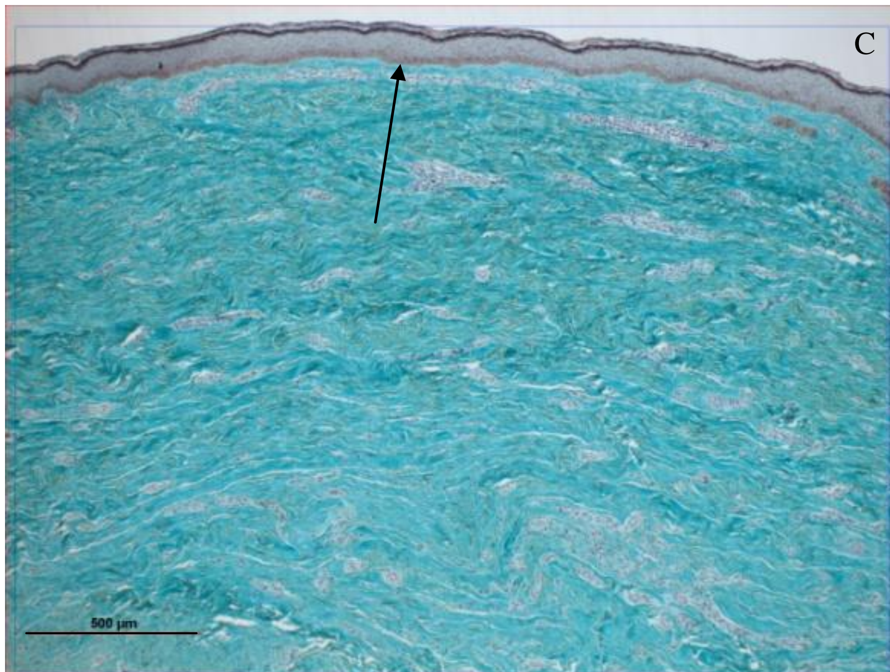
Figure 36 Histology Sections Stained with Masson's Trichrome at Month 10



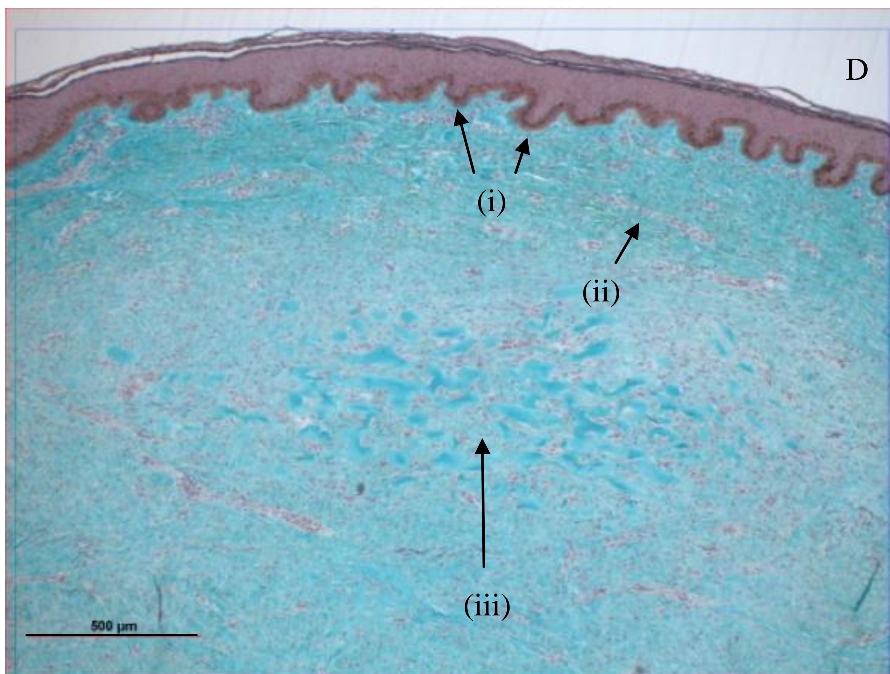
A Illustrating a Type 3 scar (Scale bar = 500µm)



B Illustrating a Type 4 scar with good collagen maturation. (Scale bar = 500µm)



C Illustrating minimal rete ridge restoration in a Type 5 scar (Scale bar = 500μm)

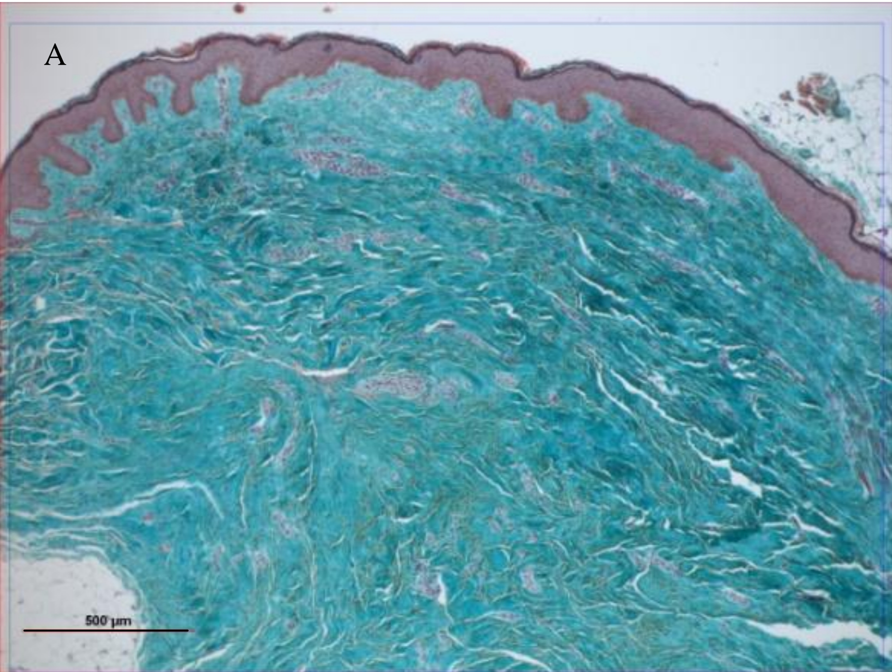


D Illustrating a Type 5 scar with (i) good rete ridge reformation, (ii) persistent inflammation and evidence of (iii) a previously very high turnover rate (Scale bar = 500μm)

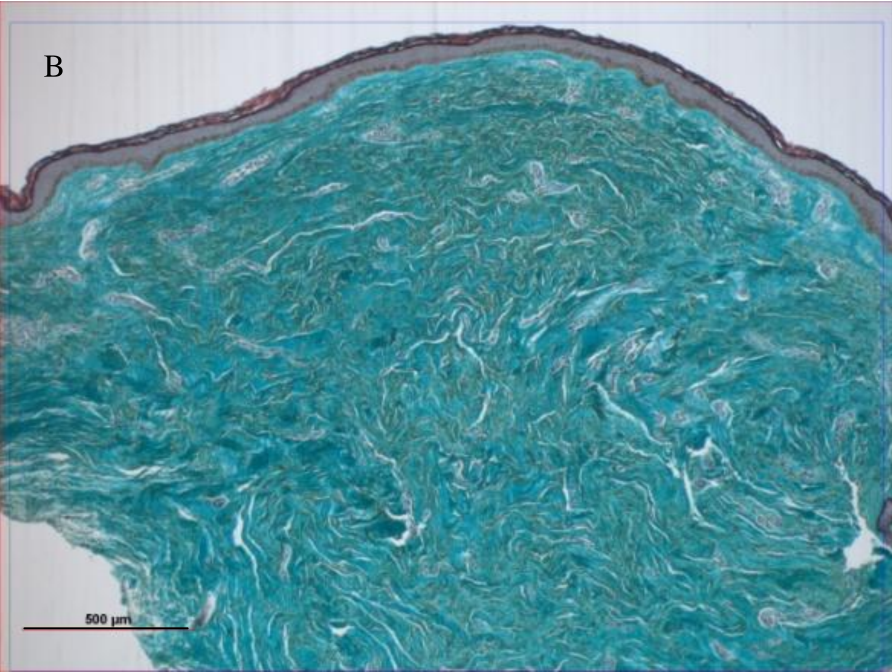
Table 33 Summary of Histological Findings - Month 11

	Month 11
Type 3	➢ Partial rete ridge reformation ➢ Pigment in the basal layer of the epidermis as in normal skin with increased pigment at wound edges ➢ Narrow scar ➢ Occasional inflammatory cell present ➢ Mature papillary dermis with good reformation almost as normal and mature reticular dermal collagen with up to 20% basket weave remodelling
Type 4	➢ Some rete ridge reformation ➢ Mature epithelium ➢ Pigment in the basal layer of the epidermis as in normal skin with increased pigment at wound edges ➢ A few inflammatory cells present ➢ Good restoration and maturity of papillary dermis, parallel bands of mature collagen with signs of remodelling and early basket weave formation in the reticular dermis ➢ Minimal undermining of the wound margins
Type 5	➢ Good rete ridge reformation in some ➢ Normal epithelial thickness ➢ Pigment in the basal layer of the epidermis as in normal skin with increased pigment at wound edges ➢ Very wide scars ➢ Significant amount of inflammatory cells even giant cells present ➢ Good restoration of papillary dermis and collagen arranged in parallel bands showing signs of maturing. Upper dermis more mature than lower ➢ Moderate undermining of the wound margins

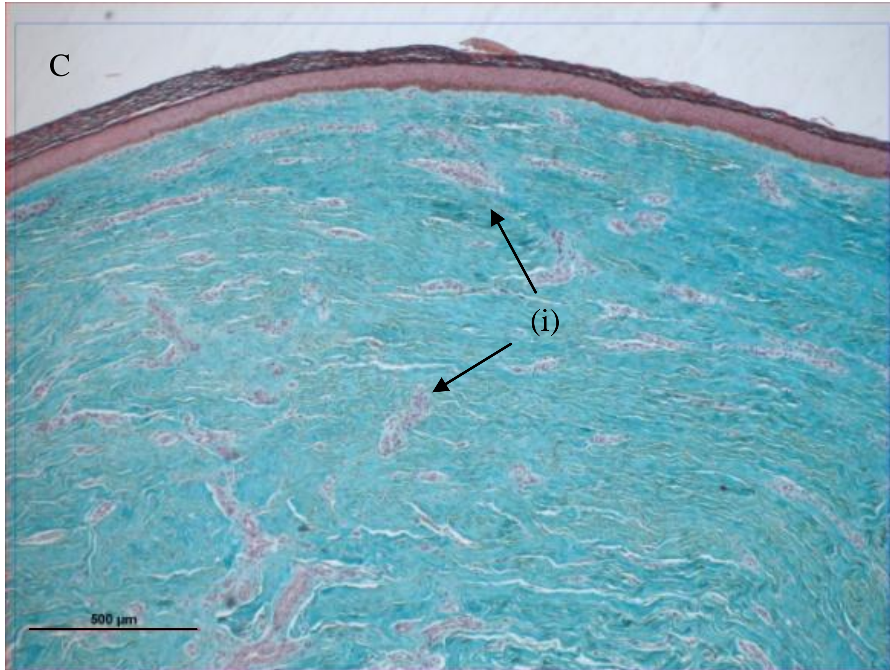
Figure 37 Histology Sections Stained with Masson's Trichrome at Month 11



A Illustrating a Type 3 scar with mature collagen and some basket weaving (Scale bar = 500μm)



B Illustrating a Type 4 scar with maturing new collagen (Scale bar = 500μm)

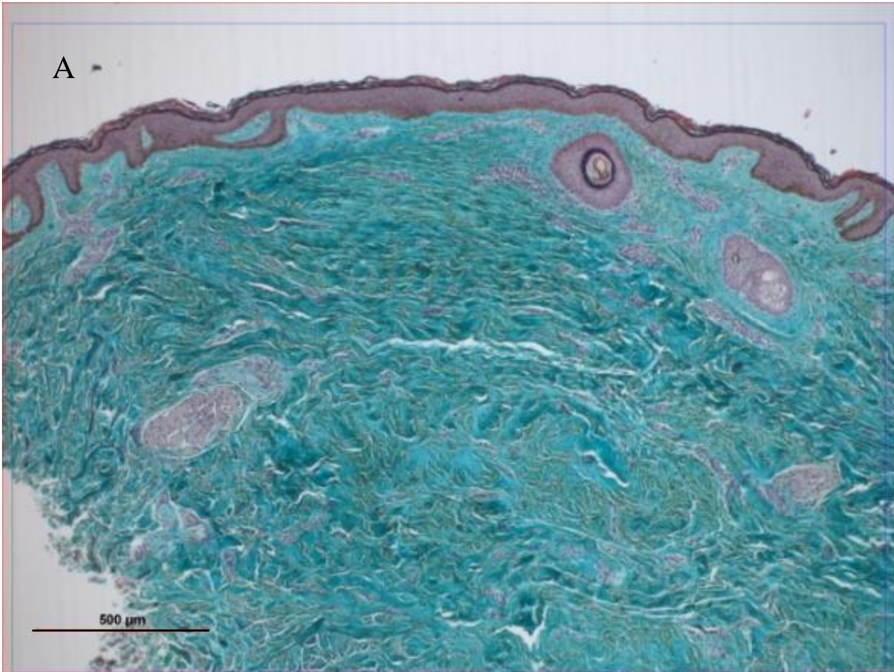


C Illustrating a Type 5 with (i) residual inflammatory cells in a very wide scar (Scale bar = 500**µm**)

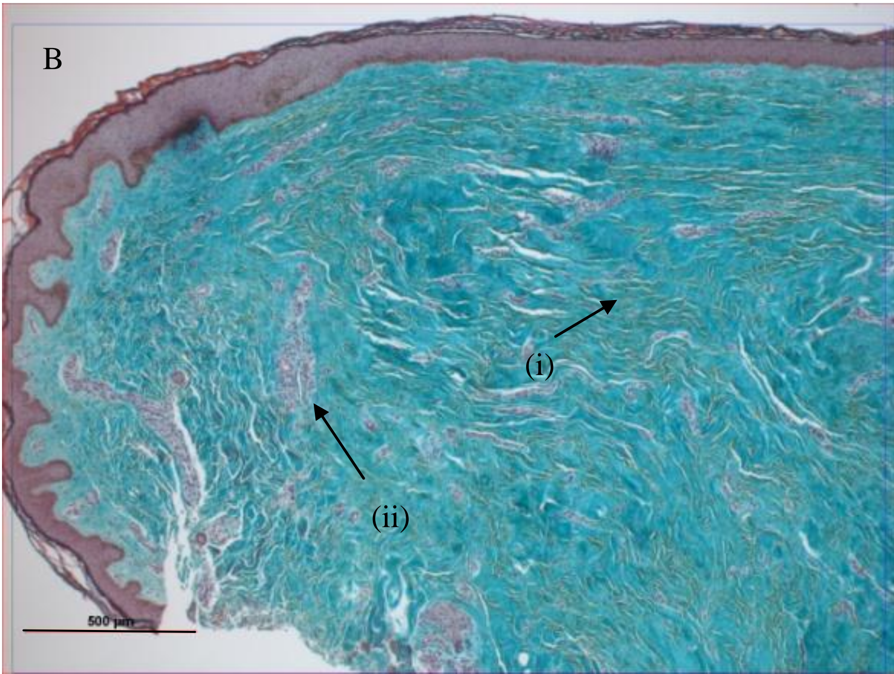
Table 34 Summary of Histological Findings - Month 12

	Month 12
Type 3	➢ Rete ridge reformation not complete ➢ Pigment in the basal layer of the epidermis almost as in normal skin ➢ Narrow scar ➢ Occasional inflammatory cells still present ➢ Mature papillary dermis with good reformation almost as normal and mature reticular dermal collagen with evidence of a basket weave pattern arrangement
Type 4	➢ Good rete ridge reformation ➢ Mature epithelium ➢ Pigment in the basal layer of the epidermis as in normal skin with increased pigment at wound edges ➢ A few inflammatory cells present ➢ Good restoration and maturity of papillary dermis, parallel bands of very mature collagen with signs of remodelling and good basket weave formation in the lower reticular dermis ➢ Minimal undermining of the wound margins
Type 5	➢ Good rete ridge reformation in some scars ➢ Normal epithelial thickness ➢ Pigment in the basal layer of the epidermis as in normal skin with increased pigment at wound edges ➢ Very wide scars ➢ Very few inflammatory cells ➢ Good restoration of papillary dermis and collagen arranged in parallel bands now mature. Upper dermis more mature than lower with some basket weaving present ➢ Moderate undermining of wound margins

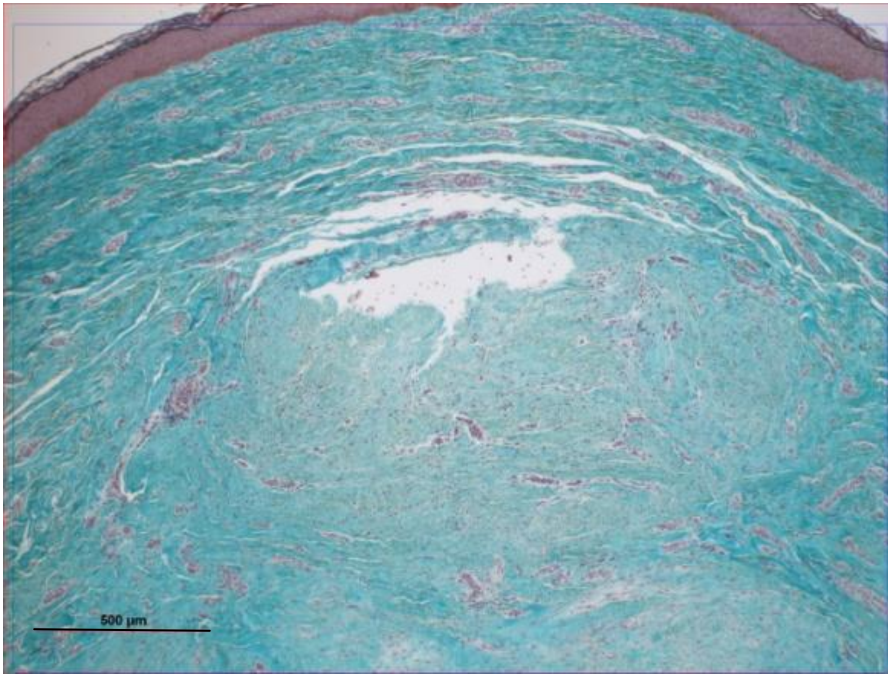
Figure 38 Histology Sections Stained with Masson's Trichrome at Month 12



A Illustrating increasing basket weaving of new collagen in a Type 3 scar (Scale bar = 500μm)



B Illustrating a Type 4 with (i) good collagen maturation and (ii) some residual inflammation (Scale bar = 500μm)



C Illustrating a Type 5 scar with a previously high turnover state (Scale bar = 500μm)

Table 35 Summary of Histological Features of Scars by Month and Scar Type (Part I)

Histological Feature	Month 1			Month 2			Month 3			Month 4		
	Type 3	**Type 4**	**Type 5**	**Type 3**	**Type 4**	**Type 5**	**Type 3**	**Type 4**	**Type 5**	**Type 3**	**Type 4**	**Type 5**
Rete Ridge reformation	**0**	**0**	**0**	+	+	**0**	+	+	**0**	++	+	+
Epithelial maturity	+	+	+	++	++	++	++	++	++	++	++	++
Pigmentation	**0**	**0**	**0**	+	+	+	++	++	++	+++	++	++
Scar Width	+	++	+++	+	++	+++	+	++	+++	+	++	+++
Inflammatory Cells	+	+++	++++	+	++	++++	+	++	++++	+	++	++++
Maturity of the Dermis	+	+	+	++	++	++	++	++	++	+++	++	++
Undermining of wound margins	+	+	+++	+	++	+++	+	+	+++	**0**	+	+++

Key:
Rete Ridge Restoration: 0 = none; + = some; ++ = partial; +++ = good
Epithelial Maturity: + = immature; ++ = maturing; +++ = mature
Pigmentation: 0 = none; + = some; ++ = present throughout scar but in an abnormal pattern; +++ = present as in normal skin
Scar Width: + = narrow; ++ = moderate; +++ = wide; ++++ = very wide
Inflammatory Cells: 0 = none; + = only a few; ++ = moderate numbers; +++ = significant numbers
Maturity of the Dermis: Range from + = immature to +++++ = good maturity
Wound Margins: 0 = no undermining; + = minimal undermining; ++ = moderate undermining; +++ = significant undermining

Table 36 Summary of Histological Features of Scars by Month and Scar Type (Part II)

Histological Feature	Month 5			Month 6			Month 7			Month 8		
	Type 3	**Type 4**	**Type 5**	**Type 3**	**Type 4**	**Type 5**	**Type 3**	**Type 4**	**Type 5**	**Type 3**	**Type 4**	**Type 5**
Rete Ridge reformation	++	+	+	++	+	+	++	+	+	++	+	+++
Epithelial maturity	++	++	++	+++	++	++	+++	++	++	+++	++	++
Pigmentation	+++	++	++	+++	++	++	+++	++	++	+++	++	++
Scar Width	+	+++	+++	+	+++	+++	+	+++	+++	+	+++	+++
Inflammatory Cells	**0**	+	++	**0**	++	+++	**0**	+	+	**0**	+	+++
Maturity of the Dermis	+++	+++	++	+++	+++	++	++++	+++	++	++++	++++	+++
Undermining of wound margins	**0**	+	+++	**0**	+	+++	**0**	+	+++	**0**	+	+++

Key:
Rete Ridge Restoration: 0 = none; + = some; ++ = partial; +++ = good
Epithelial Maturity: + = immature; ++ = maturing; +++ = mature
Pigmentation: 0 = none; + = some; ++ = present throughout scar but in an abnormal pattern; +++ = present as in normal skin
Scar Width: + = narrow; ++ = moderate; +++ = wide; ++++ = very wide
Inflammatory Cells: 0 = none; + = only a few; ++ = moderate numbers; +++ = significant numbers
Maturity of the Dermis: Range from + = immature to +++++ = good maturity
Wound Margins: 0 = no undermining; + = minimal undermining; ++ = moderate undermining; +++ = significant undermining

Table 37 Summary of Histological Features of Scars by Month and Scar Type (Part III)

Histological Feature	Month 9			Month 10			Month 11			Month 12		
	Type 3	**Type 4**	**Type 5**	**Type 3**	**Type 4**	**Type 5**	**Type 3**	**Type 4**	**Type 5**	**Type 3**	**Type 4**	**Type 5**
Rete Ridge reformation	++	+	+++	++	+	+++	++	+	+++	+++	+++	+++
Epithelial maturity	+++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Pigmentation	+++	++	++	+++	++	++	++	++	++	+++	++	++
Scar Width	+	+++	+++	+	+++	+++	+	++	+++	+	++	++++
Inflammatory Cells	+	+	+++	+	+	+++	+	+	+++	+	+	+
Maturity of the Dermis	++++	++++	+++	+++++	++++	+++	+++++	++++	+++	+++++	+++++	++++
Undermining of wound margins	**0**	+	+++	**0**	+	+++	**0**	+	+++	**0**	+	+++

Key:
Rete Ridge Restoration: 0 = none; + = some; ++ = partial; +++ = good
Epithelial Maturity: + = immature; ++ = maturing; +++ = mature
Pigmentation: 0 = none; + = some; ++ = present throughout scar but in an abnormal pattern; +++ = present as in normal skin
Scar Width: + = narrow; ++ = moderate; +++ = wide; ++++ = very wide
Inflammatory Cells: 0 = none; + = only a few; ++ = moderate numbers; +++ = significant numbers
Maturity of the Dermis: Range from + = immature to +++++ = good maturity
Wound Margins: 0 = no undermining; + = minimal undermining; ++ = moderate undermining; +++ = significant undermining

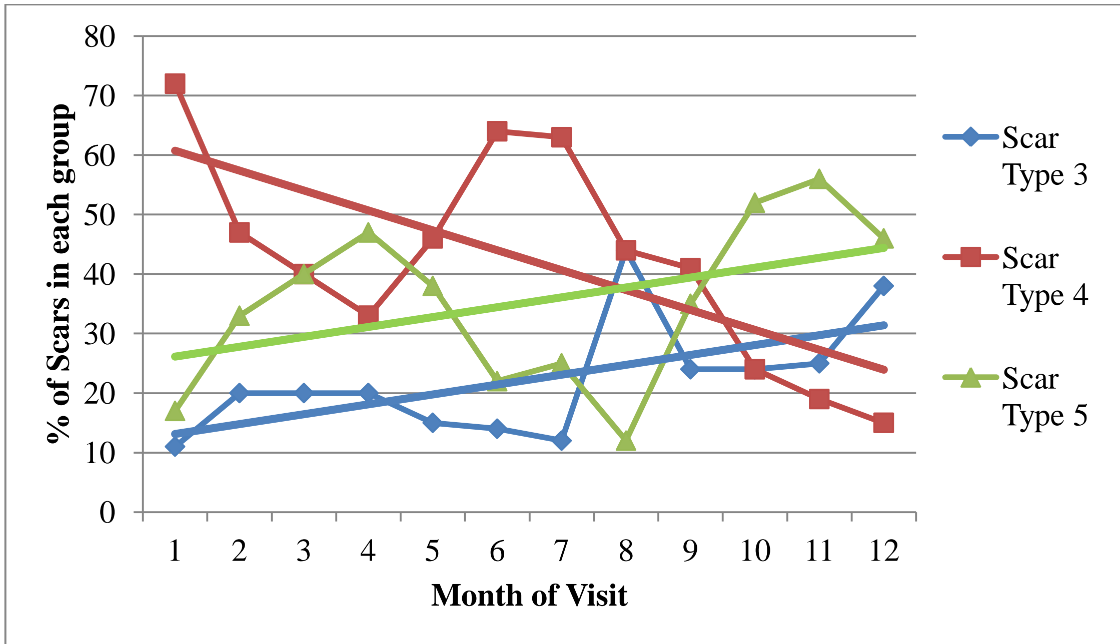
4.412 Scar Specimen Type by Month of Excision

All scar specimens were described as being Type 3, Type 4 and Type 5. Figure 39 and Table 38 consider the percentage of scars in each group at each monthly visit. At Month 1 the majority of scars have been classed Type 4 (72%). Over the 12 month period there are many fluctuations. Plotted trend lines show Type 3 and Type 5 scars steadily increase up to Month 12. At Month 12 Type 5 scars are the most common (46%) followed closely by Type 3 (38%). Only 15% of scars are classed as Type 4 at Month 12 in contrast to 72% at Month 1.

