An examination of scar modelling and assessment methods for the evaluation of treatments

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Contents

List of abbreviations

α-SMA	Alpha Smooth Muscle Actin
ADSC	Adipose Derived Stem Cells
BBSIP	Brisbane Burn Scar Impact Profile
$BBSIP_{0-8}$	Brisbane Burn Scar Impact Profile – 0 to 8 years old
$BBSIP_{8-18}$	Brisbane Burn Scar Impact Profile – 8 to 18 years old
BEST	Best Evidence for Scar Treatment
BTXA	Botulinum Toxin Type A
CO_2	Carbon Dioxide
COL1A1	Collagen type I, Alpha 1
CTGF	Connective Tissue Growth Factor
Er:YAG	Erbium-doped Yttrium Aluminium Garnet
FGF	Fibroblast Growth Factor
GCP	Good Clinical Practice
ICC	Intra-class Correlation Co-efficient
ICO	Information Commissioner
IGF-1	Insulin like Growth Factor 1
IL	Interleukin
LASER	Light Amplification by Stimulated Emission of Radiation
MMP	Matrix Protease
MSS	Manchester Scar Scale
mVSS	Modified Vancouver Scar Scale
NHS	National Health Service
PAI	Plasminogen Activator Inhibitor
PC	Panniculus Carnosus
PDGF	Platelet-derived Growth Factor
PDL	585mm Pulsed-dye LASER
PI	Perfusion Index
PIP	Pyrrole-imidazole Polyamide

PLGA	Polylactic-co-glycolic Acid
POSAS	Patient Observer Scar Assessment Scale
PROM	Patient Reported Outcome Measure
qPCR	Quantitative Polymerase Chain Reaction
RCT	Randomised Control Trial
REC	Research Ethics Committee
SEI	Scar Elevation Index
SNP	Single Nucleotide Polymorphism
TBSA	Total Burn Surface Area
TCA	Triamcinolone Acetonide
TERT	Telomerase Reverse Transcriptase
TEWL	Trans-epidermal Water Loss
TGF	Transforming Growth Factor
TNF-α	Tumour Necrosis Factor Alpha
TSC	Trial Steering Committee
TSS	Topical Silicone Sheeting
VEGF	Vascular Endothelial Growth Factor
VSS	Vancouver Scar Scale

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Abstract

Scarring is the final common pathway for healing within the skin irrespective of age, gender and race. Scars can be itchy, painful, tight and above all, cosmetically disfiguring. Despite advances in surgery and trauma management, there is currently no reliably effective treatment for reducing or preventing scarring. The primary aim of this thesis is to assess the currently available models for scarring and evaluate/further develop the utility of current assessment tools, in an effort to design a pilot randomised control trial for silicone gel treatment of scars.

Review of scar models in humans and animals demonstrated the limitations and drawbacks of many existing methods to assess scar treatments. Examination of currently used subjective and objective scar assessment tools in a plastic surgery clinic helped to develop protocols and methodology for a scar treatment research trial. Long-term scar outcomes assessed using a novel subjective patient reported outcome measure for paediatric burn patients demonstrated no statistically significant difference between those treated surgically, and those treated conservatively. A pilot randomised control trial to produce high quality evidence for silicone gel sheeting in the treatment of scars was set up and successful in recruitment.

Scarring remains a difficult condition for clinicians to manage, with many treatments utilised on a poor evidence basis. Here, we have demonstrated difficulties in establishing a scientific scar treatment model; and created a pilot study that will help to provide high quality evidence for the efficacy of silicone gel sheeting as a treatment for scars.

Chapter 1. Introduction

Scarring is the final outcome of the physiological healing process following injury. The skin is the largest organ in the human body and acts as a protective barrier amongst many other physiological functions. Damage to the skin through injury or insult leads to a physiological healing process to restore its function. Unlike the axolotl, salamanders and some reptilian species; humans do not regenerate after injury, we develop a repaired version of the tissue commonly known as scar tissue.[1] The exact mechanism by which scarring occurs is not fully understood, but the process by which a wound heals is well documented. Scarring in skin aims to heal and close a wound, seal it off and restore the skin's protective barrier function. Scar tissue can often look different to surrounding skin, appearing thicker or even continuing to grow after the wound has healed. Approximately 300 million people acquire significant scars annually across the globe.[2] Scarring is often considered problematic if patients are suffering from psychological and/or physical symptoms. Pathological scarring includes hypertrophic and keloid scars. These are often the most difficult to treat producing the most severe symptoms.

1.1 Wound Healing

The physiological process of wound healing has been well studied and can be divided into three distinct phases that are universally accepted in the process. They are the inflammatory phase, migratory (or tissue formation) phase, and the remodelling phase.[3] When it is injured, the highly vascularised nature of skin results in disruption of blood vessels and extravasation of blood constituents.[3] In the example of a laceration injury to the skin, the clotting cascade is activated and a clot is formed to prevent any further blood loss. Platelets help to form the clot and fibrin mesh which in turn acts a matrix for the healing process to begin. The platelets release platelet-derived growth factor (PDGF) that recruit macrophages and fibroblasts to the wound site.[3] In burns injuries

where bleeding is absent, the heat energy causes coagulation of dermal constituent proteins.[4] This results in recruitment of inflammatory leukocytes to the wound site which active the inflammatory pathways.[3]

Neutrophil recruitment to the wound site helps to reduce infection risk and clear cellular debris.[5] Chemoattractants released by the neutrophils and macrophages, along with the exposed extra-cellular matrix proteins result in expression of transforming growth factor (TGF) -β and infiltration of monocytes.[6] Macrophages release PDGF and vascular endothelial growth factor (VEGF) resulting in the creation of granulation tissue.[6] During this inflammatory phase, the macrophages phagocytose any damaged extracellular matrix protein and cellular debris, facilitating inflammatory cytokine release.[3] These include tumour necrosis factor-α (TNF- α), TGF- α, TGF-β and insulin-like growth factor 1 (IGF-1).[7] The exact mechanism by which these cytokines interact is not fully understood but their presence has been well documented.[3]

One to two days after the injury, the migratory phase begins which involves the proliferation and migration of certain cell types within the wound environment.[3] The proliferation of inflammatory cells is followed by migration of epidermal cells at the edge of the wound.[6] In order to enter the wound environment, the migrating epidermal cells activate plasmin by the action of plasminogen activator which also activates the collagenase matrix metalloproteases (MMP)-1.[3] These enzymes help to clear the path for the epidermal cells allowing them to migrate into the wound site and establish re-epithelialisation.[7] The exact proteins involved in activating migration are not known but are generally considered to be due to epidermal growth factor, TGF-α and keratinocyte growth factor.[3]

The formation of granulation tissue usually occurs by four days after the injury with the inflammatory action of macrophages and cytokines recruiting fibroblasts to the wound site.[6] The effect of VEGF promotes angiogenesis and neovascularisation to the wound site adequately nourishing the fibroblast cells, allowing them to lay down extra-cellular matrix and collagen.[8] The exact mechanism of how this happens is not understood but PDGF and TGF-β1 are believed to be responsible for activating fibroblasts to produce collagen.[3] Once the collagen has been laid down approximately 10 days after injury, the fibroblasts become myofibroblasts and produce actin filaments causing contraction of the wound site.[6] This aids the complete re-epithelialisation of the wound.

The final phase referred to as the remodelling phase can last for up to 18 months and involves the reshaping and restructuring of the newly formed collagen in the wound.[3] This tissue is referred to as scar tissue.

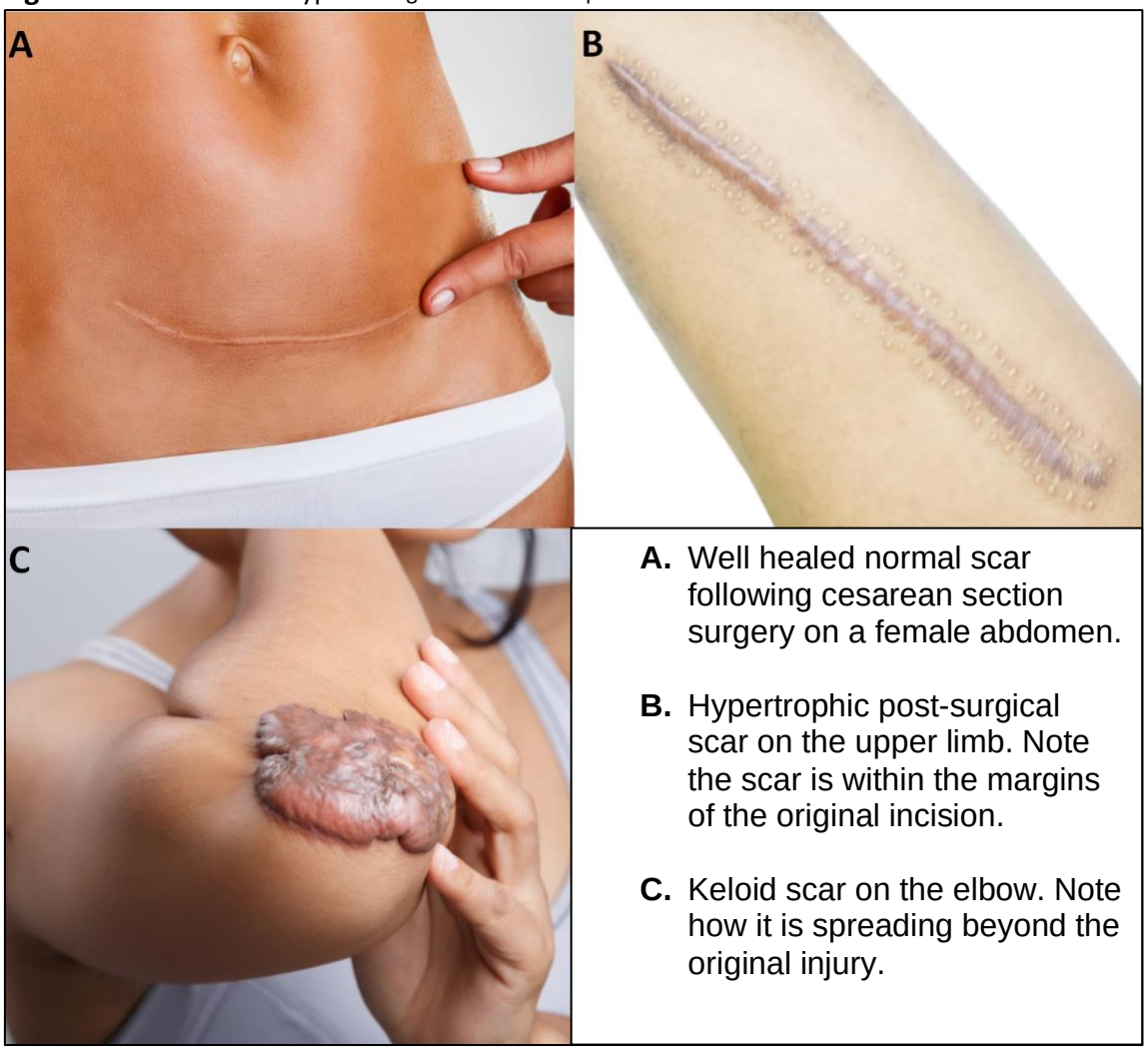
1.2 Scar Tissue

Scar tissue is comprised of collagen; and although there are 28 known forms of collagen, there are 5 types that are regarded as the most common. Type I Collagen is the most abundant and makes up the bulk of organic tissues including skin, tendon, vasculature and the organic part of bone.[9] Normal uninjured skin and scar tissue are both comprised of Type I collagen, but obvious differences in their macroscopic and microscopic appearance can be observed. Additional differences in texture, thickness and elasticity of scar tissue can affect function. These are likely due to structural differences in the way collagen in uninjured skin is arranged in a characteristic “basket weave” pattern in contrast to scar tissue collagens unorganised arrangement. Although humans do not possess regenerative ability, embryos appear to heal without scars if injured in utero.[10]

Inflammation is a key part of the healing process, but has also been reported to aid in the formation of thickened fibrotic scar tissue.[7,11] An interesting study by Martin et al showed that wound healing in PU.1 null mice (immunosuppressed mouse without macrophages and neutrophils) was significantly better than in wild type mice and produced almost scar-less healing.[11,12] Proposed theories as to the reason we mount an inflammatory response to injury include the beneficial effect this has on fighting infection.[13,14]

In today's society, scarring is generally seen as negative and can have a significant impact both physically and psychologically. Despite medicine's advances, scarring is still a difficult area in which to create effective treatments. Scarring is often classified into five separate subgroups: normal scar, widened scar, atrophic scar, hypertrophic scar and keloid scar.

Figure 1. Different scar types. Images obtained with permission from Shutterstock Inc.



1.2.1 Normal scar

Normal scarring or fine scarring (see Figure 1. A) is considered to be a cutaneous scar that is flat, pale, relatively discrete and which does not interfere with function. This could be from a well healed surgical incision or graze that has healed and scarred well. The collagen content, although not as organised as in uninjured skin, is sufficient to provide coverage with no excess and minimal interreference with skin function. The surface profile is usually flat, confined to the site of injury and may show darker or lighter coloration than surrounding skin. Normal scar tissue is not tethered to any deeper structures and moves in unison with normal healthy skin.

1.2.2 Widened scar

Widened or stretched scars have undergone a change in their structure due to the action of tension across the wound or skin.[15] They are flat scars and do not have a raised surface. Examples of where this can occur include after abdominal surgery or bilateral breast reduction where there can be tension across the wound.[2] Other examples of widened scar include stretch marks referred to as abdominal striae if on the abdomen (common during pregnancy). They can also occur in areas of muscle growth in groups such as body builders and athletes. Widened scars contain normal scar tissue, sufficient collagen content and glide/move over underlying tissues with the normal skin. Changes in pigmentation compared to surrounding skin may also be observed.

1.2.3 Atrophic Scar

Atrophic scars are referred to as scars that have a depressed appearance compared to the surrounding skin.[2] These are usually the result of skin conditions such as acne and chicken-pox, commonly referred to as pick-axe type scars due their sharp sunken in appearance .[16] The collagen content of atrophic scars is usually less than in normal scar tissue resulting in the depressed appearance. Atrophic scarring is superficial and so does not affect movement of skin. They typically appear darker than surrounding skin either due to pigmentation difference, the shadow cast by the depression or the combination of both.

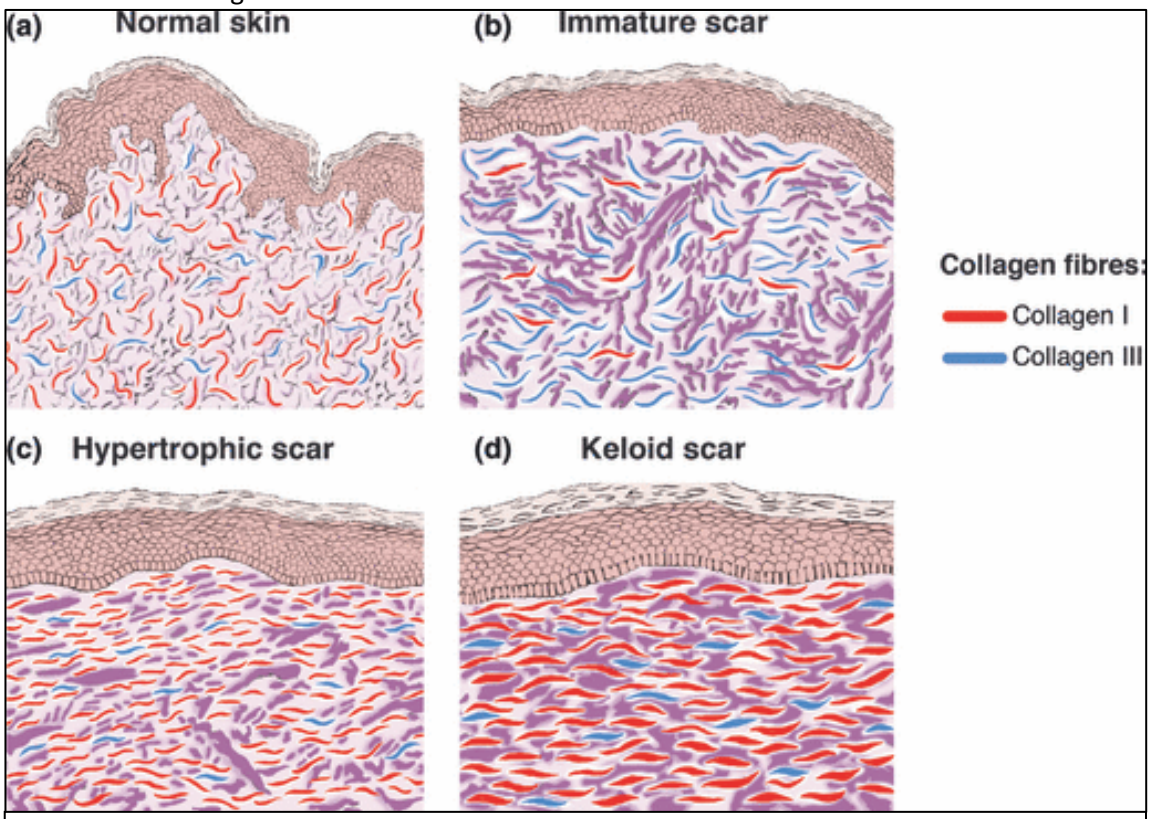
1.2.4 Hypertrophic Scars

Hypertrophic scarring is typically defined as a scar that is raised compared to the surrounding normal skin and has a typically thickened appearance (see Figure 1. B). Hypertrophic scars stay within the original margin of injury due to an excess of collage tissue. They can be more disfiguring than normal scar tissue and can be more symptomatic. Interestingly, they typically appear 3-4 months after the original injury once the overlying epidermis has healed. The exact pathophysiology of hypertrophic scarring and why they form instead of normal scar tissue is not fully understood. On a

histopathological level there seems to be upregulation of pro-inflammatory markers such as the aforementioned TNF-α, interleukin (IL)-1α, IL-1β and IL-6 and ongoing activity of fibroblasts.[17-20]

A study looking into the exact depth of cutaneous injury that results in scar tissue formation demonstrated that 33.1% depth through the skin was enough to cause a scar; and that only injuries involving the dermis results in physical scar.[21] Hypertrophic scars are thought to be more commonly seen in areas of deeper dermal injury such as in burns. This is supported by Ogawa et al who suggested that injuries involving the deeper reticular layer of the dermis promotes prolonged inflammation; which results in an increased deposition of collagen in the dermal layer, creating the raised appearance.[19] The histological profile of hypertrophic scars are classically referred to have a whorl-like pattern arrangement of the collagen matrix (see Figure 2). As hypertrophic scars are typically caused by deeper dermal injury, they may be tethered to underlying structures and demonstrate a typically darker red or purple appearance compared to surrounding skin. The thicker scar tissue can have an impact on the skins function in movement.

Figure 2. Schematic pictorial representation of collagen fibre arrangement in scar tissue. Image adapted with permission from Sidgwick et al[22]



Normal skin (a) – Collagen I and III fibres are thin and arranged in an organised weave.
Immature scar (b) – Remodelling collagen fibres laid down in immature scar.
Hypertrophic scar (c) – Collagen I and III fibres laid down in unorganised whorl pattern and thicker than in normal skin.
Keloid scar (d) – Similar to hypertrophic scar but with thicker collagen fibres and less organised.

1.2.5 Keloid Scars

Keloid scarring is defined as thickened, raised scar tissue that extends beyond the original margin of injury and begins to invade normal healthy skin (see Figure 1. C). Although traditionally referred to as a separate condition to hypertrophic scarring, the histopathological profiles of the two are near identical and are now often considered part of the same disorder.[17,19,20] Keloid scars tend to contain an abundance of thicker hyalinised collagen referred to as 'keloid collagen' (see Figure 2), but this is also seen in hypertrophic scar tissue, albeit on a smaller scale.[19] Keloid scars tend to differ from hypertrophic scars as there seems to be a genetic and anatomical site predisposition. Keloids tend to form specific shapes depending on their anatomical location, such as a butterfly appearance on the anterior chest or a dumb-bell shape on the upper arm.[23] Tension across the skin seems to be

important in the shapes keloid scars form. For instance in the anterior chest, the skin is pulled horizontally by the action of the pectoralis major muscle, possibly resulting in the horizontal butterfly shaped scar.[19] The theories behind mechanical force could suggest why keloids are rarely seen in the lower limbs as the skin is more relaxed in this area.[20] Ears are a common site for keloids and this could be due to repeated inflammation from the earring material or perhaps the action of the ear pressing on the pillow when sleeping.[19] As the thick keloid scar tissue can invade across the skin, tethering can occur if over areas of skin movement such as the neck or on the ear lobes. Histological immunostaining of keloid scar tissue has demonstrated an increase in pro-inflammatory cytokines such as IL-α, IL-1β, IL-6 and TNF-α.[17,18] Keloids like hypertrophic scar, are typically darker in colour comparing to surrounding normal skin.

1.2.6 Risk factors for developing hypertrophic and keloid scarring

Multiple systemic factors have been suggested for developing hypertrophic or keloid scars. The more potent inflammatory response seen in adolescents and adults has been suggested to cause higher rates of hypertrophic and keloid scarring.[14] Sex hormones such as oestrogens and androgens could play an important role in this as they are associated with a more aggressive inflammatory response.[24] Ogawa et al reported that a patient suffering from Castleman disease, a disorder characterised with excessive production of the pro-inflammatory IL-6; was found to have aggravated ear keloid scars when the levels of pro-inflammatory cytokines were higher.[19] This could be interpreted to suggest that a systemic inflammatory response is a risk factor for developing keloid scarring.

Genetics seem to play a role in the risk of developing keloid scarring. Certain ethnic groups are more susceptible to developing keloid scarring with reports that patients with darker skin may be up to 15 times more likely to develop these scars.[25] Keloids are also more prevalent amongst African-

American groups and certain east Asian communities.[26] Interestingly, hypertrophic or keloid scars have not been reported in albino people, but are slightly more common in those with fair skin and red hair.[27] Chromosomal changes have been associated with increased likelihood of keloid scarring. Marneros et al performed a genome-wide linkage search for genes predisposing to keloid scarring in a Japanese family with a history of keloid scarring and an African-American family with a history of keloid scarring.[26] The authors identified a potential keloid causing chromosomal change on 2q23 in the Japanese family and 7p11 for the African-American family. A similar study by Chen et al identified a population endemic to the Han region of China that are predisposed to getting keloid scarring. The cause was identified on chromosome 10q23.31.[28] Although these chromosomal changes have been detected, the specific genes responsible have not been identified.

Single nucleotide-polymorphisms (SNPs) could be responsible for a genetic cause for keloid scarring. Again, in a Japanese population, Nakashima et al performed a genome-wide associated study and identified four SNP locations on three different chromosomes.[29] The most significant locus associated with keloid scar formation was on chromosome 1 at rs873549.[29] Ogawa et al identified an SNP on chromosome 15 at rs8032158 that may cause the deviant cell proliferation witnessed in keloid scar tissue.[30] The authors of both studies however have reported that there may be other genes and factors at play.

1.3 Scar Treatments

Despite scarring being a widespread problem, many treatments are limited in their effect and range from minimally invasive such as topical agents, to more invasive surgery. The most commonly used treatments globally for scarring (summarised in Table 1) include corticosteroids, 5-fluorouracil, bleomycin, LASER therapy, topical creams, topical silicone gel ointment/lotion, silicone gel sheeting, pressure therapy, radiation and further surgery.[14,19]

Table 1. Summary of Scar Treatments

Treatments	Potential effects	Risks	Outcome Data	References
Corticosteroids	Flattening of scar, reduction in scar height and size	Subcutaneous atrophy, hypopigmentation, telangiectasia	Scar size, height, pliability. Patient opinion, histology, VSS	Darzi et al 1992[31] Mansukiatti et al 2002[32] Tan et al 1999[33] Khan et al 2014[34] Ahuja et al 2014[35]
5-fluouracil	Flattening of scar, reduction in scar height and size	Burning sensation, pain, wound breakdown, ulceration	Scar surface area, scar colour	Fitzpatrick et al 1999[36] Gupta et al 2002[37] Goldan et al 2008[38] Yang et al 2009[39] Prabhu et al 2012[40]
Bleomycin	Flattening of the scar, reduction scar in height and size	Hyperpigmentation, pain, blistering, ulceration	Scar dimensions, POSAS, VSS	Espana et al 2001[41] Naeini et al 2006[42] Payapvipapong et al 2015[43] Huu et al 2019[44]
LASER – Pulsed Dye LASER	Flattening of scar, improvement in scar texture,	Pain, swelling, itching, redness, bleeding, long recovery	Clinician assessment, patient assessment, VSS	Alster et al 1998[45] Sheridan et al 1997[46] Allison et al 2003[47] Wittenberg et al 1999[48] Hohenleutner et al 1995[49]
LASER – Carbon Dioxide	Flattening of the scar, improvement in scar colour, improvement in scar texture	Pain, swelling, itching, redness, bleeding, hypopigmentation, hyperpigmentation, long recovery	VSS, POSAS, thickness via ultrasound, scar colour with colorimetry, patient opinion	Blome-Eberwein et al 2016[50] Connolly et al 2014[51] El-Zawahry et al 2015[52] Levi et al 2016[53] Ozgog et al 2013[54] Qu et al 2012[55] Zadkowski et al 2016[56]

Topical Creams	Reduction in scar itch, pain, scar cosmesis, transepidermal water loss	Skin irritation/allergy	Clinician opinion, range of motion, scar thickness, photography, VSS	Bauman et al 1999[57] Jenkins et al 1986[58] Nedelec et al 2012[59] Phillips et al 1996[60] Klotz et al 2017[61]
Topical Silicone Gel Ointment	Reduction in redness, reduction in scar size, improvement in texture	Skin irritation	Clinician opinion, VSS, photography	Chan et al 2005[62] Signorinin et al 2007[63] Chernoff et al 2007[64]
Pressure therapy	Flattening of scar, reduction in scar redness	Pain, discomfort, tightness, restriction in movement, ulceration	VSS, scar colour, scar thickness	Li-Tsang et al 2010[65] Candy et al 2010[66] Moore et al 2000[67] Chang et al 1995[68]
Radiotherapy	Reduced recurrence of scar, flattening of scar, reduced spreading of keloid scar	Wound break down, tissue necrosis, pain, bleeding	Clinician opinion	Ogawa et al 2007[69] Flickinger et al 2011[70] Wang et al 2014[71] Shen et al 2015[72] Kovalic et al 1989[73]
Surgery	Less visible/improved appearance of scar, removal of problematic scar	Scar recurrence, wound break down, further surgery, worsening of scarring, infection, pain	Clinician opinion, patient opinion	Garg et al 2014[74] Liotta et al 2012[75]

POSAS – Patient Observer Scar Assessment Scale
VSS – Vancouver Scar Scale

1.3.1 Corticosteroids

Corticosteroids are commonly used as an intra-lesional injection into scar tissue. In clinical practice they are used to treat hypertrophic and keloid scarring with the hope of shrinking them down. Widely used since the 1960's, Triamcinolone Acetonide (TCA) is the steroid of choice in the treatment of scars.[76] Previous studies have indicated a varied efficacy of 50% to 100% in treating

hypertrophic or keloid scars such as those caused by sternotomy.[31,32] Corticosteroids are known to suppress the inflammatory response; a potential therapeutic target in hypertrophic and keloid scarring that are considered to have a prolonged inflammatory response. Corticosteroids can inhibit the expression of TGF-β1, TGF-β2, certain collagens in keratinocytes and VEGF.[77]

Treatments are typically given as a course at 4-8 weekly intervals. In the case of keloid scarring, steroids may temporarily dampen any further growth of the scar, shrink the scar, or have no therapeutic effect. Side effects can include dermal atrophy, telangiectasia, hypopigmentation at the injection site, delayed healing and a wide scar.[78] Studies for corticosteroid treatment summarised in table 1 all involve uncontrolled, non-uniform scars. Outcome measures in the form of scar size are subjective in the majority of the studies and lack description of the techniques/tools used to measure.[31,33,34] Mansukiatti et al used patient reported outcomes whilst Ahuja et al utilised the Vancouver Scar Scale (VSS – discussed in chapter 3) for their scar outcomes.[32,35]

1.3.2 5-fluorouracil

5-fluorouracil is a pyrimidine analogue that is typically used as an anti-cancer agent. Its mechanism of action is to act as a thymidylate synthase inhibitor which results in a reduction in thymidine monophosphate.[79] Thymidine is necessary for DNA replication and the dividing cells undergo cell death; this action may help to prevent excessive proliferation of fibroblasts in scar tissue.[79] Inhibition of TGF-β1 and type I collagen production caused by 5-fluorouracil has been reported in fibroblasts.[80] 5-fluorouracil's first use in the treatment of keloid scars as an intralesional injection was reported in 1999 with good results with scar size reducing by 50% in up to 75% of patients.[36,81] More recent studies report good to excellent improvement in scar size in 64% of patients; but no statistically better or worst outcome than corticosteroid.[40]

Side effects from 5-fluouracil injections include transient hyperpigmentation, pain at the injection site, tissue sloughing and a transient burning sensation.[37] It has also been reported to have a greater

efficacy when used in combination with corticosteroids or radiotherapy.[32,36] Despite relatively well reported outcomes, 5-fluouracil is not as popular as a treatment compared to steroids due to side effects being more common. It remains an alternative for many patients for whom steroids have failed. Patient reported outcome measures (PROM) have been used in the literature for the effect of 5-fluouracil, but have not been validated.[37-39] Additionally, objective measures utilised such as callipers to measure length combined with non-uniform scars can make accurately assessing outcomes difficult.[40]

1.3.3 Bleomycin

Bleomycin is an antibiotic with anti-tumour properties also commonly used as a chemotherapy agent.[41,42] It has been used as an intradermal injection in patients with hypertrophic and keloid scars. Complete regression of the scar tissue has been noted in approximately 2/3 patients who have been treated with this drug.[41,42] Despite these promising results, pain has been reported as a very common side effect in treatment along with hyperpigmentation, blistering and ulceration.[41-44] Earlier studies have relied on subjective clinical opinion on the outcome of the scar weakening the conclusions.[41,42] Better validated outcome measures such as the Patient Observer Scar Assessment Scale (POSAS) and VSS have shown a positive response.[43,44] Bleomycin has been demonstrated to inhibit collagen synthesis in fibroblasts by downregulating TGF-β1 production.[42] It is not in widespread use as the evidence base is relatively small and the mechanism is not fully understood.

1.3.4 LASER Resurfacing Therapy

LASER (light amplification by stimulated emission of radiation) devices emit light via a process of optical amplification. LASERs that are used to treat scars can be divided into non-ablative and ablative. Suggested mechanisms of LASER therapy include stimulation of the dermis to produce

collagen and remodelling of collagen fibres. [82,83] Non-ablative LASER therapy in the form of 585mm pulsed-dye laser (PDL) is widely used internationally as a treatment for hypertrophic scars. In use since the 1990's, it has been reported to improve the appearance of scars and reduce the volume of thickened scar areas.[84,85] PDL creates a controlled thermal injury to the scar microvasculature which leads to ischaemia in the scar tissue without ablating the surface of the scar.[86] This subsequently leads to a reduction in collagen production in the scar tissue. Suppression of TGF-β1, fibroblast proliferation and upregulation of MMP-13 have also been reported.[87] Although most studies have demonstrated an overall positive effect with PDL treatment; two control trials demonstrated no statistically significant difference in reduction or erythema in patients treated with PDL compared to silicone or no treatment.[47,48] One possible theory for PDL's limited effect is that in patients with thick scars, the laser may not be able to penetrate deep enough to the vessels in the scar tissue.[49] Commonly reported side effects from PDL include purpura, temporary hyperpigmentation and mild bleeding from the site.[88] LASER therapy remains a popular choice in the treatment of hypertrophic scars; it is often used when injectables have not had the desired effect.