Table 38 Percentage of Scars in Each Group by Month

MONTH	SCAR TYPE		
	3	4	5
1	11%	72%	17%
2	20%	47%	33%
3	20%	40%	40%
4	20%	33%	47%
5	15%	46%	38%
6	14%	64%	22%
7	12%	63%	25%
8	44%	44%	12%
9	24%	41%	35%
10	24%	24%	52%
11	25%	19%	56%
12	38%	15%	46%

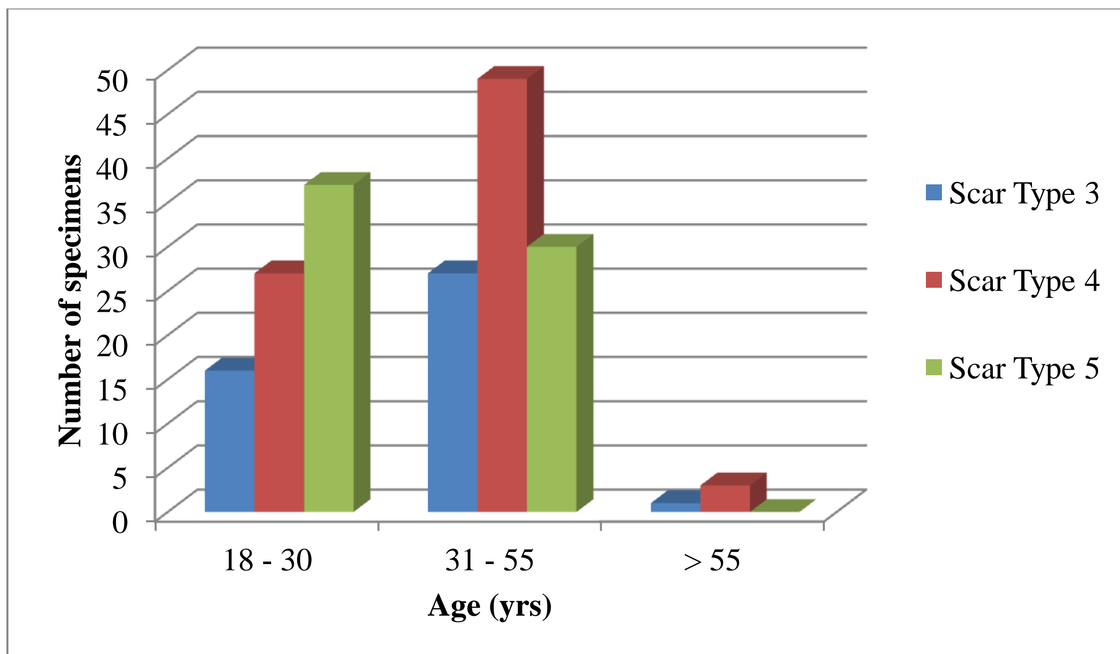
Figure 39 The Percentage of Scars in Each Group by Month



4.413 Scar Specimen Type by Age Group

In the 18-30 age group the majority of scar specimens are Type 5 scars with the minority Type 3 (Figure 40). In the age group 31-55 the majority of scar specimens are Type 4 scars with similar numbers of Type 3 and Type 4. As before there is not enough data to comment on the age group > 55.

Figure 40 Number of Scar Specimens by Scar Type in Each Age Group



4.414 Scar Specimen Type by Fitzpatrick Skin Type

For volunteers with Fitzpatrick Skin Type IV the majority of scars assessed histologically were classed as Type 4 scars, followed by Type 3 and a smaller number of Type 5 (Figure 41). For volunteers with Fitzpatrick Skin Type V the majority of scars are classed as Type 5 scars with the minority classed as Type 3 scars. For volunteers with Fitzpatrick Skin Type VI the majority of scars are classed as Type 4 scars with fewer scars classed as Type 3 than any of the other Skin Types.

Figure 41 Number of Scar Specimens by Scar Type in Each Fitzpatrick Skin Type Group

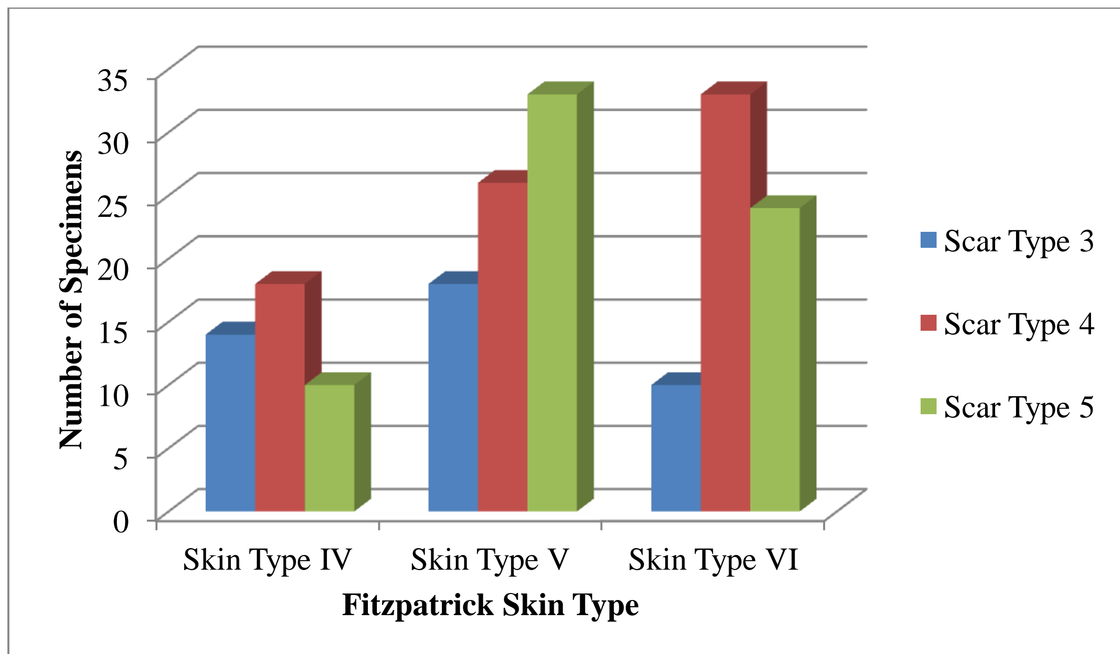


Figure 42 Percentage of Scars of Each Scar Type by Fitzpatrick Skin Type

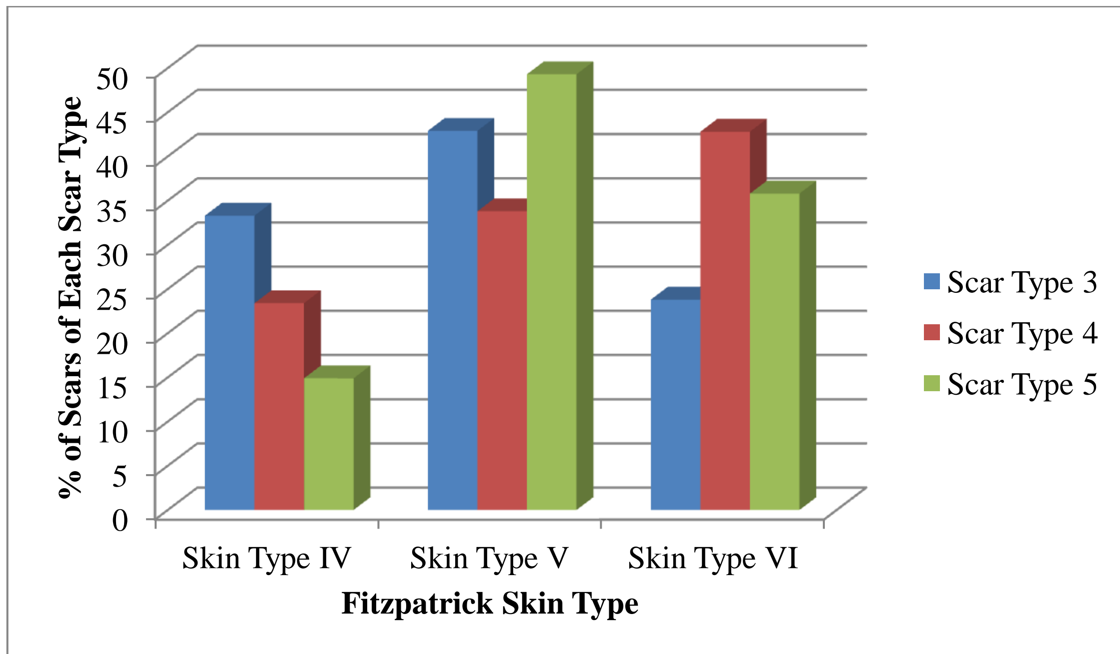


Figure 42 illustrates the percentage of each Scar Type within each Fitzpatrick Skin Type Grouping. The largest percentage of Type 3 scars was in Skin Type V and the lowest percentage is in Skin Type VI. The percentage of Type 4 scars increased as the Skin Type increased with the largest percentage in Skin Type VI. Type 5 scars were present in Skin Type IV volunteers but in much fewer numbers than Skin Type V and VI.

4.42 Histological Scar Assessments

Specimens were also assessed by 2 trained histologists according to a modified version of the microscopic VAS of Beausang et al (1998). The specimens were scored according to the following criteria which are set out in more detail in the materials and methods section 2.51:

- Epidermal Restitution
- Angiogenesis and Inflammation
- Collagen Organisation
- Visual Analogue Scale Assessment of Collagen Organisation
- Other Scar Features

4.421 Epidermal Restitution

The restoration of rete ridges and epithelial thickness at the scar area were assessed by comparing these to the surrounding normal skin. The restoration of rete ridges occurs slowly (Figure 43). At month 1 three quarters of scars show no restoration of rete ridges and one quarter some restoration. At month 12 over half of the scars have some restoration evident. However just under fifty percent of the scars continue to show no restoration and only 10% of scars are considered to have normal rete ridge restoration. The process of rete ridge restoration does not appear to be complete at month12.

Figure 43 Rete Ridge Restoration

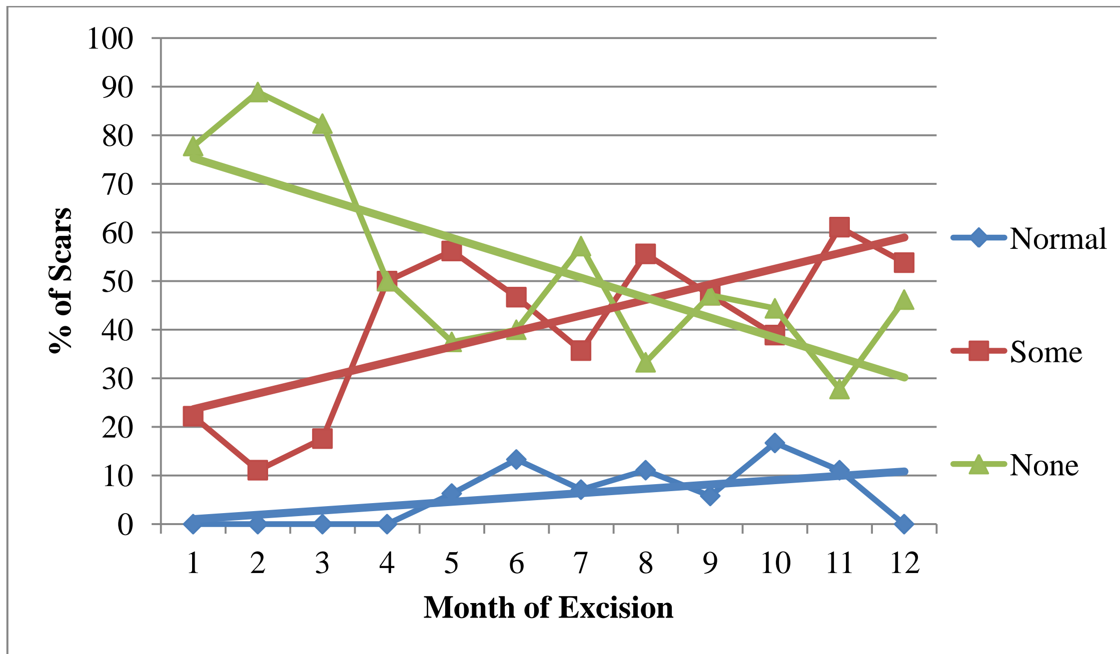


Figure 44, Figure 45 and Figure 46 illustrate rete ridge restoration in the different scar types described earlier in this Chapter. Interestingly, the scars that achieve normal rete ridge restoration were mainly classed as Type 5 (worst) scars (Figure 44).

Figure 44 Normal Rete Ridge Restoration

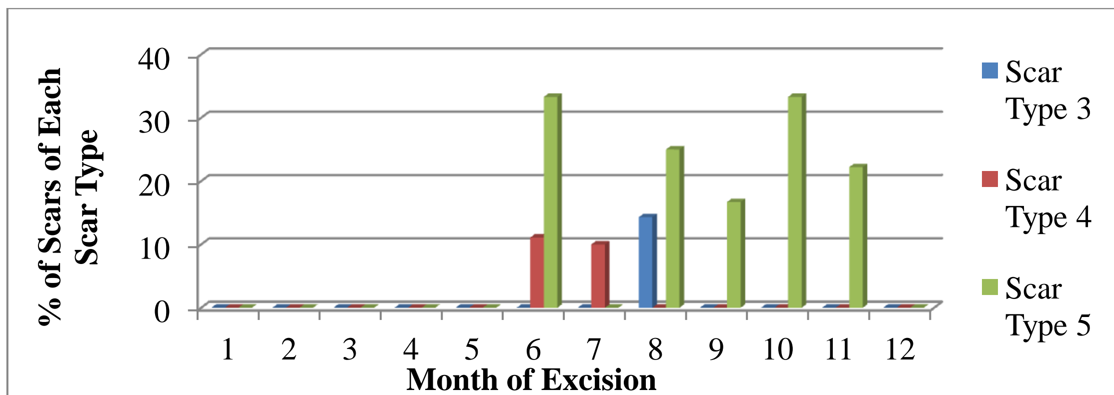
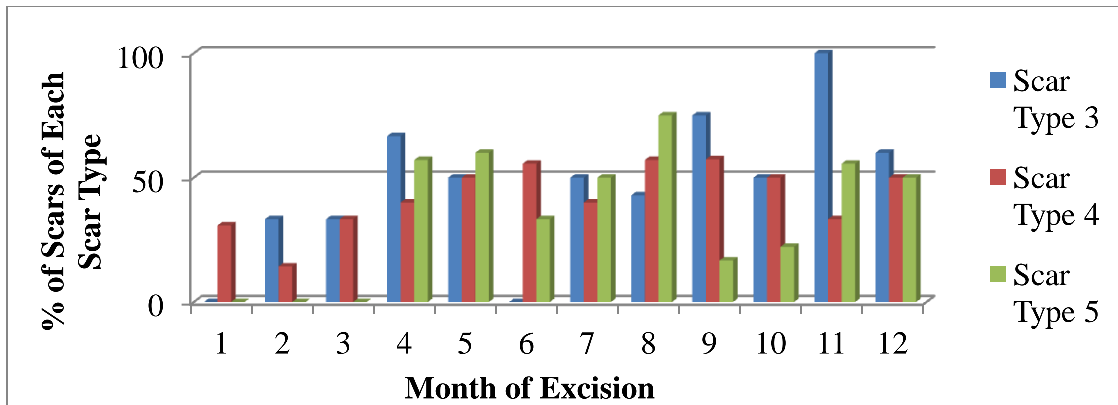
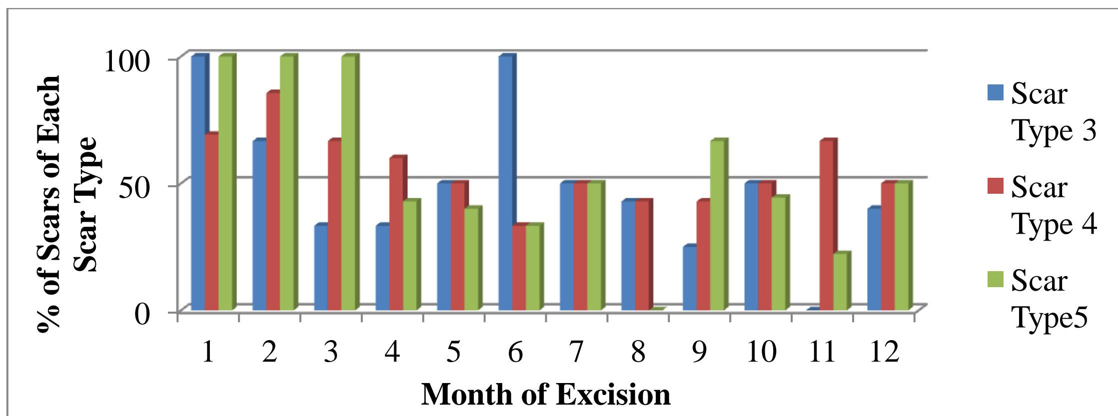


Figure 45 Some Rete Ridge Restoration



Approximately 50% of all scar types have some rete ridge restoration by month 12 (Figure 45). Type 5 scars do not show evidence of rete ridge formation until month 4.

Figure 46 No Rete Ridge Restoration



There is no rete ridge reformation in Type 5 scars until Month 4 (Figure 46). By month 12 approximately 50% of each scar type are assessed to have no rete ridge restoration.

Epithelial thickness is restored comparatively quicker than rete ridges (Figure 47). At month 1 90% of scars have a thicker epithelium than normal and 10% have normal epithelial thickness. By Month 12 the opposite is the case with over 90% of scars having normal epithelial thickness. No scars were scored as having a thinner epithelium. Restoration of epithelial thickness is almost complete by Month12.

Figure 47 Epithelial Thickness

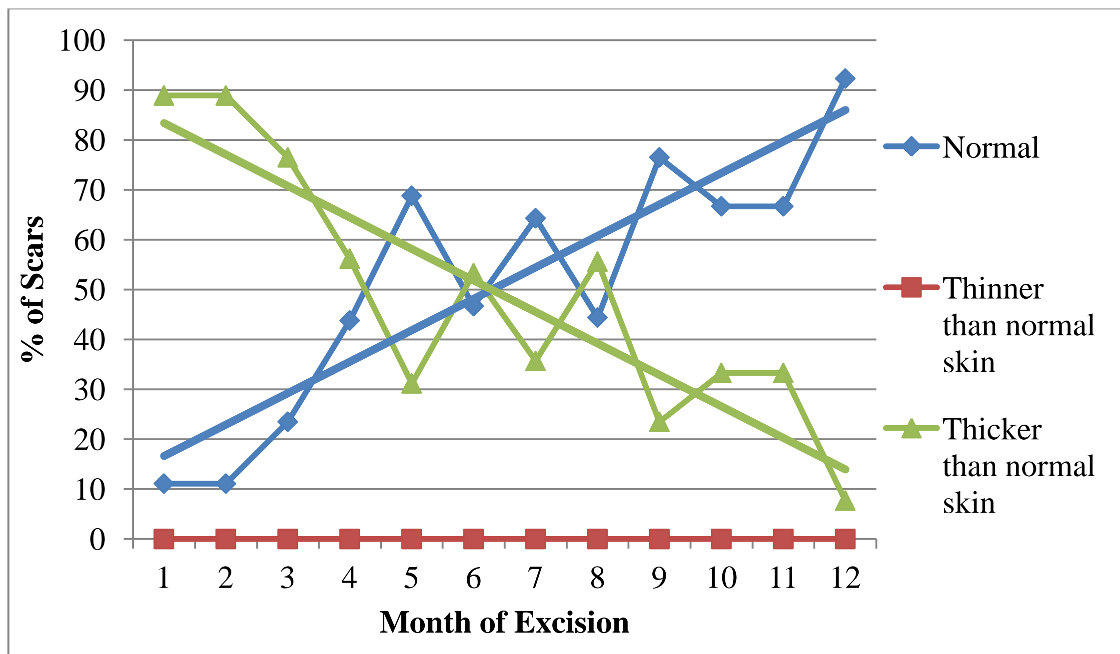


Figure 48 and Figure 49 illustrate the restoration of epithelial thickness in the different scar types. Figure 48 demonstrates the gradual return of normal epithelial thickness across the Scar Types over 12 months. However, the process appears to be slower in Type 5 scars. Figure 49 shows that at month 12 it is only Type 5 scars that continue to have a thicker epithelium Type 3 and Type 4 scars have normal epithelial thickness.

Figure 48 Normal Epithelial Thickness

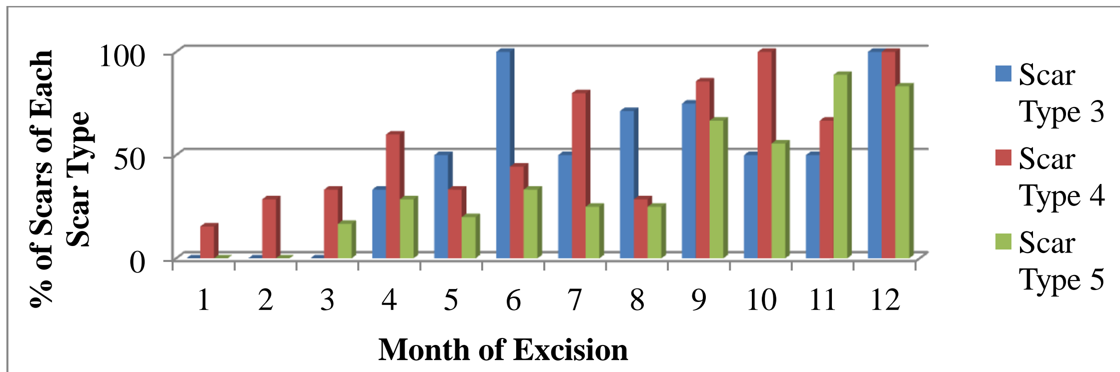
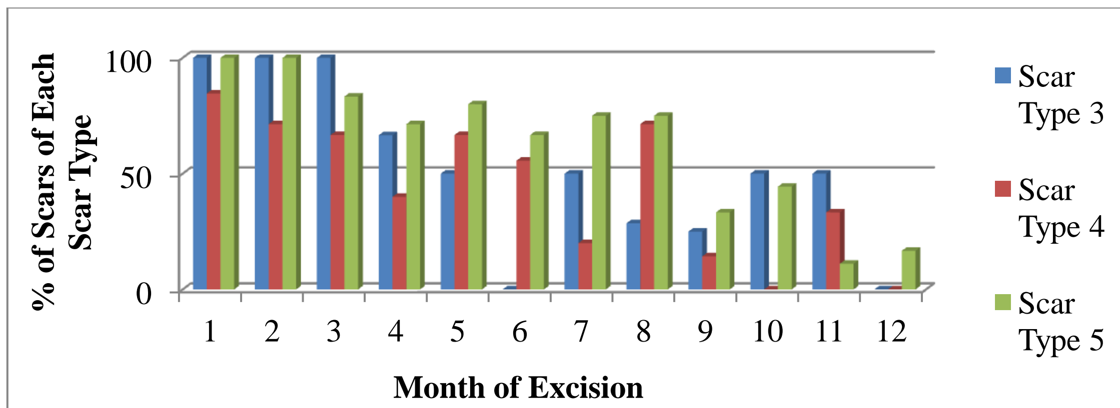


Figure 49 Thicker Epithelium



4.422 Angiogenesis and Inflammation

The number and size of blood vessels and the level of inflammation in the scar were assessed by comparing these to the surrounding normal skin. The percentage of scars, with more blood vessels than normal skin, increased steadily from 15% at month 1 to 70% at month 12 (Figure 50). On the other hand, 60% of scars had less than the normal number of blood vessels at month 1 and by month 4 this dropped to approximately 20% and continued to decline to month 12. About one third of scars showed a similar number of blood vessels to normal dermis over the 12 months of the study. Therefore at month 12 over two thirds of scars had more blood vessels and one

third a similar number of blood vessels to normal dermis. A steady state has not been reached and it is likely that increased angiogenesis will continue within the greater proportion of these scars for a number of months beyond month 12.

Figure 50 Angiogenesis - Number of Blood Vessels

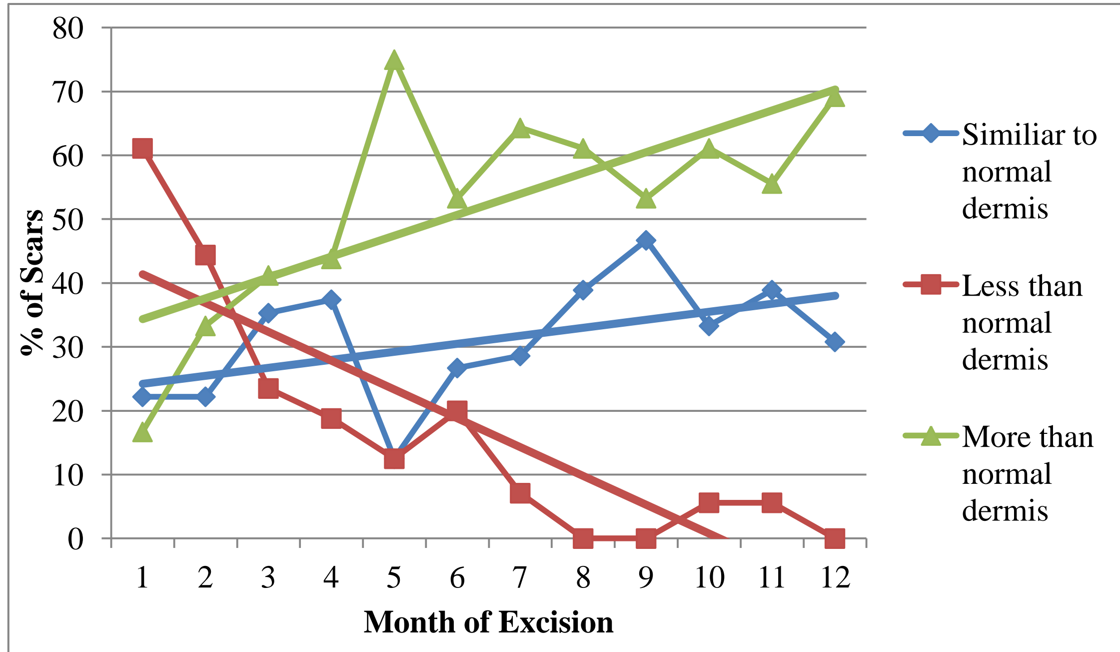


Figure 51, Figure 52 and Figure 53 illustrate the number of blood vessels according to the different scar types. A greater proportion of Type 3 scars are noted to have a similar number of blood vessels to normal dermis (Figure 51). Figure 53 shows that Type 5 scars are mostly classed as having more blood vessels than normal dermis. No clear trend is seen in Figure 52 which illustrates the data for scars classed as having fewer blood vessels than normal dermis.