Ablative carbon dioxide (CO_2) LASER resurfacing devices produce energy with a wavelength 10,600nm.[89] The mechanism by which CO_2 LASER works is by creating a pixelated pattern described as fractional photothermolysis.[90] The resulting multiple thermal treatment zones with areas of untreated tissue in-between allows for a deeper penetration of the LASER with the spared areas aiding in the recovery of the tissue.[90,91] CO_2 LASER has a similar side effect profile to PDL but these are reported to be less common than in PDL.[52,89,91] For both types of LASER, there are no blinded randomised control trials. The majority are pre-test and post-test analysis. There are more papers analysing scar outcomes with PROMs such as the POSAS and VSS for CO_2 LASER compared to the PDL. As with the injectable treatments, many of the methods for assessment in PDL have been subjective clinician opinion on non-standardised scars.[45,46,49]

1.3.5 Topical Creams

In most burn centres, a common mantra is to moisturise and massage the scar with a simple aqueous cream.[92] The combination of the two is believed to help prevent moisture loss in the scar keeping it hydrated, preventing trans-epidermal water loss (TEWL).[93] Increased TEWL is believed to aid inflammation resulting in more scarring.[94] Despite their global use, there is very little evidence behind aqueous creams mechanism of action and if it actually does provide any benefit. Multiple types of aqueous creams are available at different prices and brands yet there is little evidence over which brands are better than others.[61]

Converse to the standard dogma, a report of aqueous cream increasing TEWL was published with sodium laurel sulphate content the potential culprit in facilitating this.[94] The authors demonstrated an increase in skin thinning, TEWL and inflammatory proteins; but this was tested on normal healthy skin, not scar tissue. There is a plethora of aqueous cream type moisturisers available all with slightly different constitutions. There is no general consensus on which one is best and if they make a statistically significant difference in treating scars. A recent study looking into the most commonly used moisturisers across North America, Australia and New Zealand for burns scars indicated that availability, price and above all, recommendation by the practitioner had the biggest impact on what was used.[61] Main properties desired by the product would include hypoallergenicity, reduction in itch, unscented, intense hydration with ease of massage and low cost. In Australia Aqueous Cream BP® and Rewin Sorbolene® were the most popular whilst in the USA it was Eucerin® moisturiser. The outcome of this study indicated that there is no overall consensus on which product is best and that there is still no high quality evidence behind the treatments currently being used.[61] Like with the other studies assessing scar, those used in assessing moisturisers as a scar treatment are on

patients with non-standardised scars. Many of the outcome measures are also subjective clinician opinion.[57,58,60]

1.3.6 Silicone gel and Silicone sheeting

Silicone gel ointment and silicone gel-based sheeting are commonly used treatment modalities in burns centres globally. Similar to the topical creams, theories into how they work are focused on the prevention of TEWL from the cells within the wound and how this may prevent further inflammation. Three silicone-based treatments were evaluated in a systematic review by Mustoe et al 2008.[95] These were silicone gel sheeting, silicone oil in a moisturiser and silicone gel in a tube. Reports of the silicone gel sheeting and topical silicone gel suggested the occlusive factor of the gel helped improve the appearance of the scar tissue, yet the silicone oil in moisturiser was reported to show benefit only if it was used with an occlusive dressing. The studies assessing silicone gel in a tube utilised surgically created scars; in particular Chan et al 2005 utilised midline sternotomy scars.[62,63] This can provide a more controlled uniform scar then in the traumatic scars used to assess other treatments; however there are still many variables in a surgical scar such as length, underlying sutures and tension. Prevention of TEWL in keratinocytes has been a proposed mechanism of silicone including the ability to provide adequate but not excessive hydration.[95] The combination of occlusion, limited but not full permeability of silicone and the silicone itself are all involved in an improved scar outcome; however these reports are based off low quality evidence studies.[96] Silicone gel sheeting will be discussed in more detail in 1.4.

1.3.7 Pressure Therapy

First identified as a treatment option in the 1970's, pressure garments are still used today, often in combination with silicone gel sheeting.[97] The mechanism behind pressure therapy is not understood. The most commonly accepted theory is that the pressure occludes small vessels in the scar resulting in reduced oxygen tension.[98] Lower oxygen tension is thought to reduce collagen

production by fibroblasts and increase collagen lysis.[98] Interestingly, a randomised control trial (RCT) looking at the efficacy of pressure therapy alone in patients with burn scars reported no significant differences in scar reduction compared to controls.[68] Reported issues with pressure garments are the discomfort they can cause, the need to be worn at all times and skin ulceration in some areas. This often results in poor compliance.[99] Despite the relatively limited evidence base, pressure garments are used regularly in burns centres, often in conjunction with other treatments. Many studies are limited by the application of the pressure therapy at different times post injury and poor compliance.[68,99]

1.3.8 Radiotherapy

Radiation in conjunction with surgery has been reported as a treatment for those with severe keloid scars. Beta-radiation is used as it is absorbed superficially. The proposed mechanism is neurovascular damage to the scar tissue and inhibition of proliferating fibroblasts.[100] Surgical excision of keloids have a very high recurrence rate (45% to 100%), often making them hard to treat.[100] A pre and post-treatment study of 75 patients with 113 keloid scars that underwent surgical excision and beta-radiation treatment resulted in a regression rate of 73% at 9 and a half years with no complications compared with no regression.[73] Side effects from radiotherapy are relatively limited as the radiation used is observed superficially. Radiotherapy is not a common treatment and is only used in conjunction with surgery in patients with very difficult to manage recurring keloid scars.[72] The studies reporting its use are often in patients where previous treatments for keloids have failed.[70-72]

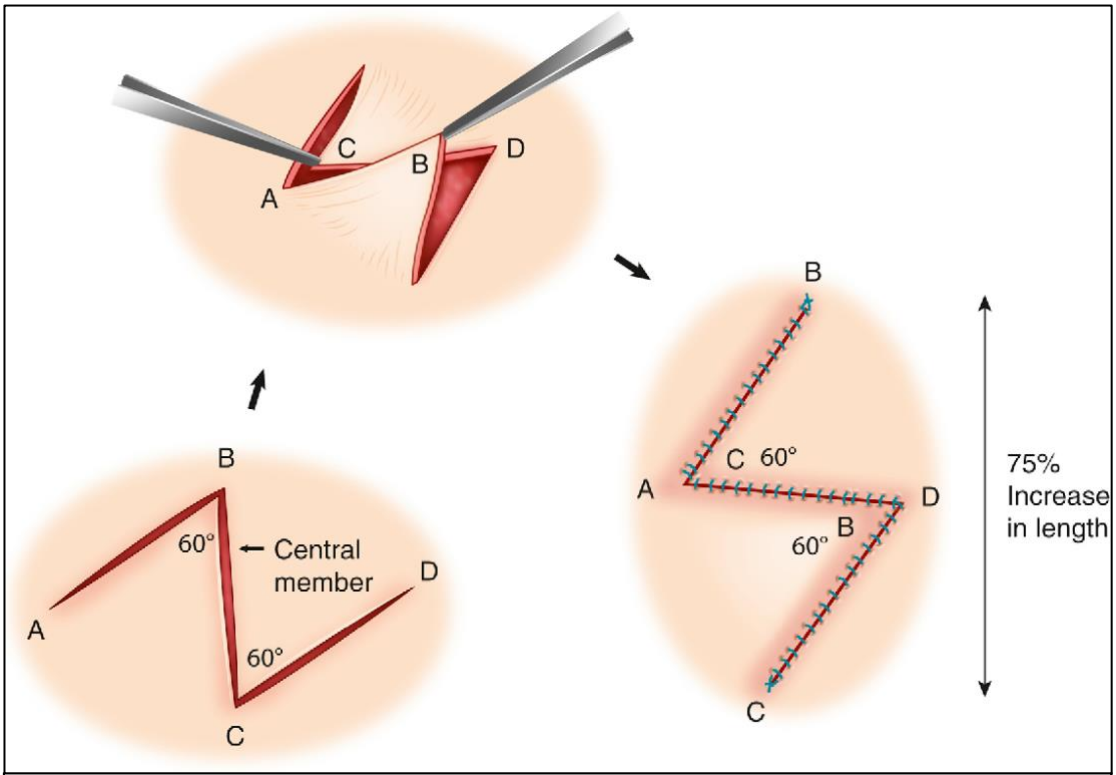
1.3.9 Surgical Treatment

Surgical scar treatment does not erase a scar, but helps to make it less noticeable. Typically performed by plastic surgeons, the techniques utilised can be categorised as excisional or incisional. Excisional scar surgery aims to remove the problematic scar, often to improve cosmesis and

symptom control.[74] Fusiform elliptical excision involves removing the scar completely from the skin. The scar is excised as part of an ellipse pattern with a 3:1 ratio of length to breadth to allow for easier closure and reduce the risk of a "dog ear".[74] Extramarginal excision includes removal of normal healthy tissue at the scar border to ensure normal tissue at the wound margins.[74] For more cosmetically sensitive and tighter areas, intramarginal excision of scar can help to improve the appearance of the remaining scar tissue[74]. Excision of larger scars that will result in tissue loss may require further procedures to close the defect.[75] These include serial small excisions, skin grafting, closure with local flaps and Integra® artificial skin.[75]

Incisional techniques involve cutting the scar and manipulating the tissue to improve the appearance of the scar.[74] The Z-plasty is the most commonly used incisional technique utilising geometric principles to revise the appearance of a scar (see Figure 3).[74] Z-plasty creates a lengthening effect in the tissue and can realign scars to make them less noticeable. An incision is made along the centre of a scar referred to as the common diagonal or central member, with two arms of the same length at either end of the diagonal at an angle typically of 60˚ in the shape of a Z (Figure 3). The two triangles of tissue are raised surgically from the underlying subcutaneous fat, rotated over each other and sutured in place. An angle of 60 ˚ will create a gain in length of 75%.[74] The newly revised scar will lie perpendicular to the original scar.[74] Different angles used will provide different percentages of lengthening. There are multiple variations of the Z-plasty, such as the Limberg flap for areas of tethered scar contracture and the half Z-plasty for regions with inelastic tissue.[74]

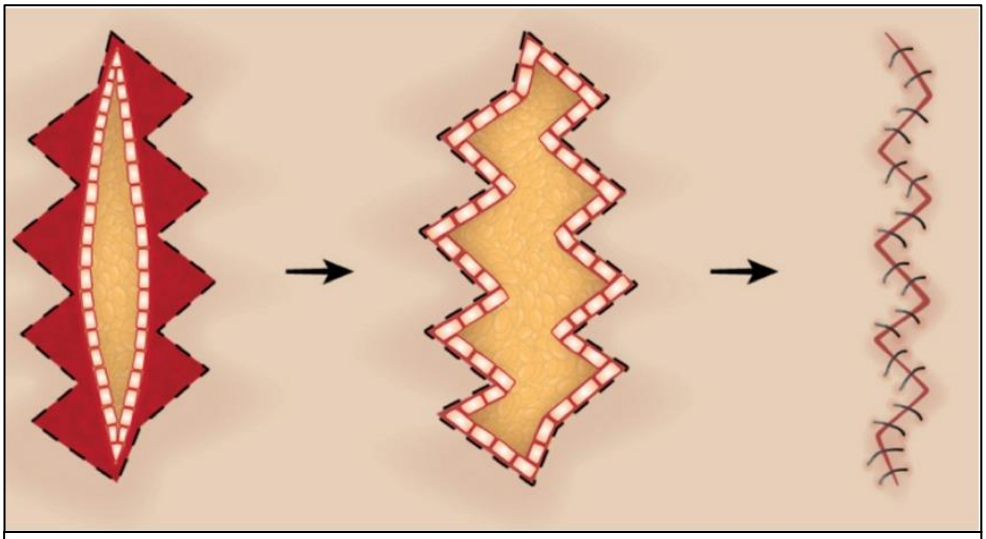
Figure 3. Z-plasty technique. Image adapted with permission from Cerrati et al[101]



Bottom left. Creation of Z with the central member running through the middle of the scar. All incisions are the same length and the angles are 60˚. **Upper central**. Triangular skin flaps raised and rotated over each other with tip C being brought to A and tip B being brought to D. **Bottom right**. Skin is sutured in place, the 'new' scar is perpendicular to the original scar and a lengthening effect of 75% is created.

The W-plasty aims to improve the appearance of a linear scar by the principle that a broken irregular line has poorer visibility.[74] A series of regular continuous triangles are made on one side of a scar with their mirror on the other side of the scar. The scar is resected and the triangles are lined up (Figure 4). The limbs of each triangle are 3-5mm with an apex angle of 60-90 ˚. The angles at the end of the W-plasty are <30 ˚ to prevent a dog ear deformity.[74] Although surgery may improve the appearance of a scar or the range of motion over a tight area, all surgery still results in a scar.

Figure 4. W-plasty technique. Image adapted with permission from Kerwin et al.[102]



Left. Central ellipse of scar removed, consecutive triangles are made either side of the scar with apex angles of 60-90˚. **Central.** The scar is surgically excised and the skin re-approximated by interdigitating the triangles. **Right**. The skin is sutured into place creating a single zig-zag shaped scar.

1.4 Silicone Gel Sheeting Treatment - Background

Use of silicone gel as a scar treatment dates back to the 1980's and is still in widespread use today.

Table 2. Summary table of studies assessing silicone gel sheeting as a scar treatment.

Study	Study Design	Patient Group	Patient Numbers	Intervention	Outcome
Ahn et al 1989[103]	RCT	Patients with hypertrophic scars	10	Silicone gel sheet applied for 12 hours per day for 8 weeks	Clinical assessment of scar appearance, elasticity
Ahn et al 1991[104]	RCT	Patient with hypertrophic scars and post-surgical scars	19	Silicone gel bandage worn for 12 hours per day	Clinical assessment of scar elasticity and volume
Carney et al 1994[105]	RCT	Patients with hypertrophic scars	42	Half assigned to Silastic Gel Sheeting and half Cica-Care®	Clinical assessment of scar appearance, elasticity and colour
Cruz-Korchin et al 1996[106]	Controlled Trial	Patients post bilateral McKissock	20	Pre-cut silicone elastomer sheet applied 12 hours/day for 2	Clinical assessment of scar hypertrophy

		reduction mammaplasty		months on one breast, untreated on the control breast	
De Oliveira et al 2001[107]	RCT	Patients with hypertrophic or keloid scars	26	Patients received silicone gel sheet and non-silicone gel sheet for control, some scars no treatment	Clinical assessment of pain and itch relief, scar dimensions, colour, intracicatricial pressure and hardness
Gold et al 1994[108]	Controlled Trial	Patients with hypetrophic or keloid scar, scars from thermal burns, keloids removed by CO2 laser	21	Scar divided in half with one half receiving silicone gel sheeting for 12 hours/day for 12 weeks, no treatment on other half (control)	Patient and physician evaluation of scar appearance and colour, recurrence of keloid, scar thickness
Gold et al 2001[109]	RCT	Post-surgical patients with a high risk of abnormal and scarring and with a low risk of abnormal scarring	66	Silicone gel sheeting for 12 hours/day for 6 months or routine post-operative care (control)	Clinician assessment, patient opinion, photographic analysis
Li-Tsang et al 2006[110]	RCT	Patients with hypertrophic scar	45	Silicone gel sheeting for 24 hours/day for 6 months + lanolin massage twice daily versus lanolin massage only	VSS Scar pigmentation Patient opinion of pain and itch
Li-Tsang et al 2010[65]	RCT	Patients with 'active' hypertrophic scars	104	Pressure therapy versus Silicone gel sheeting worn for 24 hours/day versus pressure therapy + silicone gel sheeting versus lanolin massage (control)	VSS Scar thickness by ultrasound Scar colour by colorimetry, scar pain and itch

Mercer et al 1989[111]	Observational study	Patients with hypertrophic scars	18	Silicone gel sheeting secured with tubigrip worn or 8 hours/day for 6 months	Clinician assessment of scar height, colour and texture
Niessen et al 1998[112]	RCT	Patients undergoing bilateral breast reduction	129	Areas on each breast allocated to receive silicone gel sheeting for 24 hours/day for 3 months versus micropore tape	Clinician assessment of scar height and width, Patient opinion of itch and pain
Tan et al 1999[33]	Controlled Trial	Patients with multiple keloid scars	20	Scar with no treatment (controls) versus 1 scar receiving silicone gel sheeting versus 1 scar injected with triamcinolone acetonide at 4 weekly intervals	Clinician assessment of scar length, width height, change in colour and texture Patient reported pain and itch

RCT – Randomised Control Trial
VSS – Vancouver Scar Scale

Ahn et al 1989 discussed silicone gel sheeting use in the treatment of established hypertrophic scars analysing 14 scars in 10 different patients for 8 weeks.[103] They reported that the scars in those treated with silicone had an improved appearance on standardised photography and a statistically significant improvement in the scars elasticity.[103] Biopsies taken in the same study showed no infiltration of silicone into the scar tissue. The same group did a further study assessing a larger number of patients with more established hypertrophic scars; some from surgical incisions and some from burns.[104] Their conclusions were that silicone gel sheeting seemed to have a beneficial role in both preventing hypertrophic scars in recent scars and treating hypertrophic scars in more established scars.[104] The use of silicone gel sheeting as a treatment for keloid scars was explored by Mercer et al 1989.[111] Nineteen patients with established keloid scars (33 scars in total) would wear topical silicone gel sheets for 8 hours a day for 6 months in total. In a 6 month period, they reported

an improvement in the texture, height and colour in 86% of those treated.[111] The overall conclusions for the study were that silicone gel sheeting is beneficial as a treatment in smaller keloid scars.[111]

As more burn centres went on to use silicone gel sheeting, common issues reported were getting it to stay on, flexibility of the gel and patient motivation for changing the dressings daily.[105] Smith and Nephew® developed a new version of silicone gel called Cica-care® which was assessed in an open-controlled trial by Carney et al 1994.[105] Forty-two patients with a total of forty-seven hypertrophic scars were assessed in this study comparing Cica-care® and a previous older silicone gel treatment called silastic gel sheeting against no treatment at all for 6 months. Extensibility was the primary outcome measure with the authors reporting an improvement in 93% of scars treated with Cica-care®, 100% of scars treated with silastic gel and 38% of scars not treated at all.[105] The authors concluded that silicone gel sheeting made a statistically significant difference in treating hypertrophic scars and that properties of Cica-care® such as its flexibility and ease of applying made it a popular choice.[105]

One of the first papers to include a PROM for silicone gel sheeting was by Gold et al 1994.[108] In their study, 21 patients with mixed hypertrophic and keloid scars (17 patients had hypertrophic scar, 4 had keloid scars) had half their scar treated with topical silicone gel sheeting and no treatment on the other half for 12 weeks, for a minimum of 12 hours per day. In terms of overall effectiveness, 12.1% of participants reported no improvement versus 4.8% reported by the physician. 57.1% of participants reported some improvements versus 42.9% reported by the physician; 23.8% of participants reported moderate improvement versus 52.3% reported by the physician. Silicone gel sheeting was concluded to be of benefit, but the time frame on which to begin treatment and how long to use it for is uncertain.[108]

A further follow up study by the same group assessed if silicone gel sheeting could be used to help prevent hypertrophic or keloid scars in patients known to be at risk for developing them undergoing minor dermatological surgery.[109] The cohort was split into 50 patients deemed not high risk for developing hypertrophic or keloid scars and 46 patients deemed as high risk for developing hypertrophic or keloid scars. Patients wore the silicone gel sheeting for 12 to 24 hours a day for 6 months in total. Gold et al reported no statistically significant difference in the scarring outcomes of those who were low-risk for hypertrophic or keloid scarring.[109] A statistically significant difference was observed in the scarring outcome in the high-risk group when comparing silicone sheeting versus normal treatment; however the number of participants was very small due to loss-to follow up.[109]

Cruz-Korchin et al compared silicone treatment versus no treatment on 20 women undergoing bilateral breast reduction mammoplasty.[106] The patients would act as their own control with one breast receiving a silicone dressing to wear over the scar for 2 months and the other breast to be left with no treatment. Patients were followed up at 6 months. In the untreated group, 45% developed flat scars and 55% developed hypertrophic scars; versus the treatment group where 75% developed flat scars and 35% developed hypertrophic scars.[106] The results were deemed to be statistically significant and theories were put forward about the mechanism of action.

Niessen et al performed a similar study but with 155 women undergoing bilateral breast reduction mammoplasty.[112] In the Niessen study, the silicone dressing was worn on one set of randomly allocated breast scars (right medial and left medial, or right lateral and left lateral) for a period of 3 months with a follow up period of 12 months after surgery. 129 female patients were followed up with 64.3% of them developing hypertrophic scars at 3 months. This subsequently reduced down to 35.3% at one year. The authors reported that at 6 months, 29 patients with silicone treated tissue

developed hypertrophic scars versus 12 whom developed it on control sites (p value = 0.006). At 12 months they reported 19 hypertrophic scars in those treated with silicone and 7 in those without (p value =0.02). At 3 months the numbers were almost equal at 17 and 18.[112] These results suggested that silicone gel sheeting may actually increase the risk of developing hypertrophic scar tissue. The authors discussed how their methodology was to use silicone immediately after surgery whereas studies that had all shown a beneficial effect had used silicone 2 weeks after surgery. This has led to the theory that silicone may only be effective in healed skin that has begun remodelling.[112]

Tan et al compared the use of silicone gel sheeting against injectable TCA in patients with keloid scars.[33] A total of 20 patients with 3 or more similar sized keloid scars were recruited to the study. On each patient, one scar would be treated with silicone gel sheeting only for at least 12 hours daily for 12 weeks; one scar would be injected with TCA and the other would receive no treatment. The authors reported 12% of scars treated with silicone had a >50% reduction in size which was found to be not statistically significant. In the keloid scars treated with TCA, 94% had a >50% reduction in size which was found to be statistically significant. Silicone gel sheeting was deemed to be not effective in the treatment of keloid scars, but may have a role in children or patients not able to tolerate steroid injections.[33]

A study comparing topical silicone dressings with occlusive non-silicone dressings in patients with hypertrophic or keloid scars was conducted by de Oliveria et al.[107] For this study, 26 patients with a total of 41 mixed hypertrophic and keloid scars were included. Fourteen scars received silicone dressing treatment, 14 scars received occlusive non-silicone and 11 scars received no treatment. The dressings were worn for a total of 5 months with follow up every 15 days for assessment of scar colour, size and pressure. They reported that all outcome measures were reduced in both groups, but the difference between the silicone and non-silicone group was not statistically significant.[107]

This has led to the theory that perhaps it is the occlusive nature of silicone dressings, rather than the silicone gel itself.[107]

Often in clinic, advice about pressure garments and scar massage are given to help alleviate symptoms of tightness and improve appearance. Li-Tsang et al published results of a study looking into the effect of silicone gel sheeting versus massage therapy in the treatment of hypertrophic scars.[110] A total of 45 patients with hypertrophic scars were included with 22 patients undergoing 24 hour per day silicone gel treatment for 6 months and 23 patients undergoing 15 minutes of scar massage treatment per day for 6 months. A colorimeter was used to assess pigment, an ultrasound scanner for thickness and the VSS was used which assesses scar pigmentation, height, pliability and vascularity. At 6 months they reported statistically significant differences in the scars VSS score and the scar thickness seen on ultrasound.[110]

A further study by Li-Tsang et al looked into the effect of silicone gel sheeting combined with pressure garments versus pressure garments alone.[65] 104 patients were included in this study. 30 received pressure garments only, 24 received silicone gel sheeting only, 29 received a combination of pressure garment and silicone gel sheeting with 21 patients acting as controls. The study lasted 6 months with patients being advised to wear the interventions for 24 hours per day unless bathing. The outcome measures were the same as their previous study. Patients were reported to have a statistically significant reduction in the VSS scores in all intervention groups.[65] It was found however, that a combination of pressure and silicone gave the best results.[65] They also reported that for the symptoms of pain and itch, silicone based treatments scored better.[65]

With silicone's widespread use for 30 years now as a treatment for scarring, a Cochrane review by O'Brien et al aimed to determine whether or not silicone gel sheeting is beneficial in the treatment

of hypertrophic and keloid scars.[96] In the review, a mixture of 20 RCTs, quasi RCTs and controlled clinical trials were included. This resulted in a patient total number of 873. The authors found that many RCTs were of poor quality, often with no blinding, loss to follow up bias and short follow up periods.[96] Other issues were the reproducibility of the studies and how the scars used in the research are not uniform. Conclusions were that silicone gel sheeting may be of benefit in treating scarring but there is little to no high-quality evidence of its benefit.

The aforementioned studies for silicone gel sheeting all utilised existing traumatic scars, or scars created as part of a surgical procedure. This non-uniform, non-standardised mechanism for creating the scars used can introduce a large amount of variability that can weaken the observed outcomes. Additionally, outcome measures used to assess the scar rely heavily on clinician opinion and few have utilised currently available validated subjective scar assessment tools such as the POSAS and VSS. Objective scar tools aimed at analysing specific properties of scar such as ultrasound scanners for thickness have only been used in limited studies. As concluded by O'Brien et al, there is a shortage of high-quality evidence for silicone gel sheeting in the treatment of scarring.[96,113] Scarring represents a global problem, with many treatments developed on a weak evidence basis. Despite silicone's weak evidence base, it is in widespread use in the UK as a scarring treatment.

1.5 Aim

To address the challenges in scar standardisation, evaluation, and therapy assessment. The global aim of this project is to assess the currently available models for scarring and evaluate/further develop the utility of current assessment tools, in an effort to design a pilot RCT for silicone gel treatment of scars.

1.5.1 Sub-Aims

1. To review currently available scar models in an effort to understand the tools available for studying scar therapies (chapter 2).

2. To assess methods currently used to assess scars in clinical practice in order to develop subjective and objective scar assessment methods to use in a clinical trial (chapter 3).

3. To determine the long-term scarring outcomes in paediatric burn patients treated conservatively versus those treated surgically in order to assess the use of a novel purpose created paediatric scar patient reported outcome tool (chapter 4).

4. To design and set up a RCT to assess the effect of silicone gel sheeting as a treatment in purpose created standardised cutaneous scars in healthy volunteers (chapter 5).

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Chapter 2. A Systematic Review Comparing Animal and Human Scarring Models

2.1 Introduction

Establishing an easily reproducible, standardised model for creating cutaneous scar tissue for assessment of treatments in both animals and humans has long been a difficult modality to achieve. Within the animal kingdom, there is massive variation in the physiology of healing with some animals, such as amphibians, possessing regenerative ability.[1]

Within the Mammalia class, differences in the structure and physiology of skin is observed between species. Human skin has developed to be absent of fur and the presence of sweat glands allows for thermoregulation.[2] Other mammalian species are covered in a fur which can provide a survival benefit such as a bears fur in winter; whereas humans have developed hair instead. The panniculus carnosus (PC) is a thin layer of striated muscle closely attached to skin and underlying fascia.[3] In humans the PC is restricted to the platysma, the dartos and over the palmaris brevis.[3] In other mammals, it is preserved all over the body such as in rodents, dogs and cats.[3] The PC can allows the skin to glide loosely over the underlying structures and can contract.[3] This strong contraction is believed to aid in the wound healing process. Humans have relatively thick dermis compared to other mammals.

Pigs have been used for multiple research models in human disease; this is due to their anatomical and physiological similarities to humans.[4] Although often used in models for heart disease, respiratory disease and GI disease, the skin of a pig is a very suitable model for research relating to humans. Physiological and anatomical similarities include a thicker epidermis, elastic dermis, hair instead of fur, collagen structure that is similar to humans and an epidermis turnover rate of 30 days.[5-8]

One of the more common animals used in research include mice and rats. This is often due to their small size, ease of housing and the various genetic variations readily available.

There have been multiple models used for assessing wound healing in murine models, but there are few which exist specifically for creating scar tissue.[9] Difficulties in achieving scar models have been described due to the fast healing of murine tissues, the strong contraction response of the tissue, presence of fur and the mobility of the subcutaneous fascial matrix facilitating faster healing.[10,11]

Within human research, most common scarring models use patients with already existing scars often from an uncontrolled traumatic source such as flame burns. Scars from an uncontrolled source are often limited by difficulty in obtaining an equivalent control, varying depths of injury and as discussed in chapter 1, anatomical location can influence the scar produced. This massive variation can often weaken the results of studies assessing scar treatments. Due to the lack of reproducibility of scars from traumatic sources, we will not be covering them in this review, instead we will be focusing on purposefully created scars.

Here I will discuss the currently used animal scar models for mammalian species going over their benefits and limitations. These will be compared with current human scar models. The focus will be on the methods reported to create scar tissue, rather than the results of the studies.

2.1.1 Aims

In this chapter I aim to review the currently available literature to critically evaluate scarring models in both the animal kingdom and in human research.

2.2 Methods

Two near identical literature searches were performed using Medline; one focused on animal scar models and one focused on human scar models. The search was carried out with the support of the Oxford University Bodleian Library service.

2.2.1 Eligibility Criteria

All studies that utilised a specific model for assessing scar tissue that was purposefully created (Table 1) were included. Normal scar tissue, hypertrophic and keloid scar tissue were included as part of our search. Models that were analysing the wound healing process and those in humans with scars from an existing injury such as a burn wound were excluded. Review articles were not included but their references were searched to identify any additional suitable papers.

Table 1. Inclusion and Exclusion criteria

Inclusion Criteria	Exclusion Criteria
Studies involving normal scar tissue, hypertrophic scar tissue and keloid scar tissue Human studies Animal studies Purposefully created scars	Wound healing studies Scars from uncontrolled sources – such as trauma Review articles – but references searched to find suitable studies

2.2.2 Information Sources

Ovid MEDLINE (1946 to February 2019)

2.2.3 Additional sources

The reference lists of review articles were examined to identify other suitable studies.

2.2.4 Selection of the studies

The titles and abstracts were independently screened by two reviewers Dr Riyam Mistry and Dr Mark Veres to identify any potentially suitable studies. Full texts of potentially suitable studies were obtained and analysed to assess the proposed scar model. Multiple papers with the same model were assessed to find the original article in which the model was first reported.

2.2.3 Search methods for identification of studies – Animal Models

The following search strategy was used in Medline:

1. CICATRIX, HYPERTROPHIC/
2. KELOID/
3. (scarring or scars or scars or cicatrix or hypertrophic or keloid*).ab,ti.
4. (cutaneous or skin or tissue* or dermis or dermal or epiderm*).ab,ti.
5. 3 and 4
6. 1 or 2 or 5
7. RESEARCH/
8. (lab or laboratory or model* or remodel* or assess* or creat* or controlled or experiment*).ti.
9. (current and research*).ti.
10. bench.ti.
11. 7 or 8 or 9 or 10
12. 6 and 11
13. Limit 12 to animals
14. Limit 13 to (English language and yr=”2010-Current”)

2.2.4 Search methods for identification of studies – Human Models

The following search strategy was used in Medline:

1. CICATRIX, HYPERTROPHIC/
2. KELOID/
3. (scarring or scars or scars or cicatrix or hypertrophic or keloid*).ab,ti.
4. (cutaneous or skin or tissue* or dermis or dermal or epiderm*).ab,ti.
5. 3 and 4
6. 1 or 2 or 5
7. RESEARCH/
8. (lab or laboratory or model* or remodel* or assess* or creat* or controlled or experiment*).ti.
9. (current and research*).ti.
10. bench.ti.
11. 7 or 8 or 9 or 10
12. 6 and 11
13. Limit 12 to humans
14. Limit 13 to (English language and yr="2010-Current")

2.2.5 Data Extraction and Management

Each of the identified papers were further analysed by the reviewers. The methods sections were assessed to see the techniques used and the reproducibility of the models. The data extraction table has been deposited to the Oxford Research Archive.

2.2.6 Registration with PROSPERO

The review has been registered with PROSPERO and has two ID's as follows: CRD42021237692 and CRD42021233750.