Figure 51 Number of Blood Vessels - Similar to Normal Dermis

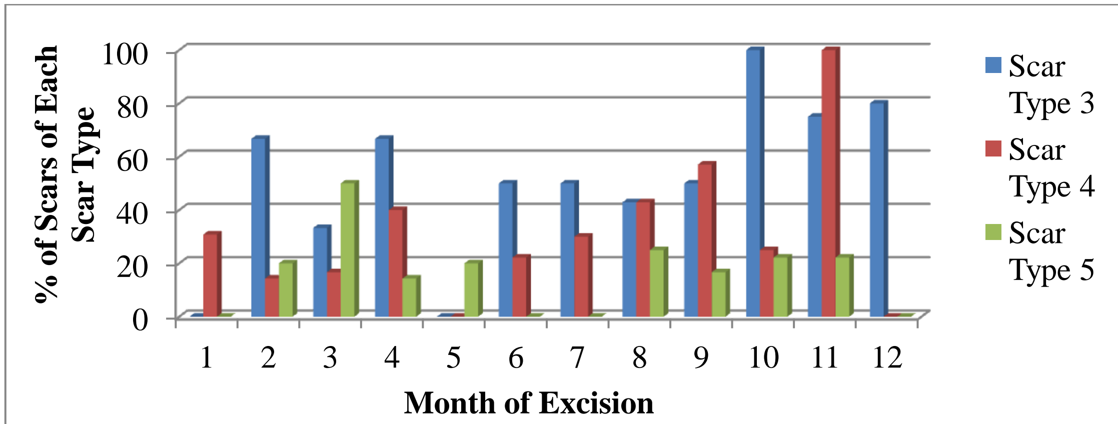


Figure 52 Number of Blood Vessels - Less than Normal Dermis

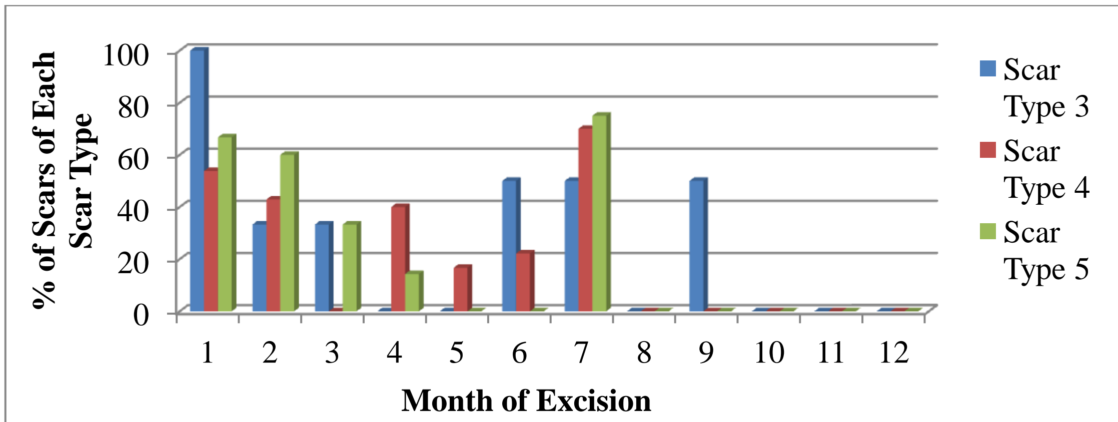
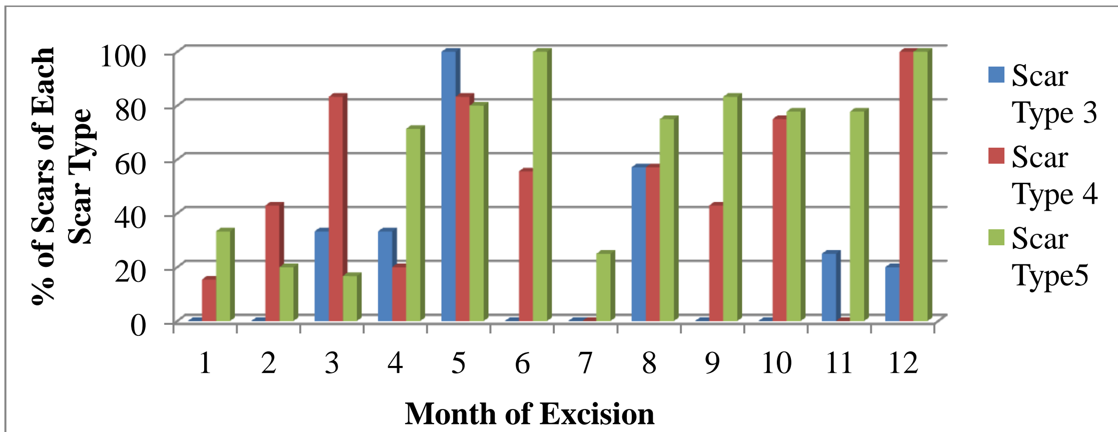


Figure 53 Number of Blood Vessels - More Than Normal Dermis



Blood vessel size in the majority of scars is similar to normal dermis (Figure 54) with about 15% of scars showing blood vessels of either greater or smaller size than normal dermis. This is seen throughout the 12 month period.

Figure 54 Angiogenesis - Size of Blood Vessels

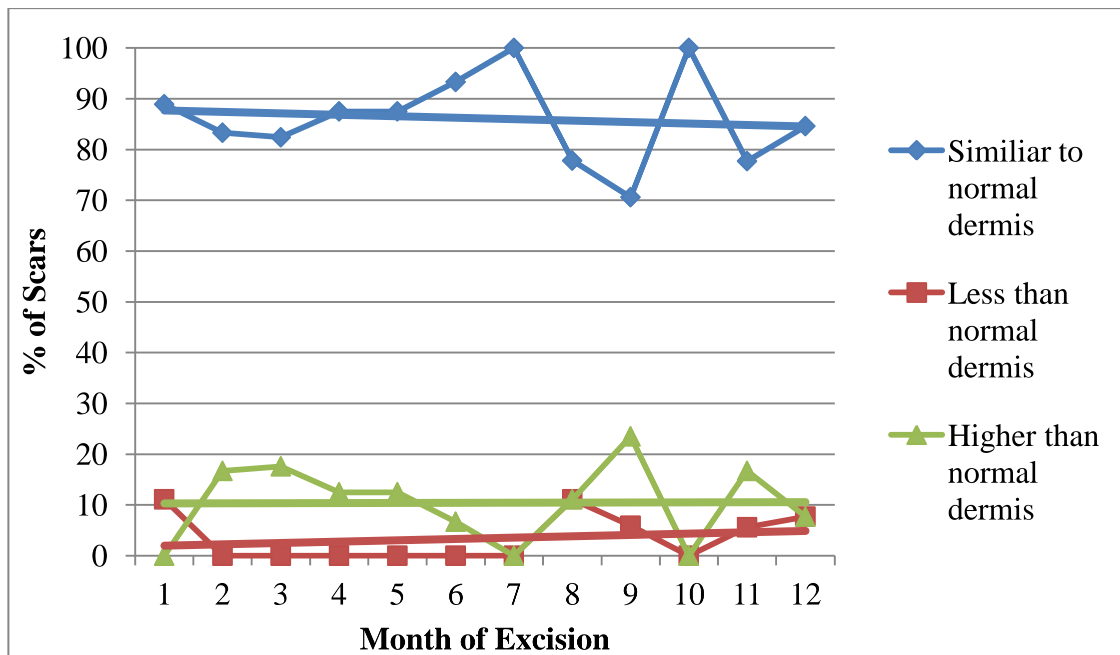


Figure 55, Figure 56 and Figure 57 illustrate blood vessel size according to the different scar types. Type 3 scars have mostly normal sized blood vessels (Figure 55). Of the scars that are assessed as having blood vessels of a greater size than normal they are mainly Type 5 scars (Figure 57). Very few scars were considered to have smaller blood vessel size that that of normal dermis but of those that did they were mainly Type 4 scars (Figure 56).

Figure 55 Blood Vessel Size Similar to Normal Dermis

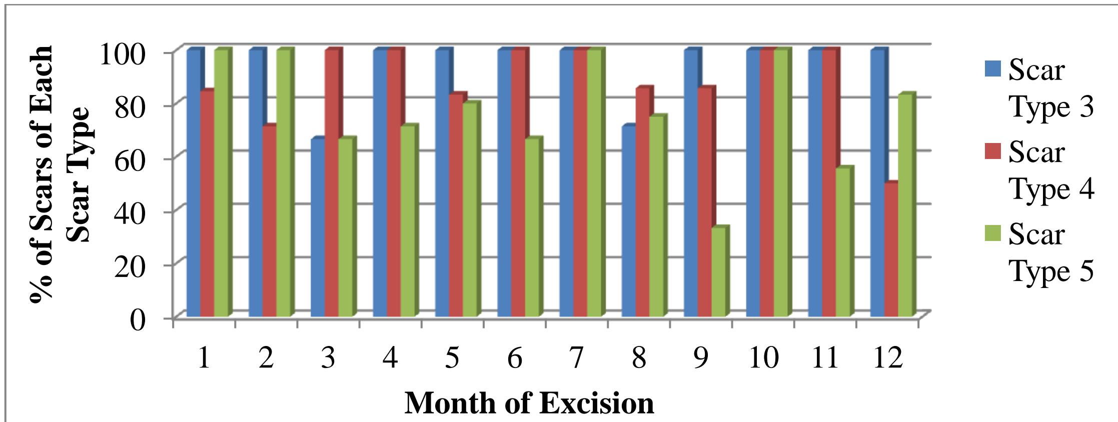


Figure 56 Blood Vessel Size Less Than Normal Dermis

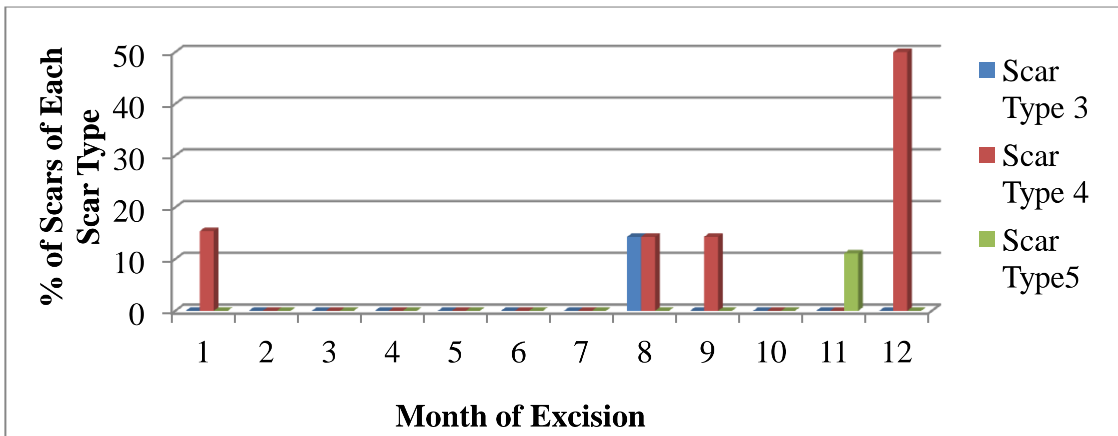
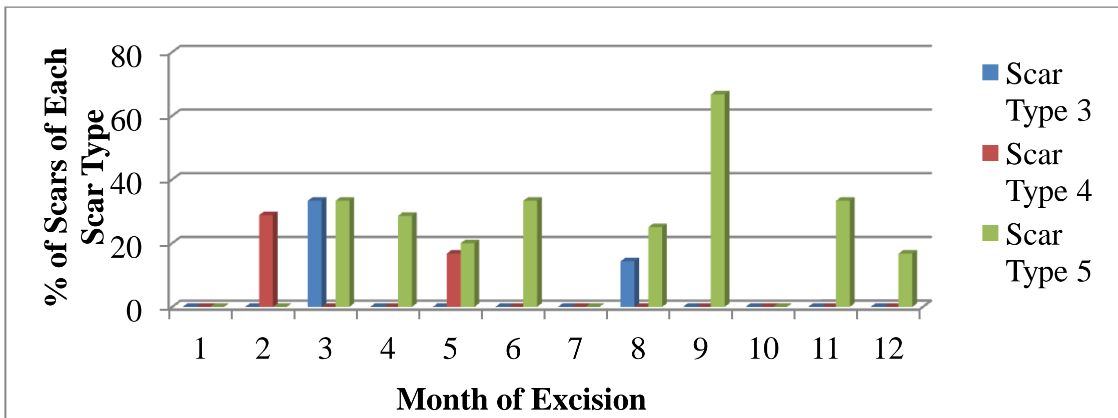


Figure 57 Blood Vessel Size Greater Than Normal Dermis



The level of inflammation in the scar appears to be similar to normal dermis by Month 7 at the latest and as early as month 4 (Figure 58). Between month 1 and 3 up to 20% of scars show levels of inflammation greater than normal. This falls to zero by month 4 and except for a small rise at month 6 remains so for the rest of the 12 months. Inflammation in the scar is similar to normal dermis by Month 7 onwards. No Type 3 scars demonstrate a level of inflammation higher than normal dermis (Figure 59).

Figure 58 Level of Inflammation

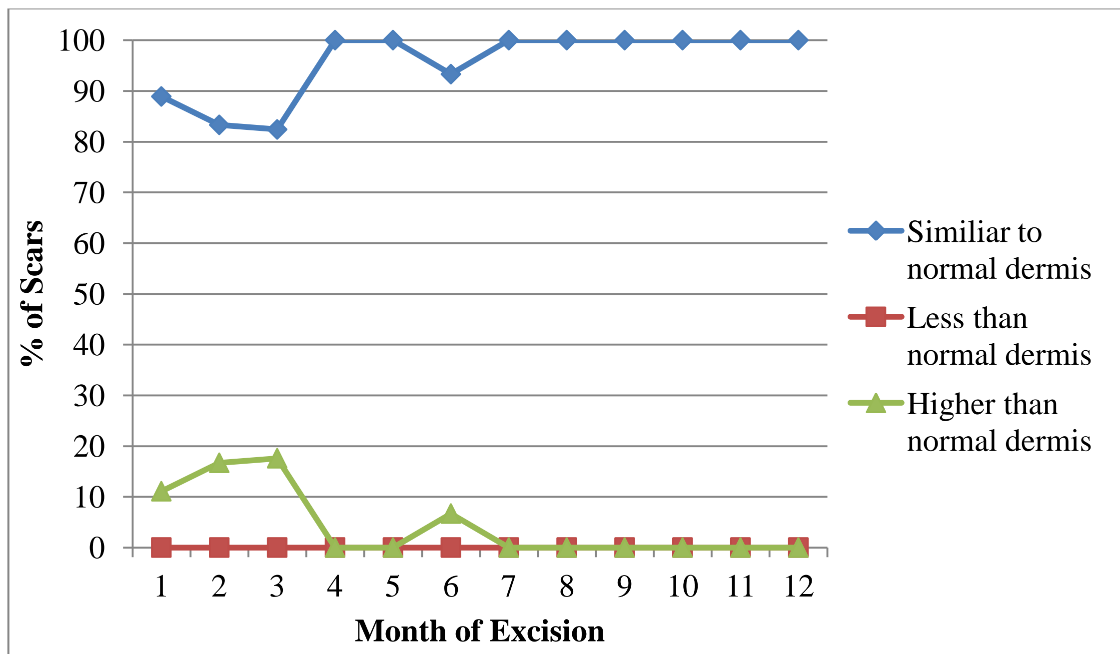
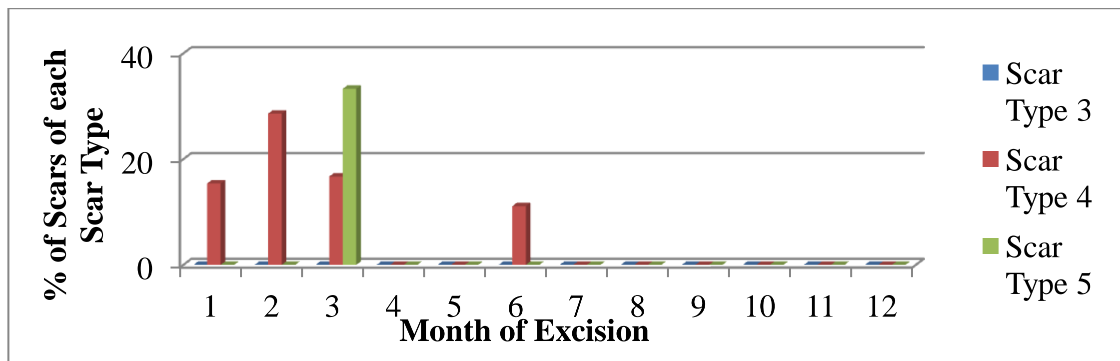


Figure 59 Level of Inflammation Higher than Normal Dermis



4.423 Collagen Organisation

The orientation, density and thickness of collagen fibres in the papillary and reticular regions of the scar were assessed compared to the surrounding normal dermis. The scores for the papillary and reticular dermis are considered separately. The scores in each of the figures to follow are defined below, in simple terms 0 is a very good score and 5 a very poor score:

0 = normal fibre orientation/density/thickness ≤ 10% abnormal
1 = 11 – 25% abnormal
2 = 26 – 50% abnormal
3 = 51 – 75% abnormal
4 = 76 - 100% abnormal
5 = Keloid-like fibre orientation/ density/thickness

Papillary dermal collagen orientation is considered first (Figure 60). The data are difficult to interpret given the variation from month to month. Data trend lines have been inserted to help in the interpretation of the results. The simplest point to note is that there have been no keloid-like fibres noted in this study population. The proportion of scars scored 0 or 1 increases over the 12 months making up about 1/3 of scars at 12 months. The proportion of scars scoring 2, 3 and 4 decreases over the 12 months but still make up approximately 2/3s of scars at month 12. Papillary dermal collagen orientation is shown to improve over the 12 months and is likely to continue to do so beyond 12 months.

Figure 61 and Figure 62 illustrate the papillary dermal collagen fibre orientation according to the different scar types. In general Type 3 scars are scored better (Figure 61). Figure 62 shows that Type 5 scars are mainly scored poorer.

Figure 60 Papillary Dermis Collagen Organisation - Fibre Orientation

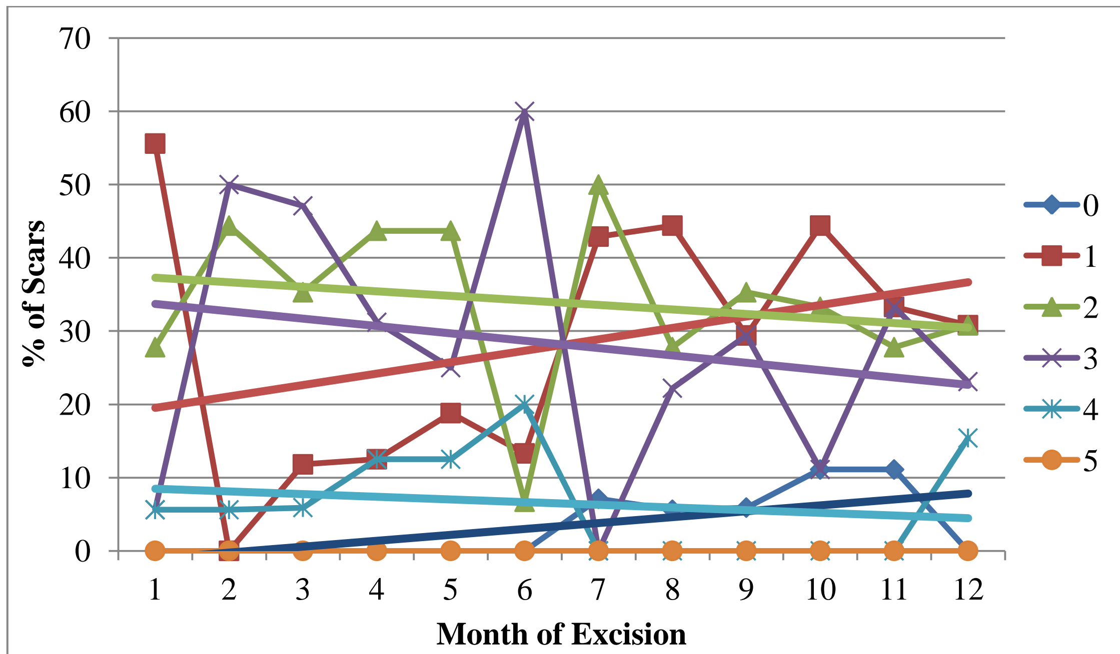


Figure 61 Papillary Dermis Collagen Orientation – Scores 0-2 (0-50% abnormal fibre orientation)

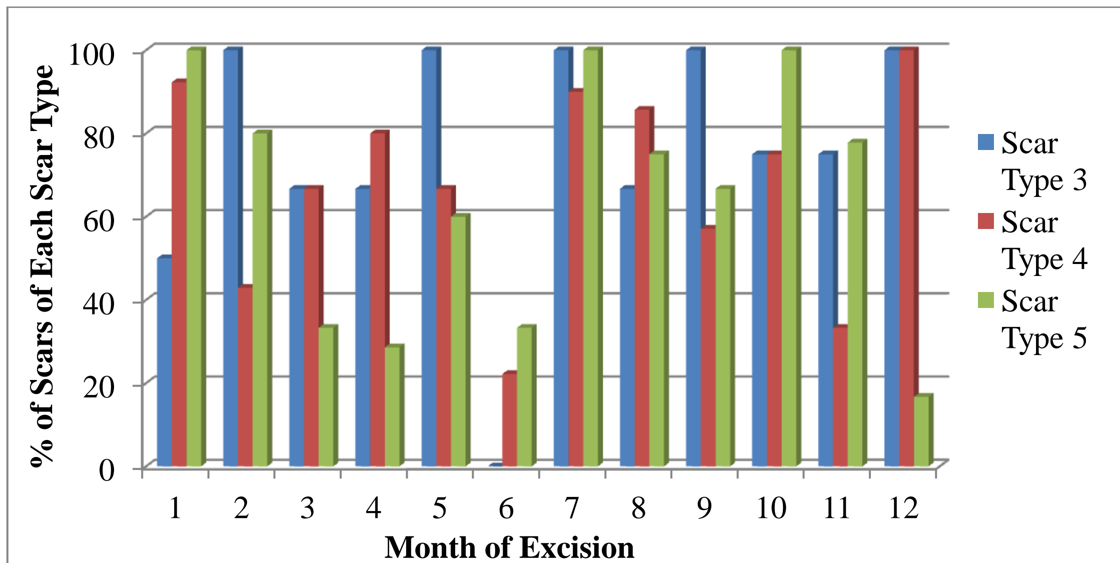


Figure 62 Papillary Dermis Collagen Orientation – Scores 3-4 (50-100% abnormal fibre orientation)

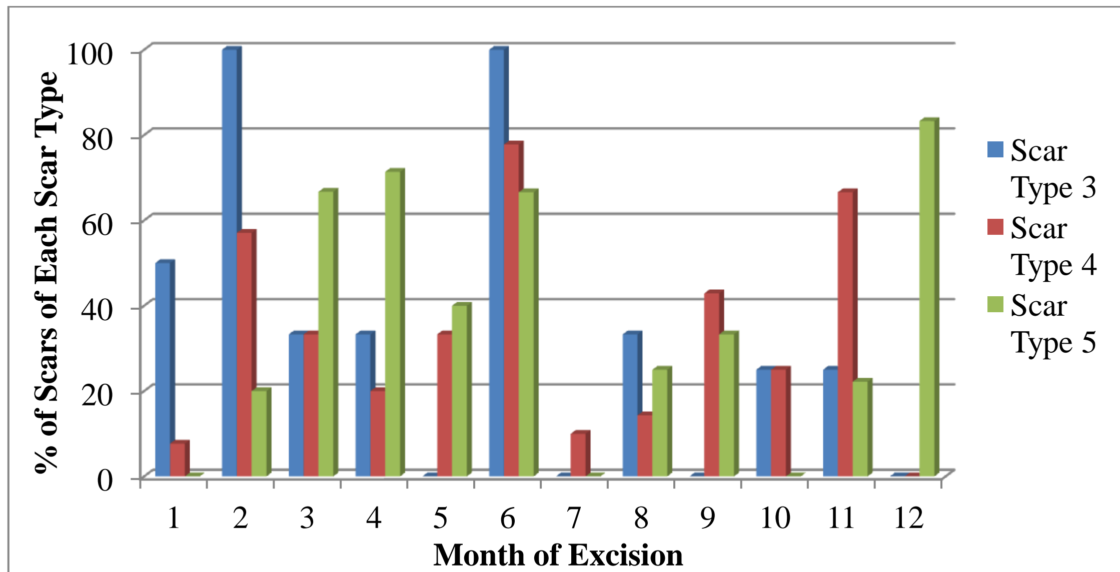


Figure 63 illustrates the papillary dermal collagen fibre density scores. Again no keloid-like fibres have been noted in this study population. There is great variation from month to month in the data. It is difficult to show any definite trend, if anything the trend over the 12 months for scores of 2 and scores of 3 would show that scars are scored worse by month 12. Other scores remain relatively static over the 12 months.