2.3 Results – Animal Scar Model Studies

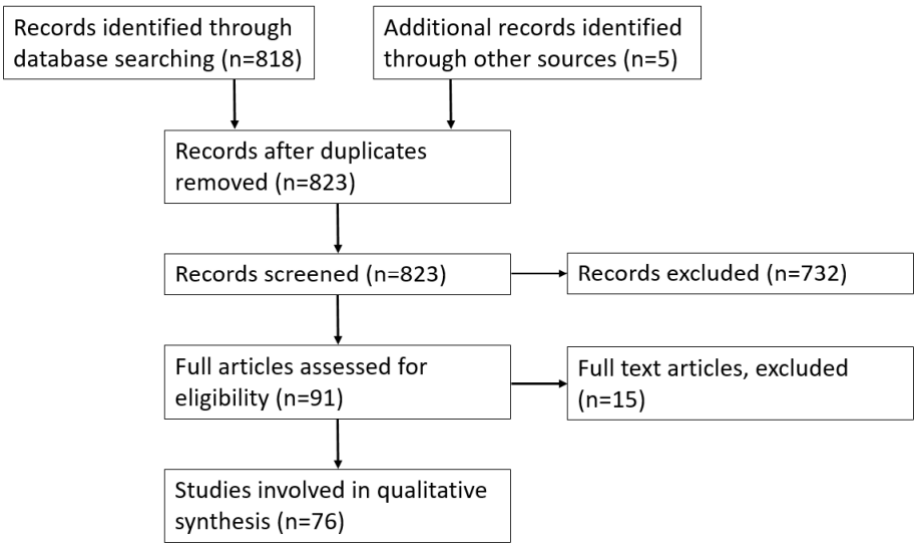

Figure 1. Flow diagram of records involved in analysis for animal scar models.

The search yielded 818 results in total. Both authors reviewed the titles and abstracts identifying 91 studies for further analysis including 4 review articles. Full analysis of the 91 studies identified 71 suitable studies along with an additional 5 studies that were identified from review articles. Rabbits were the most commonly used animal in scarring research, with 35 papers using them as a scar model. Murine models were second with 24 papers reporting them as a scar model. Fifteen papers reported using porcine scar models. There was 1 model using primates and 1 using dogs that were included.

2.3.1 Rabbit Models

The rabbit was the most common animal we came across, with all studies utilising the hypertrophic rabbit ear model first described by Morris et al 1997.[12] The technique described in all 35 studies involves creating a controlled injury on the skin of the ear resulting in a hypertrophic scar (see table 2 in appendix).[13-47] A punch biopsy was the most commonly utilised device to make the skin incisions

reported in 31 of the 35 studies. Four studies reported creating a circular incision but did not specify the type device used.[14-16,35] Friedrich et al 2017 compared the scar result from using a biopsy device with a controlled thermal injury.[19]

Of the studies we reviewed, Nabai et al 2017 gave a very in depth protocol for a hypertrophic ear scar model in rabbits using the techniques initially developed by Morris et al 1997.[47] The technique involves using a 6mm punch biopsy to make an incision in the skin over the ventral aspect of the ear going down to, but not including the surface of the cartilage. Exposed perichondral membrane is then dissected out. Paraffin gauze is placed over the wound with tegaderm and gauze sutured in place over the top for 2 days. The lesion heals over within 14 days and both sets of authors reported that hypertrophic scars reached maximum size at 3-6 months post-injury.[47] This then regressed over a period of 12 months after reaching peak size. Histological analysis by Nabai et al of the scar tissue showed increased vascularity and irregularly arranged collagen fibres with a circular whorl pattern; characteristic features of hypertrophic scar tissue.[47]

This method or slight variations of it such as size of skin excision has been mirrored in the other 35 studies reviewed. The size of the wound varied from 7mm to 10mm most commonly; the smallest reported was 5mm and the largest was a 20mm circular incision.[34,43] The average amount of time the wound was left prior to animal sacrifice for wound harvesting was 38.85 days. The majority of studies reported that the wound was fully healed at 14 days with visible scarring. Thirty of the studies reported creating injuries on both of the rabbit ears, with the remaining 5 not commenting how many ears were injured. The mean number of wounds per ear was 4.33. Every paper reported using New Zealand white rabbits, with the exception of Zhao et al 2018 who reported their rabbits as “adult laboratory white rabbits”.[16] Outcome measures for the rabbit ear model included collagen staining, vascular assessment, cytokine analysis, and histological height of the scar. Eleven of the models reported type III collagen staining as their primary outcome measure.[17,20-23,25,27,34,37,42,44]

Twelve studies involved a vascular assessment on histology with authors commenting on the visual presence and abundance of capillaries in the scar tissue sections. [13-15,17,20,22,24,25,34,36,43]

Six of the models reported quantitative polymerase chain reaction (qPCR) as an outcome tool.[19,20,22,27,29,39] Commonly investigated inflammatory proteins cytokines included TNF-α, TGF-β, MMP-1 and IL-6. qPCR was used in studies where a potential treatment for hypertrophic scarring was being assessed against a non-treatment control. The results were generally consistent with the intervention group showing a reduction in the aforementioned proteins. The exception to this was Sari et al 2017 who reported higher TGF- β1 levels in their intervention group but still reported their intervention as a potential treatment for hypertrophic scarring.[20] A total of 32 studies were assessing some form of treatment with all reporting their intervention resulted in an improvement in the resulting scar histologically or macroscopically. Four studies in particular were looking at ancient Chinese herbal medicine extracts as a scar treatment.[25,28,29,42]

Histological analysis was performed in all the studies, however Nabai et al was the only study that compared the hypertrophic scar tissue in rabbits with human hypertrophic scar tissue.[47] The height of the scar was reported using a histological approach in 29 of the 35 papers reported as the scar elevation index (SEI). The SEI is calculated by histological height of the hypertrophic scar subtracted from the histological height of the healthy skin divided by the histological height of the healthy skin.[12] All 29 studies utilising the SEI showed the intervention/treatment assessed resulted in a smaller SEI.

Limitations with this model include the difference of the rabbit immune system with that of humans. There could be genetic factors unique to the rabbit breed used that could potentially influence how

the rabbit scars. Many of the studies did not comment on blinding and those that did only blinded on the histological analysis segment when calculating SEI. Although using the ear helps to minimise contraction of the wound due to the absence of the PC at this site, the wound bed lying on top of collagen could have an effect or influence the wound healing/scarring process that is not representative of healing/remodelling on other body sites. Nabai et al put forward the theory that as the perichondral membrane is removed, an avascular surface is created increasing the risk of hypertrophic scar formation.[47] The average wound harvest of 38.5 days seems a limitation as Nabai et al left the scars for up to 12 months reporting the maximum size was reached at 3-6 months.[47] As discussed in chapter 1, human scars can remodel over a period of 18 months until they are mature. Rabbit scars may mature sooner or later, however animal studies requiring prolonged time frames may have cost implications.

Advantages to consider with the rabbit model include wide availability of the rabbit, the ease of housing, genetic reliability and traceability. The model is also relatively easy to reproduce and would produce hypertrophic scars with a similar appearance to that of human hypertrophic scar tissue.[47] The paired nature of ears allows one rabbit to act as their own control with one ear receiving intervention and the other receiving control.

2.3.2 Porcine Models

Fifteen suitable studies were identified for further analysis (see Table 3 in appendix).[48-60] Unlike with the rabbits, we came across several different methods of creating scar tissue on pigs. Eleven out of the 15 studies used the Red Duroc Pig, 1 study used both Yorkshire white pigs and Red Duroc pigs. Liu et al 2018 used the Bama mini pig and Jimi et al 2017 used the Clawn mini pig.[50,55] Although likely to be a Yorkshire white pig, Chan et al 2012 reported using “large white” pigs.[59]

For the 15 papers analysed, the post injury duration varied from 50 days at the shortest (Yun et al 2019) to 180 days at the longest (Foubert et al 2017).[49,53] The range for which the wounds were reported as fully healed was from 14 to 20 days.

All of the included studies involved histological assessment of the porcine scar tissue. Studies by DeBruler et al 2018, Jimi et al 2017 and Zhu et al 2003 all commented on the expression of collagen in the hypertrophic scar tissue created on the pigs and compared it with human hypertrophic scar tissue. They reported collagen in mature, bundle-shaped fibres that were thick and raised; with myofibroblasts expressed in a similar way to human hypertrophic scar tissue.[51,55,61] Other similarities with human hypertrophic tissues included: skin hardening, abnormal pigmentation, flattening of the epidermis, hypervascularity, longer elastic fibres, whorl-like patterns and dysregulation of TGF-β. [51,55,61] Collagen staining was performed in all but two of the studies with most authors reporting a reduction in the amount Collagen types I and III in the control/untreated hypertrophic scars they created.

The Red Duroc pig seems to have been established as a pig that produces hypertrophic scars on mid-deep dermal injury. Initially described in 1972 by Silverstein et al as producing hypertrophic scars, the model was later developed and adapted by Zhu et al 2003.[61,62] They described using an electric dermatome to create the wounds on a Red Duroc pig ranging from 0.015 inches to 0.12 inches deep. After a 5 month follow up period, immunohistochemistry analysis showed similar IGF-1 expression patterns to that of human hypertrophic scar tissue. Conversely they saw a slightly reduced TGF-β1 expression pattern in the porcine tissue compared to the expression pattern reported in human hypertrophic scar tissue but reported this could be due to a different assay used compared to those utilised by other authors.

Histological analysis of the scar tissue matched that of human hypertrophic scar tissue with unorganised collagen fibres in a characteristic whorl pattern. Red duroc pigs were concluded as able to produce thickened scar tissue with a macroscopical and histological appearance similar to that of hypertrophic scar tissue on mid to deep dermal injury.[61]

A subsequent study by Zhu et al 2004 demonstrated the expression of IGF-1, TGF-β1 and versican (an extracellular matrix proteoglycan) were increased which is the same as in human scar tissue.[63] These findings were confirmed by Gallant et al who also identified that Red Duroc pigs produced more hypertrophic like scar compared to Yorkshire pigs.[64,65]

The aforementioned dermatome model was the most common method of scar creation with eight of the studies using it. Five of the eight reported using a Zimmer® dermatome which is powered by compressed air to create the injury.[51-54,57] Two studies reported using an electric powered dermatome.[50,60] Carney et al 2017 did not comment on what type of dermatome they used.[56]

Sharp excision was reported in 3 studies; Yun et al 2019 and Yun et al 2012 reported creating a 3cm x 3cm full thickness wound using a scalpel.[49,58] Jimi et al 2017 reported a bigger incision of 7.5cm x 7.5cm that was 0.15cm deep.[55] Interestingly Yun et al 2019 and 2012 used Yorkshire pigs for their model; whilst Jimi et al 2017 used Bama pigs.[49,55,58]

Yun et al 2012 reported creating 36 full thickness skin excision on the back of the pig using a scalpel under general anaesthetic.[49] After a 50 day healing period, the interventional adipose derived stem cells (ADSC) were injected with 1cm x 1cm full thickness biopsies taken at 10 and 23 days post injection. The tissues underwent histological analysis and qPCR demonstrating reduced fibroblasts and reduced expression of TGF-β1 in the ADSC group.[58] Reported macroscopic scar outcomes included surface area for the scar itself, scar surface area, colour and pliability using a durometer.

No reason was given for using Yorkshire pigs in particular and sharp skin incision was reported as their method of choice as they felt burns are difficult to control. The sharp excision methods described both require a degree of manual dexterity and technique to perform. The dermatome benefits from a fixed depth setting that helps to create a more controlled partial thickness injury. The red duroc pig appears to be a breed that is more prone to hypertrophic scarring compared to other breeds; this could be due to a genetic cause unique to the pig which may weaken findings when compared to human hypertrophic scar.

Two different methods for creating scar via a thermal injury on the pig were identified. First described by Jandera et al 2000, a bottomless glass jug covered in waterproof tape filled with water at 82-85°C would be pressed against the skin of a pig for 10-12 seconds creating a contact burn.[66] Two studies in the literature search reported using the method developed by Jandera et al.[59,67] A variant of the Jandera model was described by Cuttle et al 2006.[67] Their model uses the breed referred to as white pig creating the thermal injury on the flank. A Pyrex® Schott Duran bottle with the base removed and replaced with plastic wrap was filled with water of various different temperatures and times. Water at 92°C held for 15 seconds was optimum for achieving a deep dermal partial thickness injury.[67] The pigs were followed up at 99 days post-burn. Scar tissue that was on average 2.2x thicker than non-injured control tissue with a histological appearance similar to that of the red duroc pig study by Zhu et al with collagen fibres arranged in an unorganised structure with a whorl pattern.[61,67] Electron microscopy of the porcine scar showed similarities with human hypertrophic scar tissue noting absence of rete peg grooves in both.[67] An increased expression of IGF-1, Ki-67 and cytokeratin (the latter two are pro-inflammatory cytokines) was reported compared to porcine control tissue; similar to human hypertrophic scar tissue.[67] The authors concluded that it was perhaps their technique and methods for creating the burn wound

that produced a hypertrophic scar, rather than the breed of pig itself.[67] Chan et al 2012 also used the Jandera model on the backs of white Yorkshire pigs to assess the correlation between time to skin grating and hypertrophic scarring following an acute contact burn.[59] Variations include using a latex membrane at the bottom of a bottomless mug and water at 92°C held for 20 seconds.[59] All the burn wounds underwent some form of surgical intervention in the study with the exception of the control burn wound that received standard burn dressings. These hot water contact burn models benefit from requiring less user skill to perform and can be done using readily available lab equipment. The depth of burn injuries is difficult to control and the temperature in this method is not kept consistent throughout the injury.

Rodriguez-Menocal et al 2018 reported a different contact burn method to create hypertrophic scarring on red duroc pigs to assess CO_2 and erbium-doped yttrium aluminium garnet (Er:YAG) LASERS as a treatment.[48]

The hypertrophic scars were created using a temperature controlled branding iron set to 300°C and held in a vertical position under gravity for 12 seconds. This resulted in a 27mm width burn wound reported to be approximately 3mm deep. Based upon clinical experience, a similar injury in humans would result in a much more substantial depth burn. The burns were dressed with a polyurethane film and assessed weekly for scar formation. Two pigs were used in this study with twenty-seven burns being created on each pig.

The authors reported that by day 70, the scars were mature and hypertrophic in nature. After this the scars were treated with either CO_2 LASER at a high or low setting; or Er:YAG at a high or low setting. Clinical assessments using a modified VSS (mVSS) and the Manchester Scar Scale (MSS) were taken on days 14, 21 and 35 with 8mm punch biopsies taken on the last day. Er:YAG LASER treated wounds had better scores in mVSS and MSS with the best in low setting Er:YAG. The most

remodelling was observed in CO_2 LASER treated scars. Decorin expression was greater in both LASERS on low setting and MMP-9 expression was greater in ER;YAG at low setting treated scars.

The temperature controlled branding iron has benefit over the aforementioned hot water bottle model in that the temperature is kept constant during the injury. The depth of burn injury is difficult to control and the 3mm depth burn seems shallow for such a hot device placed for 12 seconds.

The general consensus of the pig as a model for scarring we found was dependent on what the researchers were trying to achieve. It is apparent that the Red Duroc pig seems to have a genetic predisposition to form hypertrophic scar tissue and so represents a suitable model if this is desired. The authors that utilised the Yorkshire pig commented how the healing and scarring was similar to that of normal human scarring. Advantages of using pigs as a model include a similarity in porcine skin to human skin, a lack of fur (with hair instead) and the larger size of the animal allowing multiple scar sites. Limitations of this model is the set up required to house these animals. Several authors commented on genetic traceability of the pigs as many are obtained from the commercial farm industry.

2.3.3 Murine Models

Twenty-four papers that involved using mice or rats as a research model were identified. Twenty-two were papers from the literature search we conducted. Two papers from the literature search were a critique of already existing papers. The studies the authors were critiquing were included in our review.[68,69] Of those 24 identified, 15 involved creating some form of human based or derived scar tissue on the back of an immunosuppressed mouse kept in sterile conditions to prevent graft rejection. Four of the 15 studies involved transplanting normal human skin onto a mouse in the form of a full-thickness or split thickness skin graft (Appendix Table 4).[70-73] As discussed in chapter 1, the

inflammatory system is intimately involved with the wound healing process so conclusions from studies using immunosuppressed mice may be weakened. Four studies involved culturing of human keloid cells and subsequently injecting the culture into the mouse (Appendix Table 5).[74-77] Seven studies involved excised human keloid scar tissue and transplanting it onto a mouse (Appendix Table 6).[78-84] Four utilised a thermal injury (Appendix Table 7), 3 used incision and mechanical stretch (Appendix Table 8); we came across 1 biopsy model and 1 antibiotic injection model (Appendix Table 9).

2.3.4 Studies involving transplantation of human skin onto a mouse as a skin graft

A model using a Nu/Nu immunosuppressed mouse to receive transplanted normal human skin tissue in the form of a split thickness skin graft was described by Momtazi et al 2013.[70]

Using discarded normal human skin after cosmetic abdominoplasty, split thickness skin xenografts of set dimensions were transplanted onto the dorsums of the mice. Skin from the dorsum of the mouse was surgical excised down to the PC to receive the xenograft. Control mice were used with split thickness skin autografts. Biopsies were taken at 30, 60, 120 and 180 days post procedure. Xenograft scars were raised, thicker, pink/red in colour with a shiny appearance compared to the control autograft scars.[70] A greater average scar thickness and MSS scores (15.9±0.2 and 540.9±15.7µm respectively) were reported in the xenograft scars.[70] Presence of alpha smooth muscle actin (α-SMA) and reduced expression of Decorin was noted in the xenografts. The authors demonstrated that the human xenografts were alive and well for the 190-day duration with histological analysis showing an absence of rete pegs, loss of hair follicles and collagen arranged in the characteristic whorl pattern. All features consistent with the histological appearance of human hypertrophic scar tissue. This model was previously explored in another study by the same authors comparing the scar result with different graft thicknesses concluding that human split-thickness skin

grafts resulted in more hypertrophic scar tissue on the mice compared to human full-thickness skin grafts.[71]

A model with similar principles was utilised by Zeplin et al 2012.[73] The group wanted to assess the efficacy of an antifibrotic eluding silicone gel sheet as a treatment for a burn scar. After transplanting full-thickness normal skin xenografts onto Nu/Nu nude mice, additional scar was generated by burning the graft with a copper template heated to 80 degrees C held on for 10 seconds. They reported reduced expression of TGF-β1, collagen type 1 alpha 1 (COL1A1), connective tissue growth factor (CTGF), FGF(Fibroblast growth factor) 2, MMP-2 and 9 on qPCR in the treatment group, but no comment on the effectiveness of the model to create the scar was made.[73]

These models report creation of thickened scar that appears to show characteristics similar to that of human hypertrophic tissue; but the organisms on which the scar is developed have no immune system and are kept in artificial non-real world conditions.

As a result of this, it is plausible to suggest that the transplanted human tissue used to assess a scar treatment are not representative of human scar tissue in human skin. The burn injury created in the Zeplin model will likely heal in a different manner due to the immune suppression.

2.3.5 Studies involving cultured human scar cells implanted into mice

Supp et al 2012 developed a model of implanting engineered human keloid tissue cells into the backs of immunosuppressed nu/nu mice.[74] Fibroblasts and keratinocytes were extracted and cultured from the human tissues, then inoculated onto bovine collagen glycosaminoglycan dermal substrates in 6 different combinations. At 12 weeks the substitutes underwent qPCR showing greatest expression of COL1A1, TGF-β1, periostin gene, plasminogen activator gene (PAI) and

follistatin gene in substitutes made of deep keloid fibroblast. Histological analysis showed thick, disorganised collagen bundles observed in substitutes cultured with deep and superficial keloid fibroblasts. At 12 weeks after grafting, the bovine collagen biopolymer substrate was replaced by well organised human collagen.[74] No thick, bulging scars were observed as appear in humans suffering from keloid scarring.[74]

Wang et al 2013 created a method of implanting a cultured human keloid fibroblast polylactic-co-glycolic acid (PLGA) scaffold on BALB/c athymic mice. [75] Unlike the model by Supp et al, the engineered structures were implanted into a subcutaneous pouch within the skin of the mice, instead of an area of excised tissue. Sample collections points were 30, 60, 120 and 180 days after transplantation with the implants retrieved and fixed for immunohistochemistry and electron microscopy. The volume of tissue was noticeably larger in the keloid cultured scaffolds and that by day 180 ($12x10x2mm^3$ vs $2x2x0.4mm^3$ in the control group). Histological analysis of the keloid scaffolds showed increased staining of Type I collagen, increased number of keloid fibroblasts and the characteristic whorl pattern associated with human keloid tissue.[75] Under electron microscopy the authors visualised degradation of the PLGA scaffold in the control group by day 180.[75] A criticism of the above mentioned models is the introduction of an engineered scaffold to support the formation of the keloid scar tissue. This could be considered unnatural and may have an influence on how the scar tissue forms.

A model created by Lee et al 2016 proposed a way of implanting cultured human keloid scar onto Nu/J athymic mice without any additional scaffold within the scar tissue itself.[76] Human keloid tissue separated into epidermal keratinocytes and dermal fibroblasts were cultured in layers in a polyethylene ring to create a homotypic keloid skin implant. Mixture heterotypic implants were also created using normal human skin and fully homotypic human skin as a control. The implants were

kept on for a maximum of 18 weeks, the authors reported a greater than 90% graft survival rate 4 weeks after implantation. The polyethylene ring would detach at 2 weeks after implantation. [76] Histological analysis at 4 weeks demonstrated that collagen was more abundant in the heterotopic keloid implants than the normal tissue implants. Homotypic keloid implants showed a disrupted barrier between the epidermis and dermis, as is visualised in normal human keloid tissue Increased expression of COL1A1, PAI-1 and urokinase receptor was reported on qPCR of heterotopic keloid implants.[76] Macroscopically by 18 weeks the heterotopic and homotopic keloid implants were raised above the hosts skin.[76] This model is unique in that the cells are not cultured onto a protein based scaffold, but the polyethylene ring may still influence the healing in the area. As isolated cells are used initially, this may allow for genetic manipulation of the cells to assess potential treatments. However, the lack of the immune system limits the transferability to humans.

Shang et al 2018's model involved injecting a concentrated suspension of human keloid fibroblasts directly into a Nu/Nu athymic mouse.[77] A culture derived from the whole dermal keloid scar tissue that was cultured for 2 hours; and a culture derived from dermal keloid fibroblasts only that was cultured for 24 hours were created.

The two different cultures were injected subcutaneously into the dorsums of the mice which were subsequently euthanised 12 weeks after the injection. The group reported that by 42 days, the keloid tissue in the 2 hour group was macroscopically larger than the 24 hours cultured fibroblast group.[77] H&E staining of the tissue from both groups showed the 2 hour culture group to be more like that of human keloid scar tissue whereas the 24 hour culture group seemed to be more like normal human skin. The injection technique benefits from not creating an incision on the mouse and minimal disruption of the PC.

2.3.6 Studies involving transplanting full thickness scar tissue onto mice

Using methods initially developed by Shetlar et al 1985, we found three papers in our search by the same group who utilised a model of directly transplanting human full thickness keloid tissue onto the back of athymic BALB/c nude mice. Whole human hypertrophic scar tissue was cut into 0.5cm^3 section and implanted into a subcutaneous pocket on the dorsum of the mouse. The implanted scars would be retrieved at 1, 2 and 4 weeks. Interventions assessed include single injections of verapamil, verapamil + TCA and saline control directly to the scar.

Administering intralesional injection of verapamil and verapamil with TCA resulted in a scar that was smaller in weight, decreased fibroblast proliferation and increased decorin expression. This same model was used to assess interferon therapy.[79,80]

Chen et al 2017 also described a method of implanting dissected human hypertrophic scar tissue into a subcutaneous pocket on the dorsum of nude BALB/nu mice.[83] The authors reported stronger decorin expression in the treatment groups, with the combination group having the strongest.[83] Additionally the scar tissue weight was least in the combination group as was the reduction in fibroblast proliferation. The authors concluded intralesional combination therapy of botulinum toxin type A (BTXA) and TCA may have therapeutic potential.[83]

Fanous et al 2019 described a very similar study utilising keloid scar tissue instead of hypertrophic scar tissue.[84] For their study they implanted human keloid tissue into a subcutaneous pocket on the dorsum of nude nu/nu mice. The keloids were cut to approximately 2-3cm^3. One week after implantation, the implants would receive an injection of BTXA, saline as a control or TCA. Three weeks after implantation, the scars were removed and underwent histological analysis and weight assessments. They reported implants treated with BTXA or TCA had significantly smaller weights

than those treated with saline.[84] Blinded histological analysis revealed those treated with BTXA had a more organised collagen structure. The authors concluded BTXA may have a role as a preventative in the formation of keloid in human patients.[84]

This same model was used by Qiu et al 2015 used the same model to assess the effect of P144® an Anti-TGF-β topical agent.[81] Seven days after implantation, one group had topical placebo applied, the other had a P144® peptide applied daily for 2 weeks. The scars were extracted and underwent histological analysis. The authors reported a reduction in the expression of collagen I and collagen III in the scars treated with P144®. They concluded that P144® may have therapeutic functions in the future but more research was needed. The subcutaneous pocket model appears to facilitate human keloid tissue growth, but the sterile housing environment combined with the immunosuppression of the mouse makes it difficult to translate to human keloid tissue.

Philandrianos et al 2015 describes a model of suturing into place whole human keloid tissue onto the dorsums of immunosuppressed nude mice.[82] The keloid tissue was cut into 8mm diameter discs using a punch biopsy and sutured into place for 4 weeks when the sutures were removed. The model was used to assess 1210nm PDL treatment. The keloids were harvested at 1 month, 2 month and 3 months in all groups. The authors aimed to assess whether or not 1210nm laser could activate heat shock protein in human keloid scar tissue. Macroscopic and histological analysis showed no significant difference in the appearance of the scars.[82] This model is different in that the keloid tissue is exposed to air albeit in a sterile environment. However, healing or remodelling that may occur after a treatment such as the LASER in this case will be based upon the immunosuppressed reaction, not a human inflammatory response.

2.3.7 Studies using thermal injury to create scar on mice

Ibrahim et al 2014 developed a model to analyse scar contracture in hypertrophic tissue on immunocompetent mice.[85] The thermal injury was created using a brass metal rod 8mm in diameter heated to 100˚C in boiling water for 15 minutes, then placing the rod on the mouse for 1 second. Three days later, the burn site was excised and a full thickness skin graft from ear skin was laid over the wound. Post-grafting, tissues were excised at days 3, 7, 9, 11, 14, 28, 70 and 168. Histological analysis demonstrated increase in vascularity, macrophages and mast cells of the graft cells. The authors reported that the skin grafts contracted but did not disappear; interestingly they reported that the PC did not contribute to the contraction of the skin graft.[85] The scars were reported as flat and initially red but later becoming pale.[85] This is different to human hypertrophic scar tissue which is raised and often red or pigmented in colour.

The same model was used by Lorden et al 2016, except split thickness skin grafts were used.[86] Prior to the graft going on, the wound bed wound have a collagen coated permeable biostable polyurethane scaffold placed or a polyurethane scaffold with no collagen on it. The authors reported that the burn wounds treated with the collagen coated scaffold resulted in a reduced hypertrophic scar.[86] One could argue that once the rod is out of the water it will begin to cool introducing variability in the temperature of the burn applied. Depth of injury is difficult to control with a burn adding to variability.

A modified version of a scald model first described by Walker and Mason et al 1967 was reported by Lu et al 2014.[87,88] The thermal injury was created using a hot water bath at 100°C and placing a mouse in a plastic template that exposes 8-10% of the total body surface area and dipping into the hot water for 8 seconds. Prior to thermal injury, the mice would receive clondronate liposomes subcutaneously or intraperitoneal. Histological analysis at 15 days in untreated control mice showed

collagen arranged in the characteristic whorl patterns. [87] Quantitative PCR showed reduce TGF-β expression pattern in the mice treatment mice but did not comment on whether or not the qPCR results for the control scar were similar to those seen in human hypertrophic scar tissue.[87] This model benefits from providing a constant temperature to make the injury, however the variability of the wound site and size by placing the mouse in a template may cause differences in the resulting scar.

2.3.8 Studies involving incision and stretch

A novel model for wound healing and scarring was developed by Zhou et al 2019.[10] In order to counter the effect of the PC, Zhou et al created a novel model to create scar tissue on the tail skin of rats. Full thickness tail skin excisions including the PC were made on rats using a scalpel and iris scissors at three different sizes (3mm x 3mm, 6mm x 6mm and 9mm x 9mm). To establish the effect of mechanical strain on the wound site on the formation of scar tissue, the tails were wrapped around steel rings of set diameters of 2cm (high strain) or 3 cm (low strain) along with a control group with no steel ring attached.

Wounds were harvested at 0, 2, 6, 12 and 24 weeks post re-epithelialisation.

The morphology of the scars on the tail wounds demonstrated no wound contraction compared with control scars on the dorsum of the rats back.[10] Rat tail wounds that were put under higher strain exhibited noticeably thicker elevated scar tissue compared to the wounds under low strain.[10] The wounds under no strain had the flattest scars. Histological analysis at 12 weeks of the scar tissue under strain showed signs similar to human hypertrophic scar tissue including unarranged collagen fibres in a whorl like pattern. Expression patterns of TGF-β1 and α-SMA in the scar tissue placed under high strain were equivocal to the expression patters in human hypertrophic scar tissue.[10] This model of creating a hypertrophic scar on a strained rat tail offers advantages over the other animals

as the rat is immunocompetent, is easy to house and offers a way to minimise the effect of the PC. It is cheaper than porcine models and is relatively easy to standardise and reproduce. How transferrable results of treatments on rat tail skin are will require further investigation.

A different incision and mechanical stress model was put forward by Murphy et al 2019.[89] The authors wanted to assess the effect of angiotensin type 1 receptor blocker losartan in the resulting healed cutaneous scar. Scars were created using a scalpel to make a full skin thickness 2cm linear incision on the dorsum of the mouse and then sutured. Three days after the procedure, the sutures were removed and the wounds edges attached to a mechanical loading device. The device would stretch the wound by a further 4mm every 2 days to a maximum total of 2.4cm. At 28 days the mice were killed and the resulting scar excised for histological analysis. The authors reported a reduction of the scar area in those treated with losartan along with a reduction in expression of α-SMA, macrophages and collagen I fibres.[89] Shan et al also performed an incision and mechanical stretch model for creating hypertrophic scars on mice similar to the Murphy et al model.[90] They did not specify the extent to which the device stretched the wound over the ten day period. Topical naringenin was assessed as a scar treatment against non-treatment control. On day 14 the mice were euthanised and the scars excised for histology, qPCR and western blot analysis. The authors reported that naringenin inhibited fibroblast activation, suppressed inflammatory cell infiltration and reduced the expression of the inflammatory cytokines IL-1β, IL-6, TGF-β1 and TNF-α.[90]

The stretch models use immunocompetent rats/mice resulting in a normal murine inflammatory and healing response. In humans it is acknowledged that healing wounds under tension (such as over a joint) can result in thicker more hypertrophic scars. The techniques used to minimise the effect of the PC may help make the resulting scar more relatable to human scar.

2.3.9 Studies involving other methods

A punch biopsy method was described by Shahin et al 2012 to assess the use of a commercial topical scar treatment.[91] Scars were harvested on the 30th day post injury. The authors reported reduced expression of TGF-β, fibronectin and laminin in scars treated with the topical agent. This model is not often used as the rats superior healing ability aided by the PC and the small scars that result due to its contraction.

One paper was found that described a method of injecting bleomycin, an antibiotic known to cause lung fibrosis when inhaled, into the skin to create scar tissue.[92,93] Cameron et al 2014 described subcutaneous infusions of the antibiotic bleomycin to create hypertrophic scarring in immunocompetent mice.[93]. An osmotic pump sutured into a subcutaneous pocket between the muscle and the skin delivered bleomycin at a constant rate of 0.11(mu)l/hour for 28 days. Scar samples were harvested at the end of the infusion and a further set 28 days post the end of the transfusion. The histological appearance of the bleomycin murine skin was similar in appearance to human hypertrophic scar tissue.[93] The samples taken at 56 days had a significantly increased dermal thickness but a thinner epidermis than the 28 day models.