Figure 64 illustrates the papillary dermal collagen fibre thickness scores. The data for fibre thickness are similar to that for fibre density. No keloid-like fibres are noted and the trend for proportion of scars scored as 2 decreases while the trend for the proportion of scars scored as 3 increases. As the other scores remain relatively static over the 12 months this indicates that scar papillary dermal collagen fibre thickness is scored worse by month 12.

Figure 63 Papillary Dermis Collagen Organisation – Fibre Density

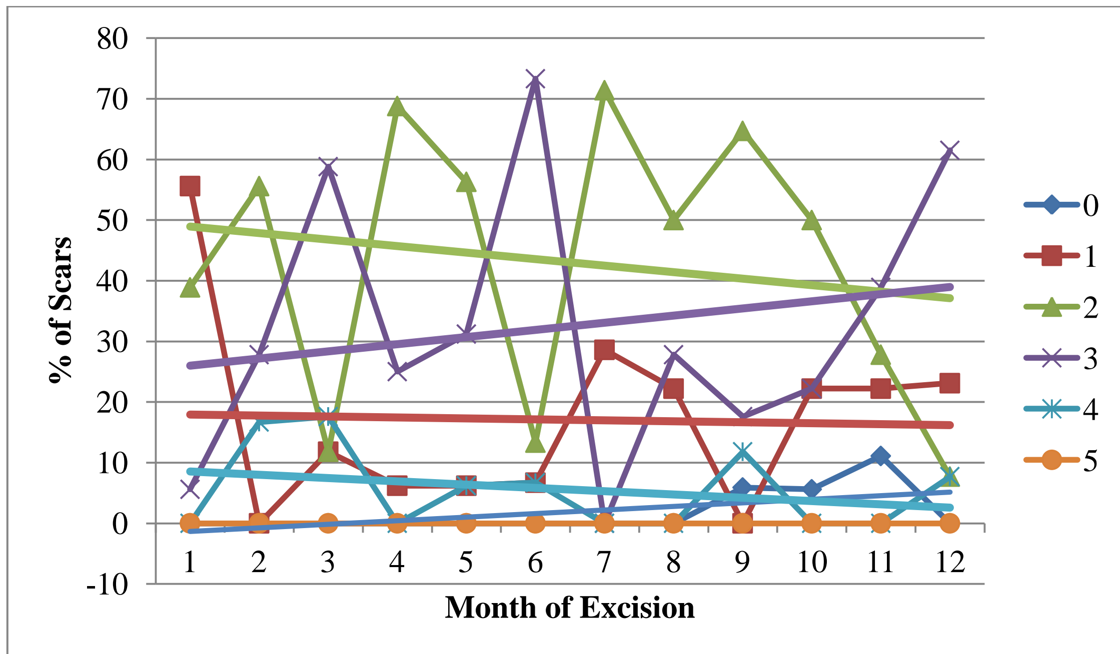
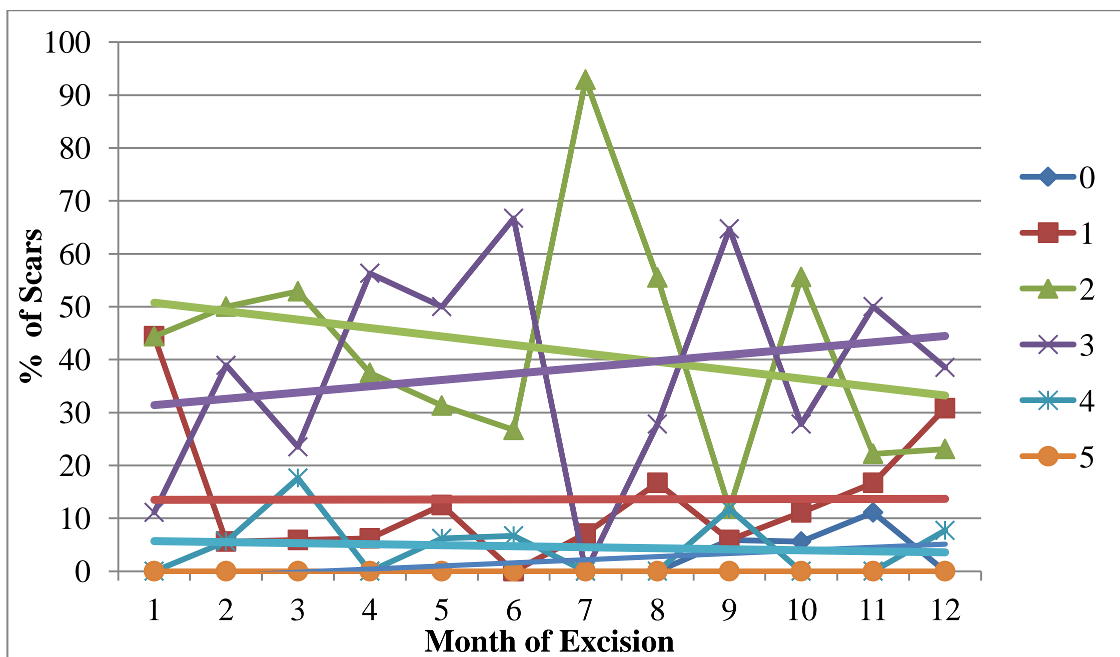
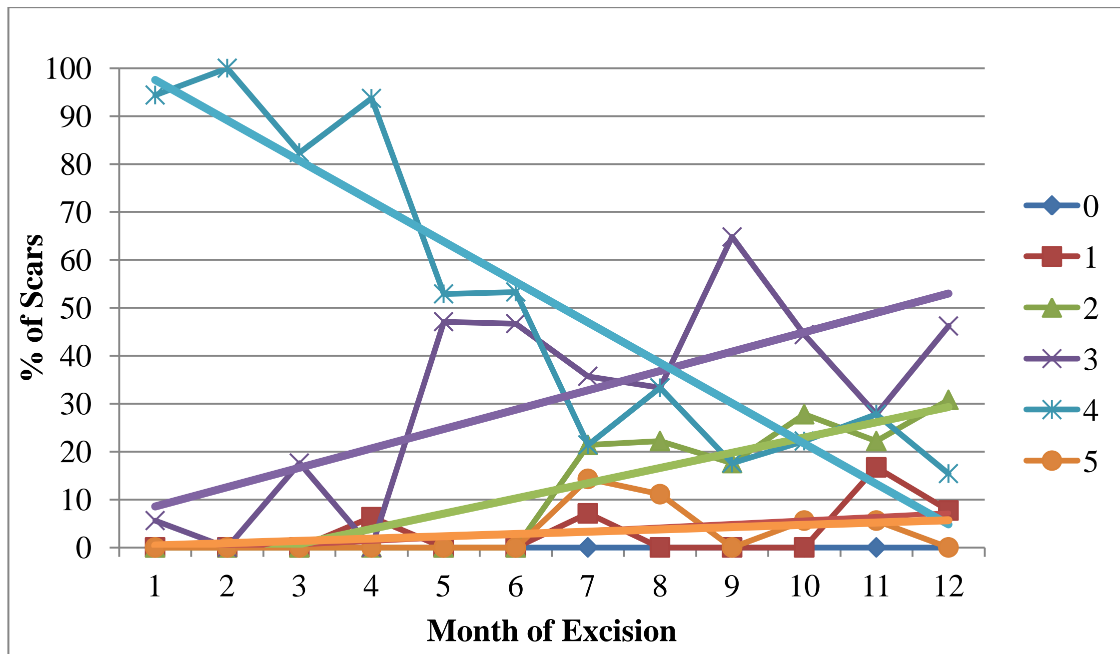


Figure 64 Papillary Dermis Collagen Orientation - Fibre Thickness



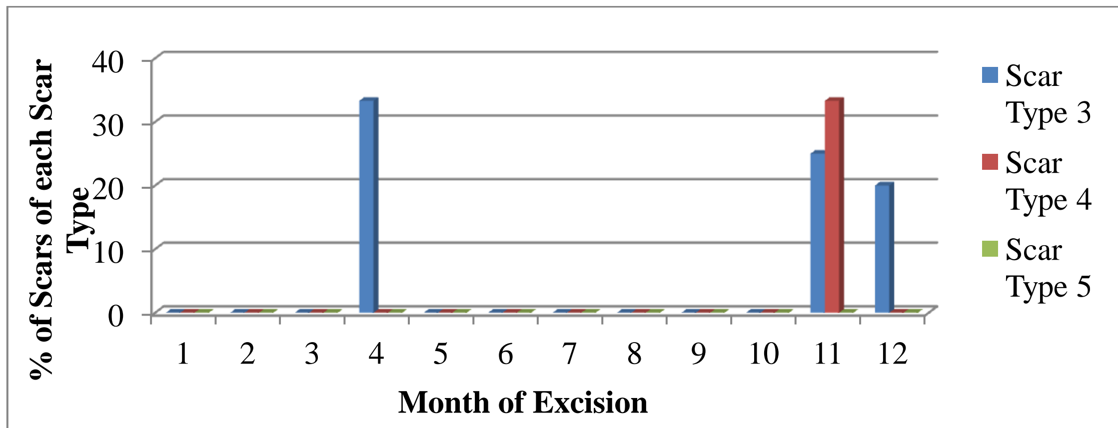
Reticular dermal collagen fibre orientation scores are illustrated in Figure 65. Scars are shown to be scored very poorly at month 1 but to improve significantly over the 12 months. The proportion for scars scored as 4 falls from almost 100% at month 1 to approximately 10% by month 12. By month 12 approximately 90% of scars are still scoring 2, 3 and 4 which is worse than the papillary dermal collagen scores for fibre orientation. Also, in contrast to papillary dermal collagen fibres a small proportion of scars were noted to have keloid-like reticular dermal collagen fibres. No scars were scored 0.

Figure 65 Reticular Dermis Collagen Organisation - Fibre Orientation



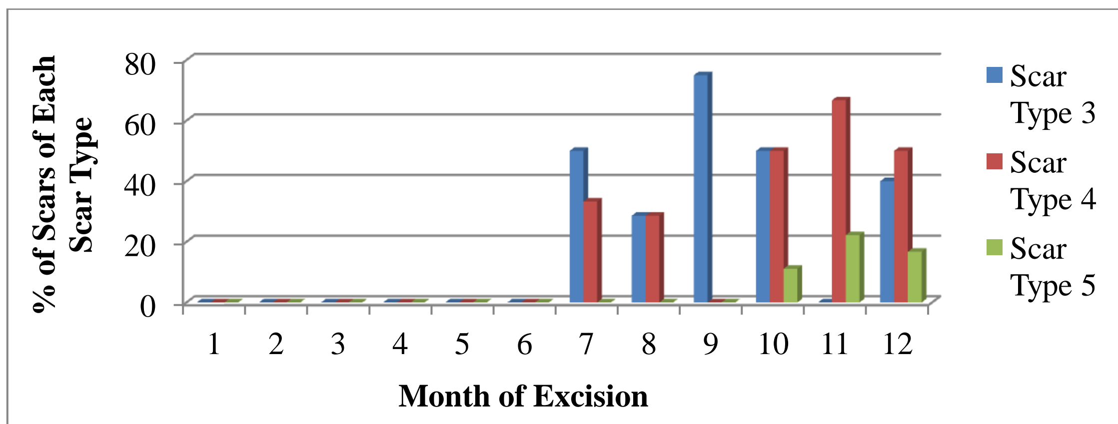
Only from month 7 do some scars score a 5 for keloid-like fibre orientation. Only scars previously described as Type 5 scars were noted to have keloid-like fibre orientation.

Figure 66 Reticular Dermis Collagen Fibre Orientation - Score 1 (11-25% abnormal fibre orientation)



No Type 5 scars were assessed as a score 1 (11-25% abnormal fibre orientation) by month 12 (Figure 66).

Figure 67 Reticular Dermis Collagen Fibre Orientation - Score 2 (26-50% abnormal fibre orientation)



The proportion of scars scored as 2 increased over the 12 months (Figure 65). In Figure 67 no scar achieved a score of 2 (26-50% abnormal fibre orientation) until Month 7 and the Type 3 scars achieved this score first along with Type 4 in fewer numbers. No Type 5 scar achieved this score until Month 10.

Figure 68 Reticular Dermis Collagen Fibre Orientation - Score 3 (51-75% abnormal fibre orientation)

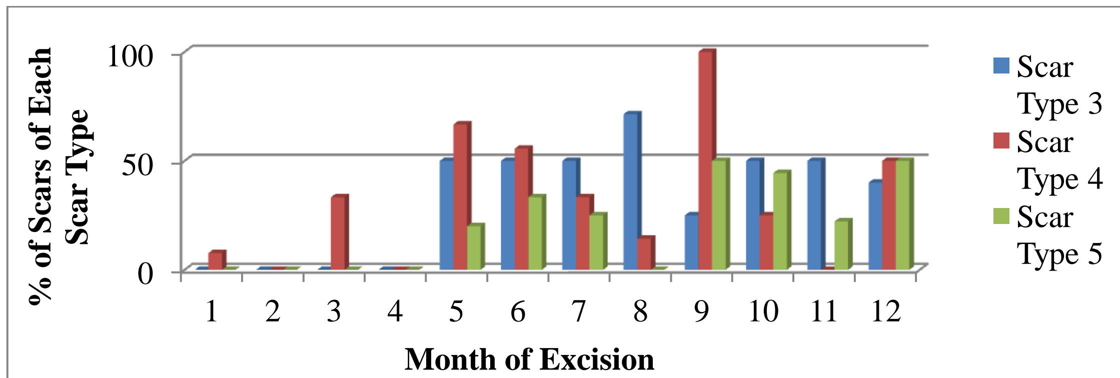


Figure 68 demonstrates that at month 12 approximately 50% of Type 4 and 5 scars are still scoring 3 (51-75% abnormal fibre orientation).

Figure 69 Reticular Dermis Collagen Fibre Orientation - Score 4 (76-100% abnormal fibre orientation)

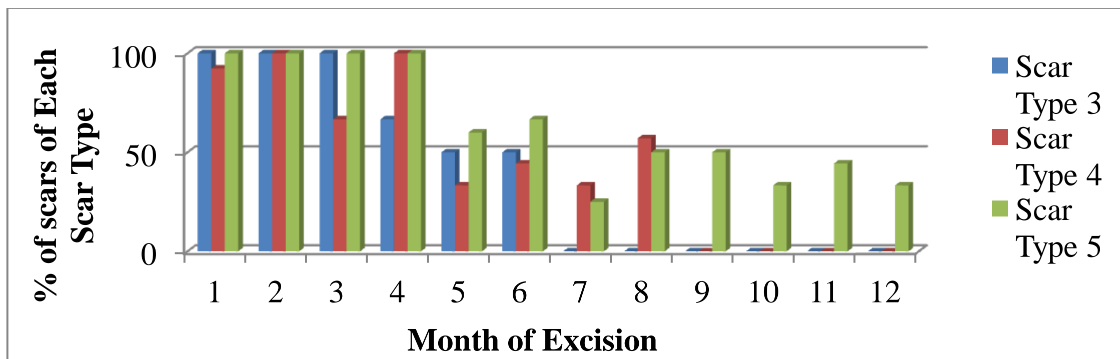


Figure 69 illustrates that most scars are scoring 4 (76-100% abnormal fibre orientation) in the first 4 months following wounding. The proportion of Type 3 scars scored 4 decreases first, followed by a decrease in proportion of Type 4 scars. Only Type 5 scars have a reticular dermal collagen fibre orientation score of 4 from month 9 onwards.

Figure 70 illustrates reticular dermal collagen fibre density scores. The scores for fibre density are very similar to those for fibre orientation with scars scored very poorly at month 1 but improving significantly over the 12 months. By month 12 approximately 90% of scars are still scoring 2, 3 and 4. In contrast to the papillary dermal collagen scores there are a few scars scoring 5 for keloid-like fibres and no scores of 0. In general the reticular dermis scores worse in comparison to the papillary dermis in relation to collagen fibre density.

Figure 70 Reticular Dermis Collagen Organisation - Fibre Density

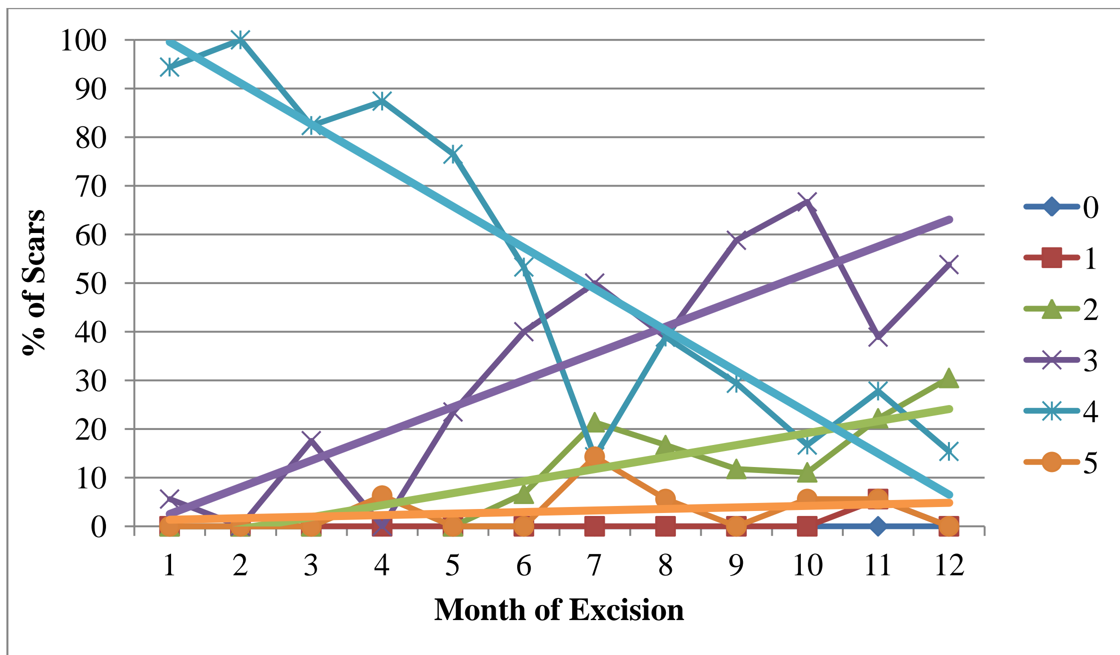
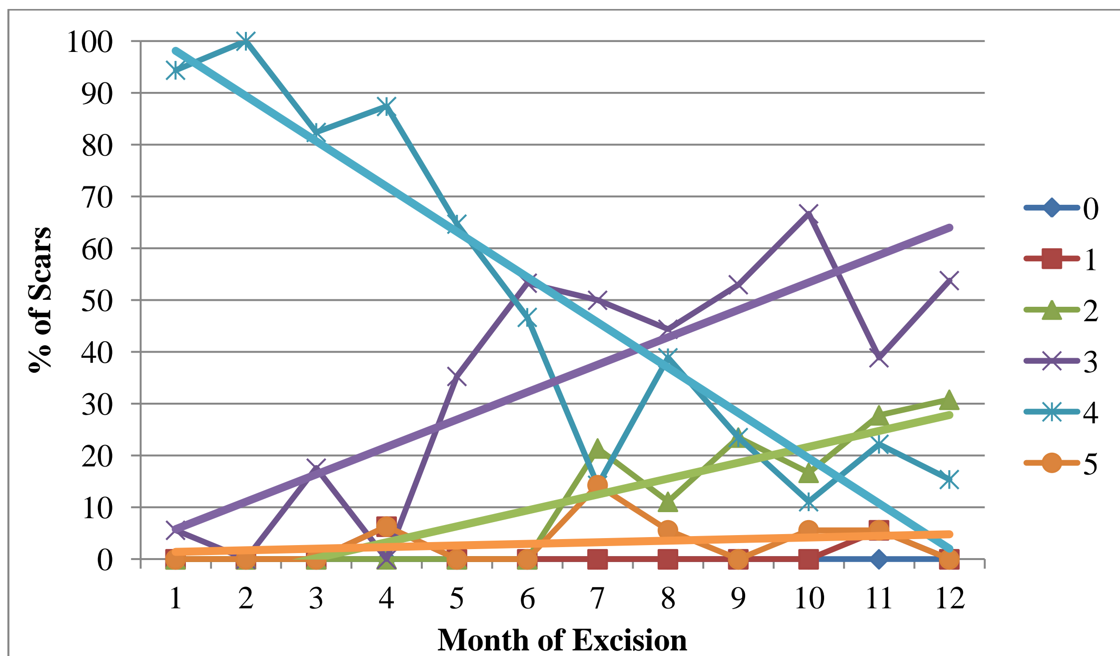


Figure 71 illustrates the reticular dermal collagen fibre thickness scores. The data for fibre thickness in the reticular dermis are similar to that for fibre density and orientation. Again, in contrast to the papillary dermal collagen scores there are a few scars scoring 5 for keloid-like fibres and no scores of 0. In general, the trend in scores for fibre thickness show improvement over the 12 months in that the proportion of 4 scores decreases and the proportion of 2 and 3 scores increases. This is in contrast with the papillary dermis. So it seems that the reticular dermal collagen fibre thickness at month 1 scores poorly but it shows greater improvement over the 12 months than the papillary dermal fibres.

Figure 71 Reticular Dermis Collagen Organisation - Fibre Thickness



4.424 Visual Analogue Scale Assessment of Collagen Organisation

The overall quality of the collagen fibre organisation within the scar area compared to the surrounding normal dermis was scored on a Visual Analogue Scale (VAS). We have already shown the collagen orientation results in detail but the data showed a lot of variation from month to month and illustrating data trends clearly has been difficult. It would seem so far that the reticular dermis scores are poorer than the papillary dermis scores but that the reticular dermis tends to show a greater improvement in scores than the papillary dermis over the 12 month period. The VAS scores (Table 39 & Table 40) are very useful in that they provide a score of the overall quality of the collagen fibre organisation and enable us to further qualify the results in section 4.423.

Table 39 Papillary Dermis Histological VAS Scores by Visit

Month	N	Mean VAS (cm)	SD	Min	Median	Max	95% CI Mean
1	18	3.69	1.991	0.8	3.6	8.6	(2.8, 4.6)
2	18	6.23	1.357	3.6	6.2	9.8	(5.6, 6.9)
3	17	6.00	1.832	1.7	5.9	9.8	(5.1, 6.9)
4	16	5.69	1.189	3.6	5.9	7.8	(5.1, 6.3)
5	16	6.07	1.607	2.4	6.1	8.6	(5.3, 6.9)
6	15	6.47	1.064	3.8	6.5	8.7	(5.9, 7.0)
7	16	4.30	1.270	1.8	4.4	7.4	(3.7, 4.9)
8	18	4.47	1.996	1.0	4.5	7.0	(3.5, 5.4)
9	17	4.92	1.953	0.2	5.0	8.9	(4.0, 5.8)
10	18	4.03	1.622	1.1	4.4	6.3	(3.3, 4.8)
11	18	4.49	2.250	0.6	5.3	7.5	(3.5, 5.5)
12	13	5.08	2.213	1.5	6.0	7.3	(3.9, 6.3)

Table 40 Reticular Dermis Histological VAS Scores by Visit

Month	N	Mean VAS (cm)	SD	Min	Median	Max	95% CI Mean
1	18	9.33	0.787	6.6	9.6	10.0	(9.0, 9.7)
2	18	9.66	0.399	8.6	9.8	10.0	(9.5, 9.8)
3	17	9.06	1.188	6.3	9.7	10.0	(8.5, 9.6)
4	16	9.09	1.114	4.6	9.3	9.9	(8.5, 9.6)
5	17	8.46	0.947	6.6	8.2	10.0	(8.0, 8.9)
6	15	8.18	1.078	6.6	8.3	9.8	(7.6, 8.7)
7	16	6.86	1.534	4.3	6.8	9.5	(6.1, 7.6)
8	18	7.57	1.553	5.1	7.7	10.0	(6.9, 8.3)
9	17	7.05	1.250	4.8	6.8	9.3	(6.5, 7.6)
10	18	6.82	1.156	4.6	6.7	9.4	(6.3, 7.4)
11	18	6.67	1.878	3.2	6.7	9.6	(5.8, 7.5)
12	13	6.20	1.347	3.7	6.3	8.9	(5.5, 6.9)

Figure 72 Mean Histological VAS Scores by Month of Excision

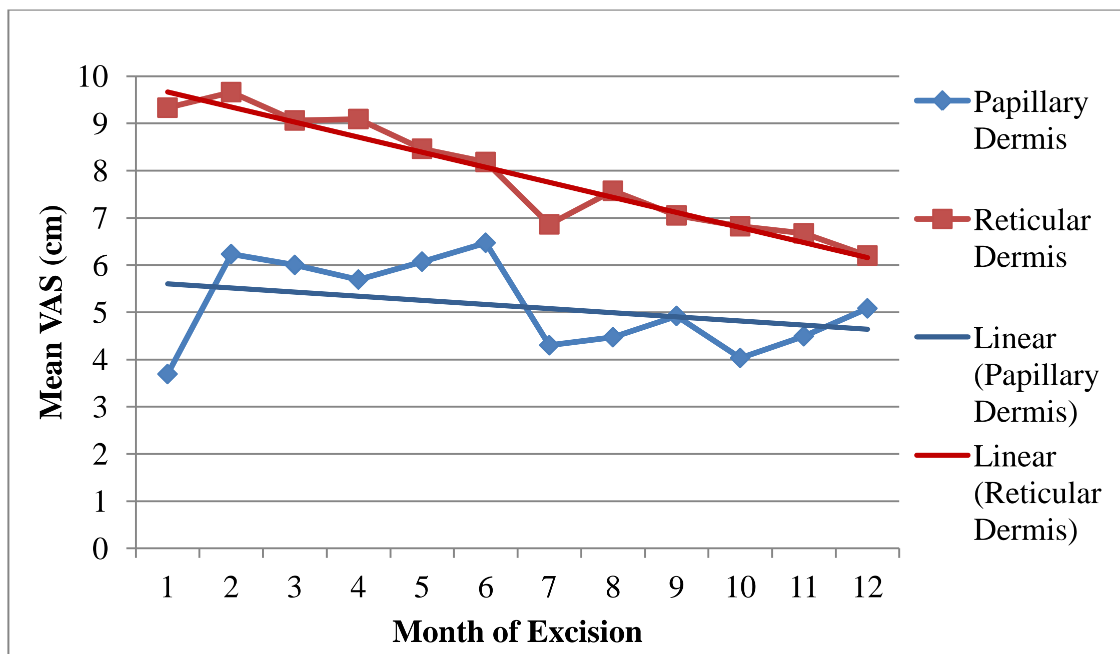


Figure 72 illustrates the histological VAS scores for the 12 months of the study. The VAS scores for both the papillary and reticular dermis improve over time with the reticular dermis VAS scores improving to a greater extent than those for the papillary dermis. This is in agreement with the results for collagen organisation set out in section 4.423. A steady state is not seen and further improvement is likely to occur beyond month 12.