A unique feature of this model is the lack of damage to the epidermis in creating the scar that helps to avoid the contraction created by the PC. The excessive fibrosis caused by the antibiotic may be sustained in the mouse's system which could alter the scarring in the longer term.

2.3.4 Other animal models

One study that reported using primates and one reported using a dog in the included articles (Appendix Table 9). Igarashi et al 2015 used marmosets to analyse the effect of a pyrrole-imidazole polyamide (PIP) that targets the human TGF-β gene to reduce its expression.[94] For this study, marmosets received a full thickness linear incision down to the PC, 2cm in length on the abdomen.

Prior to the incision, the area to be incised would receive an injection of one hundred micrograms of the PIP agent GB1101 dissolved in H_2O with the scars harvested at 35 days. Another set of incisions received GB1101 as an ointment rubbed under the skin around the incision site prior to suturing, with the scar harvested at 42 days. For both injection and ointment GB1101, histological analysis showed a thinner epidermis and a thinner dermis resulting in a flatter scar than the control group. This was the only model we found using primates which require specialist housing requirements and set up. Although humans may be more closely related to primates, the relative difficulty in obtaining primates for research and ethical concerns due to their intelligence are limitations.

Kimura et al 2011 used the Mexican hairless dog, creating 3cm x 3cm full thickness skin incisions to generate hypertrophic scar.[95] Histological analysis at 90 days demonstrated well-organised collagen with elastin present. Macroscopically, the author reported that the scarring pattern was unique in that the dogs formed hypertrophic, hyperpigmented scars that are different to those of dogs with fur.[95] As discussed in chapter 1, pathological scar tissue is thick and has disorganised collagen. The organised nature of the collagen observed in the dog model and the housing requirements make this a less than ideal animal model.

2.4 Results – Human Models

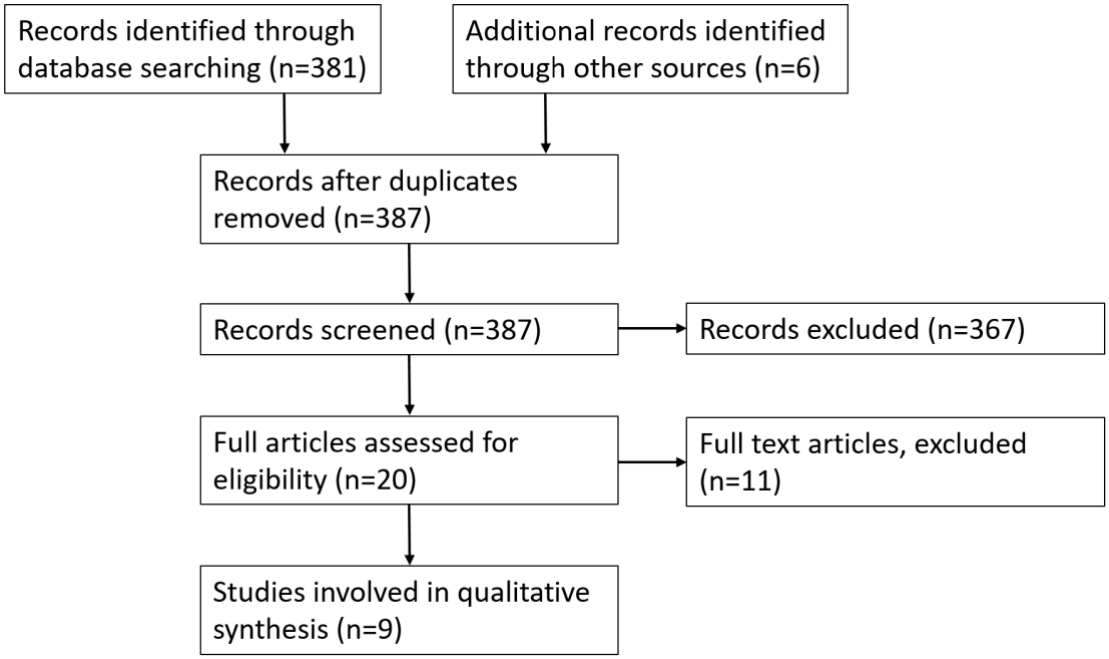

Figure 2. Flow diagram of records involved in analysis for human scar models

The search yielded 381 results in total (Figure 2). Both reviewers analysed the titles and abstracts identifying 20 studies for further analysis. Full analysis of the 20 studies identified 3 suitable studies and an additional 6 studies were identified by reviewing references of review articles (Appendix Tables 11 and 12). Reliable, easily reproducible human scar models are very rare. There are multiple models for patients that have already got a scar from a previous injury such as a burn. Very few models are present that utilise a standardised, purposefully created scar. Of the limited models that are particularly looking at scar tissue, they are typically used to assess treatments for scarring used in clinical practice.

2.4.1 *In vivo* Human Models

A similar model was reported by Cruz-Korchin et al 1996 and Niessen et al 1998 on female patients undergoing bilateral breast reduction.[96,97] Cruz-Korchin et al randomised 20 patients 2 weeks post

bilateral breast reduction surgery to wear silicone gel sheeting on one breast.[96] The patients would act as their own control and the silicone dressing would be worn for 2 months. Niessen et al used the same scar model; in their study they included 155 patients who would wear a silicone dressing on a randomly allocated breast scars (right medial and left lateral or right lateral and left medial) for 3 months.[97] The patients also acted as their own control. The follow up period for the Cruz-Korchin et al study was 6 months where they reported breast scars left untreated; 45% developed flat scars and 55% developed hypertrophic scars compared to the silicone treated group; 75% developed flat scars and 25% developed hypertrophic scars.[96]

The follow up period for the Niessen et al study was 12 months, the authors were assessing the total number of hypertrophic scars. They reported at 3 months, 64.3% of patients had at least one hypertrophic scar which then dropped to 35.3% at 12 months.[97] At 6 months, 29 patients with silicone treated tissue developed hypertrophic scars versus 13 whom developed it on control sites (p value = 0.006).[97] At 12 months they reported 19 hypertrophic scars in those treated with silicone and 7 in those without (p value = 0.02). At 3 months the numbers were almost equal at 17 and 18.[97] Although these studies used different methods for assessing the treatments, the underlying model of creating the scar was similar. However, there are several issues that make this model difficult to reproduce. There is an element of surgeon error in the model, the tensions across the wounds will be different depending on the volume of breast removed and there are sutures in the skin which could all impact how the scar tissue forms. The Niessen et al study suffered from loss to follow up bias and both studies did not comment on whether patients and observers were blinded. The Cruz-Korchin study consisted mainly of Hispanic patients that have been reported to be at increased risk of hypertrophic scarring compared to Caucasian patients which may influence the findings.[98]

A different scar model was utilised by Kong et al 2004 to assess the efficacy of liquid silicone gel on pain and itch after elective total knee replacement surgery.[99] The 100 patients involved in the study received surgery to one knee only. Five days after the surgery, patients were randomised to receive either silicone gel or placebo for one month. They were subsequently followed up at 3 months, 6 months and 12 months. They reported thinner and lighters scars in those treated with silicone (silicone gel 1.5±0.61 vs 1.92±0.8 p-value = 0.004 in VSS pigmentation score) and (silicone gel 0.86±0.6 vs 1.14±0.75 p-value = 0.044 in VSS height score). Note the VSS will be discussed in Chapter 3.

This model again relies on the same surgeon making the same cut to reduce the error. The length of the incisions could vary and like with the breast, there will be sutures holding the skin together which could affect the scarring process. The authors reported this study was double blinded with the patients unaware of whether they were applying silicone gel or placebo. Assessors of the scar were also blinded reducing bias in the study. Placebo controls were on different patients to those having intervention. Scarring is so unique to the individual, it is difficult to assess whether the differences observed between scar in intervention vs control are accurate.

In a similar suit to the aforementioned studies, Sproat et al 1992 used cardiac patients with established midline sternotomy scars from previous surgery.[100] Fourteen patients were included in this study, one half of the scar received a TCA injection, the other half had silicone gel sheeting applied for 12 hours a day for 12 weeks. Outcomes were the patients' preference in terms of the appearance of the scar, pain, itch and ease of the treatment. The authors reported that 11 of the 14 patients preferred silicone gel sheeting, 2 preferred the TCA injection and 1 had no preference.[100] There was no comment on blinding in the study and the small sample size suggests it is underpowered. Although patients would act as their own control, there may be differences in

tension and scar quality along the scar that influence its structure. Subjective PROMS alone provide patient opinion of the scar and do not include objective measures.

All models used in the aforementioned studies have limitations including ease of reproducibility of the scar, underlying materials such as sutures which could change the scar, inconsistency of the underlying mechanism of injury (as in burns) and the difficulty of establishing a reliable control.

A novel jig was developed by Dunkin et al to create a graduated precise depth injury in the skin of healthy volunteers.[101] The jig used in the study was designed to help establish a critical depth of skin injury that would result in scar tissue formation. The authors included 113 healthy volunteers who underwent a graduated skin incision using the jig on the lateral side of the hip between the anterior superior iliac spine and the greater trochanter. The patients would be followed up weekly for 1 month, then at 6, 10, 18, 24 and 36 weeks. Outcome measures were standardised photographs (assessors blinded), a high-frequency ultrasound scanner and a dedicated image analysis software package. The jig produced a wound 51.3±0.6mm in length, at 36 weeks the mean length of 34.9±1.0mm with approximately 68% of the original wound length healing with a visible scar, the remainder without.[101] The jig was designed to produce a wound with a maximum depth of 1.6mm after previous work by the authors demonstrated that skin thickness on the lateral hip is 1.6±0.1mm.[101] Using trigonometry, the authors demonstrated that the mean threshold depth on the lateral aspect of the hip that resulted in visible scar formation was 0.56±0.03mm or 33.1% of the skin thickness.[101] The jig is unique in that it provides a method of creating a standardised scar in a healthy human.

An interesting model was developed by Lanier et al 2016 utilising the skin on the lower abdomen of patients due to undergo abdominoplasty.[102] Patients would receive a series of discrete 2cm full thickness incision on the lower abdomen under local anaesthetic. The incision would be in parallel to each other with patients receiving up to 20. The incisions were sutured and left to heal leaving a scar. The 20 scars were used to assess a drug in phase 2 trial designed to help reduce scarring. The drug is given as an intradermal injection. The authors did not specify at what point after incision the drug was given, but one side of the abdomen was randomised to receive the drug and the other randomised to receive placebo. The scars were analysed over a 13-week period with the most lateral scars being biopsied for histological and mRNA analysis. The results from the study using that drug have not been published but the model is an interesting use of human skin that is planned to be discarded with elective abdominoplasty. Limitations could include how long the tissue is available to assess scar treatments and the close proximity of the scars may lead to cross contamination in studies.

2.4.2 *In vitro* human models

Although the studies predominantly discussed in this review are *in vivo* human and animal scar models; *in vitro* scar models that use human cells are also used in research (Appendix Table 12).

Ex-vivo skin cultures use human skin derived structural cells such as keratinocytes, fibroblasts, melanocytes and Langerhans cells. When cells such as keratinocytes are cultured and placed on a fresh culture media plate, they can be “wounded”. A sterile device such as a pipette tip can be used to create the wound through the cell culture. This technique is relatively simple and is well documented. Although predominantly used in healthy normal cells, there are some culture models that specifically culture cells from hypertrophic scar tissue.

Lee et al 2013 created a culture using human keloid tissue, specifically from the dermis of human participants with ongoing active keloid scars.[103] As dermis is not usually exposed to air, the keloid tissue was cultured submerged at 37°C in a humidified atmosphere at 5% CO_2. The keloid tissue was cut into identical spheres prior to being cultured. These spheres underwent immunohistochemical analysis which showed high levels of expression of collagen I and TGF-β just like in normal keloid scar tissue. Interestingly, the authors injected some of the cultured keloid sphere with TCA. They reported after injection with the steroid, the cultured keloid spheres regressed and expression of collagen I, collagen III, elastin and fibronectin was reduced just like in keloid tissue in the skin.[103]

Another technique used to co-culture keratinocytes, fibroblasts and use them to form a 3D structure was reported by Chawla et al 2018.[104] They used a collagen based gel enriched with fibroblast culture from hypertrophic scar tissue to create a 3D structure. These cultured scar tissue structures have similar α-SMA expression to that of non-cultured hypertrophic scar tissue.[104]

There are various methods reported for producing a full thickness human-skin equivalent. These involve using cultured fibroblasts and keratinocytes on a collagen based structure to form a human-skin equivalent. All of these rely on fresh human tissue to get the cells.

The literature search found a novel technique utilised by Reijnders et al 2015 aimed to use immortalised fibroblast and keratinocyte cell lines to produce a human skin equivalent, instead of cells from fresh human tissue.[105] Human telomerase reverse transcriptase (TERT) immortalised fibroblasts and keratinocytes were used to construct a human skin equivalent.

The 3D construct for the skin equivalent is a bovine matrix which lacks a basement membrane. The matrix is made up of collagen I, III and IV and elastin. The fibroblast cells were seeded onto this matrix and submerged for 3 weeks in culture. Keratinocytes were then seeded onto the matrix and

submerged for 4 days in culture and then later cultured for 14 days exposed to air. The TERT cell human skin equivalent was compared with normal human skin and with human skin equivalent derived from primary cell culture. The different tissues all went histological and immunohistochemical analysis. The authors reported the morphology of the TERT cell engineered tissue closely resembled that of real human skin and the skin equivalent derived from primary cell culture. This includes a distinct epidermis and a fibroblast populated dermis. Further electron microscopy of the TERT cell line human skin equivalent demonstrated a well-developed stratum corneum layer made of corneosomes. The authors also showed well-developed dermosomes within the stratum granulosum, stratum spinsoum and stratum basale. A lamina lucida and lamina densa were seen on the electron microscopy images of the TERT cell line human skin equivalent suggesting formation of basement membrane. This was also confirmed with expression of basement membranes laminin 5 and collagen IV staining on immunohistochemistry.

To demonstrate if this model can be used to analyse injury and healing of human skin, the authors performed a cold injury and a burn injury to the engineered TERT cell line human skin equivalent. They reported that the epidermis was able to re-epithelialise and produce wound-healing mediators. They found that burn injuries would typically disrupt the basement membrane whereas in cold injuries the basement membrane would remain intact.[105] The authors concluded that the human skin equivalent they have developed through immortalised cell lines could be a very useful model as it does not rely on fresh human tissues and can be relatively easily created.

2.5 Discussion

Trying to find a reproducible, standardised model for investigating and assessing scar tissue has long been difficult. The majority of research into scarring aims to find underlying mechanisms of how it occurs; and in patients that already have established scars to develop treatments. Within the animal

kingdom, there have been multiple models for creating a wound in mammals, analysing the healing process and to identify therapeutic targets.[9]

On review of the literature, the most popular animal model was the rabbit ear model. The use of the rabbit ear for the creation of hypertrophic scars was discussed.[12] Strengths include rabbits are relatively inexpensive to house compared to larger animals such as pigs, ease of reproducibility and a lack of the PC means no strong contraction of tissue. The authors demonstrated histological analysis of the rabbit ear scar tissue to be similar in appearance to that of human hypertrophic scar tissue. A critique of this technique could be how ear skin is different to skin elsewhere on the body as it has a layer of cartilage underneath it. This could have an impact in terms of how the skin heals, how treatments respond and absorb. Rabbits also have a layer of fur on their ears, which is different to humans.

As mentioned earlier, pigs could be a much more suitable model for scarring research as their skin anatomy and physiology is very similar to human tissue such as a thick dermis and hair instead of fur. In terms of scarring models, the Red Duroc pig has been extensively investigated by Zhu et al.[61,63,106] The scarring model using a dermatome at specific depths to create a wound that subsequently scars is easily reproducible and relatively straight forward. The histological and immunohistochemical analysis by Zhu et al demonstrated scar tissue similar in appearance and with a similar inflammatory protein profile to that of human hypertrophic scar tissue. Further study into red duroc pigs has led to the suggestion that there may be a genetic element specific to the red duroc breed itself that makes it more likely to form thickened scar tissue.[65] The red duroc pig has the potential to be a specific breed used to investigate scarring due to its particularly aggressive scarring response. Limitations include the variability in pressure applied with the dermatome,

suggesting an element of user error. Also, pigs are much larger than rats and rabbits making them more difficult and expensive to maintain in a research facility.

The contact burn technique demonstrated by Cuttle et al highlights a simple, easily reproducible, cost-effective method of producing a burn injury. Interestingly, they used a different breed referred to as the white pig that was able to produce hypertrophic scar tissue similar to that seen in the red duroc pig models. Limitations of this model include a degree of user error ensuring the temperature is correct, as it is hot water of a specific temperature it will begin cooling once removed from the heat source. The aforementioned studies show that pigs are a very suitable animal based research model for scar tissue. Due to the size, agricultural gene pool and maintenance of a pig, they are a more challenging animal research model than smaller mammals.

Murine models, as with porcine and lagomorph models have been used to study the wound healing process itself, but very few have been used to produce scar tissue for analysis. As discussed earlier, one of the main reasons rats and mice are unsuitable for scar research is due to the differences in anatomy and physiology. The prominent PC causes contraction of wound sites, they heal very fast and are covered with fur. The model developed by Momtazi et al and its subsequent use by Yang et al is a very interesting method of generating scar tissue. The transplantation of human tissue on to an immunosuppressed mouse was shown to create human tissue scars in mice that would survive. This model is reproducible and relies on both human tissue and murine tissue. As it is human tissue on the mouse, one could argue that results/effects of treatments observed could be transferrable to humans. However, the lack of inflammatory response in the mouse means reaction to treatments would likely not be similar to those seen in humans. Unlike with the pig models, the Nu/Nu nude mouse is available from a specific genetic lab grown line for use in research ensuring little genetic variation. Limitations of this model include the sterile facilities required to keep the mice and the many steps in creating human grafts increasing the risk of error.

The scalding burn model described by Lu et al is a relatively simple and easy to reproduce method; that would result in scar tissue with a histological appearance like that of human hypertrophic scar tissue.[87] The water can be maintained at a constant temperature to cause the burn and the template allows a standardised area in which the scar can form. Limitations from this model include the burns location being on the dorsum of the mouse means the PC may still have an impact on the tissue. The mice may vary in size slightly and ensuring the scald is in exactly the same spot on each mouse is difficult as it is placed dorsum side down. It is a relatively cost-effective model and the mice used can be kept in normal conditions. The model described by Zhou et al aimed to address the issue that scar models on rats are restricted due to the activity of the PC. As they demonstrated, performing the wound on the tail seemed to create more of a skin healing response similar to that of humans with less contraction. The technique described to put the tail under strain led to the creation of hypertrophic like scar tissue which could be utilised for treatments. This model is cost-effective, easily reproducible and does not require any special conditions to keep the mouse in. Limitations could be ensuring the tension circle stays on the rat and that humans don't have a tail covered in skin which could be more directly relatable. The main benefits of the mice models are they are a much easier animal to keep and get hold of with a reliable genetic background.

The human models explored are often more difficult to establish due to ethical approval and follow up constraints. The bilateral breast reduction models discussed by Cruz-korchin et al and Niessen et al are good as they allow patients to act as their own control and are in patients already undergoing elective procedures. Limitations are the variability in the surgical techniques in that it is difficult to reproduce someone else's incisions by hand as well for the surgeon to reproduce it exactly the same every time. With the model relying on breast tissue, there is some variation between each breast on women and the underlying suture material could influence the scarring process. The issues are

similar with the knee model proposed by Kong et al except there is no other scar on the same patient to act as a control. The sternotomy scar model by Sproat et al has similar limitations to the aforementioned studies. The Northwestern abdominoplasty scar model proposed by Lanier et al is similar to the aforementioned in that it utilises patients undergoing an elective procedure and in this model patients act as their own controls. Limitations of this model include longer term outcomes cannot be assessed as the tissue is discarded when the abdominoplasty takes place and a high degree in user error when creating the scars as it is done by hand with a scalpel. The model used to create a scar by Dunkin et al is unique in that it is the first of its kind to utilise a device that makes a standardised incision in the skin as the blade is on the jig. The jig means there is control in the depth and length of the incision making it significantly more reproducible than the other scar models in humans. In the study, the scar was only put on one lateral side of the hip. If a scar was created on either hip or in other body areas which allows for another scar to be placed on the contralateral side, then the model would allow patients to act as their own controls if testing treatment versus placebo. Limitations with this study include creating a scar on otherwise healthy people. All human studies are limited by issues with compliance of treatment, loss to follow up and difficulties getting biopsy for scientific analysis.

Included in the review were *in vitro* human models as a basis of studying scarring. Culturing cells on collagen and creating a wound in the cell monolayer can demonstrate the migration of the cells, but the conditions are not like that of human skin. The model proposed by Lee et al is good in that it is using human keloid tissue, maintained to assess treatments on. Similar to how Chawla et al used keratinocyte and fibroblast co-culture from hypertrophic scar tissue to create 3D structures. With both these models, it is very hard to recreate the scar micro-environment present *in vivo*. These models also rely on human tissue to be obtained first then cultured.

The model proposed by Reijnders et al counteracts this by utilising immortalised cell cultures to recreate a human skin. Their use of cultured immortalised fibroblasts and keratinocytes on a 3D bovine collagen matrix was able to generate a human skin equivalent with similar properties to that of human skin. This model will require further development to replicate the *in vivo* environment and demonstrate a scarring response but could prove useful in the future.

2.6 Conclusion

Despite being multiple models to create wounds and analyse them in animals, few exist specifically looking at scar tissue. Although porcine models are the closest animal skin to human skin, the high cost, genetic variability and large size makes them difficult to work with. The mouse model using grafted human skin is promising as a future model as the results from its use may be more translatable to human research. However, the lack of immune system of the mice in this model requires special facilities to house them and may affect the healing. Despite these limitations, it may emerge as a very reliable and relevant model. Within the human models, reproducibility and underlying factors that could influence the healing process are often an issue. The jig developed by Dunkin et al has given a promising future model to create standardised scar which could be used to assess treatments for scarring. The *in vitro* models are of benefit but lack the ability to replicate the human skin environment. They may be of benefit for testing treatments before they are tested on live human subjects.

Chapter 2 Appendix

Table 2. Results from rabbit models

Studies	Intervention	Punch Biopsy size (mm)	Data collection points (days)	Outcomes	Results	Authors comments
Caliskan et al 2016[1]	Intralesional injection TCA vs 5-FU vs BTXA	8	30, 60	Histological analysis	SEI - TCA 1.41± 0.17 vs 5-FU 1.02± 0.22 vs BTA 0.98±0.3 5-Fu reduces fibroblast count.	TCA and 5-Fu are effective monotherapies for HS, BTXA no effect on established HTS
Chavez-Munoz et al 2012[2]	Indoleamine 2,3-dioxygenase (IDO) transduced fibroblasts vs non-transduced fibroblasts vs non-treated (control)	8	35	Histological analysis, western blot	SEI – Transduced IDO 1.4±0.04 vs non-transduced IDO 2.3±0.25 vs control 2.3±0.22	IDO transduced fibroblast skin substitutes provide a wound coverage that results in a better scar
Demir et al 2012[3]	Intralesional Enalapril vs Candesartan vs TCA vs non-treatment control	6	28, 40	Histological analysis of arrangement of collage fibres	SEI - Lanapril 1.46±0.29 vs candesartan 1.62±0.35 vs steroid 1.26±0.15 vs control 1.97±0.35	Better scar appearance macroscopically and microscopically in those treated with ACE-I and steroid
Diao et al 2013[4]	Intradermal 0.02% trichostatin vs intradermal saline (control)	10	16, 23, 45	Histological analysis, qPCR, western blot	Decreased Collagen 1, fibronectin in treatment group SEI – 0.02% trichostatin 1.45±0.09 vs control 2.07±0.10	Histone deacetylase inhibitors may be an effective therapeutic strategy for HTS
Fang et al 2015[5]	Topical opuntia extra vs topical saline control	7	22, 39, 54	Histological analysis, qPCR, scar thickness assessment	Improve histological appearance and collagen deposition, decreased MMP-1 expression	Opuntia extract decreases the formation of HTS
Friedrich et al 2017[6]	Burn injury + debridement vs surgical excision (control)	10	1 hour, 3, 28, 35	Histological analysis, qPCR	SEI - Surgical (control)1.34 ± vs Burn wound 0.22 1.63 ± 0.37, no difference in TNF-α on qPCR	Thermal injury via brass rod for 20 seconds produces HTS

Gisquet et al 2011[7]	Intradermal tacrolimus vs non-treatment injection (control)	10	14, 28, 60	Histological analysis, Bimodal spectroscopy,	SEI - Intradermal tacrolimus 1.5 ± 1.5 vs Control 3.1 ± 1.7	Intradermal tacrolimus prevents HTS, bimodal spectroscopy may have a role in characterising physiopathology
Gong et al 2016[8]	Cultured HTS fibroblasts cultured cells treated with RHE	7	28	Histological analysis and qPCR	Down regulation of cyclinD1, cyclin-dependent kinase 4, proliferating cell nuclear antigen	RHE inhibits HTS fibroblast proliferation
Gong et al 2017[9]	RHE intradermal injections vs TCS vs saline control	7	28, 47	TEM, flow cytometry	Increased apoptosis of HTS fibroblasts on electron microscopy	RHE inhibits hypetrophic scar fibroblast proliferation
Hartwell et al 2015[10]	IDO secreting fibroblast scaffold vs acellular scaffold	6	1, 3, 5, 7, 20, 35	Histological analysis, IHC, immunofluorescence, macroscopic analysis	Reduced contraction, reduced inflammatory proteins SEI - Gel 1.24 ± 0.05 vs Gel IDO 1.25 ± 0.03	Application for a gel scaffold to facilitate healing
Jia et al 2011[11]	Topical silicone gel vs silicone gel + silver vs untreated control	10	35	Histological analysis, wound size assessment	SEI - Given as a chart, silicone gel and silicone gel+silver both had lower SEI	Silicone gel is as effective as SGS at reducing HTS
Ko et al 2012[12]	Intralesion inection of simvastatin vs lovastatin vs pravastatin all at high, medium or low dose (as control)	7	15, 21, 35	Histological analysis, qPCR	No significant differences in SEI Report simvastatin, lovastatin, pravastatin reduce SEI by 21.9%, 25.8% and 22.8% respectively Reduced CTGF expression in low dose statin	Statins reduce HTS formation via inhibition of CTGF
Liu et al 2014[13]	Intra-arterial delivery of control MSC vs p53 MSC vs no treatment control	9	3, 10, 14, 21,	Histological analysis for inflammatory proteins and mesenchymal stem cell presence	Treatment down regulated α-SMA, TGF-β1 SEI - Given as a chart, SEI lower in treatment group	Wild-type MSC engraftment could inhibit HTS in a p53-dependent manner
Liu et al 2017[14]	Intralesional BTXA vs TCA vs no treatment control	7	Daily for 60 days	Histological analysis of collagen	SEI - Control 2.368 ± 0.1986 vs botulinum toxin A 1.431 ± 0.0977 vs Triamcinalone 1.630 ± 0.0768	Botulinum toxin-A improves appearance of HTS, suppresses collagen deposition and fibroblast proliferation

Menezes et al 2019[15]	Tacrolimus ointment vs Vaseline control	10	30	Histological analysis	Scar thickness in Tacrolimus group 656.69±226.94µm vs control 929.66±505.25 µm	Tacrolimus ointment helps to produce a thinner scar
Nabai et al 2017[16]	Biopsy device to create HTS	10		Protocol only		Description of rabbit ear HTS model
Rahmani-Neishaboor et al 2010[17]	Topical stratifin vs ASA incorporated CMC gel vs untreated control	8	5, 20, 28	Histological, immunohistochemistry for inflammatory proteins, collagen profile	Increased MMP-1 in stratifin and reduced collagen density SEI - CMC gel1.79 ± 0.19 vs ASA-CMC gel 1.23 ±0.07 vs Stratifin 1.15 ±0.14 vs Control 1.84 ±0.15	Topical application of stratifin and aspirin can improve or prevent HTS
Ren et al 2013[18]	Intraperitoneal endostatin injection vs intraperitoneal injection of saline	7	15, 21, 28, 35	Histological analysis, western blot	Type I collagen, Bcl-2 supressed SEI - Endostatin 1.09 ± 0.19 vs control 1.36±0.28	Systemic endostatin application inhibits angiogenesis reducing HTS
Rha et al 2016[19]	SGS+0.25mg/g verapamil vs SGS+2.5mg/g verapamil vs SGS+25mg/g verapamil vs SGS only (control)	6	17, 26, 53	Histological analysis of fibroblast and capillary count	SEI - SGS 2.2(control) vs SGS+ 0.25mg verapamil 1.3 vs SGS + 2.5mg verapamil 1.2 vs SGS+25mg verapamil 1.1 Fibroblast and capillary counts lower in verapamil groups	SGS containing verapamil microparticles at an optimum dose of 2.5mg/g improves HTS according to SEI
Sari et al 2017[20]	Intralesional DMSO vs TCA vs saline control	5	28	Histological analysis of epithelial thickness, collagen and vascularity, inflammatory gene qPCR	TGF-β1 expression greater in DMSO treated	DMSO is an alternative to steroid in the treatment of HTS
Song et al 2018[21]	Intralesional Usnic acid vs TCA control	10	23, 35	Histological analysis of inflammatory markers	Reduced CD31 staining, improved collagen appearance SEI – Usnic acid group 1.76 ± 0.31 treated vs TCA control2.85±0.82	Usnic acid inhibits HTS formation
Tark et al 2015[22]	Intradermal injection ginsenoside Rb1 0.07mg vs 0.28mg vs 0.56mg vs saline control	8	21, 35	Histological analysis of inflammatory proteins and collagen profile, qPCR	Reduced MMP, TIMP1, α-SMA and TGF-β1 SEI - Control 4.22±0.63 vs ginsenoside 0.07mg 3.40 ± 0.32 vs 0.28mg 3.09 ± 0.24 vs 0.56mg 1.21 ± 0.06	Ginsenoside Rb1 suppresses HTS in rabbit ear model

Tolefsen et al 2012[23]	SGS vs Paper tape vs Untreated (control)	6	7, 14, 28, 44	Histological analysis, photographic analysis	SEI - Paper tape 1.32±0.2 vs SGS 1.41±0.18 vs control 1.35±0.23	Paper tape and SGS show equal effectiveness in prevention of HTS on visual analysis, but no difference histologically
Tunca et al 2019[24]	Cryotherapy compared with surgical removal of skin	8	16 & 28	Histological analysis	SEI - Excision group 1.52 ± 0.4 vs Cryogroup 1.63 ± 0.5	Cryotherapy as an alternative to making surgical removal of skin
Uzun et al 2013[25]	Early oral enalapril vs later oral enalapril vs intralesional steroid injection vs no treatment control	5	14, 28, 40	Histological analysis	Improved collagen profile in treatment group SEI - Early elanpril 1.3 vs late elanapril 1.56 vs steroid group 1.25 vs control 1.98	Earl oral administration of enalapril after dermal injury reduce HTS
Wang et al 2015[26]	ASMq 400 vs 800 vs 1200 mg/kg bodyweight vs saline control	7	15, 40,	Histological analysis	Reduced collagen, pro-collagen in treatment SEI - Utilised but exact figures not given, lower in all treatment groups	Orally administered ASMq improves the appearance of HTS
Wang et al 2015[27]	Intradermal TSG-6 injections vs Saline control	7	21, 28, 42	TNFα stimulated gene 6 protein (TSG-6). Histological analysis, qPCR, collagen profile	TSG-6 lower levels if IL-1β, IL-6, TNF-α, reduced collagen SEI - Day 21 Control 1.13 ± 0.09 vs TSG-6 1.31±0.11 Day 28 Control 1.29 ± 0.15 vs TSG-6 1.92±0.18 Day 42 Control 1.34 ± 0.18 vs TSG-62.93±0.38	Immediate dose of TSG-6 during healing can reduce the severity of HTS
Wang et al 2016[28]	RHE intralesional injection vs saline control	11	25, 30	Histological analysis, qPCR of VEGF and tissue inhibitor of metalloproteinase -1	Fewer collagen fibres, smoother in treatment group, Reduced protein expression of VEGF and TIMP-1 SEI – RHE 1.37 ± 0.21 vs control 2.65±0.21	RHE reduces formation of HTS
Wei et al 2011[29]	Topical OA 2.5% vs 5% vs 10% vs untreated control	7	28	Histological analysis, Immunohistochemistry of inflammatory proteins	TGF-β1, MMP-1 collagen I and III SEI - OA 2.5% 2.63 ± 0.20 vs OA 5% 2.12 ± 0.32 vs OA 10% 1.87 ± 0.24 vs Control 3.09±0.32	OA suppresses HTS in the rabbit ear model
Wo et al 2014[30]	Topical EG loaded with 5-FU vs EG loaded with saline control	10	1, 30	Western blot and histological analysis	SEI - Given as a chart, SEI lower in treatment group	EG as a successful delivery mechanism of 5-Fu to a wound

Wu et al 2011[31]	EO from ligusticum chuanxiong at 5% vs 10% vs 20% vs topical contractubex® vs untreated (control)	7	22, 28	Histological analysis for inflammatory proteins	SEI - 5% essential oil (EO)2.95 ± 0.33 vs 10% EO 2.49 ± 0.27 vs 20% EO 2.07 ± 0.25 Control 3.48 ± 0.34	EO probably becomes an effective cure for human HTS
Yagmur et al 2011[32]	Denervated skin vs Innervated skin (Control)	20	14, 28	Histological analysis	SEI - Denervated 1.26 ± 0.22 vs Control 1.6±0.34	Surgically denervated skin results in reduced scarring
Zhang et al 2012[33]	Topical LEO 2.5% vs 5% vs 10% vs liposome without EO (control)	10	28, 56	Histological analysis, ELISA, qPCR	Collagen I, III reduced in LEO group SEI - LEO 2.5% 2.39 ± 0.21 vs LEO 5% 1.96 ± 0.20 vs LEO 10% 1.34 ± 0.11 vs control 2.74 ± 0.24	LEO reduces HTS by inhibiting hypertrophic fibroblasts and inducing their apoptosis
Zhang et al 2015[34]	Intralsional injection ADSC vs ADSC conditioned medium (CM) vs DMEM vs untreated (control)	10	14, 21, 28, 35	Adipose derived stem cells. Histological analysis	SEI - ADSC 1.08 ± 0.05 vs DMEM 1.93 ± 0.09, ADSC-CM 1.33 ± 0.10 vs DMEM 1.97 ± 0.11, Control1.90 ± 0.12 vs DMEM 1.94 ± 0.06	Adipose derived stem cells (ADSC) can suppress HTS
Zhao et al 2018[35]	High-ESWT (0.2 $mJmm^2$) vs Low-ESWT (0.1$mJ/mm^{2)}$ vs sham ESWT (control)	15	1, 4, 7, 10, 14, 21, 28, 35	Histological analysis of arrangement of collage fibres	Improved collagen appearance SEI – low-ESWT 2.32±0.15 vs high-ESWT 2.34±028 vs sham ESWT control 2.71±0.2	Extra-corporeal shock wave therapy suppresses hypertrophy scar formation

Abbreviations

5-FU – 5- fluorouracil

α-SMA – alpha smooth muscle actin

ACE-I – Angiotensin converting enzyme inhibitor

ADSC – Adipose derived stem cells

ASA - Acetysalicylic acid

ASMq - Abnormal savda munziq

Bcl2 – B-cell lymphoma 2 protein

BTXA – Botulinum toxin A

CMC - carboxymethyl cellulose

CTGF – connective tissue growth factor

DMEM - Dulbeccos modified eagle medium

DMSO – Dimethylsulfoxide

ELISA – enzyme linked immunosorbent assay

EO – essential oils

ESWT – Extra-corporeal shockwave therapy

HTS – hypertrophic scar

IDO - Indoleamine 2,3-dioxygenase

LEO - Liposome-enveloped essential oil

MMP – matrix metalloproteinases

MSC - mesenchymal stem cell

qPCR – Quantitative polymerase chain reaction

RHE – recombinant human endostatin

SEI – scar elevation index

TCA – triamcinolone acetonide

TEM – Transmission electron microscopy

TGF – Transforming growth factor

TIMP – tissue inhibitor of metalloproteinases

TNF-α – tumour necrosis factor alpha

TSG-6 - TNFα stimulated gene 6 protein

VEGF – vascular endothelial growth factor

Table 3. Results from pig models

Study	Pig Breed	Scar Method	Wound number	Data Collection Point (Days)	Intervention	Outcomes	Results	Authors comments
Bailey et al 2018[36]	Red Duroc	Contact burn, 1"x1", 200 ˚C, 40 secs, 3lbs of pressure, burn eschar then excised and covered with STSG autograft	4 on each pig, 8 pigs total	7, 28, 56, 84, 112	PDL vs CO_2 laser vs PDL and CO_2 laser vs untreated control 28 days post injury	Photography, Erythema via image software, Scar biomechanics, Histology, Scar roughness (imaging software), immunohistochemistry, qPCR	Laser intervention resulting in less scar contraction compared to control, laser treated scars were redder, CO_2 Laser scars were smoother, Co_2 laser scar strongest	PDL ad CO_2 laser therapy within 1 month of auto STSG helps to reduced scar contraction
Blackstone et al 2018[36]	Red Duroc	Zimmer® dermatome 5x5cm 0.06" deep or 0.075" deep, Contact burn, 1"x1", 200 ˚C, 40 secs, 3lbs of pressure	2 burn wounds on each pig, 1 deeper and 1 shallower dermatome injury on each pig, 4 pigs total	0, 10, 28, 90, 150	Scar formation with dermatome vs burn	TEWL, scar erythema, scar contraction, histological analysis, scar biomechanics, qPCR	2 fold greater TEWL in burn group, greater contraction in burn group, scar thickness greater in burn group, stiffer and weaker, TGF-β1 expression greater in burn scar	Burn scars produce hairless, hyper/hypopigmented, thicker, weaker, less elastic scars that are more similar to human HTS compared to dermatomal injury.
Carney et al 2017[37]	Red Duroc	Dermatome 4"x4", 0.09" deep	2 on each pig, 2 pigs total	70, 84, 126 (pressure applied from day 70 for 14 days)	Automated pressure delivery to one scar 30mmHg vs control	VSS, qPCR, Elastin protein, histological analysis	VSS pliability score greater in treatment group, decrease in elastin transcript, elastin protein and staining greater in treatment group	Pressure treatment results in higher protein level expression of elastin compared to control, correlated with VSS scores clinically

Chan et al 2012[38]	Large White Pig	Contact burn, bottomless mug 300ml water at 92˚C applied for 20 seconds, set pressure	4 burns approx. 5cm in diameter on each pig, 5 pigs total	0, 99	Burn dressing only vs STSG day 3 post-burn, STSG day 14 post-burn, STSG day 21 post-burn	VSS, histological analysis, α-SMA level, microbiological analysis	Wounds grafted at day 3 post-burn had the lowest α-SMA, VSS score on day 3 grafts closer to normal skin, degree of fibrosis greater in wounds with positive bacterial culture	Early grafting is associated with a better histological and clinical scar outcome, infection may contribute to a greater degree of fibrosis
DeBruler et al 2018[39]	Red Duroc	Contact burn, 1”x1”, 200 ˚C, 40 secs, 3lbs of pressure	8 per pig, 32 total (4 pigs used)	7, 28, 63, 119	STSG autograft 0.026” or 0.058” thick, thinner grafts meshed	Photography, TEWL, Immunohistochemistry, Gene expression, scar biomechanics	Meshed grafts greater TEWL, thicker grafts less deep scar, thick graft scar greater biomechanical strength, thick graft reduced TGF-β1 expression	Thick graft scars decreased contraction, reduced scar depth, mesh on thin grafts did not affect the scarring.
Engrav et al 2011[40]	3x Red Duroc and 3 x White Yorkshire	Padgett® dermatome 7cm x 7cm, 10, depth 0.02” and 0.06”	5 of each depth per pig, 6 pigs total	7, 14, 21, 84	Comparison of collagen genes expressed porcine HTS with that of human HTS	qPCR, Porcine GeneChip®,	11 collagen genes and 7 collagen types identified in human and duroc pig hypertrophic tissue	Collage I, II, IV, V, VI, VII, XIV, XVI are involved in the process of fibroproliferative scarring.
Foubert et al 2017[41]	Red Duroc	Electric dermatome, 7.6cm x 7.6cm , 2mm depth	4 on each pig, 12 pigs total	14, 56, 182	Autologous ADRC’s delivers as a spray onto the wound immediately post-injury vs untreated control	Photography + imaging analysis software, histology, Immunohistochemistry, skin hardness via durometer	Treatment group presence of rete pegs, better vascularity, more normally organised collagen. Upregulation of IL-6 expression then downregulation in intervention group	Delivery of ADRC’s at the time of injury improves scarring outcome
Jimi et al 2017[42]	Clawn mini-pig	Sharp excision 7.5cmx7.5cm depth 0.15cm	4 on each pig, 8 pigs in total	15, 30, 60, 90, 120, 150	Use of the Clawn minipig as a scarring model	Histological analysis, water content, chymase activity,	Scar thickened up to 90 days then decreased, TGF-β1 greater in scar, peaks at day 15 then decreases, water content peaks at day 15 in scar, chymase activity rises in scar to day 90	Chymase plays an important role in scar thickening and Clawn pigs are a useful animal model for HTS.
Liu et al 2018[43]	Bama mini-pig	Electric dermatome, 8x8cm, 1.8mm deep	4	60 (pressure application) 90, 120	Pressure of 3.4kPa	RNA analysis, gene expression, qPCR	568 DEG at 90 days, 365 DEG at 120 days, GO the DEG’s have 50 functional categorical in cellular function. α-SMA decreased after pressure therapy	Genes associated with transporter activity and signal transducer activity participate in the treatment of pressure for HTS

Rodriguez-Menocal et al 2018[44]	Red Duroc	Branding iron, 300˚C, 12 seconds, 27mm diameter	27	14, 21, 35	Erg:YAG laser treatment high, low vs CO_2 laser high, low and control (no treatment)	mVSS, MSS, Histological assessment, Western blot, qPCR	Er:YAG laser treated wounds had better scores in mVSS and MSS, low Er:YAG best, Thinner dermis in control, most remodelling observed is CO_2 laser qPCR – Decorin expression great in both lasers on low setting, western blot – MMP-9 increase in ER;YAG at low setting	Model produces hypertrophic scar that mimics human burn scar.
Travis et al 2015[45]	Red Duroc	Zimmer® dermatome 3”x3” depth 0.06” or 0.03” and 4”x4” depth 0.09”	3 partial depth, 2 full depth on each pig (number of pigs not given)	2, 4, 7, 9, 11, 14, 28, 35, 42, 56, 70, 113	Presence of Fibrocytes in healing skin	Histological analysis, immunohistochemistry,	Presence of LSP-1, CD45, procollagen-1, increase fibrocyte staining from day 56, upregulation of COL1A1 from day 56	Biphasic presence of fibroblasts initially at acute response to healing and then a second peak during remodelling
Yun et al 2012[46]	Red Duroc	Excision 3cmx3cm full thickness excision,	36 on each pig, 2 pigs total	ADSC given 50 days post injury, 0, 10, 20, 30, 40, 50 days post ADSC	Adipose derived stem cells (ADSC)	Scar photography and image analysis, Histological analysis, qPCR	Experimental group surface area smaller, colour and pliability closer to normal skin. Experimental reduced fibroblasts, and suppression of TGF-β1	ADSC produce significantly smaller scars that appear more similar to normal skin. Work by reducing mast cell and myofibroblast activity.
Yun et al 2019[47]	Red Duroc	3x3cm^2 sharp excision – full thickness	36	50, 60 (10 days post intervention)	Injection of relaxin in aliginate gel-encapsulated virus in established HTS	Photographic analysis, histological analysis, immunohistochemistry, qPCR	Relaxin treated group had decreased scar size, colour index, pliability, reduced MMP-1 inhibitor and α-SMA, downregulated TGF-β1, upregulated TGF-β3	Relaxin may have a prominent role in scar remodelling
Zhu et al 2003[48]	Red Duroc	Padgett® dermatome 8cm x 8cm, depth 0.015 to 0.12”, 0.015” intervals	41, 6 pigs total	Weekly to 20 weeks	Assess methodology for creating HTS	Macroscopic analysis, histological analysis, immunohistochemistry	Increased expression of IGF-1, reduced expression of TGF-β1 in deeper injuries. Histologically similar appearance to human HTS at deeper injury.	Further study required to see if Red Duroc pig acceptable as a human model for HTS
Zhu et al 2004[49]	Red Duroc	Padgett® dermatome 7cm x 7cm,	8 wounds on each pig, 2 pigs total	10, 30, 60, 90 and 150	Evaluate TGF-β1, IFG-1, decorin and versican	Histological analysis, immunohistochemistry, qPCR	Increased expression of TGF-β1, IFG-1, decorin reduced in deeper wounds.	Findings correlate with literature on human HTS tissue

		depth 0.015", 0.030", 0.045" and 0.06"		post-wounding	expression in porcine HTS			

Abbreviations

α-SMA – alpha smooth muscle actin

ADSC – adipose derived stem cells

ADRC - adipose derived regenerative cells

COL1A1 – Collagen type 1 Alpha 1 gene

DEG - Differentially expressed genes

Erg:Yag - erbium-doped yttrium aluminium garnet laser

GO - Gene ontology

HTS – Hypertrophic Scar

IGF-1 – Insulin like growth factor -1

LSP-1 – Lymphocyte specific protein - 1

MSS – Manchester scar scale

MMP – Matrix metalloproteinases

mVSS – modified Vancouver scar scale

PDL – Pulsed dye laser

qPCR – Quantitative Polymerase chain reaction

STSG – split thickness skin graft

TEWL – Transepidermal water loss

TGF – Transforming growth factor

VSS – Vancouver Scar Scale

Table 4. Results from murine human skin graft models

Study	Murine type	Scar method	Data Collection Point (Days)	Intervention	Outcomes	Results	Authors Comments
Ding et al 2017[50]	Nu/nu mouse	X	X	X	X	X	Description of protocols from Momtazi et al and Wang et al
Momtazi et al 2013[51]	Nu/Nu mouse	2.0cm x 1.5cm sharp excision + Human Xenograft	30, 60, 120, 180	Comparison of xenograft with human HTS tissue.	MSS, histological analysis, immunohistochemistry	MSS score 15.9±0.2 xenograft vs 8.2±0.1 autograft control, α-SMA present in xenograft	Model creates viable tissue that is morphologically, histologically and immunohistochemically identical to human HTS
Wang et al 2011[52]	Nu/nu mouse	2.0cm x 1.5cm sharp excision + Human Xenograft	0, 28, 56, 112, 196	FTSG human vs STSG (autograft) vs FTSH (autograft)	Macroscopic analysis, histological analysis, Immunohistochemistry	Scar hypertrophy develops over 4 months, upregulation COL1α1, TGF-β1, CTGF, More macrophages and mast cells in STSG	Human skin grafted onto nude mice develops fibrotic scars which resemble HTS. STSG fibrose more than FTSG
Zeplin et al 2012[53]	Nu/nu mouse	1.5cm x 1.5cm sharp excision + Human Xenograft - additional contact burn 80˚C for 10 secs	84	Halofuginone-eluting SGS vs regular SGS	qPCR	Treatment group reduced expression of TGF-β1, COLA1A1, CTGF, FGF2, MMP-2 and 9	Halofuginon-eluting hybrid surface SGS increase anti-scarring effect by normalising inflammatory scar gene expression

Abbreviations

α-SMA – alpha smooth muscle actin

COL1A1 – Collagen type 1 Alpha 1 gene

CTGF – connective tissue growth factor

FGF2 – Fibroblast growth factor 2 gene

FTSG – Full thickness skin graft

HTS – Hypertrophic Scar

MMP – Matrix metalloproteinases

MSS – Manchester scar scale

qPCR – Quantitative polymerase chain reaction

SGS – Silicone gel sheet

STSG – Split thickness skin graft

TGF – Transforming growth factor

Table 5. Results from murine cultured human keloid models

Study	Murine type	Delivery method of keloid tissue	Data Collection Point (Days)	Outcomes	Results	Authors Comments
Lee et al 2016[54]	Nu/J athymic mouse	Cultured human keloid tissue *in vivo* – transplanted onto the dorsum Normal human skin implants cultured as control	14, 28, 42, 56, 70, 84, 98, 112	Survival rate, histological analysis, qPCR, Macroscopic analysis, Immunofluorescence	90% survival rate at 4 weeks. Elevated Col1A1, PAI-1, uPAR elevated in keloid, keloid implants larger at 8 weeks than control, GFP tagged human keloid cells bifringence observed at 16 weeks	Keloid cultures take on athymic mice. Opportunity to genetically manipulate cells used to make the graft
Shang et al 2018[55]	Nu/Nu mouse	Subcutaneous injection of cultured human dermal keloid whole tissue vs cultured human keloid fibroblasts only	12, 28, 42, 84	Histological analysis, macroscopic analysis, cytokine analysis	Whole dermal keloid tissue grafts more like human keloid scar compared to keloid fibroblast only grafts	Easily reproducible model to study human keloid tissue.
Supp et al 2012[56]	Nu/Nu mouse	GAG scaffold impregnated with cells:	0, 14, 28, 42, 56, 70, 84	Histological analysis, immunohistochemistry, real time qPCR	COL1A1, TGF-β1, POSN, PAI2, FST, expression highest in DKF,	Keloid grafts survive and could an easily scalable model.

		K+F vs K+DKF vs K+SKF vs KK+F vs KK+DKF Grafted onto mouse			followed by SKF. No differences observed in COL1A1 expression. Thick, disorganised collagen bundles observed in DKF or SKF containing grafts	
Wang et al 2013[57]	BALB/c athymic mouse	PLGA scaffold cultured with keloid fibroblasts – implanted into subcutaneous pocket	30, 60, 120, 180	Volume analysis, histological analysis, immunohistochemistry	Volume of keloid scaffold 12x10x2mm^3 vs control scaffold 2x2x0.4mm^3 Presence of keloid fibroblasts in scaffold at day 180. Formation of collagen spindles	Human keloid PLGA scaffold model could be used to study fibroblast function and effect of drugs

Abbreviations

COL1A1 – Collagen type 1 Alpha 1 gene

DKF – deep keloid fibroblasts

F – normal fibroblasts

FST – Follistatin gene

GAG – Glycosaminoglycan

GFP – Green fluorescence protein

K – normal keratinocytes

KK – keloid keratinocytes

PAI2 – plasminogen activator inhibitor gene

PLGA - Polylactic-co-gylcolic acid

POSN – Periostin gene

qPCR – Quantitative polymerase chain reaction

SKF – superficial keloid fibroblasts

TGF – Transforming growth factor

uPAR – Urokinase receptor

Table 6. Results from murine whole keloid tissue graft models

Study	Murine type	Delivery method of keloid tissue	Data Collection Point (Days)	Intervention	Outcomes	Results	Authors Comments
Chen et al 2017[58]	BALB/c-nu mouse	Whole transplanted human keloid tissue graft into subcutaneous space	7, 14, 28	No injection control vs BTXA vs TCA vs TCA + BTXA	Histological analysis, fibroblast proliferation, immunohistochemistry, scar weight	Combination group greatest reduction in weight, and greatest reduction in fibroblast proliferation	Potential for combined BTXA and TCA intralesional therapy in treating HTS
Fanous et al 2019[59]	Nu/nu mouse	Whole transplanted human keloid tissue graft into subcutaneous space	7, 14, 28	Saline control vs BTXA vs TCA	Histological analysis, scar weight	Treatment groups had reduced weight BTXA group better organised collagen	Botox has a role in HTS prevention
Philandrianos et al 2015[60]	Nu/nu mouse	Full thickness 8mm human keloid tissue surgically sutured to mouse	0, 28, 56, 84, 112 (all 4 weeks post graft)	No treatment control vs LASER treatment, vs 4mm resection vs 4mm resection + LASER	Macroscopic and Histological analysis for heatshock protein	No differences observed between scars	Further studies required to determine effect of LASER on keloid
Qiu et al 2015[61]	BALB/c-nu mouse	Whole transplanted human keloid tissue graft into subcutaneous space	7, 14	P144® topical vs topical placebo	Histological analysis	Reduction in collagen I and III expression in P144® group	P144® may have future applications but further research required
Yang et al 2010[62]	BALB/c-nu mouse	Whole transplanted human keloid tissue graft into	7, 14, 28	Saline control vs Verapamil vs Verapamil + TCA	Fibroblast proliferation, Immunohistoche	Combination decreased fibroblast proliferation, scar weight and increased decorin expression	Combination therapy more effective in reducing scar – more work needed

		subcutaneous space		intralesional injections	mistry, scar weight		
Yang et al 2015[63]	BALB/c-nu mouse	Whole transplanted human keloid tissue graft into subcutaneous space	7, 14, 28	Saline control vs TCA vs TCA + verapamil + IFN	Fibroblast proliferation, histological analysis, scar weight	Fibroblast proliferation least in TCA group, combination group less disorganised collagen and smallest scar in size and weight	Combination of TCA, verapamil and IFN effective in reducing HTS tissue.
Yang et al 2017[64]	BALB/c-nu mouse	Whole transplanted human keloid tissue graft into subcutaneous space	7, 14, 28	No injection control vs TCA vs verapamil vs IFNα2b	qPCR	INFα2b group strongest expression of decorin and MMP13, TCA stronger expression of decorin compared to verapamil, stronger expression of MMP13	Suggest maintain dose of INFα2b along with high dose verapamil to improve HTS

Abbreviations

BTXA – Botulinum toxin A

HTS – Hypertrophic scar

INF – interferon

MMP - Matrix metalloproteinases

qPCR – Quantitative polymerase chain reaction

TCA – triamcinolone acetonide

Table 7. Results from murine thermal injury models

Study	Murine type	Thermal injury method	Data Collection Point (Days)	Intervention	Outcomes	Results	Authors Comments
Ibrahim et al 2014[65]	Nu/nu mouse	Thermal injury brass rod, 100˚C for 1 second, followed by excision at 3 days at auto FTSG	3, 7, 9, 11, 14, 28, 70, 168	Burn only	Macroscopic analysis, histological analysis,	Skin grafts contracted, increased vascularity, macrophages and mast cells	Resulting scar was different to human hypertrophic scar tissue.

					immunohistochemistry		
Lorden et al 2016[66]	C57BL/6 mouse	Thermal burn unspecified, excised 3 days later	7, 14, 30	Effect of microfibrous scaffold in mitigating HTS contraction	Histological analysis, biomechanics, macroscopic appearance	Scaffold group improved collagen arrangement, reduced contraction and stronger scar	Collagen coated scaffold may have merit in preventing HTS
Lu et al 2014[67]	Immunocompetent mouse	Submersion into 100˚C water for 8 seconds	15	CL injection subcutaneous vs intraperitoneal vs no intervention control	Histological analysis, qPCR	Control scars developed collagen whorl patterns, less in CL groups, reduced TGF-β expression in CL	CL may be suitable treatment for HTS
Zeplin et al 2012[53]	Nu/nu mouse	1.5cm x 1.5cm sharp excision + Human Xenograft - additional contact burn 80˚C for 10 secs	84	Halofuginone-eluting SGS vs regular SGS	qPCR	Treatment group reduced expression of TGF-β1, COLA1A1, CTGF, FGF2, MMP-2 and 9	Halofuginon-eluting hybrid surface SGS increase anti-scarring effect by normalising inflammatory scar gene expression

Abbreviations

COL1A1 – Collagen type 1 Alpha 1 gene

CL - Clondronate liposome

CTGF – connective tissue growth factor

FGF2 – Fibroblast growth factor 2 gene

HTS – Hypertrophic scar

MMP – Matrix metalloproteinases

qPCR – Quantitative polymerase chain reaction

SGS – Silicone gel sheet

TGF – Transforming growth factor

Table 8. Results from murine incision and stretch models

Study	Murine type	Scar method	Data Collection Point (Days)	Intervention	Outcomes	Results	Authors Comments
Murphy et al 2019[68]	Immunocompetent mouse	Full thickness excision followed by mechanical loading of wound edges	Every 2 days until 28 days	Oral losartan via water vs normal water control	Histological analysis, qPCR, macroscopic appearance	Losartan treated rat had smaller scars and reduced α-SMA and CD68 expression	Losartan has potential as a novel therapy for preventing HTS
Shan et al 2017 [69]	Immunocompetent mouse	Full thickness excision followed by mechanical loading of wound edges	0, 10, 14	No treatment control vs 25μM	Histological analysis, qPCR, Western blot	Naringenin inhibits fibroblast activation, reduced inflammation Reduced expression of IL-1β, IL-6, TGF-β1 + TNF-α	Naringenin could have use a scar treatment
Zhou et al 2019[70]	Immunocompetent rat	Excision of rat tail skin + application of steel ring to add strain Dorsal skin injury as control	0, 2, 10, 16, 20,42, 84, 168	3x3mm vs 6x6mm vs 9x9mm, each with no, little or high strain	Histological analysis, qPCR, macroscopic appearance	No wound contraction in tail wounds, high strain wounds produced thicker HTS. TGF-β1 and α-SMA expression equivocal to human HTS	Future HTS model, more cost effective, faster and easily reproducible

Abbreviations

α-SMA – alpha smooth muscle actin

HTS – hypertrophic scar

IL – Interleukin

qPCR – Quantitative polymerase chain reaction

TGF – Transforming growth factor

Table 9. Results from murine biopsy and antibiotic models

Study	Murine type	Scar method	Data Collection Point (Days)	Intervention	Outcomes	Results	Authors Comments
Cameron et al 2014[71]	Immunocompetent mice	Subcutaneous infusion of bleomycin	0, 28, 56	28 day bleomycin infusion vs control Saline at constant rates	Histological analysis, qPCR	Bleomycin group thicker dermis, thinner dermis – akin to human HTS. Increased expression of TGF-β	Potential model for HTS, avoids contraction created by panniculus carnosus injury
Sahin et al 2012[72]	Immunocompetent rats	Full thickness skin biopsy – size not given	0, 10, 30	Topical heparin vs alantoin vs Contractubex® vs control	Histological analysis, Immunohistochemistry, TEM	Contractubex® group thinnest epidermis, completely keratinised on TEM	Contractubex® improves quality of wound healing and resulting scar

Abbreviations

HTS- Hypertrophic scar

qPCR – Quantitative polymerase chain reaction

TEM – Transmission electron microscopy

TGF – Transforming growth factor

Table 10. Results from other animal models

Study	Animal	Scar Method	Data Collection Point (Days)	Intervention	Outcomes	Results	Authors Comments

Igarashi et al 20[73]	Mexican hairless dog	3x3cm^2 full thickness excision	0, 30, 90	X	Macroscopic appearance, histological analysis	Macroscopic pigmented HTS, well-organised collagen present on histology	Mexican hairless dog may be a suitable alternative model for HTS
Kimura Et al 2011 [74]	Primate Marmoset	2cm long full thickness abdominal incision	0, 35, 42	Control vs GB1101 injection prior to incision vs GB1101 topical post-incision (once only)	Macroscopic appearance, histological analysis	Treated scars had a thinner epidermis and dermis with a resulting flatter scar	GB1101 may have a role in preventing HTS in surgical patients.

Abbreviations

GB1101 – pyrrole-imidazole polyamide

HTS – Hypertrophic scar

Table 11. Results from human *in vivo* models

Study	Study design	Patient Group	Intervention	Data Collection Points	Outcomes	Results	Author Comments
Cruz-Korchin et al 1996 [75]	RCT	Patients post bilateral McKissock reduction mammaplasty	SGS vs No treatment	Dressing worn for 2 months – follow up at 6 months	Clinical assessment of scar hypertrophy	Untreated scars; 45% flat scars, HTS Silicone group; 75% flat scars and 25% HTS	SGS helps reduced the presence of HTS
Dunkin et al 2007[76]	Clinical study	113 healthy volunteers	Controlled standardised scratch injury on hip skin to determine depth at which scar occurs	1, 2, 3, 4, 6, 10, 18, 24, 36 weeks post-injury	Scar surface area, scar thickness (USS), histological analysis	36 weeks – mean scar length of 34.9±1.0mm (68% of the original wound length) Visible scar formed from 0.56±0.03mm or 33.1% depth injury	Jig is a well-tolerated standardised method of creating scar.
Kong et al 2014[77]	RCT	Knee replacement patients	Liquid silicone gel vs placebo gel	Gel applied 5 days post op daily for 1 month, follow	VSS	Thinner and lighter scars in treatment group, no other difference	Silicone gel has no beneficial effect on scar pain and itch during post-op period

				up 3, 6 and 12 months			
Lanier et al 2016[78]	Clinical study	Abdominoplasty patients – skin planned for excision	20 full thickness incisions over abdomen - 2cm in length - sutured	Left to heal for 12 weeks	-	-	Model offers a unique, cost-effective method for assessing scar treatments
Niessen et al 1998 [79]	RCT	Patients undergoing bilateral breast reduction	SGS vs Micropore® tape	Dressings worn for 3 months, follow up at 3,6 and 12 months	Clinician assessment of scar height and width, Patient opinion of itch and pain	3 months 64.3% of patients at least 1 HTS 6 months – 29 HTS in SGS vs 13 control 12 months – 19 HTS in SGS vs 7 control	SGS may in fact increase risk of HTS
Sproat et al 1992[80]	RCT	Established symptomatic mid-line sternotomy HTS	1 TCA injection vs SGS worn for 12 hours/day for 12 vs no treatment	12 weeks	Patient preference appearance of the scar, pain, itch and ease of the treatment	11/14 preferred silicone, 2 preferred TCA, 1 had no preference	SGS is preferred treatment by patients, better scar and relief

Abbreviations

HTS – Hypertrophic scar

RCT – Randomised Controlled Trial

SGS – Silicone gel sheeting

TCA – triamcinolone acetonide

USS – Ultrasound Scanner

VSS – Vancouver Scar Scale

Table 12. Results from human *in vitro* models

Study	**Human Tissue Type**	**Scar method**	**Data Collection Point (Days)**	**Intervention**	**Outcomes**	**Results/Authors Comments**

Chawla et al 2018[81]	HTS fibroblast culture	3D collagen structure	X	Histological analysis, qPCR	Structure, histology and PC	In vitro created dermal scar showed α-SMA expression, excessive collagen and contraction like that of HTS. Model may serve as an alternative for test scar treatments.
Lee et al 2013[82]	Human keloid tissue	Spheres of tissue cultured	Injection with TCA	Histological analysis, Immunohistochemistry	Intervention group – tissue regressed, reduced expression of collagen I, III and elastin	Immunohistochemistry profile like that of TCA treated keloid in human,
Reijnders et al 2015[83]	Immortalised fibroblasts and keratinocytes	3D bovine collagen matrix	Injured with heat and cold injuries	Histological analysis, Immunohistochemistry, TEM	Morphology similar to that of human skin, has the ability heal	Useful as a model that does not rely on fresh human tissue.

Abbreviations

α-SMA – alpha smooth muscle actin

HTS – Hypertrophic scar

qPCR – Quantitative polymerase chain reaction

TCA – triamcinolone acetonide

TEM – Transmission electron microscope

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Chapter 3. A pilot study of scar assessment methods for use in a clinical service

3.1 Introduction

A number of methods are available for the assessment of scars to help evaluate the long term outcomes of treatments.[1-4] The resulting scar after injury can be influenced by a multitude of factors including anatomical location, mechanism and physiology.[5-7] An accurate method for assessing scars, that is both valid, reliable and clinically feasible is hard to achieve.[2] Often, scar assessment methods are separated into subjective measures and objective measures.[2] Subjective outcome measures are often in the form of questionnaires and can be filled in by the patient or the clinician. Objective outcome measures tend to assess a certain quality of the scar itself and can include various tools to do so, such as a chromameter for the colour of the scar. In this chapter I aim to explore current scar assessment tools and perform a pilot study to determine their efficacy and feasibility in a clinical setting.