Figure 73 Mean Clinical and Histology VAS Scores by Month of Assessment

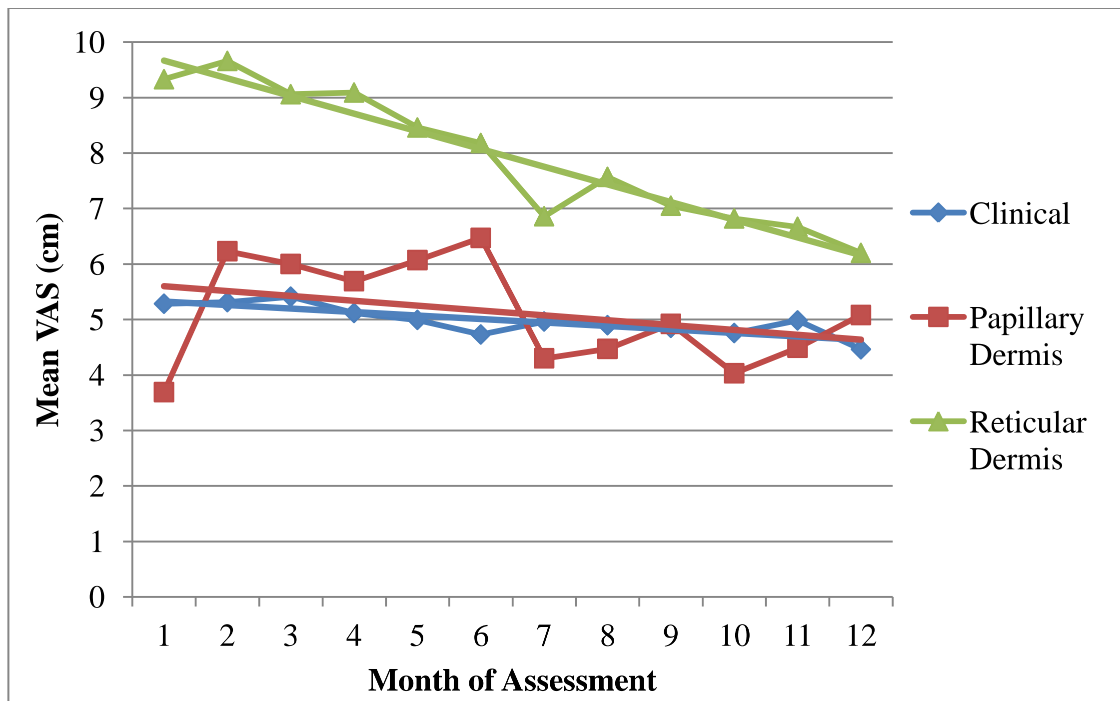


Figure 73 Mean Clinical and Histology VAS Scores by Month of Assessment includes the mean Clinical VAS scores reported in Chapter 3 to compare with the mean Histology VAS scores. There is a strong correlation shown between the Clinical VAS

scores and the Histology VAS scores for the papillary dermis. The reticular dermis VAS scores are not shown to correlate with the Clinical VAS scores.

Figure 74 and Figure 75 illustrate the mean Histology VAS scores for the overall quality of the collagen fibre organisation in the papillary and reticular dermis respectively. No clear trend is demonstrated in Figure 74 but at month 12 they do fall into order with the Type 3 scars scoring best and the Type 5 scars scoring worst.

Figure 74 Mean Histology VAS Scores for Papillary Dermis by Scar Type over 12 Months

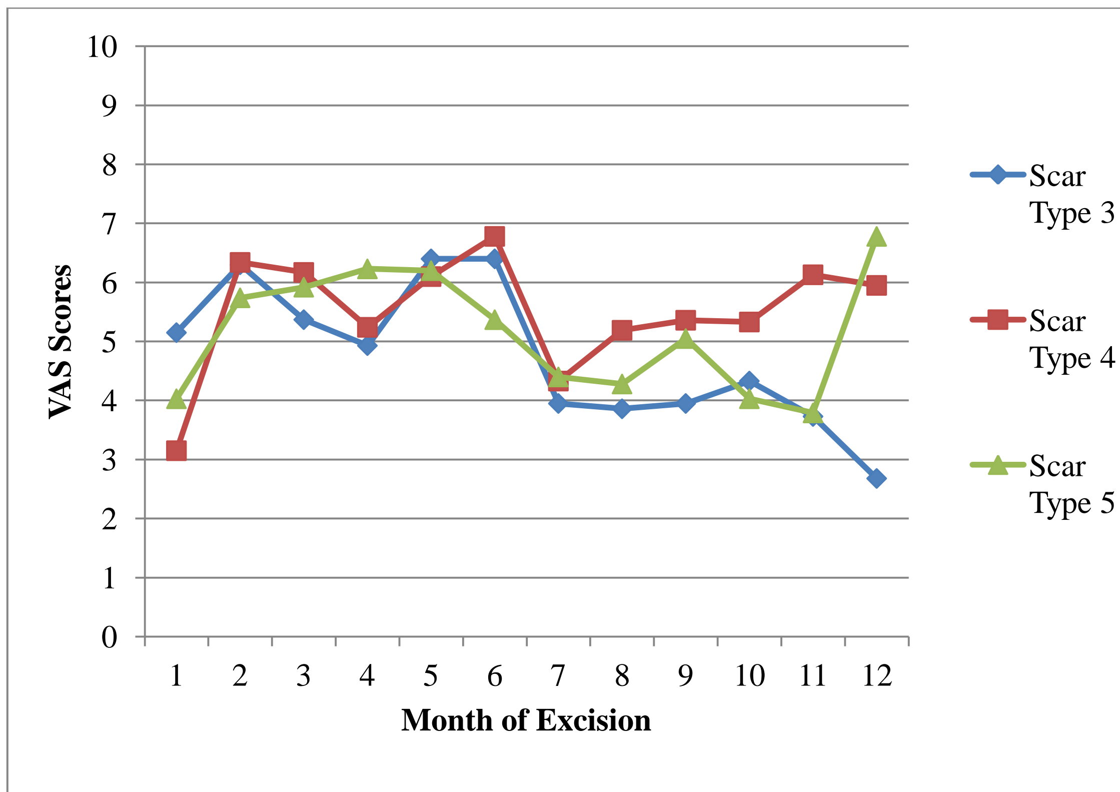
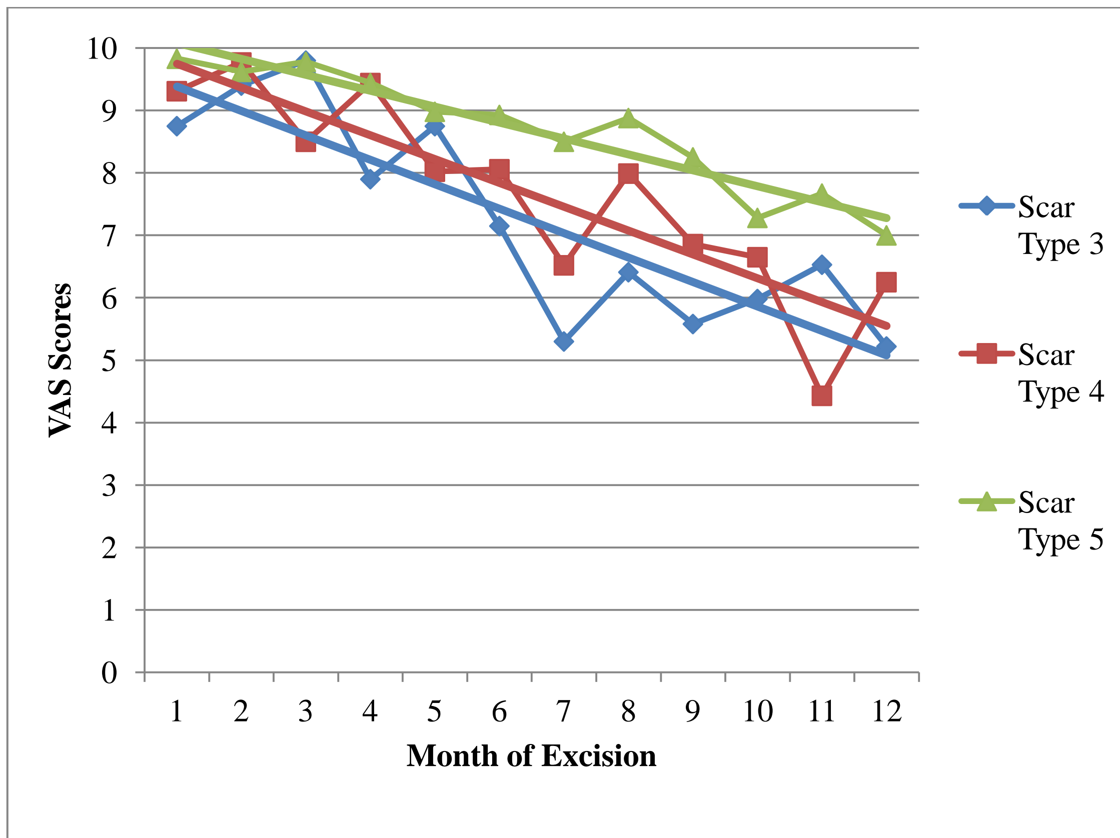


Figure 75 shows a clear trend in the reticular dermal collagen organisation VAS scores according to Scar Type. Type 5 scars tended to be scored the poorest, followed by Type 4 scars, and Type 3 scars were scored best.

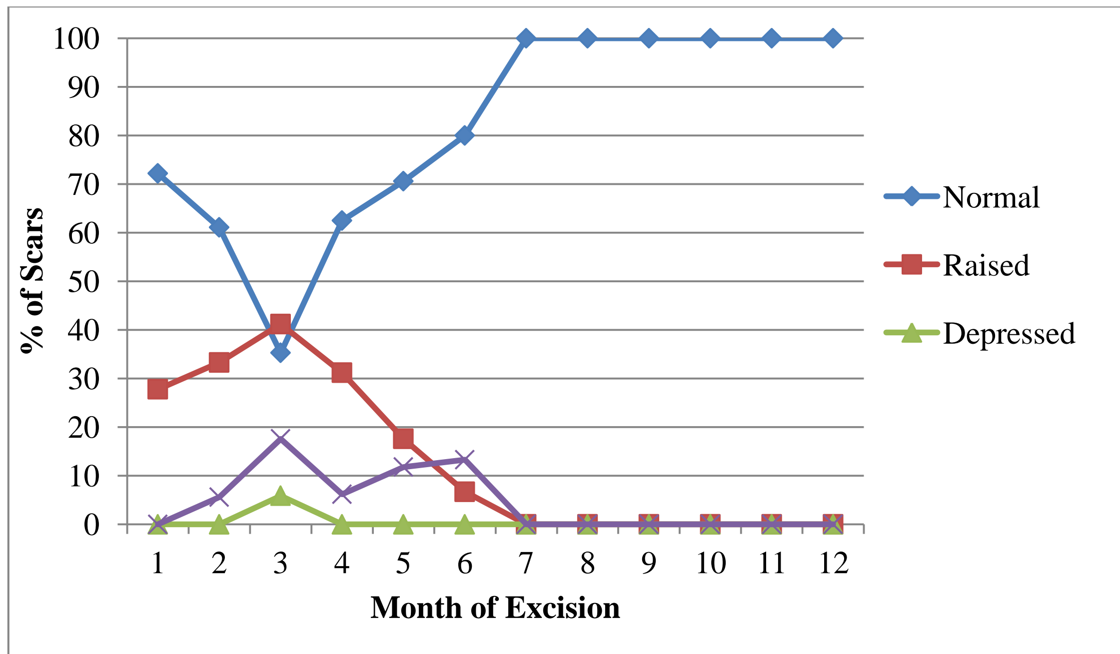
Figure 75 Mean Histology VAS Scores for Reticular Dermis by Scar Type over 12 Months



4.425 Other Scar Features

Scar elevation was assessed. Scars were scored as normal (in line with normal dermis); raised (above normal dermis); depressed (below normal dermis); or not interpretable.

Figure 76 Scar Elevation



Scar elevation scores are illustrated in Figure 76. In the first month two thirds of scars are classed as normal with one third of scars raised. There are only a small percentage of scars scored as depressed between months 2 to 6. There is an increase in the percentage of raised scars up to month 3. From month 3 onwards the percentage of scars which are raised steadily declines as the percentage of normal scars increases. All scars are scored as normal form month 7 onwards with a steady state achieved.

4.5 Discussion

This chapter describes for the first time the histology of the process of scar maturation in an African Continental Ancestry Group of volunteers over a 12 month period. The results presented are from histological examination of scar specimens harvested according to the study methods described in Chapter 2. There were between 13 and 20 specimens available each month for assessment. The length of the process of scar maturation is shown to vary for different histological aspects of scar maturation.

Two methods of assessing scar histology have been used in this study. The first method is a descriptive method. The histological appearance of each scar was described providing description of scar maturation in volunteers of African Continental Ancestry over the course of 12 months. Three groups were identified from within the study group. The second method involves the use of a modified version of the microscopic scar assessment scale of Beausang et al (1998). This scale has been previously validated and has been shown to be a reliable, consistent tool in histologic assessment of human scars (Beausang et al., 1998). This involved quantification of the major architectural abnormalities that are known to occur in scars. The epidermis was assessed according to the degree of restoration of rete ridges and epithelial thickness. Angiogenesis, including blood vessel size and numbers, and inflammation were considered. The papillary dermis and reticular dermis were scored separately according to collagen organisation in comparison with normal surrounding skin. The overall quality of the collagen fibre organisation within the scar area compared to the

surrounding normal dermis was scored on a Visual Analogue Scale. An additional feature assessed was scar elevation, scored on a simple 4-point scale.

Three groups, each displaying a different rate of longitudinal progression of scar maturation, were identified from within the study group. A prolonged high turnover state, persistence of inflammation and invasion of wound margins, were identified in scars up to Month 12. The spectrum of scar quality observed did not overlap greatly with that observed in European Continental Ancestry Group studies. Bond et al. (2008b) described three groups in the ECAG: a representative group, a group of excellent outliers and a group of poor outliers. The 'best' scar in this study was only as good as a 'poor' scar seen in ECAG studies. For this reason the Typing of the ACAG scars starts with Type 3 proposing that Type 1 and 2 scars would be the excellent outliers and representative groups respectively described by Bond et al (2008b).

The subset of 'Type 3' scars demonstrated rete ridge reformation from month 2 onwards but it was not complete by Month 12. Scars were relatively narrow. The numbers of inflammatory cells decreased in the first 5 months and from then onwards only the occasional inflammatory cell was present. Minimal collagen dissolution occurred at the wound margins. Restoration of the papillary dermis was noted from Month 4 as was the presence of melanocytes throughout the scar. By Month 12 a mature papillary dermis was noted with good reformation almost as normal and mature reticular dermal collagen with greater than 20% basket weave remodelling. Melanocytes were seen throughout the scar almost as the normal adjacent skin.

The subset of ‘Type 4’ scars also demonstrated rete ridge restoration as early as Month 2. Scars were wide. From the outset the scars were noted to be highly cellular with lots of mixed types of inflammatory cells especially surrounding blood vessels and a high turnover state with some collagen dissolution at wound margins. No melanocytes were noted in the scar until Month 3 and by Month 7 they were noted throughout the scar with increased melanin at wound edges. The numbers of blood vessels were noted to be increased from Month 5. Inflammatory cells were noted to be decreased but still present by Month 7. By Month 4 evidence of early restoration of the papillary dermis was noted and the high turnover state had resolved with minimal collagen dissolution and undermining of the wound margins. In the following months even though the high turnover state had resolved, there was evidence of previous expansion of wound margins and occasional degradation/synthesis nodules were present. By Month 9 collagen was maturing with signs of remodelling and early basket weave formation and good restoration and maturity of the papillary dermis. By Month 12 there was good restoration and maturity of the papillary dermis which was nearing normal. Parallel bands of very mature collagen with signs of remodelling and good basket weave formation were noted in the lower reticular dermis.

The subset of ‘Type 5’ scars did not show any rete ridge reformation until Month 4. Epithelium was noted to be thickened until Month10. Pigment seen in supra basal parts of epithelium at Month 1, at Month 2 no melanocytes were seen in the scar but increased melanin was present at the wound margins (dermis undermined beneath). Melanocytes were noted in the scar at Month 3 and throughout the scar by Month 5.

Melanin was noted in the supra basal layers of epithelium at Month8 to Month10. Scars were wide and wound elevation was present. Normal skin at the wound margins was invaded by the wound granulation tissue causing expansion of the wound i.e. increased width. A massive inflammatory cell infiltrate was noted from the outset with a persistent significant amount of inflammatory cells seen throughout the 12 months with giant cells present up to Month11. They continued to be vascular scars until Month 12 with increased numbers of blood vessels. A high turnover state was noted at Month 1 this was slow to resolve with significant collagen dissolution at wound margins and swirls of degraded old collagen noted in scars. By Month 6 the high turnover state was mostly resolved with evidence of previous significant collagen dissolution at wound margins shown as undermined wound margins and remnants of degraded old collagen in the scar arranged in degradation nodules. At Month 4 early restoration of the papillary dermis was noted but the collagen was immature and in parallel bands. By Month 10 good restoration of the papillary dermis was seen but collagen was slow to mature and arranged in parallel bands. By Month 12 collagen was maturing with the upper dermis more mature than the lower with some basket weaving present.

The scars in subset ‘Type 5’ follow the same pattern as the others at an aggressive but slower rate. Rete ridge reformation doesn’t start until Month 4 compared to Month 2 in the other 2 groups. They are more vascular and have many more inflammatory cells present and for longer (until Month12). A high turnover state is present until Month 6 compared to Month 4 for ‘Type 4’ scars. Dissolution of the wound margin and

consequently scar width is most significant in ‘Type 5’ scars. Interestingly, commencement of restoration of the papillary dermis is noted in all scar types at Month 4. However, by Month 12 the restoration of the papillary dermis is classed as near normal in ‘Type 3’ and ‘Type 4’ scars and good in ‘Type 5’ scars, revealing that scar maturation is slower in this scar type. All 3 scar types demonstrate a common trend in that the upper dermis matures at a faster rate than the lower dermis.

A representative group was not identified indeed the best (Type 3) and worst (Type 5) scars became the most common scars by Month 12. This contrasts with the findings of Bond et al. (2008b) where in the ECAG a representative group was described with excellent and poor outliers. No hypertrophic or keloid scars were identified. It is likely they could be classed as Type 6 and Type 7 scars respectively.

Histological analysis of excised scar tissue from ACAG populations indicated a strong association between the microscopic and macroscopic data in respect of scar width. In general, scars from ACAG populations were much wider, due in a large part to an erosion of the wound margins brought about by an aggressive remodelling of the scar margins. In scoring the scar histology scar width was a major factor with wider scars tending to be scored poorly. Bond (2009) also found in the ECAG that the ‘poor’ subset of scars tended to be wider and that there was increased cellular activity at the scar margins which was suggested to be due to the later turning over rapidly and so widening the scar. A persistent high turn-over state similar to the proliferative phase, is seen in both the European and African Continental Ancestry Groups further

contradicting the commonly held view of wound healing, whereby cellular proliferation is thought to cease early during the granulation phase (Bond et al., 2008b, Singer and Clark, 1999).

Melanocytes were noted in the scars around Month 3 to 4. Hyperpigmentation surrounding the scar may be due in part to rete-ridge fusion at the scar margins, leading to a greater density of melanocytes. This may explain in part the halo seen on macroscopic images of scars in darker skin types. There is also likely to be increased melanin synthesis, stimulated by inflammatory mediators released from the inflammatory cells at the wound margins. Undermining of the wound margins is also likely to play a part. In addition, increased cellular activity was observed and many immature (nucleated) epithelial cells were observed in the epidermis. Moreover, the epidermis may have lost some of its barrier properties, with the invasion of inflammatory cells into the epidermis.

When age is considered in relation to the assessed scar type there are more scars rated as the worst type of scar (Type 5) in the 18-30 age group than the 31-55 age group. These findings are similar to that of Bond et al (2008b) who found that the poor subset of scars identified invariably contained individuals younger than 30 years of age. However, it is not possible to comment on the age group > 55 in this study. During the recruitment process we aimed to recruit to different age groups in order to be able to illustrate histological differences in scar maturation between the young, middle-aged and older population of volunteers. Unfortunately, only 1 volunteer was recruited to

the over 55 age group. It is therefore difficult to determine whether older volunteers have better scars as has been found in similar studies in volunteers of European Continental Ancestry (Bond et al., 2008b). It is interesting to note that on clinical assessment of the scars of this population group few observable differences between subjects aged 18-30 years and 31-54 years were shown and yet it has been illustrated in the histological assessment of the scars that young people have poorer scars. Bond (2009) proposed that older people appear to lack the prolonged high turnover phase seen in young people, which was felt to result in a better quality scar. On the other hand, the high turnover state is likely to increase the resilience of normal healing in the young albeit resulting in a poorer scar. A high turnover state has been shown to be present in many scars in the African Continental Ancestry population group up to month 12. This may be indicative of more resilient wound healing process. In certain parts of the world rapid healing after injury is essential to survival. Hostile environments with exposure to pathogens etc. mean that resilient wound healing, with a high turnover state and ability to protect against secondary insults such as wound infection, foreign bodies and wound trauma, is essential. This may in part explain the greater tendency towards aggressive scar maturation at the expense of quality in this population group. This population group is comprised of individuals whose ancestral origins are in the continent of Africa which represents a hostile environment. The ECAG ancestrally have been exposed to less environmental risks and loss of function mutations may have occurred within this population resulting in less excessive scar maturation.

When Fitzpatrick Skin Type is considered alongside the scar typing we are able to see some evidence of scars being graded as poorer in volunteers with darker Skin Types. There are no studies in the literature to compare these findings to. They can be considered in relation to the clinical findings of this study presented in Chapter 3. The histology results point towards poorer scaring in the darker skin types but lack the consistency of the clinical findings. Mean clinical and lay panel VAS are both shown to be influenced by a subject's Fitzpatrick Skin Type. Scars of subjects with Fitzpatrick Skin Type IV (medium skin tone) scored consistently better at every month of assessment on the Clinical VAS in comparison to those subjects with Fitzpatrick Skin Type V (dark/olive skin tone) and Type VI (very dark skin tone) (Figure 5).

Re-epithelialisation is widely accepted to be one of the major processes in wound healing that ensures successful repair (Braiman-Wiksman et al., 2007). Epidermal restitution is considered in terms of rete ridge restoration and epithelial thickness. These two aspects of epidermal restitution progress at different rates. At the end of the 12 months epithelial thickness has returned to normal in the majority of scars. The minority of scars with thickened epithelium are Type 5 scars. Rete ridge restoration is much slower with almost half of the scars showing no evidence of rete ridge restoration. Interestingly, Type 5 scars are the slowest to commence rete ridge restoration but of the scars noted to achieve normal rete ridge formation they are mostly Type 5 scars.

Angiogenesis, is a vital part of tissue repair (Brown et al., 2002) with ineffective angiogenesis resulting in impaired healing and a chronic wound (Ferguson and Leigh, 1998). Angiogenesis is still ongoing in the majority of scars at month 12 with over two thirds of scars having more blood vessels and one third a similar number of blood vessels to normal dermis. The scars with similar numbers and size of blood vessels to normal dermis are mainly Type 3 scars. Bond (2009) in a similar study in the ECAG found that the overall density of blood vessels was similar to normal dermis by months 10-12. Other, human scar vascularity studies have demonstrated increased numbers of blood vessels in scars up to 12 months following wounding (Brown et al., 2002). The level of inflammation is recorded as normal by month 7 at the latest. Type 3 scars are consistently recorded as normal over the 12 months. Neither the histologic assessment of angiogenesis nor the level of inflammation correlates with the macroscopic assessment of scar redness in Chapter 3 where it was found that by Month 9 almost all scar redness had faded. Bond et al (2008a) first described the natural history of scar redness in humans in the ECAG where they found that scar redness fades on average at Month 7. They also found a correlation between those individuals with a red scar at Month 12 following wounding and the presence of an increased size and number of scar blood vessels. Bond et al (2008a) propose the use of the term "rubor perseverans" rather than erythema to describe the physiologic redness of a normal scar as it matures beyond the first month, a process that does not involve inflammation and attribute persistent redness to the numbers of blood vessels within the scar. This is not clearly seen within this population group. At Month 12 macroscopically scar redness has faded yet microscopically an increased number of blood vessels remain in many scars.

However, it is not only vascular factors which determine the colour of a scar or indeed the presence of redness as optical factors relating to the characteristics of the new epidermis and dermis must also be considered (Velangi and Rees, 2001). Edwards and Duntley (1939) described the phenomenon of light scattering and its importance in skin colour. They found that if light scattering was absent skin would generally speaking be much redder than it is. It is likely that as the scar dermis matures and increases in density visualisation of redness due to increased vascularity is more difficult.

There is great variation from month to month in the data for papillary dermal collagen organisation. Papillary dermal collagen orientation is shown to improve over the 12 months and is likely to continue to do so beyond 12 months. There is some evidence that Type 3 scars score better in terms of papillary collagen fibre orientation but it is not a clear trend. Scar papillary dermal collagen fibre thickness and density in general is scored worse by month 12. The reticular dermal collagen fibre organisation in general scored more poorly than the papillary dermis. Scars are shown to be scored for organisation, fibre thickness and density very poorly initially but to improve significantly over the 12 months. Also, in contrast to papillary dermal collagen fibres a small proportion of scars were noted to have keloid-like reticular dermal collagen fibres. A steady state was not shown to have been reached in the assessment of collagen fibre organisation. When the reticular dermal collagen fibre orientation was considered according to Scar Type a clear trend was evident with Type 3 scars scoring

best, followed by Type 4 scars and Type 5 scars scoring worst. This helps to qualify the scar type classification described earlier in this chapter.