3.11 Subjective Outcome Measures

The most commonly discussed and utilised subjective scar assessment scales are the VSS and its associated modified versions (mVSS), the POSAS, the MSS and the Brisbane Burn Scar Impact Profile (BBSIP).

3.12 Vancouver Scar Scale

First introduced in 1990 by Sullivan et al, the VSS is often considered to be one of the original subjective scar assessment methods.[8] The VSS involves assessing a burn scar in the following parameters: pigmentation, vascularity, pliability and height. The maximum score is 13, with 0 being like normal skin and 13 being a thick, contracted, hyperpigmented purple scar. The user of the VSS

has to identify which set category the scar fits into when assessing it against each of the parameters. Sullivan et al reported using three pairs of occupational therapists; with each pair assessing the same scar on three different patients.[8]

The authors reported a mean Cohens kappa co-efficient of 0.5 across the 3 assessment pairs and a standard error of 0.1.[8] The Cohens kappa co-efficient value can be interpreted as demonstrating moderate inter-rater reliability as defined by Landis and Koch.[9] Inter-rater reliability is the degree of agreement amongst two raters. No evidence for intra-rater reliability was given which is defined as the degree of agreement between repeated assessments by a single rater. There have been further follow-ups and assessments of the VSS.

Nedelec et al tested the validity of the VSS and proposed a modified version of the VSS.[3] The mVSS scores of scars were compared with the objective volume created using elastomer moulds to create a negative cast, that was then subsequently filled with plaster to create a positive cast.[3] They commented on the construct validity which can be defined as "the degree to which a test measures what it claims, or purports, to be measuring" of the VSS.[10] Divergent construct validity was reported due to a lack of correlation between the scar volumes with vascularity, pigmentation and pliability. As with other studies looking at scar volumes, the authors reported that scar volume seemed to increase and then decrease over the time period.[11-13] Convergent construct validity was not demonstrated as there was low correlation between the height with the other parameters. Nedelec et al suggested modifying the VSS to alter the parameters.[3] These are: changing the pliability score to 0-4 instead of 0-5, changing the height score to 0-4 instead of 0-3, changing pigmentation to 0-3 instead of 0-2 and the addition of a visual analogue score assessing pain and itchiness as reported by the patient.

A further study by Draijjers et al 2004 demonstrated convergent construct validity between quantitative measures of redness (via a chromameter) and the vascularity parameter of the VSS with a Spearman ρ co-efficient of 0.42 (two-tailed significance criteria set at 0.05).[14] They reported little correlation between the pigmentation parameter and the objective colour measures of the scar hypothesising that vascularisation of the scar could mask pigmentation of the scar making it difficult to reliably assess pigmentation. This difficulty trying to distinguish between pigmentation and vascularity whilst using the VSS has resulted in modifications as put forward by Nedelec et al and also by Baryza et al 1995 who added an additional 'mixed pigmentation' category on their version of the VSS.[3,15]

The original VSS has been utilised in multiple studies and is often regarded as a reliable subjective outcome measure. A recently published paper assessing the intra and inter-rater reliability of subjective and objective burn scar measurement tools by Lee et al has demonstrated more limitations of the VSS.[2]

Intra-class correlation coefficient (ICC) can be used to assess the consistency or reliability of quantitative measurements made by different raters measuring the same quantity.

The authors utilised the ICC to measure reliability of the mVSS defining an ICC of >0.70 to be acceptable. A total of 55 patients were included in the study with 3 assessors utilising the mVSS by Nedelec et al. They reported a total single ICC of 0.415 and an average ICC of 0.68 for the mVSS.[2]

As 3 non-paired raters were used, Fleiss Kappa values were given which can be defined as the reliability of agreement between a fixed number of raters. The Fleiss' Kappa values for the different parameters involved in the mVSS all scored below 0.7 with pigmentation scoring -0.020.[2] The authors concluded the mVSS to have poor reliability when performed by three or less assessors. This finding somewhat supports Draaijers et al 2004 who reported inter-rater reliability ICC of 0.69 with 1 rater and 0.9 for 4 assessors.[16]

3.13 Patient Observer Scar Assessment Scale

The POSAS introduced by Draaijers et al 2004 consists of both a subjective observer scar assessment scale and a subjective patient scar assessment scale.[16] The POSAS scores each of its parameters from 1 (best/normal) to 10 (worst) in both the observer and patient element. The observer scale is made up of the following parameters: vascularity, pigmentation, thickness, relief, pliability and surface area. The patient scale is made up of: pain, itch, colour difference, scar stiffness, thickness, irregularity and overall opinion. Each parameter is scored out of 10 and a total score from both the observer and patient scale is given. Internal consistency which can be defined as measure based on the correlations between different items on the same test is commonly reported as Cronbach's α, where a value greater than 0.7 is deemed acceptable.[17] The Cronbach's α score for internal consistency for POSAS has been reported as 0.76 for the patient scale and 0.69 for the observers scale, compared to the VSS's Cronbach's α score of 0.49.[16] Although the calculation was not given, concurrent validity of the observer scale and the VSS showed a significant correlation between the two with Spearman's $\rho = 0.89$ and $p < 0.001$.[16]

Draaijers et al reported the POSAS' inter-rater reliability ICC was 0.92 for 4 raters and 0.73 for 1 rater, suggesting the POSAS has a slightly better inter-rater reliability than the VSS with 1 rater or 4 raters .[16] Interestingly, the inter-rater reliability for the POSAS was reported by Lee et al as a single ICC of 0.438 and average ICC of 0.7 (only marginally better than the aforementioned mVSS scores of 0.415 and 0.68 respectively).[2] The POSAS has been used in multiple studies as an outcome measure including assessment of long-term scar following wide local excision of melanoma, predicting longer term scar outcomes in burn scars, evaluating facial skin graft scars, and linear facial scars.[18-21] In these studies, authors have commented on the reliability and validity of the POSAS in a positive light. Interestingly, Lee et al 2019 concluded that both the POSAS and the mVSS are not

adequately reliable despite the POSAS's use in other studies.[2] They reported that both the POSAS and the mVSS are only reliable if 3 or more assessors use them which is often not clinically feasible. The newer BBSIP was suggested as a better subjective test.[2]

3.14 Brisbane Burn Scar Impact Profile

The novel BBSIP was introduced in 2015 by Tyack et al as a purposely designed health related quality of life measure to assess the impact of burn scarring in adults and children.[22] Following the methodology of Gehlback and Brinkworth, the creators of the BBSIP created specific sets of questions after conducting multiple qualitative interviews to identify areas of concern/difficulty that burn scars may have on an individual.[23] Using reviews of literature and patient story experience, the authors utilised a Q-sort method to establish 17 words/phrases related to burn scars and asked participants to rate importance with a 5-point scale. The questions in the BBSIP are split with one third focusing on social interaction, relationships, appearance, body esteem, emotional reactions; one third focusing on physical symptoms and one third focusing on the activities of daily living including the overall impact of the burn scars.

The authors of the BBSIP subsequently conducted long-term prospective longitudinal cohort studies to assess the validity, reproducibility and responsiveness of a BBSIP designed for caregivers of 0-8 year olds ($BBSIP_{0-8}$) and one for young people aged 8-18 years old ($BBSIP_{8-18}$).[24,25]

The methodology for both studies were similar with participants completing the age appropriate BBSIP, the POSAS and the Paediatric Quality of Life (a 23-item generic health status instrument with parent and child assessing physical functioning, emotional functioning, psychosocial functioning, social functioning, and school functioning).[26] The time points for collecting data was a baseline, 1 to 2 weeks and then 1 month. All participants had at least 85% re epithelialisation at the time of the baseline measurements. The measurement properties for both studies were: internal consistency,

test-retest reliability, measurement error (in 8 to 18 year old group only), construct validity and responsiveness.

When reporting on the analysis for 8 to 18 year old participants, sufficient internal consistency of Cronbach's α ≥0.7 in the majority of item groups (sensory intensity, mobility, daily living, appearance, emotional reactions and physical symptoms) and a lower Cronbach's α in sensory frequency and friendship/social interaction groups (α= 0.6 and 0.67 respectively) was observed.[25]

In the analysis of caregivers of 0 to 8 year old participants, sufficient internal consistency of Cronbach's α ≥0.7 in the ten item groups (overall impact, frequency of sensory symptoms, mobility, daily living, friendships and social interaction, appearance, emotional reactions, physical symptoms, parent worry and family concerns) was reported.[24]

Test re-test reliability was reported as acceptable in the majority of groups (ICC of 0.71 or greater) in the $BBSIP_{8\text{-}18}$ with the exception of friendship/social interaction, mobility, sensory frequency and sensory reliability (ICC=0.45 to 061) in the 8 to 18 year participant group.[25]

The test-retest reliability coefficients were reported as 0.6 or higher for the majority of items (ICC=0.49 to 0.9), with acceptable test-retest reliability (ICC of 0.7 or greater) reported in items reflecting impact on the child's life, appearance, friendship, social interaction, family and parent concerns in the caregivers participant group.[24] Regarding measurement error in the 8 to 18 year old group, figures to detect the smallest detectable change were reported as 2 points (on the 5 scale items) and 4 points (on the 11 scale items) for the majority of groups (>80%). [25] In the 8 to 18 year old group, support for construct validity with moderate to large correlations (Spearman's ρ =0.50 to 0.62)in itch frequency, intensity of itch, tight feelings and pain as hypothesised with POSAS scale; and daily living items including total score (Spearman's ρ= -0.52 to -0.62) as hypothesised with the PedsQL score.[25]

There was reported support for construct validity in itch, stiffness and overall opinion (Spearman's ρ = 0.67 – 0.77) as hypothesised with POSAS scale in the caregiver group.[24] Responsiveness was reported as area under curve >0.7 in all item groups able to be tested by external criterion in the 8 to 18 group and >0.7 in the item groups of overall impact, sensory frequency, emotional reactions, physical symptoms and parent impact for the caregiver group.[24,25]

They concluded that the $BBSIP_{8-18}$ is suitable for use in clinical practice and cohort studies as a patient self-reported quality of life outcome measure for burns scar patients aged 8 to 18.[25] For the caregiver group they reported there is support for reproducibility, validity, responsiveness and interprability of most item groups in the $BBSIP_{0-8}$.[24]

For both versions of the BBSIP, the authors concluded there needs to be further studies of their use in the longer term post burn phase (minimum 18 months).[24,25] This is explored in Chapter 4.

3.15 Manchester Scar Scale

The MSS was originally developed by Beausang et al 1998.[27] The scale consists of 3 elements; a clinical assessment, an image panel assessment (from photographs) and a histological assessment. The clinical assessment scale would assess the scars colour, finish (matte or shiny), contour, distortion and texture, with each section being scored from 1-4 (best to worst); with the exception of the scar finish which was 1-2 (matte – shiny). For the image panel assessment, photographs were taken under standardised conditions, converted to 24-bit targa files and printed. The image assessment panel would consist of 10 people who score the scar on colour, finish, contour and distortion. Like the clinical assessment, the scores would be from 1-4 with the exception of the scar finish (1-2). The histological assessment of the scars would be scored on the appearance of the epidermis and the dermis. The epidermis would be scored from 0-3 based upon the appearance of rete ridges, whilst the dermis would be scored on collagen fibre orientation, collagen fibre density

and collagen fibre maturity. Each section on the dermis section would be scored from 0-5 with each score based upon the % of abnormal collagen. The papillary and reticular dermis would be scored separately forming a score range of 0 to 32. The authors recruited 69 patients for the study, of which 55 full data sets were available as not all underwent histological analysis.

Spearman's rank correlations between the clinical assessment scores and the histological assessment scores (R^2) were reported with ≥0.7 deemed as significant. Overall histological scores and overall clinical scores were compared and reported to have good correlation (R^2 = 0.76, p < 0.001).[27] The authors subsequently compared specific subdivisions of the histologic score with the overall clinical score. The histological reticular dermal scores showed less correlation with overall clinical score compared to overall histological score and overall clinical score (R^2 = 0.65, p < 0.001).[27] The authors reported no significant correlation between either the epidermal or papillary dermal scores individually (R^2 = 0.46) when compared with total clinical score; however good correlation was seen with the sum of papillary and dermal scores compared with total clinical score (R^2 = 0.80, p < 0.001).[27] When the five sections from the clinical scar score were compared with each other and subsequently with the visual analogue scale scores (assessed as part of the clinical assessment), good correlation was found (mean R^2 = 0.69 and 0.72 respectively).[27]

In the photographic assessment section, for the 10 observers used, intra-observer variability was reported between 7.8% and 14.8%.[27] The inter-observer variability showed consistent scoring with a Spearman's Coefficient of 0.87 (range 0.83 to 0.99, p < 0.005).[27] The overall image panel scores were reported as consistent with the clinical score for each scar reporting an overall mean Spearman's Coefficient of 0.9 (range 0.85 to 0.95, p <0.001).[27]

The authors concluded that quantitative scar assessment remains difficult and their scale provides a relatively quick and easy to use scale with the scores correlating with histological findings.[27]

3.2 Objective Scar Assessment Tools

Methods to objectively assess a scar are difficult to come across in a clinical setting. On reviewing studies that have objectively assessed scars, the reported areas in which they can be objectively assessed are: colour/pigment, perfusion/blood flow, pliability and thickness.

3.21 Chromameter

The colour/pigment in a scar can make it stand out if it is different to that of the surrounding skin. Scar colours can range from a darker brown, purple to pink and pale. The device that can pick up and quantify colour is referred to as a chromameter. These devices have been reported in studies assessing the colour of scars.[14,28] A chromameter objectively quantifies the colour red, blue and yellow. This is achieved by shining a bright white light against an object and quantifying the ratio of three different reflected colours. Both Merman et al 2013 and Draaijers et al 2004 reported the reliability of the chromameter and that it accurately provides quantitative information on the colour of the scar. They are quick, easy to use and painless for the patient.

3.22 LASER Doppler

The LASER Doppler is commonly used in burns clinic to assess the blood flow/perfusion through a burn wound for the purpose of determining burn thickness. The blood flow through a scar has been reported as increased in hypertrophic scar tissue, leading to the hypothesis that increased blood flow may be associated with pathological scarring.[29] A LASER Doppler will project a visible-to-infrared LASER beam into the surface of a tissue. Moving blood cells in the area of projected light with cause a shift in the lights frequency accord to the Doppler effect which is subsequently detected. The LASER Doppler machine creates images that demonstrate the blood flow in different areas of the tissue. The machine has been described as easy to operate and well validated.[30] The visual representation can be quantified by the imager as a perfusion index (PI), with areas of higher

perfusion scoring a higher PI. Interestingly Mermans et al 2013 demonstrated that post-surgical scars with a higher PI on laser Doppler, did not correlate with a scar that is more red in colour.[28] They also demonstrated that post-surgical wounds that were closed under higher mechanical pressure, showed scars that have a greater PI.[28]

3.23 Pliability

The pliability of skin has been reported as objectively assessed by the pneumotonometer and the cutometer.[1,31-33] The pneumotonometer relies on air pressure to measure the pliability of the skin. This works by recording the amount pressurised air required to lock the system when it is applied to the skin.[31] Spann et al 1996 demonstrated that the results from the pneumotonometer can be used to calculate cutaneous compliance reporting less compliance in regions of burn scar compared to normal control skin.[31] The cutometer has also been reported as a measure of pliability by objectively measuring the elasticity of skin. The cutometer works by creating controlled negative pressure on the skin and recording the vertical deformation of the skin in millimetres. Draaijers et al 2004 assessed the reliability of the cutometer against a subjective 10-step pliability score. They reported an ICC of 0.92 for the cutometer amongst four observers.[33] They used the data from the cutometer to quantify the pliability, elasticity, retraction, visco-elacity and extension of the skin. They reported correlations for concurrent validity were moderate and statistically significant for each parameter ($r \geq -0.46$) except for visco-elasticity which had low correlation ($r= -0.29$).[33] The authors concluded that the cutometer is a reliable piece of equipment for objectively quantifying the pliability of skin or scar tissue.[33]

3.24 Thickness

The thickness of a scar can be visually easy to identify if the scar is raised above the surface of the skin. Histologically it can be relatively easy to measure on a stained section of scar tissue. On a

patient in clinic however, it is more difficult to objectively quantify, especially as much of the scar tissue will be within the dermis. Ultrasound scanners that are designed for use on skin have been reported as a method of measuring the thickness of a scar within the skin.[1,34,35] Fong et al 1997 compared the use of an ultrasound scanner with that of a cutometer reporting higher levels of specificity and sensitivity in the ultrasound scanner.[1] Lau et al 2005 used an ultrasound scanner to assess established scars on patients and to see if the results of the scar showed correlation with the thickness assessment part of the VSS.[34] They reported a Spearman's correlation of 0.42 ($p < 0.01$) between the VSS thickness score and the ultrasound scanner measurement.[34] An ultrasound scanner was also used by Dunkin et al 2007 to assess the thickness and depth of the scar within the dermis of standardised scars.[35] The authors of the two aforementioned studies commented on the limitation of the ultrasound scanner in that it requires a degree of training and technique to use effectively, however it does produced high quality accurate images.[34,35]

3.3 Aim

After exploration of the currently available tools and equipment to assess scars, I set out to create pilot data for their use in a plastic surgery scar clinic. By using these tools, I aim to determine any statistically significant differences in the scar outcomes of patients depending on treatments they have had. I also aim to comment on how feasible these scar assessment tools are in a time pressured clinic and what their value may be in planning and assessing treatments.

3.4 Methods

Patients in scar clinic are often reviewed for treatment of problematic scars; typically after burn injuries or after surgical treatment such as skin grafting. Patients underwent scar assessment as part of their routine scar clinic appointment at Stoke Mandeville Hospital. The chosen subjective outcome measures were the POSAS and mVSS both completed by me. The patient section of POSAS was completed by the patient in clinic.

Objective outcome measures were scar pigment using a chromameter, scar thickness using an ultrasound scanner and scar blood flow using a LASER Doppler. All subjective measures were taken by me. LASER Doppler training was provided by the lead nurse at the Stoke Mandeville Hospital burns unit. Training for the ultrasound scanner was provided by an ultrasonographer from the hospital radiography department.

When using the chromameter and the ultrasound scanner, readings were taken on 4 equally spaced points along the length of the scar (referred to as zone 1-4 in the results) like in the study performed by Dunkin et al 2007.[35] The chromameter would provide pigment readings based upon black to white (L), red to green (a) and yellow to blue (b). These would be quantified as percentage reflectance. For L, the range would be 0.01% to 160% with the higher figure representing more white. For a, the range would be -160% to +160% with a negative value representing green reflectance and positive value representing red reflectance. For b, the range would be -160% to +160% with a negative value representing blue reflectance and positive value representing yellow reflectance.

We had organised sessions as part of the Buckinghamshire Healthcare NHS Scar Clinic service, unfortunately due to the Covid-19 outbreak, clinics were changed to telephone based. As a result, data was not collected from further clinics. I was therefore only able to obtain data from 2 patients; however, this exercise provides a useful proof of concept for the use of these assessment tools within a clinical service.

Research ethics was not required for this study. Buckinghamshire NHS Trust supported this study as service evaluation as patients were being monitored as part of routine scar clinic.

3.5 Results

The two patients reviewed in scar clinic were both over 60 and had scars that were less than 18 months old (see Table 1). Patient 2 had been treated surgically when I saw him in scar clinic. His burn site had been treated with a split thickness skin graft with the donor skin being harvest from his left thigh.

Table 1. Demographics of the patients in scar clinic

Patient	Age	Gender	Age of injury	Location	Mechanism	Original TBSA	Scar Shape	Scar Length	Scar Width	Previous Treatment	Planned Treatment
1	65	Female	7 months	Abdomen	Scald	7%	Knots, right abdomen	10cm	1.5cm	Nil	Laser and steroid
2	68	Male	4 months	Left foot dorsum	Chemical - grafted	0.5%	Round	4cm	2cm	Split thickness skin graft	Nil
				Left thigh	Dermatome		Rectangle	8cm	4cm	Nil	Nil

The Total Burn Surface Area (TBSA) of the original burn was greater in Patient 1 at 7% whilst patient 2 had a TBSA of 0.5%. The scar dimensions were greater in Patient 1 compared to Patient 2's burn site (10cm x 1.5cm vs 4cm x 2cm). Patient 2 had already been treated with a split thickness skin graft over the site of the burn injury whilst Patient 1 had a steroid injection in the clinic with further laser planned.

Table 2. POSAS scores of the patients in scar clinic.

Patient Scar Assessment Scale							
Patient	**Painful**	**Itching**	**Colour**	**Stiffness**	**Thickness**	**Irregularity**	**Overall**
1	5	6	8	8	9	9	9
2 graft	1	1	10	1	1	1	2
2 donor	1	1	10	1	1	1	1

Observer Scar Assessment Scale							
Patient	**Vascularity**	**Pigmentation**	**Thickness**	**Relief**	**Pliability**	**Surface Area**	**Overall**
1	8	8	9	6	7	5	7
2 graft	3	3	2	2	2	2	2
2 donor	2	2	1	1	1	1	1

The POSAS scores for Patient 1 demonstrated high scores in the areas of vascularity, pigmentation, thickness in the observer scale and high scores in colour, stiffness, thickness and irregularity in the patient scale (Table 2). For Patient 2, the graft site demonstrated a high score in the colour section (10/10) for the patient scale and a score of 3/10 in vascularity and pigmentation for the observer scale. In the donor site for Patient 2, the colour score was also maximum at 10/10 in the patient scale and 2/10 for vascularity and pigmentation for the observer scale. The overall scores for both scars in Patient 2 were the same between the patient and observer scales (2/10 for graft site, 1/10 for donor site).

Table 3. mVSS scores of the patients in scar clinic.

Modified Vancouver Scar Scale (mVSS)					
Patient	**Vascularity**	**Pigmentation**	**Pliability**	**Height**	**Total**
1	3/3	2/3	2/5	2/4	9/15
2 graft	1/3	3/3	1/5	1/4	6/15
2 donor	1/3	3/3	0/5	0/4	4/15

The mVSS scores (see Table 3) were high in Patient 1 for vascularity (3/3) and pigmentation (2/3) and demonstrated a raised a scar (Height 2/4). Patient 2 scored higher on the graft site overall compared to the donor site (Graft site 6/15 versus donor site 4/15). Pigmentation (3/3) and vascularity (1/3) scores were the same for both the graft and donor site in patient. Patient 2's graft site scored higher in pliability and height (1/5 and 1/4 respectively), whilst the donor site was reported as like normal skin in these two areas.

When using the subjective outcome measures, both the POSAS and the mVSS were easy and relatively fast to complete in the scar clinic. The POSAS was good in that it gave patients an opportunity to report how they felt about their scars.

The chromameter readings would vary at the different sites where they were taken on the scar (Table 4 in Appendix). For patient 1, the L value demonstrated the greater standard deviation of the

mean (L=58.98±279%), with the a value demonstrating red light reflectance (a=10.17±1.91%) and the b value demonstrating yellow light reflectance (b=8.77±1.35%). For patient 2, the a value demonstrated red light reflectance (a=8.92±2.54%) and the b value demonstrated yellow light reflectance (b=10.03±0.81%) for the graft site. For the donor site, the a and b values also demonstrated red and yellow light reflectance respectively. The standard deviation in all the donor site values was smaller than in the graft site values (L=58.74±0.75%, a=8.35±0.44%, b=7.79±0.45%). The chromameter is a very quick and easy piece of kit to use and gave instant readings. For the patient the procedure is painless. The results in the form of L, a and b may be difficult to interpret, but I think it could have value in monitoring the long term pigmentation in scars.

Table 5. Ultrasound image measurements of the patients in scar clinic

Ultrasound Scan										
Patient	**Zone 1**		**Zone 2**		**Zone 3**		**Zone 4**		**Mean**	
	Depth	**Width**	**Depth**	**Width**	**Depth**	**Width**	**Depth**	**Width**	**Depth**	**Width**
1	2.60mm	11.53mm	1.84mm	7.61mm	2.56mm	5.08mm	1.48mm	11.05mm	2.12±0.48mm	8.82±2.63mm
2 graft	1.94mm	12.32mm	2.53mm	9.68mm	1.79mm	10.34mm	2.01mm	11.72mm	2.07±0.28mm	11.02±1.05mm

The ultrasound imaging software allowed measurements of the scar tissue within the skin (Table 5). For patient 1 the mean depth of scar tissue was 2.12±0.48mm and mean width was 8.82±2.63mm. For patient 2 at the grafted site the mean depth of scar tissue was 2.07±0.28mm and mean width was 11.02±1.05mm. The skin graft donor site on Patient 2 would show up the same as the normal uninjured skin and so was not included. The ultrasound scanner takes a while to set up and required training on how to use it effectively. Once set up, the images were produced quickly and the procedure was painless for the patient. I found the scanner to be very useful for determining the depth of a scar within the dermis and the software allowed for easy measurements. The scan produced high resolution images where the difference in normal dermis and scar tissue was easily

visualised. Difficulties with the scanner were using it in areas where the scar tissue is wider than the head of the probe.

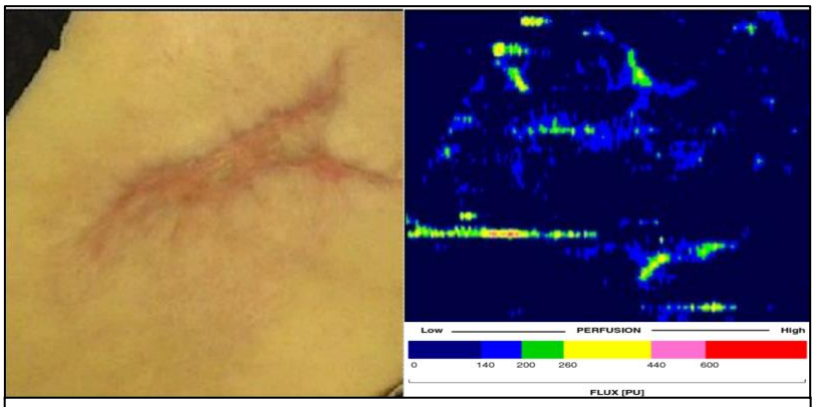

Figure 1. Patient 1 burn site demonstrates perfusion similar to surrounding normal skin with some areas of increased perfusion on the scar.

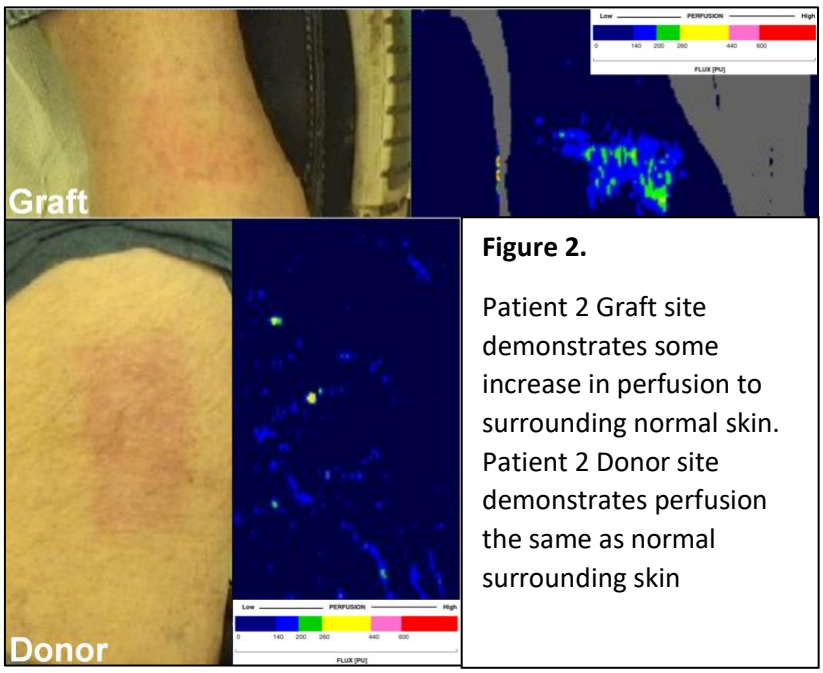

Figure 2.

Patient 2 Graft site demonstrates some increase in perfusion to surrounding normal skin. Patient 2 Donor site demonstrates perfusion the same as normal surrounding skin

The LASER Doppler images for Patient 1 (see figure 1) showed a perfusion pattern similar to that of the surrounding skin. There were some areas of higher perfusion on the thicker hypertrophic scar. For Patient 2 (see figure 2), the grafted site showed up as having a higher perfusion than the surrounding normal skin, with the centre and inferior left side of the graft showing the highest areas

of perfusion. Interestingly, the donor site images showed perfusion the same as the surrounding normal skin. The LASER Doppler is a large piece of equipment and is relatively time consuming to set up and use effectively. The images created were difficult to interpret and as the scars were over 2 months old, the differences in blood flow may have been smaller.

3.6 Discussion

For the limited patients we had in our study, the POSAS scores for the observer and patient scales were higher in the areas assessing vascularity, pigmentation, thickness, colour and surface area. The areas related to difference in colour (vascularity, pigmentation in observer and colour in patient scale) were the higher scoring items for both patients. A similar pattern was seen with mVSS scores, with pigmentation scores being high in both patients along with a high vascularity score in patient 1. The chromameter readings were taken at 4 different sites across the patients' scars. All the sites showed reflectance of red light (a value greater than 0) and yellow light (b value greater than 0). The mean for the L value in patient 1 and patient 2's graft site were 58.98±2.79 and 60.49±2.26 respectively. The ultrasound scanner readings demonstrated similar depths of scar tissue (2.12±0.4mm and 2.07± 0.28mm respectively) whereas width tended to be more variable. Images created by the LASER Doppler showed areas of increased perfusion in the hypertrophic scar of patient 1 and increased perfusion in the graft site of patient 2.

For both patients 1 and 2, the high scores for difference in colour of the scar were found in both the POSAS and mVSS. Interestingly patient 2 reported the thickness and irregularity of the graft and donor sites the same on the POSAS, whereas I reported a slight difference between the two on the observer POSAS. The mVSS scores were near identical for patient 2 between the graft and donor site, with the graft site scoring higher on pliability and height. The observer POSAS scores for both of patient 2's scar sites were the same.

As the clinician assessing patient 2's graft site scar in clinic, I found it to be raised and so scored 2/10 (graft scar) and 1/10 (donor site) on the observer POSAS; and 1/4 (graft scar) and 0/4 (donor site) on the mVSS. For the Patient POSAS, patient 2 scored the thickness the same on both sites (1/10 for both). This demonstrates the degree of subjectivity with PROMs and how it personally affects a patient; as something that may seem an obvious difference or concern to a clinician may be interpreted differently by the patient.

Previous studies discussed earlier reported how subjective scar assessments such as the mVSS are more suitable when there are multiple assessors.[3,16] This may not be practical in a busy clinic where often patients and staff are time restricted.[3,16] Compared to the BBSIP explored in Chapter 4, both the POSAS and the mVSS are quicker and easier to complete with a patient. I feel the POSAS will be a suitable subjective outcome measure in the planned randomised control trial in Chapter 5.