The overall quality of the collagen fibre organisation within the scar area compared to the surrounding normal dermis was scored on a Visual Analogue Scale (VAS). The papillary dermis is seen to be of better quality in terms of collagen fibre organisation compared to the reticular dermis. It is clear from the results that the process of scar maturation is still ongoing with no steady state reached at month 12. The Clinical VAS scores are presented alongside the Histology VAS scores in Figure 73. There is a strong correlation shown between the Clinical VAS scores and the Histology VAS scores for the papillary dermis over the entire 12 months of the study. Beausang et al (1998) also found a ‘strong correlation between the macroscopic and microscopic appearance of scars, particularly between the clinical appearance and histologic scores of features in the epidermis and papillary dermis’. In their opinion this finding validates the use of the Beausang scale as sensitive scar assessment tool and also indicates that macroscopic scar appearance is a reflection of the underlying histologic abnormalities. The Histology VAS scores were also presented according to the Scar Type. This revealed a good correlation between VAS scores and Scar Type for the reticular dermis. This provides some qualification of the Scar Typing presented in this Chapter. The same correlation was not demonstrated within the VAS scores of the papillary dermis. It is possible that in the process of scar typing a greater weight was placed on the assessment of the reticular dermis.

Scar elevation was assessed and all scars were scored as normal form month 7 onwards with a steady state achieved. This occurs early in comparison to the clinical findings of scar contour and scar height. The percentage of scars deemed flush with the surrounding skin remains relatively unchanged from Month 9 onwards achieving a steady state with over 95% of scars rated as flush with the surrounding skin at Month 12. This finding is consistent with the measures of scar height. Therefore the process of scar maturation that allows a scar to become flush with surrounding skin has completed within the study period. No scars in this study have been described as keloid or hypertrophic.

4.6 Summary

In summary this Chapter has described for the first time the longitudinal process of scar maturation in individuals of African Continental Ancestry, in terms of its histological appearance over 12 months. Scars can be classified into three groups. All scars undergo a similar sequence of changes but they do so at different rates as previously shown by Bond et al (2008b) in individuals of European Continental Ancestry. None of these scars reached a state of maturity by Month 12. In fact the description of scar maturation in this population group presented here is not complete as the process of scar maturation is still ongoing at Month 12. A prolonged high turnover state, persistence of inflammation and invasion of wound margins, were identified in scars up to Month 12. No hypertrophic or keloid scars were identified.

The papillary dermis is of better quality compared to the reticular dermis in terms of collagen fibre organisation. There is a strong correlation shown between the Clinical VAS scores and the Histology VAS scores for the papillary dermis. There is also a correlation between the results of the different individual histology assessments and the Scar Typing of the specimens. The process of collagen maturation is still ongoing with no steady state reached. At the end of the 12 months few processes are complete or in a steady state except for epithelial thickness, scar elevation and the level of inflammation recorded on the Beausang assessment scale. In addition to collagen maturation, rete ridge restoration and angiogenesis are still ongoing.

In the histological assessment of the scars young people (ACAG) have poorer scars. When Fitzpatrick Skin Type is considered alongside the scar typing we are able to see some evidence of scars being graded as poorer in volunteers with darker Skin Types. In general, these findings have shown scars in the ACAG to be poorer than those in the ECAG and suggest a spectrum of wound healing, with the best scars (Type 1) occurring in elderly ECAG individuals with optimal wound healing conditions through to scarring in young individuals of the ACAG (Type 5). Hypertrophic and keloid scars could be classed as Type 6 and Type 7 respectively.

5 SKIN TYPING

5.1 Introduction

In this study we have used Continental Ancestry and Fitzpatrick Skin Typing to categorise individuals according to skin colour. There are no objective measures of skin typing.

The Fitzpatrick Skin Typing scale is a recognised tool for dermatological research into the colour of skin. However, there can be problems in its use mainly due to inconsistency. There is potential for different investigators to score the same individual with a different skin typing.

Reflectance spectrometry is now available as a quantitative method of assessing colour. Dermatologists tend to use the tristimulus systems whereby colour is represented according to one of a number of CIE indexes such as the Lab score, where colour is defined on three axes, light/dark, red/green and yellow blue (Shriver and Parra, 2000). Li-Tsang et al (2003) validated the use of the spectrocolorimeter as an instrument to quantify scar pigmentation. This device was used in this study as an objective measurement of scar colour.

This Chapter will consider if there is a relationship between the Lab score and the Fitzpatrick Skin Type in the study group.

5.2 Methods

At each monthly visit a spectrophotometer measurement for normal skin was taken. In addition, at commencement of the study each volunteer was interviewed using the questionnaire in Appendix 6 and the Fitzpatrick Skin Type was recorded.

Volunteers attended every month for scar assessments. At each visit a spectrophotometry reading was taken of a control area of normal skin in addition to the scar colour assessment. The colour was assessed using an X-rite™ SP62 Spectrophotometer with a 4mm aperture. The spectrophotometer measures lightness (L*) redness/greenness (a*) and yellowness/blueness (b*) in accordance with the Commission Internationale de Eclairage (CIE) international standards on colour perception.

The measurement used for each volunteer in this Chapter was an average of the normal skin measurements taken from the dominant arm over the 12 months of the study. The skin typing for each volunteer was compared to the spectrophotometer reading for that individual.

5.3 Study Subjects - Results

5.31 Time period of the study

Sixty patients were successfully recruited between February 2006 and January 2007.

5.32 Disposition of Subjects

All 60 volunteers attended for scar assessment. Spectrophotometer readings were available for each volunteer. Volunteers were Fitzpatrick Skin Type IV, V or VI. No volunteers were assessed as Fitzpatrick Skin Type I, II or III.

5.33 Demographic and Other Baseline Characteristics

As documented in Chapter 3.

5.4 Skin Colour Assessment - Results

An average of spectrophotometer readings of normal skin for each volunteer was used in the analysis. They were compared to the Fitzpatrick Skin Type. Descriptive statistics are presented. In addition, Delta-E has been calculated to compare the three Fitzpatrick Skin Types. Delta-E is a single number defined as the difference between two colours in a L a b colour space. A Delta-E of 1.0 is the smallest colour difference the human eye can see.

Table 41 Summary Statistics of Spectrophotometer Readings of Normal Skin by Fitzpatrick Skin Type

Fitzpatrick Skin Type	Statistic	L	a	b
IV	**number**	14	14	14
	mean	55.062	8.906	18.662
	standard deviation	6.757	1.233	1.486
	median	55.79	8.975	19.19
	min	41.59	6.63	19.59
	max	64.32	10.69	20.15
V	**n**	24	24	24
	mean	45.728	9.703	17.236
	standard deviation	9.969	0.845	3.065
	median	43.74	9.865	17.57
	min	32.07	6.85	8.55
	max	58.13	11.07	21.45
VI	**n**	23	23	23
	mean	39.941	9.007	13.875
	standard deviation	6.500	1.385	3.917
	median	38.545	9.335	13.365
	min	29.06	5.06	5.81
	max	55.02	10.81	20.5

Figure 77 Summary of Mean Spectrophotometer Values for Normal Skin

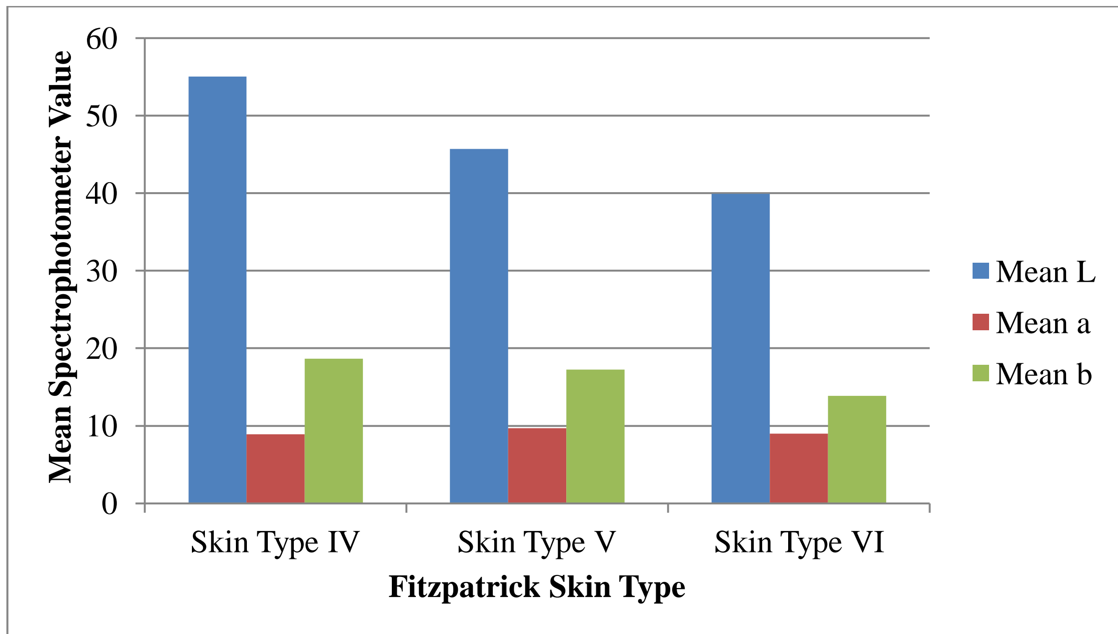


Table 41 and Figure 77 illustrates how the mean Lab values vary between Fitzpatrick Skin Types IV, V and VI. The mean L value was greater in Fitzpatrick Skin Type IV and decreased in Fitzpatrick Skin Type V and further decreased in Fitzpatrick Skin Type VI.

L value is a measure of lightness and the data show that with increasing Fitzpatrick Skin Type score there is decreased lightness on spectrophotometric analysis. The mean a value, which is a measure of redness/greenness, is similar between the Fitzpatrick Skin Type Groups. The mean b value was greater in Fitzpatrick Skin Type IV and decreased in Fitzpatrick Skin Type V and further decreased in Fitzpatrick Skin Type VI, a similar to trend to the L value. The b value is a measure of yellowness/blueness and the data demonstrate that with decreasing Fitzpatrick Skin Type score there is an increase in yellowness/blueness on spectrophotometric analysis.

Figure 78 Spectrophotometer Readings (Lightness) for Normal skin by Fitzpatrick Skin Type

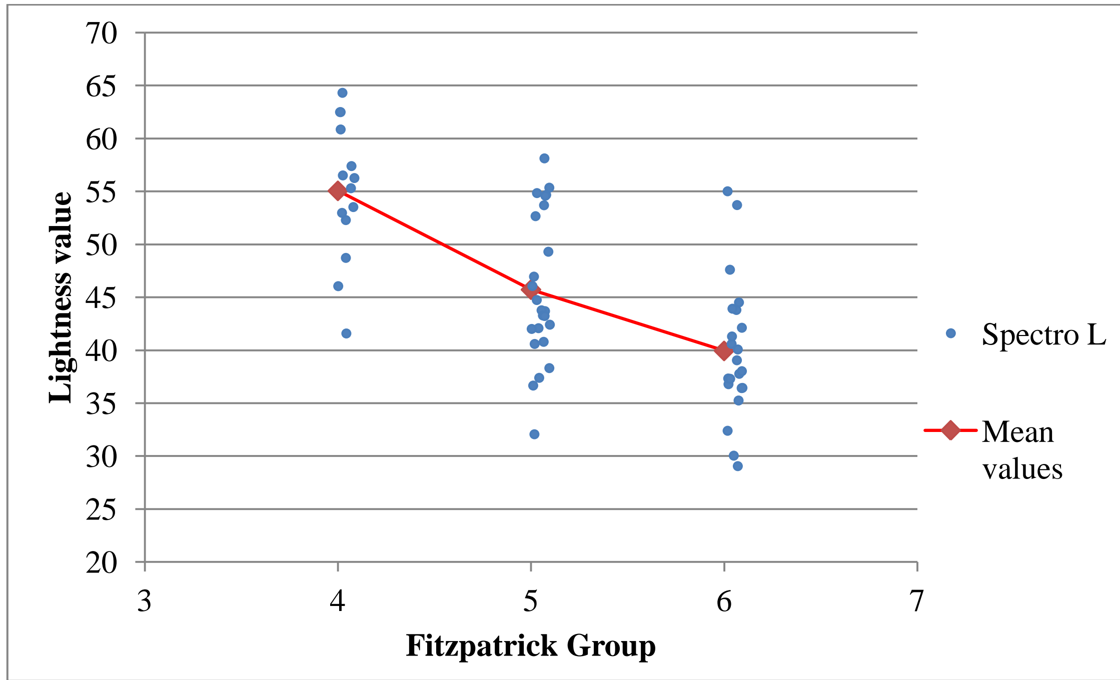


Figure 79 Spectrophotometer Readings (Red/Green) for Normal skin by Fitzpatrick Skin Type

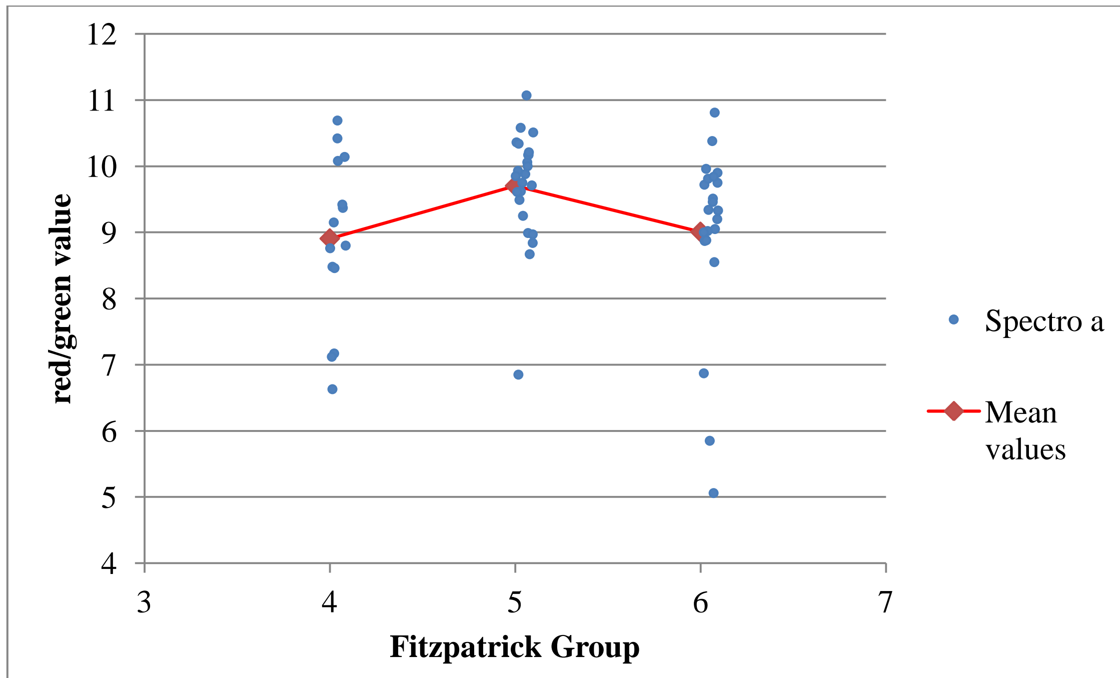


Figure 80 Spectrophotometer Readings (Blue/Yellow) for Normal skin by Fitzpatrick Skin Type

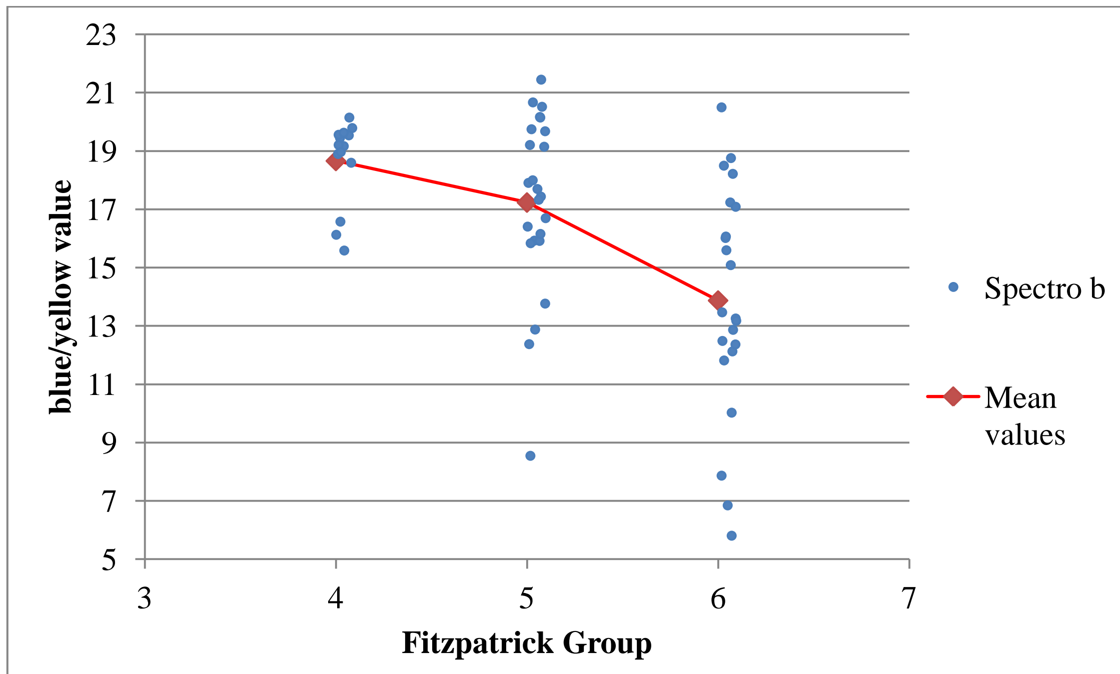


Figure 78, Figure 79 and Figure 80 further illustrate the results. The L values show a clear trend. Volunteers classed as Fitzpatrick Skin Type VI have darker skin as measured by the spectrophotometer than those of Fitzpatrick Skin Type V. Those of Fitzpatrick Skin Type IV have the lightest colour of skin of the 3 Skin Types considered. However the correlation noted between the b values and Fitzpatrick Skin Type is less striking. The b values do decrease as Skin Type increases but a lot of variability is seen within the study group especially as Fitzpatrick Skin Type increases. a values are not demonstrated to vary according to the Fitzpatrick Skin Type.

Table 42 presents the Delta-E values which represent the colour difference if any between each of the Fitzpatrick Skin Types. Table 43 describes the meaning of the calculated Delta-E value.

Table 42 Delta-E of Means and Medians

Fitzpatrick Skin Type	Delta E of Means	Delta E of Medians
V vs. IV	9.476	12.191
VI vs. IV	15.861	18.206
VI vs. V	6.729	6.705

Table 43 The Meaning of Delta-E Values

Delta E Value	Meaning
0-1	A normally invisible difference
1-2	Very small difference, only obvious to a trained eye
2-3.5	Medium difference, also obvious to an untrained eye
3.5-5	An obvious difference
>6	A very obvious difference

Table 42 and Table 43 illustrate that there is a very obvious difference in Lab values between the Fitzpatrick Skin Types according to the calculated Delta-E values. Not surprisingly the biggest difference is between Type IV and Type VI. The difference between Type IV and Type V is greater than the difference between Type V and Type VI.

The data was analysed using ANOVA analysis of variance. ANOVA produces an F-statistic which is used to calculate a p-value. Because ANOVA is carried out with three independent variable, in this case Fitzpatrick Skin Type, a p-value of <0.05 shows that at least 2 groups are different from each other. In order to determine which groups are different from which, post-hoc one-way ANOVA (equivalent to a t-test) tests have been performed.

Table 44 ANalysis Of VAriance in L values between Fitzpatrick Skin Type Groups

ANOVA	F	P-value	F critical
IV – V - VI	21.77647	9.42E-08	3.158843
IV – V	16.57395	0.000245	4.113165
V – VI	8.439568	0.005725	4.061706
IV-VI	46.0862	8.3E-08	4.130018

L values subjected to ANOVA testing are shown in Table 44 to be significantly different (p< 0.05) between all the groups. Table 45 illustrates ANOVA testing of the a values. A significant difference between the 3 groups is not noted (p > 0.05). When Skin Type IV and V are compared there is a significant difference noted (p-0.03) and when Skin Type V and VI are compared there is a significant difference noted (p-0.04). There is not a significant difference noted between Skin Type IV and Skin Type V (p-0.8). Subjects with Skin Type V were shown earlier in the chapter to have a higher mean a value than Skin Type IV and Type VI.

Table 45 ANalysis Of VAriance in a values between Fitzpatrick Skin Type Groups

ANOVA	F	P-value	F critical
IV – V - VI	2.897667	0.063314	3.158843
IV – V	5.446279	0.025310	4.113165
V – VI	4.314463	0.043660	4.061706
IV-VI	0.048479	0.827049	4.130018

Table 46 ANalysis Of VAriance in b values between Fitzpatrick Skin Type Groups

ANOVA	F	P-value	F critical
IV – V - VI	11.50723	6.34E-05	3.158843
IV – V	2.656349	0.111857	4.113165
V – VI	10.60090	0.002179	4.061706
IV-VI	19.06098	0.000112	4.130018

Table 46 illustrates that b values of all three Skin Types subjected to ANOVA testing are significantly different ($p< 0.05$). However whenever the groups are considered in pairs, Skin Type IV and Skin Type V are not shown to be significantly different in terms of b values. The other pair wise tests comparing b values for Skin Type V and VI, and Skin Type IV and VI, were both significant ($p<0.05$).

5.5 Discussion and Summary

This chapter describes for the first time the Fitzpatrick Skin Type and its correlation with spectrophotometric measurements of skin colour in normal skin. The skin measurements are in the form of Lab scores which define colour on three axes, light/dark, red/green and yellow/blue. The results demonstrate that volunteers described as having Fitzpatrick Skin Type IV in this study had the lightest skin. Volunteers described as having Fitzpatrick Skin Type V had darker skin than those described as Fitzpatrick Skin Type 4 and lighter skin than those described as having Fitzpatrick Skin Type 6. Volunteers described as having Fitzpatrick Skin Type VI had the darkest skin. The a values were not shown to vary according to Fitzpatrick Skin Type. The b values varied with Fitzpatrick Skin Type but the variation was not consistently significant across the Skin Types.

These results are relevant to this group of volunteers and show that the Fitzpatrick Skin Type Classification of volunteers was an effective way of classifying the varying skin colour of the population group in terms of lightness/darkness.

Further work would be necessary to validate the Fitzpatrick Skin Type Classification system. Greater numbers would be required, including females and individuals from all of the Fitzpatrick Skin Type Groups.

6 THESIS SUMMARY

Until recently the natural history of scar maturation in humans had not been formally described. Bond et al. (2008b) carried out an observational study of scar maturation from both a clinical and histologic standpoint over a 12 month period. This study considered a male European Continental Ancestry Group (ECAG). Scar severity is variable and is dependent on numerous factors such as scar position, age, gender and race (O'Kane, 2002). It is important that the natural history of scar maturation in humans is established for all skin types. This study considered the maturation of scars in male volunteers of African Continental Ancestry.

Sixty male subjects of African Continental Ancestry between the ages of 18-85 years, with a median age of 32 years who had given written informed consent were recruited to take part in this study. The primary objective of this trial was to describe the process of scar maturation in volunteers of African Continental Ancestry. The study was designed in a similar fashion to a previously reported clinical trial (Bond, 2009) which described the maturation of the human scar in volunteers of European Ancestry (Caucasian). The African Continental Ancestry Group was considered in this study, and in order to maintain a standardised approach to collecting race and ethnicity information, FDA guidance on ethnic groupings was followed. These guidelines also reflect the 2004 changes to MeSH headings for race and ethnic groups, with terms such as race, Caucasian and black no longer being used. Volunteers self-ascribed to the appropriate grouping. This trial involved the wounding of volunteers with a greater

risk of poor scarring than the previous European Continental Ancestry Group. Arrangements were made therefore for review of the scars, by a Consultant Plastic Surgeon, of any volunteer that during the course of the trial developed a problematic scar. Such volunteers could then be excluded from further surgical interventions and referred to an appropriate person for further follow-up or treatment as appropriate. Fortunately we did not have to avail of this service as no volunteer developed a problematic scar.

Three incisions and a punch biopsy where carried out on each arm according to a standardised technique and under standardised conditions. The incisional scars and punch biopsy scar were assessed clinically on a monthly basis for 12 months using subjective and objective clinical assessment tools. These included: investigator scar assessments according to a Clinical Scar Assessment Scale and Global assessment scoring; scar photography; VAS scoring by an Independent External Scar Assessment Panel (IESAP); colour assessment with spectrophotometry; and assessment of mechanical properties with the use of ballistometry. At various time points scars were excised for histology providing information at each month of scar development. Following scar excision the resultant excisional scar continued to be assessed every month. One of the three incisions was not excised but assessed monthly for 12 months.

Chapter 3 described how the appearance of a scar in male volunteers of African Continental Ancestry improves with time and is still ongoing at the end of a 12 month time period. With the exception of scar contour and scar redness, a steady state was not

been achieved. The results demonstrate that scar colour mismatch decreases and the mechanical properties of scars improve with time. The scar data was not shown to plateau or equilibrate with normal unwounded skin which would suggest that scar maturation is still ongoing. Scar width was shown to increase over the 12 months of the study, with younger volunteers having a greater propensity to develop wide scars. Volunteer skin type was shown to influence the resulting scar appearance. Age was not shown to exert a significant influence on scar quality but the interpretation of the results was limited by the lack of subjects recruited from the oldest age group (>55).However, in general, an improvement in scar appearance was observed over the course of 12 months, with maturation still ongoing but largely complete by 12 months.

Chapter 4 described for the first time the histology of scar maturation in individuals of African Continental Ancestry over 12 months. Scars were classified into three groups each displaying a different rate of longitudinal progression of scar maturation. Histology assessments were also carried out and some correlation between the results of the different individual histology assessments and the Scar Typing of the specimens was seen. None of these scars reached a state of maturity by Month 12 with only a few processes complete or in a steady state such as epithelial thickness and scar elevation. The process of collagen maturation is still ongoing at month 12; indeed many scars demonstrate a prolonged high turnover state of collagen synthesis and degradation. In general, scars were quite wide, due in large part to an erosion of the wound margins brought about by an aggressive remodeling of the scar margins. In addition to collagen maturation, rete ridge restoration and angiogenesis are still ongoing with persistent

inflammation identified in scars up to Month 12. However, no hypertrophic or keloid scars were identified.

The papillary dermis is of better quality compared to the reticular dermis in terms of collagen fibre organisation and there is a strong correlation shown between the Clinical VAS scores and the Histology VAS scores for the papillary dermis. In the histological assessment of the scars young people (ACAG) have poorer scars. However, the conclusions are limited by a lack of subjects from the older age group (>55). When Fitzpatrick Skin Type is considered alongside the scar typing we are able to see some evidence of scars being graded as poorer in volunteers with darker Skin Types but not with the consistency seen in the clinical findings. In general, findings have shown scars in the ACAG to be poorer than those in the ECAG.

Chapter 5 describes for the first time the Fitzpatrick Skin Type Classification and whether it correlates with spectrophotometric measurements of skin colour. The results demonstrate that there is correlation with values for lightness/darkness. According to the spectrophotometry values, volunteers described as having Fitzpatrick Skin Type IV had the lightest skin and volunteers described as having Fitzpatrick Skin Type VI had the darkest skin. These findings are limited in value. The Fitzpatrick Skin Type Classification would seem to have been an effective way of classifying the varying skin colour for this group of volunteers. However, further work would be necessary to fully validate the Fitzpatrick Skin Type Classification system.

Skin colour is one of the most obvious ways in which humans vary. This variation has been attributed to many factors, but most agree that the variations observed reflect adaptation to the environment (Jablonski and Chaplin, 2000). There are many theories regarding the evolution of human skin colour. Rogers et al (2004) propose that the common ancestors of all humans had light skin that was covered by dark hair. Jablonski and Chaplin (2000), on review of the literature, highlight that a darkly pigmented skin evolved quickly following hair loss and for two main reasons. Firstly, in order to protect sweat glands from UV-induced injury and secondly to prevent photolysis of folate which is essential for normal development of the embryonic neural tube and spermatogenesis. Jablonski and Chaplin (2000) describe the leading hypothesis for the evolution of human skin colour starting with a now hairless ancestral group of dark skinned individuals. As populations began to migrate, skin coloration evolved to accommodate the physiological needs of humans. The evolutionary constraints changed, resulting in a range of skin tones within northern populations where melanisation was no longer important and positive selection for lighter skin occurred most likely in order to permit UVB-induced synthesis of Vitamin D3.

Research by Elias et al (2010) proposes that a superior barrier function in darkly pigmented skin has driven, in part, the evolution of skin colour. The pigmentation of epidermis improves permeability barrier function, stratum corneum integrity and enhances cutaneous innate immunity (Elias et al., 2010). In hot humid climates there are a lot of potential pathogens and the benefits associated with increased pigmentation

would have provided a large adaptive advantage. In reviewing the literature there is no mention of response to injury and how it may have evolved in line with skin coloration. Undoubtedly, repeated microbial, parasitic and physical assaults will have prevailed in the tropical climates during early human evolution. The ability to fight infection and heal quickly will also have been important in terms of adaptive advantage. This is one possible reason for the strong cutaneous inflammatory response to injury and high turnover state observed during this study in volunteers of African Continental Ancestry. The result of aggressive wound healing is a cosmetically poorer scar. As humans migrated north the advantage of aggressive wound healing would have decreased in the less hostile colder climates and this function could have been lost without environmental pressures. A less aggressive form of wound healing provides a cosmetically good scar. These thoughts propose a reason for the association seen in this study between increased skin pigmentation and quality of wound healing especially in terms of appearance.

The study had two main limitations. The first limitation was the fact that only one volunteer was recruited from the age group 55-85. This meant that we were unable to make meaningful comparisons with previous work, in volunteers of European Continental Ancestry, which suggested that older people have a better quality of scar (Table 47). Secondly, the fact that the study length is 12 months is a limitation. Many of the results presented show that a steady state has not been reached by month 12 and it would be interesting to have data for later time points.

Table 47 Comparison of Study Findings with Findings of Bond (2009)

European Continental Ancestry Group	African Continental Ancestry Group
The clinical appearance of a scar correlates with its histological appearance.	The clinical appearance of a scar correlates with its histological appearance.
Human scars improve with time as they mature but in 12 months a steady state has not been being reached.	Human scars improve with time as they mature but in 12 months a steady state has not been being reached.
Three distinct groups, each displaying a different rate of longitudinal progression of scar maturation were identified from within the study group. Most subjects belonged to a "representative" subset.	Three groups were identified which displayed a different rate of longitudinal progression of scar maturation. No representative group was identified.
Healthy older people produce scars of better clinical quality than young people.	Subject age was not shown clinically to exert significant influence on scar quality.
Healthy older people (>55 years) produce scars which mature more quickly than scars of young people.	Lack of older subjects in this study, therefore no comparison can be made.
Younger subjects (<30 years) displayed a prolonged high turnover state and a retarded rate of maturation.	In the histological assessment of scars young people have poorer scars.
Clinical scoring of scars was influenced by scar type, position on the arm, subject age and subject BMI (volunteer skin type was not assessed in this study).	Clinical scoring of scars was influenced by volunteer skin type and not by age (scar type, position and subject BMI were not assessed in this study).
Clinical scoring of scars was influenced mainly by scar characteristics of scar colour and scar distortion.	Clinical scoring of scars was influenced mainly by scar characteristics of scar colour and scar distortion.
Scar redness faded at an average of seven months following wounding.	Scar redness faded at an average of nine months following wounding.
Scar mechanical properties improved with time but did not return to those of normal skin in 12 months.	Scar mechanical properties improved with time but did not return to those of normal skin in 12 months.
Ongoing inflammatory processes absent in all scars at month 12.	Ongoing inflammatory processes still noted in scars at month 12.

Several similarities are present between the African and European Continental Ancestry Groups as illustrated in Table 47. There are novel findings of prolonged scar redness and prolonged inflammatory processes in the African Continental Ancestry Group. There is no data available on scar width in the study of the ECAG to compare with the interesting finding in this study that scar width increased over the 12 month period. It appears clear that whilst the process of scar maturation slows with time, a steady state is not achieved within one year irrespective of skin type. The end of scar maturation occurs at a later time point. This has an important clinical application. In considering whether a patient should undergo scar revision surgery it is important that an adequate time as been given for scar maturation to have occurred before embarking on further surgery.

In conclusion, scar maturation in the African Continental Ancestry Group occurs as a series of defined macroscopic and microscopic stages over the course of 1 year. All scars showed evidence of clinical and histological improvement over the course of the study influenced in part by volunteer skin type and age. The process of scar maturation is not complete at 12 months. Results suggest that scar maturation in this study group occurs at a different rate and is of a different quality macroscopically and microscopically, compared to current knowledge of scar maturation in individuals of European Continental Ancestry (Caucasians).

REFERENCES

(2005) Compact Oxford English Dictionary of Current English. 3rd ed., Oxford University Press.

ABDEL-MALEK, Z., SWOPE, V., COLLINS, C., BOISSY, R., ZHAO, H. & NORDLUND, J. (1993) Contribution of melanogenic proteins to the heterogeneous pigmentation of human melanocytes. *J Cell Sci,* 106 (Pt 4)**,** 1323-31.

ALALUF, S., ATKINS, D., BARRETT, K., BLOUNT, M., CARTER, N. & HEATH, A. (2002) Ethnic variation in melanin content and composition in photoexposed and photoprotected human skin. *Pigment Cell Res,* 15**,** 112-8.

ALHADY, S. M. & SIVANANTHARAJAH, K. (1969) Keloids in various races. A review of 175 cases. *Plast Reconstr Surg,* 44**,** 564-6.

ANDERSEN, K. E. & MAIBACH, H. I. (1979) Black and white human skin differences. *J Am Acad Dermatol,* 1**,** 276-82.

ARNOLD, F. & WEST, D. C. (1991) Angiogenesis in wound healing. *Pharmacol Ther,* 52**,** 407-22.

ASHCROFT, G. S., HORAN, M. A. & FERGUSON, M. W. (1997) Aging is associated with reduced deposition of specific extracellular matrix components, an upregulation of angiogenesis, and an altered inflammatory response in a murine incisional wound healing model. *J Invest Dermatol,* 108**,** 430-7.

ASPINALL, P. J. (2005) The operationalization of race and ethnicity concepts in medical classification systems: issues of validity and utility. *Health Informatics Journal,* 11**,** 259-274.

BAIN, B. J. (1996) Ethnic and sex differences in the total and differential white cell count and platelet count. *J Clin Pathol,* 49**,** 664-6.

BARRICK, B., CAMPBELL, E. J. & OWEN, C. A. (1999) Leukocyte proteinases in wound healing: roles in physiologic and pathologic processes. *Wound Repair Regen,* 7**,** 410-22.

BARSH, G. S. (2003) What controls variation in human skin color? *PLoS Biol,* 1**,** E27.

BARYZA, M. J. & BARYZA, G. A. (1995) The Vancouver Scar Scale: an administration tool and its interrater reliability. *J Burn Care Rehabil,* 16**,** 535-8.

BAYAT, A., ARSCOTT, G., OLLIER, W. E., FERGUSON, M. W. & MC GROUTHER, D. A. (2004a) Description of site-specific morphology of keloid phenotypes in an Afrocaribbean population. *Br J Plast Surg,* 57**,** 122-33.

BAYAT, A., ARSCOTT, G., OLLIER, W. E., MCGROUTHER, D. A. & FERGUSON, M. W. (2005) Keloid disease: clinical relevance of single versus multiple site scars. *Br J Plast Surg,* 58**,** 28-37.

BAYAT, A., BOCK, O., MROWIETZ, U., OLLIER, W. E. & FERGUSON, M. W. (2004b) Genetic susceptibility to keloid disease: transforming growth factor beta receptor gene polymorphisms are not associated with keloid disease. *Exp Dermatol,* 13**,** 120-4.

BEAUSANG, E., FLOYD, H., DUNN, K. W., ORTON, C. I. & FERGUSON, M. W. (1998) A new quantitative scale for clinical scar assessment. *Plast Reconstr Surg,* 102**,** 1954-61.

BERARDESCA, E. & MAIBACH, H. (2003) Ethnic skin: overview of structure and function. *J Am Acad Dermatol,* 48**,** S139-42.

BOND, J. S. (2009) The Maturation of the Human Scar: A Clinical and Histological Study. *Faculty of Medical and Human Sciences.* Manchester, University of Manchester.

BOND, J. S., DUNCAN, J. A., MASON, T., SATTAR, A., BOANAS, A., O'KANE, S. & FERGUSON, M. W. (2008a) Scar redness in humans: how long does it persist after incisional and excisional wounding? *Plast Reconstr Surg,* 121**,** 487-96.

BOND, J. S., DUNCAN, J. A., SATTAR, A., BOANAS, A., MASON, T., O'KANE, S. & FERGUSON, M. W. (2008b) Maturation of the human scar: an observational study. *Plast Reconstr Surg,* 121**,** 1650-8.

BOTELLA-ESTRADA, R., SANMARTIN, O., SEVILA, A., ESCUDERO, A. & GUILLEN, C. (1999) Melanotic pigmentation in excision scars of melanocytic and non-melanocytic skin tumors. *J Cutan Pathol,* 26**,** 137-44.

BOWEN, R. L., STEBBING, J. & JONES, L. J. (2006) A review of the ethnic differences in breast cancer. *Pharmacogenomics,* 7**,** 935-42.

BRAIMAN-WIKSMAN, L., SOLOMONIK, I., SPIRA, R. & TENNENBAUM, T. (2007) Novel insights into wound healing sequence of events. *Toxicol Pathol,* 35**,** 767-79.

BROUGHTON, G., 2ND, JANIS, J. E. & ATTINGER, C. E. (2006) Wound healing: an overview. *Plast Reconstr Surg,* 117**,** 1e-S-32e-S.

BROWN, N. J., SMYTH, E. A., CROSS, S. S. & REED, M. W. (2002) Angiogenesis induction and regression in human surgical wounds. *Wound Repair Regen,* 10**,** 245-51.

BURCHARD, E. G., ZIV, E., COYLE, N., GOMEZ, S. L., TANG, H., KARTER, A. J., MOUNTAIN, J. L., PEREZ-STABLE, E. J., SHEPPARD, D. & RISCH, N. (2003) The importance of race and ethnic background in biomedical research and clinical practice. *N Engl J Med,* 348**,** 1170-5.

BURT, V. L., WHELTON, P., ROCCELLA, E. J., BROWN, C., CUTLER, J. A., HIGGINS, M., HORAN, M. J. & LABARTHE, D. (1995) Prevalence of hypertension in the US adult population. Results from the Third National Health and Nutrition Examination Survey, 1988-1991. *Hypertension,* 25**,** 305-13.

BUSCA, R. & BALLOTTI, R. (2000) Cyclic AMP a key messenger in the regulation of skin pigmentation. *Pigment Cell Res,* 13**,** 60-9.

CARR, T., HARRIS, D. & JAMES, C. (2000) The Derriford Appearance Scale (DAS-59): A new scale to measure individual responses to living with problems of appearance. *British Journal of Health Psychology,* 5**,** 201-215.

CHIEN, C., MORIMOTO, L. M., TOM, J. & LI, C. I. (2005) Differences in colorectal carcinoma stage and survival by race and ethnicity. *Cancer,* 104**,** 629-39.

COON, C. S. (1962) The Problem of Racial Origins. *The Origin of Races.* London, Jonathan Cape.

COX, L. (2003) Present angiogenesis research and its possible future implementation in wound care. *J Wound Care,* 12**,** 225-8.

DANIELS, F. & JOHNSON, B. E. (1972) Normal, Physiologic, and Pathologic Effects of Solar Radiation on the Skin. IN FITZPATRICK, T. B. (Ed.) *Sunlight and Man.* Univeristy of Tokyo Press.

DESMOULIERE, A., CHAPONNIER, C. & GABBIANI, G. (2005) Tissue repair, contraction, and the myofibroblast. *Wound Repair Regen,* 13**,** 7-12.

DIEGELMANN, R. F., COHEN, I. K. & KAPLAN, A. M. (1981) The role of macrophages in wound repair: a review. *Plast Reconstr Surg,* 68**,** 107-13.

DIEGELMANN, R. F. & EVANS, M. C. (2004) Wound healing: an overview of acute, fibrotic and delayed healing. *Front Biosci,* 9**,** 283-9.

DIERICKX, C., GOLDMAN, M. P. & FITZPATRICK, R. E. (1995) Laser treatment of erythematous/hypertrophic and pigmented scars in 26 patients. *Plast Reconstr Surg,* 95**,** 84-90; discussion 91-2.

DOVI, J. V., HE, L. K. & DIPIETRO, L. A. (2003) Accelerated wound closure in neutrophil-depleted mice. *J Leukoc Biol,* 73**,** 448-55.

DOVI, J. V., SZPADERSKA, A. M. & DIPIETRO, L. A. (2004) Neutrophil function in the healing wound: adding insult to injury? *Thromb Haemost,* 92**,** 275-80.

DRESSLER, J., BUSUTTIL, A., KOCH, R. & HARRISON, D. J. (2001) Sequence of melanocyte migration into human scar tissue. *Int J Legal Med,* 115**,** 61-3.

DUNCAN, J. A., BOND, J. S., MASON, T., LUDLOW, A., CRIDLAND, P., O'KANE, S. & FERGUSON, M. W. (2006) Visual analogue scale scoring and ranking: a suitable and sensitive method for assessing scar quality? *Plast Reconstr Surg,* 118**,** 909-18.

EDWARDS, E. A. & DUNTLEY, S. Q. (1939) The pigments and color of living human skin. *American Journal of Anatomy,* 65**,** 1-33.

ELIAS, P. M., MENON, G., WETZEL, B. K. & WILLIAMS, J. J. (2010) Barrier requirements as the evolutionary "driver" of epidermal pigmentation in humans. *Am J Hum Biol,* 22**,** 526-37.

EMING, S. A., KRIEG, T. & DAVIDSON, J. M. (2007) Inflammation in wound repair: molecular and cellular mechanisms. *J Invest Dermatol,* 127**,** 514-25.

FDA (2005) Guidance for Industry: Collection of Race and Ethnicity Data in Clinical Trials.

FERGUSON, M. W. & LEIGH, I. M. (1998) Wound Healing. IN BURTON, J. L., BURNS, D. A. & BREATHNACH, S. M. (Eds.) *Textbook of Dermatology.* 6th ed., Blackwell Science.

FERGUSON, M. W. & O'KANE, S. (2004) Scar-free healing: from embryonic mechanisms to adult therapeutic intervention. *Philos Trans R Soc Lond B Biol Sci,* 359**,** 839-50.

FERGUSON, M. W., WHITBY, D. J., SHAH, M., ARMSTRONG, J., SIEBERT, J. W. & LONGAKER, M. T. (1996) Scar formation: the spectral nature of fetal and adult wound repair. *Plast Reconstr Surg,* 97**,** 854-60.

FITZPATRICK, T. B. (1988) The validity and practicality of sun-reactive skin types I through VI. *Arch Dermatol,* 124**,** 869-71.

FLEMING, I. D., BARNAWELL, J. R., BURLISON, P. E. & RANKIN, J. S. (1975) Skin cancer in black patients. *Cancer,* 35**,** 600-5.

FREEDLAND, S. J. & ISAACS, W. B. (2005) Explaining racial differences in prostate cancer in the United States: sociology or biology? *Prostate,* 62**,** 243-52.

FURIE, B. & FURIE, B. C. (1988) The molecular basis of blood coagulation. *Cell,* 53**,** 505-18.

GIRARDEAU, S., MINE, S., PAGEON, H. & ASSELINEAU, D. (2009) The Caucasian and African skin types differ morphologically and functionally in their dermal component. *Exp Dermatol,* 18**,** 704-11.

GODING, C. R. (2007) Melanocytes: the new Black. *Int J Biochem Cell Biol,* 39**,** 275-9.

GURTNER, G. C. (2007) Wound Healing: Normal and Abnormal. IN THORNE, C. (Ed.) *Grabb & Smith's Plastic Surgery.* 6th ed. Philadelphia, Lippincott Williams & Wilkins.

HAASS, N. K., SMALLEY, K. S., LI, L. & HERLYN, M. (2005) Adhesion, migration and communication in melanocytes and melanoma. *Pigment Cell Res,* 18**,** 150-9.

HAMMAR, H. (1993) Wound healing. *Int J Dermatol,* 32**,** 6-15.

HART, J. (2002) Inflammation. 1: Its role in the healing of acute wounds. *J Wound Care,* 11**,** 205-9.

HARTLAPP, I., ABE, R., SAEED, R. W., PENG, T., VOELTER, W., BUCALA, R. & METZ, C. N. (2001) Fibrocytes induce an angiogenic phenotype in cultured endothelial cells and promote angiogenesis in vivo. *FASEB J,* 15**,** 2215-24.

HEIAT, A., GROSS, C. P. & KRUMHOLZ, H. M. (2002) Representation of the elderly, women, and minorities in heart failure clinical trials. *Arch Intern Med,* 162**,** 1682-8.

HINZ, B. (2007) Formation and function of the myofibroblast during tissue repair. *J Invest Dermatol,* 127**,** 526-37.

HIROBE, T. (2005) Role of keratinocyte-derived factors involved in regulating the proliferation and differentiation of mammalian epidermal melanocytes. *Pigment Cell Res,* 18**,** 2-12.

JABLONSKI, N. G. & CHAPLIN, G. (2000) The evolution of human skin coloration. *J Hum Evol,* 39**,** 57-106.

JEMEC, G. B., SELVAAG, E., AGREN, M. & WULF, H. C. (2001) Measurement of the mechanical properties of skin with ballistometer and suction cup. *Skin Res Technol,* 7**,** 122-6.

JIMBOW, K., QUEVEDO, W. C., JR., FITZPATRICK, T. B. & SZABO, G. (1976) Some aspects of melanin biology: 1950-1975. *J Invest Dermatol,* 67**,** 72-89.

KAIDBEY, K. H., AGIN, P. P., SAYRE, R. M. & KLIGMAN, A. M. (1979) Photoprotection by melanin--a comparison of black and Caucasian skin. *J Am Acad Dermatol,* 1**,** 249-60.

KARCH, F. E. & LASAGNA, L. (1977) Toward the operational identification of adverse drug reactions. *Clin Pharmacol Ther,* 21**,** 247-54.

KAWADA, A. (2000) Risk and preventive factors for skin phototype. *J Dermatol Sci,* 23 Suppl 1**,** S27-9.

KEAST, O. & ORSTED, H. (2009) The Basic Principles of Wound Healing. Canadian Association of Wound Care.

KETCHUM, L. D., COHEN, I. K. & MASTERS, F. W. (1974) Hypertrophic scars and keloids. A collective review. *Plast Reconstr Surg,* 53**,** 140-54.

LA RUCHE, G. & CESARINI, J. P. (1992) [Histology and physiology of black skin]. *Ann Dermatol Venereol,* 119**,** 567-74.

LEE, J. Y., YANG, C. C., CHAO, S. C. & WONG, T. W. (2004) Histopathological differential diagnosis of keloid and hypertrophic scar. *Am J Dermatopathol,* 26**,** 379-84.

LEIBOVICH, S. & ROSS, R. (1975) The role of the macrophage in wound repair. A study with hydrocortisone and antimacrophage serum. *Am J Pathol,* 78**,** 71-100.

LI-TSANG, C. W., LAU, J. C. & LIU, S. K. (2003) Validation of an objective scar pigmentation measurement by using a spectrocolorimeter. *Burns,* 29**,** 779-84.

LIN, J. Y. & FISHER, D. E. (2007) Melanocyte biology and skin pigmentation. *Nature,* 445**,** 843-50.

MARCUS, J. R., TYRONE, J. W., BONOMO, S., XIA, Y. & MUSTOE, T. A. (2000) Cellular mechanisms for diminished scarring with aging. *Plast Reconstr Surg,* 105**,** 1591-9.

MCDONALD, C. J. (1988) Structure and function of the skin. Are there differences between black and white skin? *Dermatol Clin,* 6**,** 343-7.

MCGREGOR, A. D., & MCGREGOR, I.A. (2000) *Fundamental Techniques of Plastic Surgery and Their Surgical Applications,* London, Churchill Livingstone.

METCALFE, A. D. & FERGUSON, M. W. (2005) Harnessing wound healing and regeneration for tissue engineering. *Biochem Soc Trans,* 33**,** 413-7.

MILLER, M. C. & NANCHAHAL, J. (2005) Advances in the modulation of cutaneous wound healing and scarring. *BioDrugs,* 19**,** 363-81.

MONTAGNA, W. & CARLISLE, K. (1991) The architecture of black and white facial skin. *J Am Acad Dermatol,* 24**,** 929-37.

MONTAGNA, W., PROTA, G. & KENNEY, J. A. (1993) *Black Skin: Structure and Function*, Academic Press, Inc.

MORGAN, J. E., GILCHREST, B. & GOLDWYN, R. M. (1975) Skin pigmentation. Current concepts and relevance to plastic surgery. *Plast Reconstr Surg,* 56**,** 617-28.

NISSEN, N. N., POLVERINI, P. J., KOCH, A. E., VOLIN, M. V., GAMELLI, R. L. & DIPIETRO, L. A. (1998) Vascular endothelial growth factor mediates angiogenic activity during the proliferative phase of wound healing. *Am J Pathol,* 152**,** 1445-52.

O'KANE, S. (2002) Wound remodelling and scarring. *J Wound Care,* 11**,** 296-9.

OCCLESTON, N. L., LAVERTY, H. G., O'KANE, S. & FERGUSON, M. W. (2008a) Prevention and reduction of scarring in the skin by Transforming Growth Factor beta 3 (TGFbeta3): from laboratory discovery to clinical pharmaceutical. *J Biomater Sci Polym Ed,* 19**,** 1047-63.

OCCLESTON, N. L., METCALFE, A. D., BOANAS, A., BURGOYNE, N. J., NIELD, K., O'KANE, S. & FERGUSON, M. W. (2010) Therapeutic improvement of scarring: mechanisms of scarless and scar-forming healing and approaches to the discovery of new treatments. *Dermatol Res Pract,* 2010.

OCCLESTON, N. L., O'KANE, S., GOLDSPINK, N. & FERGUSON, M. W. (2008b) New therapeutics for the prevention and reduction of scarring. *Drug Discov Today,* 13**,** 973-81.

PARKS, W. C. (1999) Matrix metalloproteinases in repair. *Wound Repair Regen,* 7**,** 423-32.

PEPPER, F. J. (1954) The Epithelial Repair of Skin Wounds in the Guinea-Pig with Special Reference to the Participation of Melanocytes. *Journal of Morphology,* 95**,** 471-499.

QUAN, T. E., COWPER, S., WU, S. P., BOCKENSTEDT, L. K. & BUCALA, R. (2004) Circulating fibrocytes: collagen-secreting cells of the peripheral blood. *Int J Biochem Cell Biol,* 36**,** 598-606.

QUEVEDO, W. C., FITZPATRICK, T. B. & JIMBOW, K. (1985) Human-Skin Color - Origin, Variation and Significance. *Journal of Human Evolution,* 14**,** 43-56.

QUEVEDO, W. C., FITZPATRICK, T. B., PATHAK, M. A. & JIMBOW, K. (1972) Light and Skin Colour. IN FITZPATRICK, T. B. (Ed.) *Sunlight and Man.* University of Tokyo Press.

QUEVEDO, W. C., FITZPATRICK, T. B., PATHAK, M. A. & JIMBOW, K. (1975) Role of Light in Human Skin Color Variation. *American Journal of Physical Anthropology,* 43**,** 393-408.

QUEVEDO, W. C. & HOLSTEIN, T. J. (2006) General Biology of Mammalian Pigmentation. IN NORDLUND, J. J. (Ed.) *The Pigmentary System: Physiology and Pathophysiology.* 2nd ed., Blackwell Publishing Ltd.