The chromameter provided quantitative data on the colour of a specific area of the scar tissue. The studies reporting the use of a chromameter are very limited. Draajers et al 2004 reported on the reliability and concurrent validity of the chromameter and a colorimeter. We could not find any studies where chromameters have been used for the longer term evaluation of pigmentation of a scar. The mean scores in Patient 2's donor site demonstrated smaller standard deviations compared to the graft site. This was to be expected as a skin graft donor site tends to be more uniform in depth and colour compared to a burn scar. Draajers et al reported that chromameters do not provide more information than the pigment sections on the POSAS and mVSS; however I think the chromameter provides an accurate way of quantifying the colour of a scar which will be valuable when assessing a scar treatment.[14]

The ultrasound scanner was used to take cross sectional images of the scar site like in a previous study by Dunkin et al 2007.[35] Using the dedicated software for the scanner we could take

measurements on the images. The width of a scar can be more easily viewed on the surface of the skin but the depth of the scar within the dermis can be more difficult with the naked eye. The ultrasound scanner allowed imaging of the scar within the dermis. Although the average widths varied between the two patients (8.82±2.63 and 11.02±1.05), the depths were more similar (2.12±0.48 and 2.07±0.28). No detectable difference in the images of the donor site of patient 2 compared to their normal skin was observed and therefore was not included in the analysis. Difficulties with the scanner were using it in areas where the scar tissue is wider than the head of the probe. In scar clinic, the scanner may not be a practical tool due to the set up requirements and the training required, however, like the chromameter, it has value in assessing a scar treatment. I feel that on larger scars, it will be more useful for determining depth only instead of depth and width of scar.

The LASER Doppler was used to provide images demonstrating blood flow to the scar tissue. For patient 1, the scar was 7 months old and demonstrated increased perfusion in certain regions of hypertrophic scar tissue. This could suggest that the increased in perfusion demonstrates an ongoing remodelling or inflammatory process to the region. For patient 2 the graft site which was 4 months old demonstrated an increased perfusion over the whole site with certain areas having a higher blood flow. This also could suggest an ongoing remodelling or inflammatory process. The donor site showing normal perfusion could suggest only deeper dermal injuries will have a longer term increase in perfusion. I do not think the Doppler will have value in the long term scar treatment clinic. During the healing period of a wound and the initial scar remodelling phase, it may have value in monitoring the blood flow over time. For the proposed BEST study, the LASER Doppler will be good for monitoring blood flow through the scar over the duration of the study. Whilst increase in blood flow is known to increase during the initial wound healing phase, there is not data on the long

term effect of blood flow and the final scar result. It may be useful to see if there is correlation between blood flow and the final POSAS results.

Unfortunately due to the very small sample size, it is difficult to make comparisons to other literature. All three analysed scars have different mechanisms of injury, treatment and location. For Patient 1 the scald mechanism resulted in a macroscopically thickened hypertrophic scar. The assessment tools reflect this with high scores for thickness in both sections of the POSAS and the higher mean thickness measured with the ultrasound scanner. For Patient 2, the original mechanism of injury was a chemical burn that was treated with a split-thickness skin graft. The assessment tools reflect the differences in the two different scars on patient 2. Thickness of the graft site scored slightly higher in the observer section of the POSAS, the mVSS and ultrasound scanner. The ultrasound scanner was unable to pick up any difference in thickness to that of normal skin at the skin graft donor site with the low POSAS and mVSS scored also supporting this.

Scar colour and pigmentation was an area that scored highly for both patient 1 and patient 2.

For patient 1, high scores on the vascularity, colour and pigmentation sections of the POSAS and mVSS were observed. Skin and pigment are unique to individuals, however the higher vascularity scores seemed to be supported by the higher average score for red light reflectance using the chromameter.

For patient 2, higher scores in pigmentation and colour were quantified with the chromameter. The lower vascularity scores in the mVSS and POSAS were supported with a lower red light reflectance score on the chromameter. Interestingly, the uniform controlled depth and mechanism of injury created by the dermatome resulted in a scar with a more uniform colour.

The higher subjective vascularity scores for patient 1 were only weakly supported by the LASER Doppler with the images showing only a very small increase in blood flow to certain areas of the scar. By contrast, the graft site on patient 2 scored lower on the subjective vascularity score but

demonstrated an increase in blood flow on the LASER Doppler. This could potentially be due to the age of the scar being 4 months old and still in a healing/remodelling process.

3.7 Conclusion

In conclusion, there are a multitude of outcome measures to assess scar tissue in clinic, but finding one that is well validated, allows for clinical time restraints and provides reliable data is difficult. Here we have discussed the merits and difficulties in using a combination of subjective and objective outcome measures in scar clinic and how they may be used to analyse scar tissue. Identifying what property of the scar a patient has concern with and using a specific tool to assess this pre and post treatment may have value.

For the proposed BEST study in Chapter 5, the POSAS will be a suitable subjective outcome measure; with the ultrasound scanner, chromameter and laser Doppler providing additional quantitative data on how the scars evolve with or without treatment.

Chapter 3 Appendix

Table 4. Chromameter readings of the patients in scar clinic

Chromameter Readings															
Patient	**Zone 1**			**Zone 2**			**Zone 3**			**Zone 4**			**Mean**		
	L (%)	**a (%)**	**b (%)**	**L (%)**	**a (%)**	**b (%)**	**L (%)**	**a (%)**	**b (%)**	**L (%)**	**a (%)**	**b (%)**	**L (%)**	**a (%)**	**b (%)**
1	61.02	9.21	11.06	62.28	8.1	8.05	55.38	13.23	7.61	57.23	10.15	8.36	58.98±279	10.17±1.91	8.77±1.35
2 graft	57.69	9.14	8.68	62.17	7.45	10.81	63.18	6.17	10.19	58.91	12.93	10.43	60.49±2.26	8.92±2.54	10.03±0.81
2 donor	59.57	8.12	7.82	58.21	8.09	8.46	57.8	8.84	7.2	59.38	9.09	7.69	58.74±0.75	8.35±0.44	7.79±0.45

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Chapter 4. Retrospective Analysis of Long Term Scarring Outcomes in Paediatric Burns Patients

4.1 Introduction

As discussed in Chapter 3, the novel BBSIP is the first quality of life based PROM specifically for burns scar patients. In this chapter, I report my experience of using the paediatric specific BBSIP in an outpatient setting. Paediatric burn injuries present a worldwide problem and can often be challenging to treat and prevent. The global number of reported cases presenting to a hospital in 2005 was 505,276, with a then paediatric population of 1.843 billion.[1] This figure is likely to be much larger today due to population growth and the under reporting of paediatric burn injuries in lower income countries.

Although the aetiology of the paediatric burn injury varies across nations; it is often reported that in developed western countries, scalds from hot drinks are the commonest cause; whilst in lower income developing countries, flame burns are more common.[2,3] There is no defined 'gold standard' for treating paediatric partial thickness burns. The general dogma involves acutely cooling the site with water to dissipate any thermal energy followed by providing a moist wound environment, physiologically stabilising the patient, removing exudate, preventing infection and minimising pain.[4,5]

The Stoke Mandeville Hospital Burns unit receives approximately 450 paediatric burns referrals per year. The most common presentation is of scald injuries to the chest, typically from hot drinks. The thinner dermis in younger children can often result in a deeper more substantial burn compared to a similar injury in adults.[4] The majority of patients can be treated conservatively although those with deeper burns may require surgical intervention. The unit's policy is to admit all children with a TBSA greater than or equal to 5%, and depending on the presentation, this threshold can be lower.

Although both conservative and surgical treatments aim to get the child healed, longer term scar outcomes for this very common injury are relatively unknown. Management of the acute burn can depend on the extent of tissue damage suffered. The three zones of the burn by Jackson describes areas of a thermal injury and the likelihood of the area to recover.[6] The zone of coagulation describes the area of maximum damage where there is irreversible tissue loss, whilst the zone of stasis describes an area with reduced tissue perfusion that is potentially salvageable.[6] The zone of hyperaemia is the area with the least amount of damage, is reversible and can be treated conservatively.[6]

Acute burn injuries can either be managed conservatively with dressings or surgically with debridement. Established for over 30 years, Biobrane® (UDL Laboratories, Rockford, IL, USA) is a popular treatment choice for superficial and partial thickness burns. It consists of a semipermeable silicone membrane combined with a Type I collagen (of porcine origin) coated nylon mesh. Multiple studies have demonstrated that Biobrane® can protect against infection, reduce pain, improve healing time resulting in a shorter inpatient stay, reduced dressing changes and better cost-effectiveness.[7-13] These qualities have made it a popular dressing choice around the UK. Despite its widespread use, there is a lack of data on longer term scar outcomes of patients treated with Biobrane.[13]

Silver sulfadiazine and silver nitrate impregnated dressings such as Acticoat®, are another popular treatment for partial thickness burns.[9,10,14] The silver is reported to provide an anti-microbial surface helping to reduce the risk of infection of the burn wound.[15] Acticoat® is a popular choice in UK burn centres, consisting of a silver nano-crystalline mesh that can be manipulated over the burn site.[15,16] Other reported benefits of Acticoat® include fewer inflammatory reactions compared to silver sulfadiazine and prolonged silver release.[15,16]There is conflicting information between topical silver

dressings compared with Biobrane®, but the few available studies report no observed differences in longer term outcomes.[14,17,18]

Depending on the depth of the burn, a surgical approach may be required to remove necrotic non-viable tissue referred to as debridement. An issue with surgical debridement is removal of normal healthy viable tissue along with non-viable tissue which can result in more prominent scarring.[19,20] The Versajet® (Smith and Nephew, Key Largo FL, USA) hydrosurgery system has been used as a burn debridement method for 12 years.[21] Its mechanism is to use high pressure sterile saline to create a controlled cutting field with a built in suction system. Due to its ability to debride in a very controlled manner, it is regarded as a very useful tool for debriding burnt tissue whist preserving the dermis.[22] The thinner dermis observed in the paediatric population means this greater level of control is advantageous.[20] Cubison et al 2006 reported using Versajet® debridement and subsequent Biobrane® dressings in paediatric patients with good healing and scar outcomes.[20]

There is a plethora of studies reporting on the short term outcomes of paediatric burn wounds focusing on factors such as hospital stay, time for healing and cost-effectiveness.[4,5,13,17,18] Longer term scar outcomes are less commonly reported with discharge from outpatient clinic reported as a common contributory factor.[4] In our experience as a regional burns unit, once children have acutely healed after burn injury, they are followed up in 6 weeks. After this period, if there are no concerns or pathological/problematic scarring, they are discharged from our care. This can serve as a barrier in obtaining information on longer term scar outcomes. Fan et al 2018 commented on the longer term scarring outcomes of paediatric burns treated with Biobrane® compared to those treated with silver based dressings.[18] No difference in the longer term scarring outcomes was observed when using the patient reported outcome section of the POSAS.[18] For the Versajet® debridement, there is limited literature observing the longer term scarring outcomes in paediatric

burn patients. Legemate et al 2018 put forward a research protocol to compare the longer term scarring outcomes in patients of all ages with burns treated with conventional surgical debridement versus Versajet® debridement.[23] Although the results have not been published yet, they will be following up patients at 12 months post-surgery with the POSAS as their primary outcome measure.

4.1.1 Assessing Paediatric Burns Scars

There are multiple subjective outcome measures to assess the results of a scar as discussed in chapter 3. The most commonly used in academic literature include the VSS and its various modifications, the MSS and the POSAS. The POSAS is a unique outcome assessment tool as it includes both an assessors score and a patient reported score.

Fan et al 2018 reported using the patient reported score of the POSAS as a longer-term outcome measure by conducting telephone interviews with the parents of children included in the trial. The POSAS patient section assesses the scars on pain, itch, colour, stiffness, thickness and irregularity. Although utilised in their study, the POSAS is not specifically targeted at children. The defined criteria for a PROMs quality is based on content validity, internal consistency, criterion validity, construct validity, reproducibility, longitudinal validity, responsiveness, floor and ceiling effects and interpretability.[24] The defined COSMIN criteria can be used to assess the methodological quality of studies assessing a PROM.[25] A suitable PROM for children with burn scars would include assessment of how the scar affects all elements related to quality of life (physical, mental and social well-being).[26] Paediatric specific PROMs would ideally use language that is age-appropriate, not include elements irrelevant to children (such as driving and financial) and be focused on factors important in a child's development such as play. For younger children with a burn injury, the PROM would be more appropriate to be aimed at the primary caregiver of the child. Given the aesthetic as well as functional impact of scarring, a suitable PROM would be able to distinguish from patients that have undergone surgery and those that haven't.[27,28] It would additionally allow for comparison of techniques and identify patients likely to benefit from the procedure.[28]

The BBSIP discussed in chapter 3 is a PROM purposefully created to assess scar outcomes based on the aforementioned elements related to quality of life in patient with scars from burn injuries.[29] The BBSSIP has been further developed to create versions dedicated to young children aged 8 to 18 ($BBSIP_{8-18}$) and the caregivers of younger children aged 0 to 8 ($BBSIP_{0-8}$).[30,31] A recent study measured the internal consistency, test-retest reliability, longitudinal validity and responsiveness of the BBSIP in post-acute burn period.[31] The content and construct validity had been reported in another study.[26,29] The authors concluded that the BBSIP is a suitable PROM in the post-acute burn period and that future studies will help evaluate its use as a longer term scar outcome tool.[31] The aforementioned POSAS is often considered to be a well validated PROM for scarring. The observer element filled in by an assessor has demonstrated acceptable reliability for the vascularity section and total score; along with internal consistency and construct validation.[32,33] Test-retest reliability of the POSAS has been reported as acceptable for all items (except vascularity and relief) in the observer section and the pain section of the patient version.[34] The internal consistency for the patient scale has been reported as generally acceptable.[32]

4.2 Aim

The aim of this study was to determine if there were any long term differences in scarring outcomes of paediatric burn patients treated with surgical Versajet® and Biobrane® compared to conservative Acticoat® and non-adherent dressings. Our hypothesis was that patients treated with Versajet® and Biobrane® would have superior long-term scar outcomes compared to those treated conservatively.

4.3 Methods

A retrospective review was performed of all paediatric burns patients admitted to the Stoke Mandeville Burns Unit from October 2014 to September 2017. The data collected from the

patients' medical records included: age, gender, TBSA, mechanism of burn (e.g. scald), number of follow up appointments, further treatments, the location of the burn, injury date, assessment date, admission date and treatment received.

Table 1. Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
-All paediatric burn referrals requiring admission -Patients treated with Versajet® and Biobrane® or Acticoat®	- Patients referred but not admitted - Patients receiving treatment other than Versajet® and Biobrane® or Acticoat®

A total of 107 patients satisfied our inclusion and exclusion criteria (Table 1) and were included in the study. As the focus was on longer term scar outcomes, many patients were no longer being followed up in outpatient clinic and so scar evaluation was conducted via telephone interview using the age appropriate version of the BBSIP.

The responses were analysed using IBM SPSS Statistics for Windows version 26 (IBM Corp, New York USA). Data was presented as mean ± standard deviation. A two-tailed Student's *t*-test was performed for the continuous variables; for categorical response variables a Fisher's exact test was used.

The study was supported by Buckinghamshire NHS Trust as a service evaluation study. Research ethics was not required as previous treatments that followed trust protocol were being evaluated.

4.4 Results

The dataset included all children admitted to the ward with a burn injury between October 2014 and September 2017. All 107 children admitted met our inclusion criteria. There were 37 patients (34.6%) treated surgically with Versajet® debridement and Biobrane® dressings; and 70 (65.4%) patients treated conservatively with Mepitel® and Acticoat®. The majority of the paediatric patients in our study were pre-school (Versajet and Biobrane vs. Mepitel and Acticoat: mean age 3.25[±0.93]

versus 2.79[±0.85]). The overwhelming majority (n=105 98.1%) of patients had scalds whilst 1 patient had flame burns and 1 patient had chemical burns (Table 2).

Table 2. Patient Demographics

Variable	**No. (%)/mean ± standard deviation**	
	Versajet + Biobrane (n=37)	**Non-adherent dressing + Acticoat (n=70)**
Age (yr)	3.25[±0.93]	2.79[±0.85]
Gender		
Male	18 (48.6%)	46 (65.7%)
Female	19 (51.4%)	24 (34.3%)
Total Body Surface Area %	6%[±0.77]	7%[±1.32]
Mechanism of burn		
Scald	36 (97.3%)	69 (98.6%)
Chemical	0	1 (1.4%)
Flame	1 (2.7%)	0
Further treatments	16 (43.2%)	10 (14.3%)

Telephone interviews resulted in a total of 34 responses (Versajet® and Biobrane® vs. Acticoat®: 13 versus 21); 39 of the phone numbers contacted were no longer in service and a further 34 phone numbers resulted in no response or refusal to participate (Figure 1).

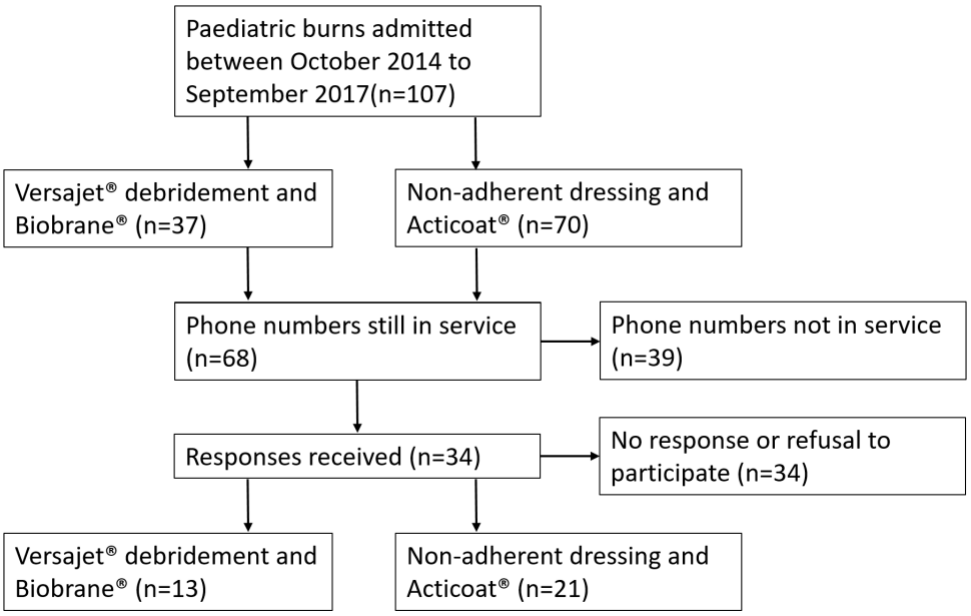


Figure 1. Flowchart of patient responses

For the responses received on telephone interview, the average interval post-injury was three years (Versajet® and Biobrane® vs. Acticoat®: 34.19[±7.71 months] versus 36.85[±7.36 months]). The age demographics of respondents were all 8 years old or younger and so the $BBSIP_{0-8}$ was used (see Appendix).

Of the 58 questions that make up the $BBSIP_{0-8}$, a two-tailed students t-test was performed on the results from 57 of them (Table 3). For 31 of the 57 questions, both treatment groups scored the same lowest possible score indicating no impact on the child's life/best possible outcome.

For the remaining 26 questions, there was no statistically significant difference observed between the groups. The lower and upper 95% confidence intervals spanned across zero in all questions. Although a small sample size, the p-values for all questions were greater than 0.05.

Table 3. Long term BBSIP outcomes for Patients

Variable	No.		95% Confidence Intervals		
	Versajet + Biobrane (n=37)	Non-adherent dressing + Acticoat (n=70)	p value	Lower	Upper
Overall, how much do your child's burn scars impact on their life now?*	1.23[±0.60]	1.00[±0]	0.84	-0.03297	0.49451
Itch, pain, sensitivity to touch, or other sensations from your child's scars*	1.31[±1.11]	1.05±[0.22]	0.301	-0.24379	0.76393
Physical scar symptoms (like thick, tight scars)*	1.08[±0.28]	1.38[±0.80]	0.2	-0.77738	0.16932
Scar treatments (like pressure garments, exercises, creams)*	1.46[±1.20]	1.05[±0.22]	0.13	-0.12795	0.95579
School, play and daily activities*	1.00[±0]	1.00[±0]	0	N/A	N/A
Peer relationships and social interaction*	1.00[±0]	1.00[±0]	0	N/A	N/A
Your child's emotional reactions or mood*	1.15±[0.55]	1.00[±0]	0.209	-0.09033	0.39803
Your child's appearance*	1.00[±0]	1.14[±0.48]	0.292	-0.41456	0.128841
During the last week, how often has your child reported itch, pain or other sensations or shown signs of sensations in their scars(like scratching, grabbing at their scars or facial grimaces)?*	1.31[±1.11]	1.05[±0.22]	0.301	-0.24379	0.76393
During the last week, on average how many times each day did your child scratch or rub their scars more than their normal skin?*	1.15[±0.55]	1.00[±0]	0.209	-0.9033	0.39803

During the last week, how many times did your child scratch or rub their scars so much that other problems happened to their scar (like wounds opened or sores developed)?*	1.00[±0]	1.00[±0]	0	N/A	N/A
Rate the severity of sensitivity of your child's burn scars to be to light touch or clothing.*	0.54[±1.40]	0.19[±0.51]	0.304	-0.33004	1.02601
Moving easily*	1.00[±0]	1.00[±0]	0	N/A	N/A
Climbing during play or up or down stairs*	1.00[±0]	1.00[±0]	0	N/A	N/A
Walking short distances*	1.00[±0]	1.00[±0]	0	N/A	N/A
Getting in and out of a chair*	1.00[±0]	1.00[±0]	0	N/A	N/A
Physical activities like swimming, riding a bike, ball games or sport*	1.00[±0]	1.00[±0]	0	N/A	N/A
Schoolwork*	1.00[±0]	1.00[±0]	0	N/A	N/A
Play*	1.00[±0]	1.00[±0]	0	N/A	N/A
Dressing and undressing*	1.00[±0]	1.00[±0]	0	N/A	N/A
Showering or bathing*	1.00[±0]	1.00[±0]	0	N/A	N/A
Eating or drinking*	1.00[±0]	1.00[±0]	0	N/A	N/A
Self-care activities (like brushing their teeth, doing their hair)*	1.00[±0]	1.00[±0]	0	N/A	N/A
Getting to sleep*	1.00[±0]	1.00[±0]	0	N/A	N/A
Staying asleep*	1.00[±0]	1.05[±0.22]	0.44	-0.17163	0.07639
Your child’s daily routine*	1.00[±0]	1.00[±0]	0	N/A	N/A
Developing new skills or becoming more independent*	1.00[±0]	1.00[±0]	0	N/A	N/A

Your child's friendships or interaction with children their age*	1.00[±0]	1.00[±0]	0	N/A	N/A
Your child's interaction with family members*	1.00[±0]	1.00[±0]	0	N/A	N/A
Family activities (such as meals or outings)*	1.00[±0]	1.05[±0.22]	0.44	-0.17163	0.07639
Parent bothered by appearance of child's scars*	1.08[±0.28]	1.14[±0.48]	0.655	-0.3638	0.23193
Parent bothered by the look of your child's worst scar*	1.23[±0.44]	1.10[±0.30]	0.292	-0.12232	0.39338
Parent bothered by looks or comments you or your child got from other people because of your child's scars*	1.00[±0]	1.05[±0.22]	0.44	-0.17163	0.07639
How bothered has your child been by the appearance of their scars, during the last week*	1.00[±0]	1.00[±0]	0	N/A	N/A
Irritable or cranky*	1.15[±0.55]	1.00[±0]	0.209	-0.09033	0.39803
Anxious or nervous*	1.00[±0]	1.00[±0]	0	N/A	N/A
Worried*	1.00[±0]	1.00[±0]	0	N/A	N/A
Sad*	1.00[±0]	1.00[±0]	0	N/A	N/A
Angry*	1.00[±0]	1.00[±0]	0	N/A	N/A
Embarrassed or self-conscious*	1.00[±0]	1.05[±0.22]	0.44	-0.17163	0.07639
Upset*	1.00[±0]	1.00[±0]	0	N/A	N/A
Worst part – Tight*	1.00[±0]	1.00[±0]	0	N/A	N/A
Worst part – Thick*	1.69[±1.25]	1.33[±0.66]	0.28	-0.30664	1.02458
Worst part – Wrinkled*	1.31[±0.63]	1.10[±0.30]	0.194	-0.11348	0.53839
Worst part – Dry*	1.15[±0.55]	1.10[±0.44]	0.734	-0.28944	0.40666

Worst part – Hard*	1.15[±0.55]	1.00[±0]	0.209	-0.09033	0.39803
Worst part – Rough*	1.00[±0]	1.00[±0]	0	N/A	N/A
Worst part - A different colour*	2.08[±0.95]	1.71[±0.72]	0.216	-0.22258	0.94786
Did your child have open wounds or sores in their scars, during the last week?*	0	0	0	N/A	N/A
Parent worry - whether the lack of your child's scars will bother them in the future?*	1.23[±0.44]	1.10[±0.30]	0.292	-0.12232	0.39338
Parent worry - the effect of your child's scars on other family members*	1.08[±0.28]	1.00[±0]	0.209	-0.4517	0.19901
Parent worry - the way others treated your child*	1.00[±0]	1.10[±0.30]	0.265	-0.26618	0.0757
Parent - ability to work, study, or complete household jobs*	1.00[±0]	1.00[±0]	0	N/A	N/A
Parent - relationship with family members*	1.00[±0]	1.00[±0]	0	N/A	N/A
Parent - you getting together with friends*	1.00[±0]	1.00[±0]	0	N/A	N/A
Parent – mood*	1.00[±0]	1.05[±0.22]	0.44	-0.17163	0.7639
Parent - family routine*	1.00[±0]	1.05[±0.22]	0.44	-0.17163	0.7639

*Data presented as mean ± standard deviation

Part 7 of the BBSIP focuses on physical symptoms with question 15 asking parents to rate how much their child's worst area of scar was affected by a physical property in the last week.

Physical properties of thickness (question 15B), wrinkling (question 15C) and difference in colour (question 15G) demonstrated the larger differences (Figure 2).

Responses for question 15B relating to scar thickness in the conservative treatment group were; 16 (76%) conservative versus 9 (69%) surgical reported not thick, 3 (14%) conservative versus 1 (8%)

surgical reported a little bit thick, 2 (10%) reported a bit thick in conservative and 1 (8%) reported really thick in surgical.

Question 15C focused on wrinkling with responses as; 19 (91%) conservative versus 10 (77%) surgical reported not wrinkled, 2 (9%) conservative versus 2 (15%) reported a little bit wrinkled with 1 (8%) report of a bit wrinkled in the surgical group. Scar colour asked in question 15G had the largest difference in responses; 9 (43%) conservative versus 3 (23%) surgical reported not different, 9 (43%) conservative versus 8 (62%) surgical reported a little bit different, 3 (14%) conservative reported a bit different and 2 (15%) surgical reported quite different. In the surgical treatment group, 6 of the 13 had further treatments in clinic, whilst 3 of the 21 children in the conservative group had further treatments.

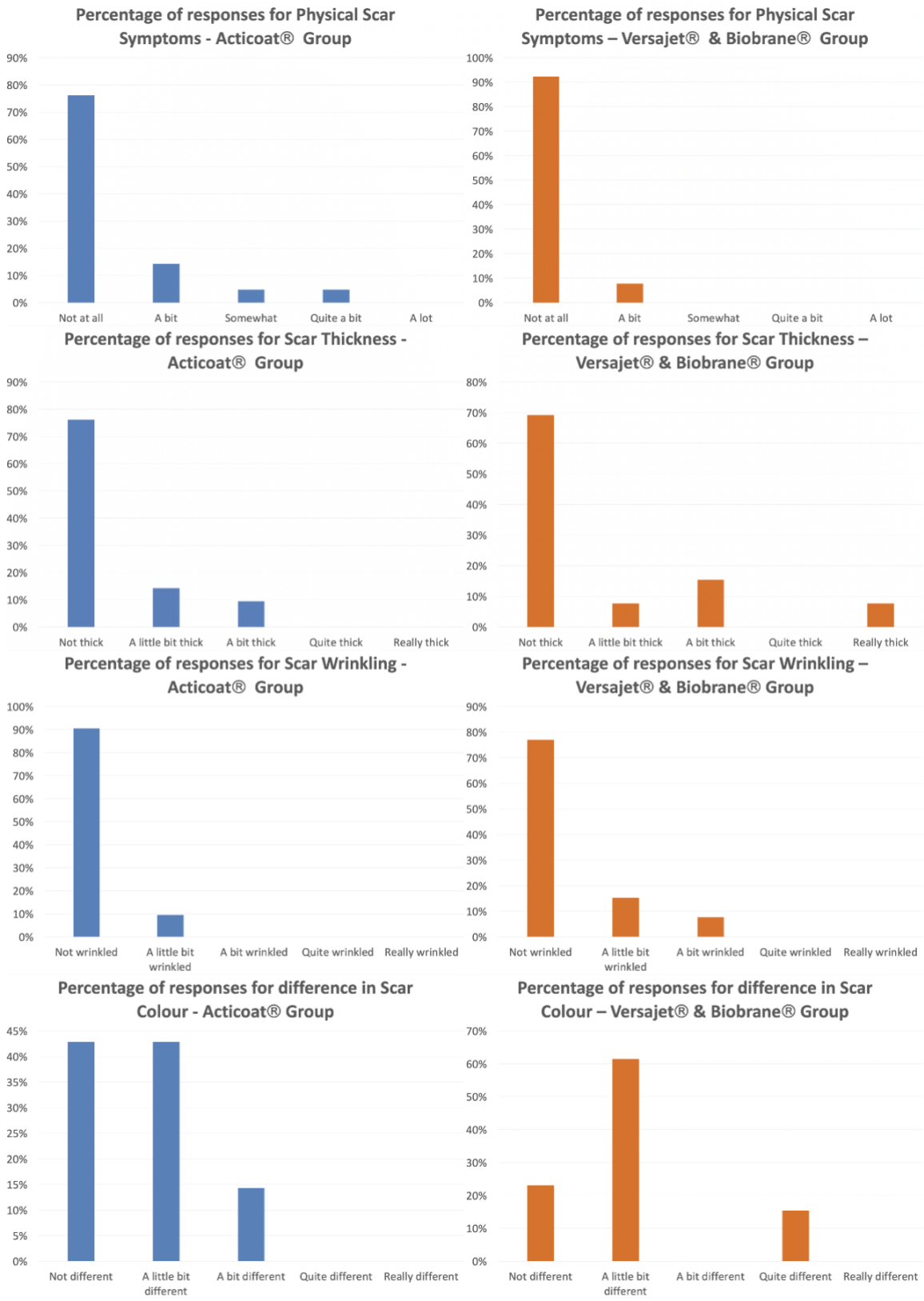


Figure 2. Percentage Responses for Worst Scar Physical Properties

Question 14 in part 7 of the $BSIP_{0\text{-}8}$ asked to describe the worst part of the child's scar by anatomical location. The Fishers exact test was used for any correlation between worst areas of burn and

treatment group. No statistically significant difference was demonstrated between the two groups (Fishers Exact Test = 0.817).

4.5 Discussion

The results show that there are no statistically significant differences in the longer-term scar outcome as assessed by the $BBSIP_{0-8}$ in paediatric burn patients treated with surgical Versajet® debridement and Biobrane® versus those treated conservatively with Acticoat® and non-adherent dressings. For over half of the questions asked as part of the BBSIP, all responses in both groups were the lowest possible score indicating the least/no impact of the scar on the child's life. For part 3 of the $BBSIP_{0-8}$ which focuses on school, play and daily activities, 14 of the 15 questions in this section all scored the lowest possible score in both groups. Part 6 of the $BBSIP_{0-8}$ focuses on emotional reactions; we found that 5 out of the 7 questions in the sections had scored the lowest possible score in both groups (irritable or cranky and self-conscious). The area that had the most variation was part 7 of the $BBSIP_{0-8}$ which focuses on physical symptoms. The questions asking about the worst areas of scar tightness and roughness scored the lowest possible in both groups. Thickness, wrinkling, dryness, hardness and difference in colour all had the greater variety of responses in both groups. These properties are assessed as part of the POSAS patient scale referred to as scar colour, stiffness, thickness and irregularity.

In terms of clinical practice, there are more risks associated with treating a child surgically. These include but are not limited to anaesthetic risk, surgical procedure risk and post-procedure risks such as infection and wound breakdown. In the acute burn phase, the priority is to get the child physiologically stable and healed as fast as possible. The zone of coagulation can become necrotic and increase the infection risk. Plastic surgeons will use clinical judgement to decide whether or not

to debride an area of burnt tissue weighing up the risks of treating surgically versus conservatively. Our findings suggest that plastic surgeons have the flexibility to choose whichever approach will get the child healed fastest, acknowledging that the longer-term scarring outcomes would likely be the same.