QUINN, J. V., DRZEWIECKI, A. E., STIELL, I. G. & ELMSLIE, T. J. (1995) Appearance scales to measure cosmetic outcomes of healed lacerations. *Am J Emerg Med,* 13**,** 229-31.

RAMPEN, F. H., FLEUREN, B. A., DE BOO, T. M. & LEMMENS, W. A. (1988) Unreliability of self-reported burning tendency and tanning ability. *Arch Dermatol,* 124**,** 885-8.

RAWLINGS, A. V. (2006) Ethnic skin types: are there differences in skin structure and function? *Int J Cosmet Sci,* 28**,** 79-93.

REES, J. L. (2000) The melanocortin 1 receptor (MC1R): more than just red hair. *Pigment Cell Res,* 13**,** 135-40.

REES, J. L. (2003) Genetics of hair and skin color. *Annu Rev Genet,* 37**,** 67-90.

ROBERTS, W. E. (2008) The Roberts Skin Type Classification System. *J Drugs Dermatol,* 7**,** 452-6.

ROBINS, A. H. (1991) *Biological Perspectives on Human Pigmentation*, Cambridge University Press.

ROGERS, R., ILTIS, D. & WOODING, S. (2004) Genetic Variation at the MC1R Locus and the Time since Loss of Human Body Hair. *Current Anthropology,* 45**,** 105-108.

ROQUES, C. & TEOT, L. (2007) A critical analysis of measurements used to assess and manage scars. *Int J Low Extrem Wounds,* 6**,** 249-53.

ROSS, R. & BENDITT, E. P. (1961) Wound healing and collagen formation. I. Sequential changes in components of guinea pig skin wounds observed in the electron microscope. *J Biophys Biochem Cytol,* 11**,** 677-700.

ROUSSEAU, K., KAUSER, S., PRITCHARD, L. E., WARHURST, A., OLIVER, R. L., SLOMINSKI, A., WEI, E. T., THODY, A. J., TOBIN, D. J. & WHITE, A. (2007) Proopiomelanocortin (POMC), the ACTH/melanocortin precursor, is secreted by human epidermal keratinocytes and melanocytes and stimulates melanogenesis. *Faseb Journal,* 21**,** 1844-1856.

SCHWAHN, D. J., XU, W., HERRIN, A. B., BALES, E. S. & MEDRANO, E. E. (2001) Tyrosine levels regulate the melanogenic response to alpha-melanocyte-stimulating hormone in human melanocytes: implications for pigmentation and proliferation. *Pigment Cell Res,* 14**,** 32-9.

SCOTT, G. A. (2006) Melanosome Trafficking and Transfer. IN NORDLUND, J. J. (Ed.) *The Pigmentary System: Physiology and Pathophysiology.* 2nd ed., Blackwell Publishing Ltd.

SEIJI, M., FITZPATRICK, T. B., SIMPSON, R. T. & BIRBECK, M. S. (1963) Chemical composition and terminology of specialized organelles (melanosomes and melanin granules) in mammalian melanocytes. *Nature,* 197**,** 1082-4.

SHAH, M., FOREMAN, D. M. & FERGUSON, M. W. (1995) Neutralisation of TGF-beta 1 and TGF-beta 2 or exogenous addition of TGF-beta 3 to cutaneous rat wounds reduces scarring. *J Cell Sci,* 108 (Pt 3)**,** 985-1002.

SHRIVER, M. D. & PARRA, E. J. (2000) Comparison of narrow-band reflectance spectroscopy and tristimulus colorimetry for measurements of skin and hair color in persons of different biological ancestry. *Am J Phys Anthropol,* 112**,** 17-27.

SIMPSON, D. M. & ROSS, R. (1972) The neutrophilic leukocyte in wound repair a study with antineutrophil serum. *J Clin Invest,* 51**,** 2009-23.

SINGER, A. J., ARORA, B., DAGUM, A., VALENTINE, S. & HOLLANDER, J. E. (2007) Development and validation of a novel scar evaluation scale. *Plast Reconstr Surg,* 120**,** 1892-7.

SINGER, A. J. & CLARK, R. A. (1999) Cutaneous wound healing. *N Engl J Med,* 341**,** 738-46.

SLOMINSKI, A., TOBIN, D. J., SHIBAHARA, S. & WORTSMAN, J. (2004) Melanin pigmentation in mammalian skin and its hormonal regulation. *Physiol Rev,* 84**,** 1155-228.

SNELL, R. S. (1963) A study of the melanocytes and melanin in a healing deep wound. *J Anat,* 97**,** 243-53.

SOMMERLAD, B. C. & CREASEY, J. M. (1978) The stretched scar: a clinical and histological study. *Br J Plast Surg,* 31**,** 34-45.

SPYROU, G. E. & NAYLOR, I. L. (2002) The effect of basic fibroblast growth factor on scarring. *Br J Plast Surg,* 55**,** 275-82.

STAVROU, D. (2008) Neovascularisation in wound healing. *J Wound Care,* 17**,** 298-300, 302.

STEPHENS, P. & THOMAS, D. W. (2002) The cellular proliferative phase of the wound repair process. *J Wound Care,* 11**,** 253-61.

SULLIVAN, T., SMITH, J., KERMODE, J., MCIVER, E. & COURTEMANCHE, D. J. (1990) Rating the burn scar. *J Burn Care Rehabil,* 11**,** 256-60.

SZABO, G., GERALD, A. B., PATHAK, M. A. & FITZPATRICK, T. B. (1969) Racial differences in the fate of melanosomes in human epidermis. *Nature,* 222**,** 1081-2.

TAYLOR, A. L. & WRIGHT, J. T., JR. (2005) Should ethnicity serve as the basis for clinical trial design? Importance of race/ethnicity in clinical trials: lessons from the African-American Heart Failure Trial (A-HeFT), the African-American Study of Kidney Disease and Hypertension (AASK), and the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). *Circulation,* 112**,** 3654-60; discussion 3666.

TAYLOR, S. C. (2002) Skin of color: biology, structure, function, and implications for dermatologic disease. *J Am Acad Dermatol,* 46**,** S41-62.

THOMAS, D. W. & HARDING, K. G. (2002) Wound healing. *Br J Surg,* 89**,** 1203-5.

THONG, H. Y., JEE, S. H., SUN, C. C. & BOISSY, R. E. (2003) The patterns of melanosome distribution in keratinocytes of human skin as one determining factor of skin colour. *Br J Dermatol,* 149**,** 498-505.

TONNESEN, M. G., FENG, X. & CLARK, R. A. (2000) Angiogenesis in wound healing. *J Investig Dermatol Symp Proc,* 5**,** 40-6.

TRACY, R. P. (2006) The Five Cardinal Signs of Inflammation: Calor, Dolor, Rubor, Tumor ... and Penuria (Apologies to Aulus Cornelius Celsus, De medicina, c. A.D. 25). *J Gerontol A Biol Sci Med Sci,* 61**,** 1051-1052.

VAN ZUIJLEN, P. P., ANGELES, A. P., KREIS, R. W., BOS, K. E. & MIDDELKOOP, E. (2002) Scar assessment tools: implications for current research. *Plast Reconstr Surg,* 109**,** 1108-22.

VANCE, K. W. & GODING, C. R. (2004) The transcription network regulating melanocyte development and melanoma. *Pigment Cell Res,* 17**,** 318-25.

VELANGI, S. S. & REES, J. L. (2001) Why are scars pale? An immunohistochemical study indicating preservation of melanocyte number and function in surgical scars. *Acta Derm Venereol,* 81**,** 326-8.

WERNER, S., KRIEG, T. & SMOLA, H. (2007) Keratinocyte-fibroblast interactions in wound healing. *J Invest Dermatol,* 127**,** 998-1008.

WESTERHOF, W. (2006) The discovery of the human melanocyte. *Pigment Cell Res,* 19**,** 183-93.

WU, Y., WANG, J., SCOTT, P. G. & TREDGET, E. E. (2007) Bone marrow-derived stem cells in wound healing: a review. *Wound Repair Regen,* 15 Suppl 1**,** S18-26.

YAAR, M. & GILCHREST, B. A. (2004) Melanocyte biology: before, during, and after the Fitzpatrick era. *J Invest Dermatol,* 122**,** xxvii-xxix.

APPENDIX 1: Visit Schedule and Assessments

Procedure / Assessment	Visit														
	Screening	Day 0	Day 7	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10	Month 11	Month 12
Informed consent	X														
Medical history review	X														
Demographic data	X														
Physical examination	X	X		G1	G2	G3	G4	G5	G6	G1	G2	G3	G4	G5	G6
Blood pressure & pulse	X	X		G1	G2	G3	G4	G5	G6	G1	G2	G3	G4	G5	G6
12-lead ECG	X														
Blood sample & urine sample for laboratory safety screen	X														
Urine drugs of abuse	X														
Record body temp & room temp				X	X	X	X	X	X	X	X	X	X	X	X
Incisions and punch biopsy made		X													
Wound re-dressing			X												
Excision of 2 incisions (proximal & distal)				G1 (i)	G2 (i)	G3 (i)	G4 (i)	G5 (i)	G6 (i)	G1 (ii)	G2 (ii)	G3 (ii)	G4 (ii)	G5 (ii)	G6 (ii)
Photography		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Local tolerability assessed		X	X	G1*	G2*	G3*	G4*	G5*	G6*	G1*	G2*	G3*	G4*	G5*	G6*
Adverse events & concomitant medication		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Scar assessments and rankings				X	X	X	X	X	X	X	X	X	X	X	X
Ballistometry						X	X	X	X	X	X	X	X	X	X

G1 – G6 = Groups 1 to 6 in each population group.
(i) = First excision surgery visit (1st arm) **(ii)** = Second excision surgery visit (2nd arm) punch biopsy scar will also be excised at this visit.
***** = An additional follow-up 7 days following excision surgery will be carried out for these groups; this will be the same format as Day 7.

APPENDIX 2: Histology Sample Processing

Tissue processing is the process where the samples are dehydrated in increasing concentrations of Industrial Denatured Alcohol (IDA). Following dehydration, a clearing reagent (Xylene) is introduced to the samples. Xylene is miscible with IDA and also the eventual paraplast wax. Following the Xylene, paraplast wax is pumped into the samples under vacuum. The ultimate aim of tissue processing is to replace the water in the tissue with paraffin wax (providing stability to the tissue). The samples in this trial were subjected to the following process in the Leica TP1050 Tissue Processor:

50% IDA 0:45hr
70% IDA 0:45hr
90% IDA 1:00hr
100% IDA 0:30hr
100% IDA 0:30hr
100% IDA 1:00hr
Xylene 0:30hr
Xylene 0:30hr
Xylene 1:30hr
Paraplast plus wax 0:30hr (Vacuum)
Paraplast plus wax 0:30hr (Vacuum)
Paraplast plus wax 1:00hr (Vacuum)

The samples were embedded in Paraplast wax blocks which were then cooled and cut on a microtome. Sections were taken at 5 microns thickness and mounted on

Superfrost Plus charged slides. Once dried, the slides were ready for staining in the Autostainer XL.

Masson's Trichrome Protocol:

Xylene 3:00min
Xylene 3.00min
100% IDA 2:00min
70% 2:00min
Water 1:00min
Harris` Haematoxylin 9:00min
Wash 6:00min
Picric Acid 0.30min
Wash 4.00min
Biebrich Scarlet 1.00min
Water 0.20min
Phosphomolybdic Acid / Phosphotungstic Acid 8:00min
Fast Green 5:00min
Wash 1:40min
50% IDA 0:15min
70% IDA 0:15min
90% IDA 0:30min
100% IDA 1:00min
Xylene 1:00min
Xylene Exit

Following staining, the slides were cover slipped using pertex mountant on the CV3500 Cover slipper. Slides were viewed on LEICA DFC420 FX microscope and photographed using Leica QWin V3 software.

APPENDIX 3: Photography Procedure

The photographic procedure for this trial involved capturing a general shot of all 4 incisions and a close-up shot of each individual incision from Day 0 to Month 12. To ensure all the images remain consistent over a period of time, the equipment, lighting, exposure and magnification were all standardised.

Equipment/Materials:

- Kodak 14N digital camera
- Nikon 70-180mm macro lens
- Minolta macro twin 2400 flash body with 2 heads
- Minolta hot shoe adaptor FS1200 X2
- Sailwind Macromate Bellows with adaptor rings X2
- Large and small black plastic image mask (including Tetra adhesive ruler & white spot cut from Kodak Q13 step wedges)

Camera Exposure setting: Aperture = f38

Shutter speed = 1/90

Flash setting: Flash to fire both heads A and B

Flash set to ‘M’ manual and single flash

Lens Focal Length setting: Small image mask = 175mm.

Large image mask =95mm.

Procedure

The appropriate Image Identification Label was placed onto the image mask; this contained the Subject Number, Trial Number, Scar Site and Study Day. The image mask was dropped in place at the front of the camera. The Camera-Set-Up was then positioned at right angles to the incision site(s) and the photograph was taken.

The large image mask = general shot of all four incisions.

The small image mask = close-up of each individual incision.

After image capture, the digital card was uploaded onto the R drive of the Renovo computer network.

All Original Digital Images were burnt onto CD and stored at Renovo.

All images were then renamed and burnt to CD and stored at Renovo.

The renamed images were stored in the appropriate Subject Folder (CRF) within the Reference section.

Image processing

All images were standardised during processing. Images were taken from the Subjects CRF within Reference and loaded into Adobe Photoshop.

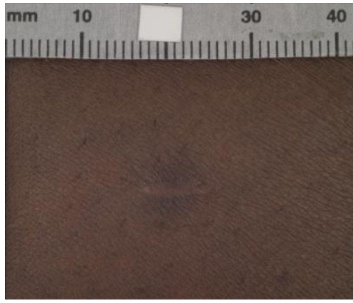
This trial had specific actions set in Adobe Photoshop, the required actions consisted of

- cropping the image to 25x20mm
- setting the resolution to 1280x1024 pixels
- altering the levels.

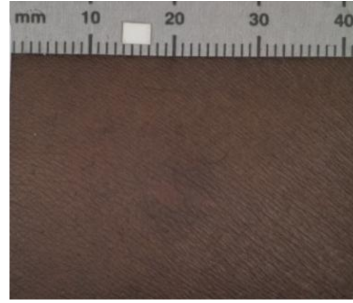
The images are calibrated and adjusted using the white spot on the scale. The white spot allows for standardisation by compensating for any fluctuations in flash or skin tone comparisons.

Figure 81 and Figure 82 provide a sample of photographic data showing monthly photography of the anterior scar of the dominant arm in 2 individuals who clinically classed as having a good/poor scar.

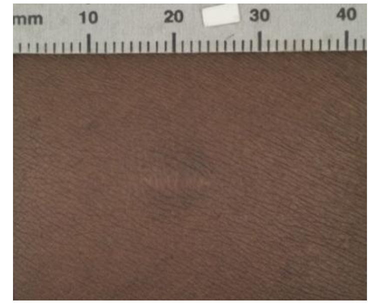
Month 1



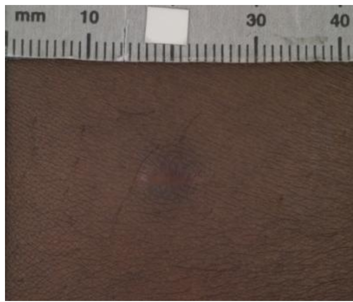
Month 5



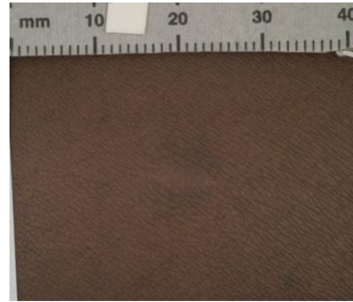
Month 9



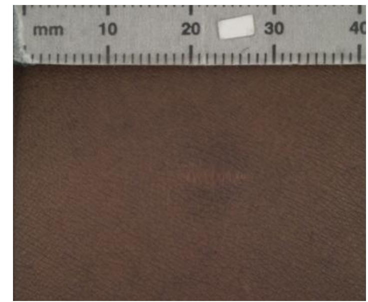
Month 2



Month 6



Month 10



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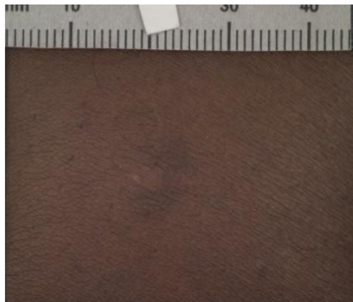

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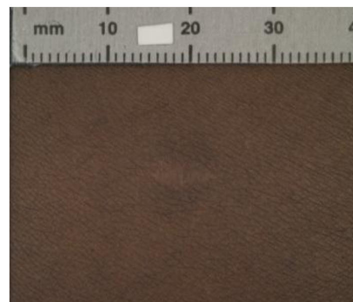

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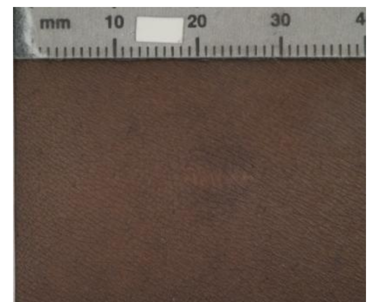

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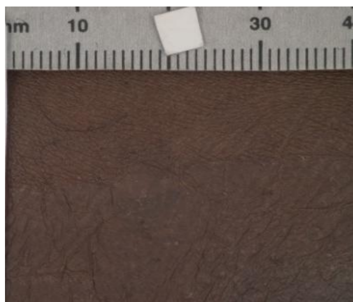


Month 8

Missed Visit

Month 12

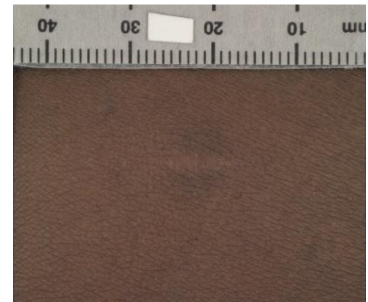
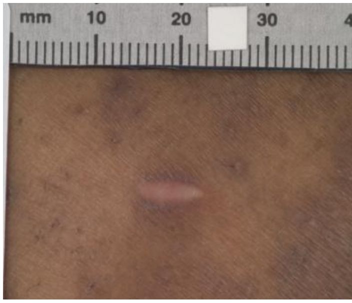


Figure 81 Sequence of Clinical Photographs - 'good' scar

Month 1



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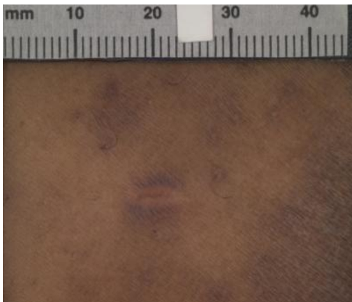

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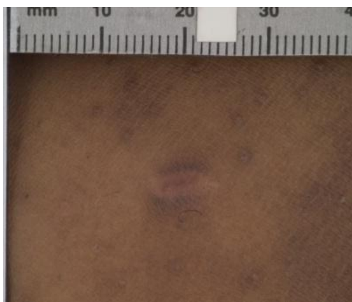

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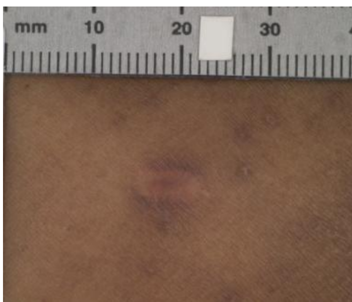
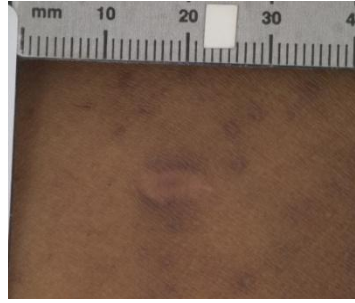
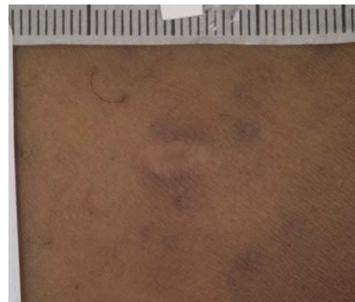


Month 5



Month 6

Month 7



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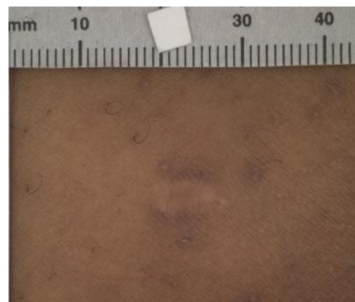

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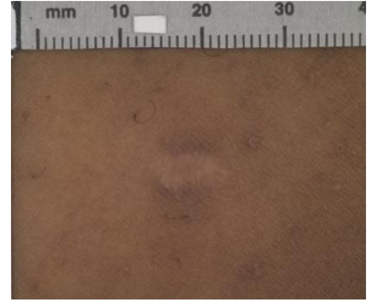

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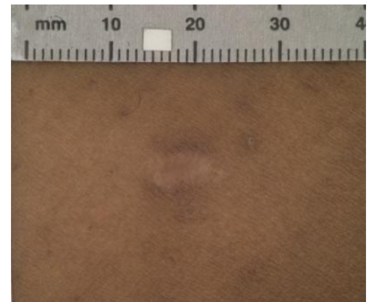
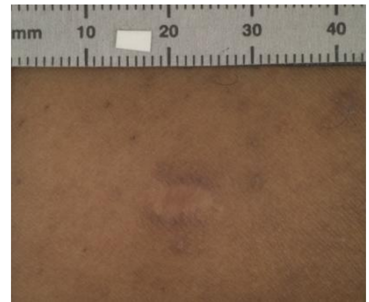


Month 11



Month 12

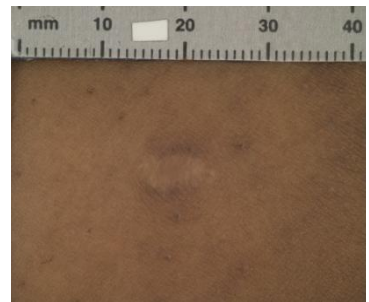


Figure 82 Sequence of Clinical Photographs – 'poor' scar

APPENDIX 4: Clinical Scar Assessment Scale

Width (mm) ___.__

Height (mm) ___.__

Colour (compared to surrounding skin)

1. ☐ Perfect
2. ☐ Slight Mismatch
3. ☐ Obvious Mismatch
4. ☐ Gross Mismatch

If 2-4 ☐ Lighter

☐ Darker

Contour

1. ☐ Flush with surrounding skin

2a. ☐ Slightly proud

2b. ☐ Slightly indented

3. ☐ Hypertrophic

4. ☐ Keloid

Appearance

1. ☐ Matte
2. ☐ Shiny

Distortion

1. ☐ None
2. ☐ Mild
3. ☐ Moderate
4. ☐ Severe

Texture

1. ☐ Normal
2. ☐ Just palpable
3. ☐ Firm
4. ☐ Hard

APPENDIX 5: Investigator Global Assessment Scale

Indicate which best indicates the appearance of each scar.

Very good scar	☐
Good scar	☐
Moderate scar	☐
Poor scar	☐
Very poor scar	☐

APPENDIX 6: Skin Type

Fitzpatrick devised a description of skin types known as the Fitzpatrick skin type classification. This classification denotes 6 different skin types, skin colour, and reaction to sun exposure.

Type I (very white or freckled) - Always burn

Type II (white) - Usually burn

Type III (white to olive) - Sometimes burn

Type IV (brown) - Rarely burn

Type V (dark brown) - Very rarely burn

Type VI (black) - Never burn

The following is a table that categorises skin from Skin Type 1 (light skin) to Skin Type VI (dark skin). The Fitzpatrick classification is determined by genetics, reaction of the skin to sun exposure and tanning habits.

The table below can be used by circling the appropriate response to each of the items. A total result can be calculated which will give Fitzpatrick skin type.

Genetic Disposition					
Score	**0**	**1**	**2**	**3**	**4**
What is the colour of your eyes?	Light blue, Gray, Green	Blue, Gray or Green	Blue	Dark Brown	Brownish Black
What is the natural colour of your hair?	Sandy Red	Blond	Chestnut/Dark Blond	Dark Brown	Black
What is the colour of your skin (non exposed areas)?	Reddish	Very Pale	Pale with Beige tint	Light Brown	Dark Brown
Do you have freckles on unexposed areas?	Many	Several	Few	Incidental	none
Total score for Genetic Disposition: _____					

Reaction to Sun Exposure					
Score	**0**	**1**	**2**	**3**	**4**
What happens when you stay in the sun too long?	Painful redness, blistering, peeling	Blistering followed by peeling	Burns sometimes followed by peeling	Rare burns	Never had burns
To What degree do you turn brown?	Hardly or not at all	Light colour tan	Reasonable tan	Tan very easy	Turn dark brown quickly
Do you turn brown within several hours after sun exposure?	Never	Seldom	Sometimes	Often	Always
How does your face react to the sun?	Very sensitive	Sensitive	Normal	Very resistant	Never had a problem
Total score for Reaction to Sun Exposure: _____					

Tanning Habits					
Score	**0**	**1**	**2**	**3**	**4**
When did you last expose your body to sun (or artificial sunlamp/tanning cream)?	More than 3 months ago	2-3 months ago	1-2 months ago	Less than a month ago	Less than 2 weeks ago
Did you expose the area to be treated to the sun?	Never	Hardly ever	Sometimes	Often	Always
Total score for Tanning Habits: _____					

Add up the total scores for each of the three sections for your Skin Type Score.

Skin Type Score	Fitzpatrick Skin Type
0-7	I
8-16	II
17-25	III
25-30	IV
over 30	V-VI

Fitzpatrick Classification for Sun-Reactive Skin Types

Skin Type	Reaction to UVA	Reaction to Sun
Type I	Very Sensitive	Always burns easily, never tans; very fair skin tone
Type II	Very Sensitive	Usually burns easily, tans with difficulty; fair skin tone
Type III	Sensitive	Burns moderately, tans gradually; fair to medium skin tone
Type IV	Moderately Sensitive	Rarely burns, always tans well; medium skin tone
Type V	Minimally Sensitive	Very rarely burns, tans very easily; olive or dark skin tone
Type VI	Least Sensitive	Never burns, deeply pigmented; very dark skin tone