As discussed in chapter 1, the physiology of scarring is unique to the individual and there are additional factors that can increase the risk of pathological scarring. Our results showed a higher proportion of the children treated surgically underwent further treatments in scar clinic compared to those treated conservatively. This could be due to deeper more severe burns in those treated surgically. Despite this, longer term scarring outcomes were similar.

This study is the first to utilise the $BBSIP_{0-8}$ as a longer-term scar outcome tool. Tyack et al 2019 described the value of the $BBSIP_{0-8}$ in the acute post burn phase and that more studies would be required to establish the $BBSIP_{0-8}$ role for longer term scar outcomes.[31] The small sample size means the study may not be adequately powered and is potentially at risk of type II error. The clinical entries used as part of the data collection would not always include the depths of the burn injury and language used to describe depth was not uniform. Additionally, there is a degree of subjectivity when assessing depths of burns clinically. Telephone interviews have generally been regarded as less-attractive than face-to-face interviews and physical questionnaires.[35] Negatives of telephone interviews in research have been reported as a lack of visual cues can result in a loss of contextual and non-verbal data which in turn may lead to lower quality data.[35] The $BBSIP_{0-8}$ is a physical questionnaire designed to be filled in by the caregiver of the patient. Whether or not telephone interviews result in lower quality data compared to physical interviews or questionnaires is still a matter of debate.[35]

Future research could focus on comparing paediatric longer term scarring outcomes with other burn units in the country that may have different standard operating procedures. It would be interesting to see if this would have an impact on the long term scarring outcome. A RCT would help to provide the best evidence for surgical versus conservative treatment in burns but often this can be hard to facilitate in a busy healthcare service. As the BBSIP is a burns scar focused assessment tool, it is not appropriate for the proposed BEST study in chapter 5.

4.6 Conclusion

In conclusion, no difference was found in the long term scar outcomes as assessed by the $BBSIP_{0-8}$ in those treated surgically with Versajet® debridement and Biobrane® compared to those treated conservatively with Acticoat® and non-adherent dressings. The BBSIP and its various versions are the only full PROM for burns scars widely available. It has value as a longer-term scar assessment tool, but a shorter more focused version may be of more value in clinic.

Chapter 4 Appendix

Brisbane Burn Scar Impact Profile (BBSIP) For Caregivers

of Children Aged less than 8 years

General Instructions:

When completing this questionnaire please think of burn scars as being in the place where your child had the burn, or where your child had skin grafts, or where your child has donor sites. For questions like those in part 1 please answer by placing a mark in one of the circles. If the item does not apply to you or your child please place a mark in the not applicable box when that option has been provided. Part 1 to 7 will mostly ask you questions about the impact of burn scars on your child and Part 8 will ask you questions about the impact of your child's burn scars on you and your family.

Part 1: Overall Impact of Burn Scars

1. Overall, how much do your child's burn scars **impact on their life** now?

Not at all *A bit* *Somewhat* *Quite a bit* *A lot*

2. How much did these aspects **impact on your child's life,** DURING THE LAST WEEK?

	Not at all	*A bit*	*Somewhat*	*Quite a bit*	*A lot*	*Not applicable*
Itch, pain, sensitivity to touch, or other sensations from your child's scars						☐
Physical scar symptoms (like thick, tight scars)						☐
Scar treatments (like pressure garments, exercises, creams)						☐

3. DURING THE LAST WEEK, how much did your child's burn scars **impact on** the following aspects?

	Not at all	*A bit*	*Somewhat*	*Quite a bit*	*A lot*	*Not applicable*
School, play and daily activities						☐
Peer relationships and social interaction						☐
Your child's emotional reactions or mood						☐
Your child's appearance						☐

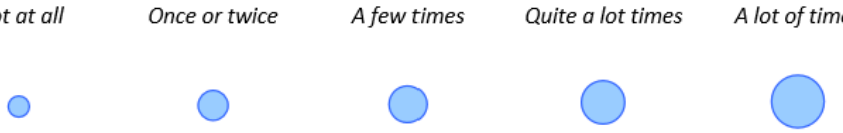
Part 2: Itch, Pain, Discomfort and Other Sensations

4. DURING THE LAST WEEK, how **often** has your child reported itch, pain or other sensations or shown signs of sensations in their scars (like scratching, grabbing at their scars, facial grimaces)?

Not at all *Once or twice* *A few times* *Almost everyday* *Everyday*

5. DURING THE LAST WEEK, **ON AVERAGE** how many times **EACH DAY** did your child **scratch or rub** their scars more than their normal skin?

Not at all *Once or twice* *A few times* *Quite a lot times* *A lot of times*



6. DURING THE LAST WEEK, how many times did your child **scratch or rub** their scars so much that **other problems happened** to their scar (like wounds opened up or sores developed)?

Not at all *Once or twice* *A few times* *Almost everyday* Everyday

*7. This question asks you to rate the severity of **sensitivity** of your child's burn scars **to light touch or clothing**, if 0 means 'not sensitive' and 10 means 'as sensitive as scars could possibly be'. Please put an X through the number that best describes the **AVERAGE** sensitivity in your child's scars* during the **last week**. *Use 0 if your child had no sensitivity to light touch or clothing.*

Not sensitive	0	1	2	3	4	5	6	7	8	9	10	As sensitive as scars could possibly be

Part 3: School, Play and Daily Activities

When completing this question think about how your child would usually complete these activities if they didn't have scars, considering the level of assistance that is appropriate for them.

8. DURING THE LAST WEEK, how much did your child's burn scars **impact on** the following aspects?

	Not at all	*A bit*	*Somewhat*	*Quite a bit*	*A lot*	*Not applicable*
Moving easily	○	○	○	○	○	☐
Climbing during play or up or down stairs	○	○	○	○	○	☐
Walking short distances	○	○	○	○	○	☐
Getting in and out of a chair	○	○	○	○	○	☐
Physical activities like swimming, riding a bike, ball games, or sport	○	○	○	○	○	☐
Schoolwork	○	○	○	○	○	☐
Play	○	○	○	○	○	☐
Dressing and undressing	○	○	○	○	○	☐
Showering or bathing	○	○	○	○	○	☐
Eating or drinking	○	○	○	○	○	☐
Self-care activities (like brushing their teeth, doing their hair)	○	○	○	○	○	☐
Getting to sleep	○	○	○	○	○	☐
Staying asleep	○	○	○	○	○	☐

For further information contact Dr Zephanie Tyack, Centre for Children's Burns and Trauma Research, email: z.tyack@uq.edu.au.

9. DURING THE LAST WEEK, how much did your child's burn scars **impact on** the following aspects?

	Not at all	*A bit*	*Somewhat*	*Quite a bit*	*A lot*	*Not applicable*
Your child's daily routine (including attending school, doing jobs at home, playing ball games or sport, going to a lesson)	○	○	○	○	○	☐
Developing new skills or becoming more independent (like being toilet trained, learning to use a spoon, completing homework)	○	○	○	○	○	☐

Part 4: Friendships and Social Interaction

10. DURING THE LAST WEEK, how much did your child's burn scars **impact on** the following aspects?

	Not at all	*A bit*	*Somewhat*	*Quite a bit*	*A lot*	*Not applicable*
Your child's friendships or interaction with children their age	○	○	○	○	○	☐
Your child's interaction with family members	○	○	○	○	○	☐
Family activities (such as meals or outings)	○	○	○	○	○	☐

Part 5: Your Child's Appearance

11. How **bothered have you been** by these things, DURING THE LAST WEEK?

	Not at all	*A bit*	*Somewhat*	*Quite a bit*	*A lot*	*Not applicable*
The **appearance of your child's scars**						☐
The look of your child's worst scar						☐
The looks or comments you or your child got from other people because of your child's scars						☐

12. How **bothered has your child been** by the appearance of their scars, DURING THE LAST WEEK?

Not at all *A bit* *Somewhat* *Quite a bit* *A lot*

Part 6: Emotional Reactions

13. How much did your child **feel like this because of their scars,** DURING THE LAST WEEK?

	Not at all	*A little bit*	*Somewhat*	*Quite a bit*	*A lot*
Irritable or cranky					
Anxious or nervous					
Worried					
Sad					
Angry					
Embarrassed or self-conscious					
Upset					

Part 7: Physical Symptoms

14. Describe the WORST part of your child's scars (for example, their left shoulder).

_____

_____

15. Think about the **WORST part** of your child's scars (that you wrote down above) compared to their normal skin when you answer the following questions. Rate how much your child's scars were like this **AT THEIR WORST** DURING THE LAST WEEK.

	Not at all tight - not restricting movement or pulling body parts	*A little bit tight* - restricting movement or pulling body parts a bit	*A bit tight* - restricting movement or pulling body parts somewhat	*Quite tight* - restricting movement or pulling body parts quite a lot	*Really tight* - restricting movement or pulling body parts a real lot
A. **Tight**					

	Not thick	*A little bit thick*	*A bit thick*	*Quite thick*	*Really thick*
B. **Thick**					

	Not wrinkled	*A little bit wrinkled*	*A bit wrinkled*	*Quite wrinkled*	*Really wrinkled*
C. **Wrinkled**					

	Not dry	*A little bit dry*	*A bit dry*	*Quite dry*	*Really dry*
D. **Dry**					

	Not hard	*A little bit hard*	*A bit hard*	*Quite hard*	*Really hard*
E. **Hard**					

	Not rough	*A little bit rough*	*A bit rough*	*Quite rough*	*Really rough*
F. **Rough**					

	Not different	*A little bit different*	*A bit different*	*Quite different*	*Really different*
G. **A different colour** (like red or darker than normal skin)					

16. Did your child have **open wounds or sores** in their scars, DURING THE LAST WEEK?

Yes	*No*
○	○

Part 8: Parent and Family Concerns

17. How **worried have you been** about the following aspects, DURING THE LAST WEEK?

	Not at all	*A little bit*	*Somewhat*	*Quite a bit*	*Extremely*
Whether the look of your child's scars will bother them in the future	○	○	○	○	○
The **effect** of your child's scars **on other family members**	○	○	○	○	○
The way others treated your child	○	○	○	○	○

18. How much did your child's burn scars **impact on** the following aspects, DURING THE LAST WEEK?

	Not at all	*A little bit*	*Somewhat*	*Quite a bit*	*A lot*
Your ability to work, study, or complete household jobs	○	○	○	○	○
Your relationship with family members	○	○	○	○	○
You getting together with friends	○	○	○	○	○
Your mood	○	○	○	○	○
Your family routine (for example, your work or other children's activities)	○	○	○	○	○

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Chapter 5. BEST-1: A single blinded, randomised trial into the efficacy of silicone sheeting for cutaneous scars. A Best Evidence for Scar Treatment (BEST) Group Trial

5.1 Introduction

In the previous chapters, I have explored methods of scar analysis and demonstrated the lack of standardisation of injury and the resulting scar. This ultimately acts as a limitation when assessing treatments for scarring and weakens the observed outcomes. In this chapter, I will discuss how I set out to create a study that would provide high quality evidence for the effectiveness of silicone gel sheeting as a treatment for cutaneous scars. In order to achieve this, we devised a standardised method of creating two scars in healthy volunteers who would then be randomised to receive silicone gel sheet treatment to one of the scars with the other scar acting as a control.

After reviewing the scar assessment tools, we developed our own primary and secondary outcome measures and developed a research protocol for the study. This protocol was reviewed and adapted over several months and submitted to a Research Ethics Committee (REC) where it gained approval. In this chapter, I will include the full research protocol (see Appendix) we developed and discuss our experience with the study to date.

5.2 Experience with the BEST Study

The aim of this study was to try and create high level evidence for the effectiveness of silicone gel sheeting in the treatment of cutaneous scars. One of the challenges with previous studies that assessed silicone gel sheeting, as well as other scar treatments is the lack of reproducibility of the scar itself. In order to create an easily reproducible scar, we used the jig scarring model developed by Dunkin et al 2007.[1] This model had previously gained ethical approval and utilised healthy volunteers as the subjects.

When developing the protocol, previous experience from the Dunkin et al study showed that wounds caused by the jig would be ready at 2 weeks after the injury. At this point, participants would be randomised to wear the silicone gel sheeting dressing on either the left hip, or the right hip. As the adhesive property of the silicone gel dressings is quite poor, a normal standard Opsite® dressing would be placed over the silicone gel sheeting. An Opsite® would also be placed on the scar that is not receiving silicone gel sheeting treatment. Volunteers would be expected to wear the dressings for the entire duration of the study (50 weeks in total) with plenty of spares given. The Opsite® being waterproof would also allow for dressings to be worn whilst showering. We wanted the dressings to minimally interfere with the lives of the participants in the study.

The initial aim was for an 18 month follow up period; this is due to most scars reaching their fully mature final appearance by 18 months. This however did not seem a feasible and fair expectation of participants so it was shortened to 12 months. The follow up periods of 3 months, 6 months and 12 months after the application of the silicone gel sheeting were chosen as it would allow adequate data collection and seemed more reasonable for participants. We used a mixture of objective and subjective scar assessment measures in our study. Our primary objective outcome measure of the POSAS was chosen as it allows subjective assessment of the scar by both the participant and the clinician. The mVSS was included as it is relatively quick and straight forward to complete in a clinical setting as discussed in chapter 3. Our secondary outcome measures are all objective and had been developed from the study by Dunkin et al 2007.[1] They are blood flow through the scar (identified with the LASER Doppler), scar thickness (measured with ultrasound), scar pigment (chromameter) and scar dimensions (photographs and dedicated software). By utilising the secondary outcome measures, we hope to strengthen our findings. Despite this study primarily aiming to assess a scar treatment, we decided not to include patients with a personal or family history of keloid scars. This

is due to ethical concerns about creating a problematic, pathological scar on a patient who is known or highly likely to produce keloid tissue.

There were several delays in obtaining ethical approval for the study. After confirming a final protocol with my supervisors, the first step was obtaining trust approval from Buckinghamshire NHS Trust. After trust approval I was able to submit the research protocol and all necessary associated paperwork such as participant information sheets for ethical approval. After obtaining ethical approval we began our recruitment strategy. This was performed by putting recruitment posters up around the trust, advertising on the trust website/newsletter and from a recruitment stand. Difficulties recruiting became apparent as people who were approaching were asking about some form of financial incentive. It was decided that in order to reach our target of participants for the study, we would have to re-evaluate to determine a suitable financial incentive. This required re-submitting again for ethical approval to have the financial incentive added to the study. After approval for the financial incentive, I successfully recruited 6 participants to the study. Unfortunately, all research was suspended as a result of the SARS 2 Covid-19 outbreak and the recruited participants therefore did not enter the trial protocol.

5.3 Conclusions from setting up the study

Setting up an RCT was an educational experience; I gained a better understanding of the practicalities of setting up a clinical research trial and the necessary steps required in obtaining ethical approval and getting to a position to begin recruitment. The study is planned to continue once the Buckinghamshire NHS Trust Research department are allowing research involving healthy volunteers to commence on site again. Prior to the Covid-19 outbreak, our recruitment strategy was successful in getting healthy volunteers. The dedicated staff, necessary equipment and funding are

in place. The project lead has organised a research fellow to take over the project. There is still a pressing need to establish a suitable treatment for scarring in skin. Trying to establish a suitable human model that is reproducible and standardised as discussed has been difficult. The progress I have made with the BEST study has brought us one step closer to creating a suitable model and determining the effectiveness of silicone gel sheeting as a scar treatment. In the future, if this model proves effective in creating and assessing standardised scars, it could be used to assess other scar treatments.

Chapter 5 Appendix

5.4 Research Protocol

Study title:

BEST-1: A single blinded, randomised trial into the efficacy of silicone sheeting for cutaneous scars. A Best Evidence for Scar Treatment (BEST) Group trial.

Protocol number:	**1-01**
Version number:	**4.1**
Date:	**3rd March 2020**

1. Dunkin CS, Pleat JM, Gillespie PH, Tyler MP, Roberts AH, McGrouther DA. Scarring occurs at a critical depth of skin injury: precise measurement in a graduated dermal scratch in human volunteers. Plastic and reconstructive surgery 2007;119:1722-32; discussion 33-4.

5.5 Signatures of Investigators

We confirm our approval of this protocol version,

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5.6 Summary

A single blinded, randomised trial into the efficacy of silicone sheeting for cutaneous scars.

The proposed trial will test the efficacy of an existing anti-scarring therapy. The treatment consists of a single blinded, randomised controlled trial of *topical silicone sheeting*, which has been adopted worldwide as a conventional treatment with very limited evidence for its benefit.

We anticipate that this study will generate the first evidence for the efficacy or lack thereof of this intervention using objective outcome measures. The null hypothesis is that topical silicone sheet does not improve clinical scarring in humans.

We will recruit 48 healthy subjects who will take part in an intra-subject, intervention versus no intervention, blinded study. All patients will receive a standardised scratch injury to the skin of the lateral hip areas under the anaesthesia of EMLA® cream. This highly stereotyped model creates a small wound that heals quickly and then results in a 2cm scar at 18 months after injury. After the acute phase of injury, the subjects will be randomised to apply topical silicone to one of the resulting scars as it matures. The application of the silicone will be according to standard clinical recommendations. The opposite side will have no intervention and will act as the control, receiving only a standard dressing. The subjects will be followed up at two weeks, three months, six months and 12 months post-injury. The subjects will be asked to indicate how compliant they have been with the scar intervention.

The healed wounds/scars will be assessed by a Lead Investigator together with a Research Nurse in the outpatient setting. Scar assessment will take place with scar assessment scales (Modified Vancouver Scar Scale, POSAS) and objective outcome measures (laser Doppler imaging for blood flow; high frequency ultrasound for scar morphometry, chromameter for fixed pigmentation; and high resolution photography). A panel of independent experts will assess standardised photographs

in a blinded manner using a modified Manchester Scar Scale and an intra-patient scar-comparison scale. The assessing experts will also be asked whether they consider the scars to be abnormally inflamed (hypertrophic scar).

On statistical advice, the data gathered will be unblinded for analysis of efficacy once 17 subjects have been recruited.

5.7 Introduction

5.71 Background

Scarring is a major clinical problem that results from many types of skin trauma. Each year, it is estimated that 300 million new scars are formed after trauma such as burns. Scarring is the final common pathway for healing within the skin irrespective of age, gender and race. The effect upon the individual can be life-changing. Scars can be itchy, painful, tight and above all, cosmetically disfiguring. There is currently no reliably effective treatment for reducing or preventing scarring. Moreover, the vast majority of treatment modalities for scarring have no evidence to confirm their benefit. Topical silicone sheeting is one such treatment that is widely available and used throughout the world.

5.72 The background to wound healing and silicone

Human wounds heal through a process of repair, leading to compromised skin structure and function. After injury, there is immediate cessation of bleeding through a process called *haemostasis* involving platelets and blood proteins. This seals the wound and initiates the release of chemical mediators which govern subsequent phases. Next, *inflammation* attracts inflammatory cells to the wound that combat infection and co-ordinate subsequent cellular recruitment. These latter cell types include fibroblasts which migrate over a provisional scaffold of molecules to deposit and remodel scar tissue. The deposition of scar tissue is dependent on numerous factors including the amount of inflammation within the skin, the genetic tendency to scarring, the site on the skin surface, and the tension within this area. Although the reconstitution of the top layer of the skin can occur within a few weeks, remodelling and maturation of the scar beneath can last for over a year. The final common pathway is the restoration of integrity of the layers of the skin but with the invariable formation of a visible scar. Clinically, the most common problematic scars are those in

which excess connective tissue is deposited and for which there is usually excessive inflammation, so called fibroproliferative hypertrophic and keloid scars.

A key process that is modifiable in the progress to scar formation is that of early inflammation. One of the drives to make the skin inflamed is the integrity of the top layer of the skin (the epidermis) and the function of cells at this site (keratinocytes). Keratinocytes migrate over the early wound to cover the site of injury, but although they may provide a visible layer, the function of the epidermis is changed for a long period of time. Its top layer, the *stratum granulosum,* is less able to waterproof the skin. Consequently water from within the body can escape – abnormally excessive 'transepidermal water loss' – and this can dehydrate the keratinocytes. In response to drying out, the keratinocytes release inflammatory chemical alarm signals. These molecules pass down into the bottom layer of the skin (the dermis) where the scar is forming to potentiate the activity of the cells which deposit scar tissue (fibroblasts). Theoretically, any therapeutic approach which can aim to lock moisture within the epidermis has the potential to limit the tendency to scar formation.

Silicones are synthetic polymer compounds made from a repeating backbone chain of silicone and oxygen atoms. The length of the backbone polymer, the nature of any side groups and the crosslinking of chains lead to a variety of different physicochemical properties. Generally, they are inert, heat resistant, impermeable, soft, flexible and mildly adherent. Dependent upon chemical structure, they exist as liquids, gels or sheets. These features are particularly relevant for medical grade silicone which has been incorporated into a range of devices, from catheter tubes to permanent implants. Liquid silicone has been used since the 1970s to treat hypertrophic scars and keloids. From the early 1980's, a change in formulation led to silicone becoming available in the

form of topical sheets and these, on the basis of anecdotal improvement, were adopted as a therapeutic modality.[1]

Topical silicone sheet (TSS) has been widely applied to scars of differing clinical subtype and maturity. It has been used with the aims of preventing the formation of hypertrophic scars and for treating mature hypertrophic scars. The most accepted theory as to how it might work is that it exerts a beneficial action by occluding and hydrating the epidermis. In so doing, inflammation is thought to be downgraded with ultimately less scar deposition. Other, less supported theories include an elevation of temperature within the skin, a change in the electrical field and the release of low molecular weight silicone as an agent to improve inflammation. Often, the TSS is held in place with a pressure garment. Pressure is another perceived modality for reducing scar formation and the combined action of silicone and pressure is felt to be synergistic.

5.73 Previous clinical trials of topical silicone

Topical silicone sheeting has become a ubiquitous treatment for scarring. It is used by all tertiary care burns scar teams within the United Kingdom with a cost of £1.2 million in England and Wales alone (NHS Business Services Authority ePACT2 October 2016). A survey of 818 GP practices within the South West found that silicone was the second most popular scar treatment prescribed (Indira Yonjan, MSc Thesis, University of London 2015). Extrapolating this work to the entire cohort of general practitioners across the UK, and with an average cost of a pack of TSS at £22.13, an estimated £12 million is spent on TSS per annum.[2] Given that topical silicone is now widely available over-the-counter, it is likely that the total UK market for TSS is worth significantly more than this figure.

However, the clinical evidence for the utility of topical silicone is lacking. A Cochrane Wound Group systematic review reviewed 20 trials involving 873 subjects who had received TSS for their scars.[3] Their conclusions were:

"There is weak evidence of a benefit of silicone gel sheeting as a prevention for abnormal scarring in high-risk individuals but the poor quality of research means a great deal of uncertainty prevails. Trials evaluating silicone gel sheeting as a treatment for hypertrophic and keloid scarring showed improvements in scar thickness and scar colour but are of poor quality and highly susceptible to bias."

There are numerous problems inherent in undertaking rigorous scar research. Almost all scar studies are confounded. In particular, non-identical wounds are compared, for example, burns at different body sites or with different depths. If an intervention is applied to a single scar, there is no way of establishing the extent to which the scar would have improved without the intervention. The patient assessment outcome measures used to assess scars are highly subjective and poorly validated. Few studies have utilised outcome measures that precisely gauge physical properties such as blood flow, colour or scar dimensions. Optimally, all scar interventions would be assessed by a 'gold standard' technique:

- Identical wounds are made at the same time on identical body sites in the same individual
- The wounds are treated identically up to the point at which scar interventions are required (usually, once the initial wound has healed)

- As both wounds develop into scars, *only one of the wounds,* determined randomly, receives the intervention under investigation
- Only the patient/subject knows which one of the wounds is being treated – everyone else is 'blinded' to the intervention (a 'single blinded study')
- The intervention is carried out by the patient/subject in an identical manner to real world usage
- The patient/subject returns to the research team on a regular basis for assessment over at least 12 months so that the scar outcome can be reassessed repeatedly and for long enough to ensure full scar maturation
- At each visit, a range of validated, subjective and objective outcome measures are used to quantify the progress of both scars
- At each visit, the research team do not know which scar is the one that is being treated by the intervention
- At 12 months, such a single blinded, randomised, controlled trial is unblinded and the research team can assess whether the intervention has really made a difference or whether enough subjects have been recruited

This is the approach that was used by the largest international study of a novel scar treatment and the one that was recommended by an international consensus panel.[4,5] Notably, the latter body stated, "A fundamental principle that should be observed in the prospective evaluation of scar prevention/reduction therapies is that, if left untreated, wounds in treatment and control groups should have healed with identical scars. Observation of this principle will allow the detection of true treatment effects."[5]

Since 2013, there have been further attempts to assess the efficacy of TSS. These include: analysis of scarring in children who have had an implantable venous access device; rhytidectomy scars in adults; knee scars.[6-8] However, none of these studies have utilised identical wounds, blinded intervention on just one wound and validated outcome measures, both subjective and objective. As Friedstat and Hultman indicated in another systematic review in 2014;

"Despite hypertrophic scars being a common occurrence in burn survivors, both the number of studies and consensus for treatment are limited. Efforts to perform larger, adequately powered RCTs are needed, specifically in the areas of silicone, compression garments, and combination therapy."[9]

5.74 Rationale

Topical silicone sheet has been adopted worldwide as a standard scar treatment on the basis of poor evidence. There is a pressing need to establish first rate evidence as to whether it is effective clinically. If effective, it can be recommended as efficacious, funding is more likely to be assured and care will improve. If it is ineffective, there will be a substantial cost saving to the NHS and equivalent health care systems as it will no longer be recommended as a standard treatment.

We propose a single-blinded, intra-patient controlled trial using the simultaneous creation of two small scratch wounds on the hip skin of healthy subjects (one scratch on each side). The device to create the skin scratch was created at Stoke Mandeville Hospital by the team at Restore Burn and Wound Research in the late 1990's and published in 2007.[10] It involves the creation of a sterile, longitudinal scratch to the skin in a region of the outer hip skin that has been anaesthetised with standard local anaesthetic cream (EMLA®). A small scar results at 18 months post-injury. The injury is well-tolerated and was not associated with any complications in 113 consecutive subjects in the first study.[10] A survey of all participants after the study indicated no concerns about the final appearance of the scar (unpublished results).

The resulting scar can be monitored in an outpatient setting using a number of subjective and objective outcome measures. The subjective outcome measure will include the two most validated scar assessment scales, The Modified Vancouver Scar Scale (see Appendix II) and The Patient and Observer Scar Assessment Scale (see Appendix III and IV).[11-13] The VSS requires an independent observer to assess scar vascularity, pigmentation, pliability and height relative to the surrounding

skin. The sum of the defined scores in each of these spheres produces a combined score out of 13, with higher scores indicating more severe scarring.

The original POSAS has been studied for suitability as a scar assessment tool on the clinimetric grounds of reliability, consistency, validity and feasibility. The current POSAS v.2.0 scale has both a 'patient' and 'observer' element and was found to be reliable, feasible, valid and correlates well with mVSS.[14,15] The subjective 'patient' scale, completed by the subject with the scar, entails an assessment of itch, pain, colour, stiffness, thickness, irregularity and a global score. The 'observer' scale assesses vascularity, pigmentation, thickness, relief, pliability and surface area. The sum of both scales gives a final score between 6 and 60 of scar severity; the greater the score, the worse the scar. The POSAS score will be the primary outcome measure of this study. Both the mVSS and POSAS take a few minutes to complete.

Frequently, existing studies to assess scars have been deficient in providing no objective measures of scar outcome. In our study, we aim to assess the scars with a comprehensive range of secondary outcome measures that relate to scar evolution: blood flow; surface dimensions; and dimension within the skin. Blood flow will be monitored with a laser Doppler imager (LDI, MoorLDI2-IR, Moor Instruments, Axminster, Devon). This is a non-invasive device that produces a standardised, two-dimensional image of blood flow across the surface of a scar. Augmented blood flow has been found to correlate with worse scarring and it is believed to be an ongoing surrogate index of continued inflammation and scar remodelling.[16] Blood flow does not completely equate to colour change in a scar, and as such, to establish the extent to which fixed pigments such as melanin and flavinoids contribute to colour, a chromameter (Minolta CM700d, Konica Minolta, Osaka, Japan) scan will be undertaken on the scars at each time point.[17] The chromameter gives a non-invasive, objective and

standardised value for the surface pigment of a scar in terms of relative colour space giving an automated reading for hue, saturation and luminance.

The surface dimensions of the scar include length of perimeter, surface area, maximal width and maximal length. In collaboration with Zeiss UK Ltd, we have created and modified a dedicated software package (Zen and AxioVision) to interpret standardised photographs in a reproducible manner such that these parameters are calculated. A program has been written that allows the assessment of the scar and which standardises for both colour and dimensions relative to scales placed within the image. With regards to the dimensions of the scar beneath the surface, these include the cross-sectional area of the scar, maximal width and depth from epidermis downwards into the dermis of the skin. These can be assessed non-invasively with a high frequency ultrasound imager (Episcan I200, Longport, Pennsylvania, USA) to give measurements down to a resolution of several microns spatially. Finally, standardised photographs of the scars will be analysed by observers blinded to the intervention/control using an established technique, The Manchester Scar Assessment Scale with an adaptation to allow direct comparison of treatment/control images (Appendix V).[18] This will provide further evidence for equivalence/non-equivalence between TSS and control.

Early versions of all of the objective monitoring tools – laser Doppler imaging, chromameter, photographic image analysis and ultrasound scanning – were used in the first study using the scratch model.[10] Combined, they took an average of 15 minutes to complete, were all non-invasive and were not associated with any complications.

48 subjects will be recruited and followed-up over a 12 month period after treatment. After baseline assessment of the skin using the objective outcome measures, the scratch injuries will be assessed at several time points. These will be: three days; two weeks; three months; six months and twelve months. This frequency of assessment worked well and was acceptable to participants.[10]

The silicone intervention (TSS) is only instigated after healing is assured at the two week appointment; this mirrors the usage of topical silicone in the clinical setting. Volunteers for the study will be offered £100.00 for their participation in the study.

5.80 Trial aim/objectives

The trial will test the efficacy of topical silicone sheet on subsequent scar formation in human skin. We anticipate that this study will provide the first valid evidence for or against the clinical usage of this widespread treatment for scars. The trial will examine if TSS is effective at improving the appearance of normal scars and secondarily, it will determine the extent of any improvement in scarring.

5.801 Trial endpoints

The primary endpoint will be the subjects' own assessments of their scars by means of the POSAS 2.0 scar scale (www.posas.org) at 12 months. Secondary endpoints will comprise an assessment of standardised photographs that will be assessed by image analysis and panel assessment at 12 months. A panel of independent experts will assess the scars by modified Manchester Scar Scale alongside an intra-patient scar comparison scale. Additionally the assessing experts will be asked whether they consider scars to be hypertrophic.

5.81 Trial design

5.811 Patient group

5.812 Inclusion criterion

1. Subjects willing to have two small but permanent scars in the lateral hip skin; this is made explicit from the time of recruitment. Potential recruits will be given a suitable period of consideration of the permanent nature of scarring before enrolling.
2. Subjects willing and able to attend for all follow up appointments for the full 12 months of the study.

5.813 Exclusion criteria

1. Volunteers with a history of psychological illness including depression, body dysmorphia or personality disorder.
2. Any evidence of infection, either localised to the skin in the hip area, or systemic.
3. Volunteers with either personal or family history or suspected to have keloid scarring.
4. Age younger than 18 or older than 70 years.
5. Any systemic illness that could significantly impair wound healing including end stage liver, renal or respiratory impairment, immunosuppression or metastatic cancer, uncontrolled diabetes
6. Lacking capacity to consent *e.g.* inability to weigh up the merits of the study or retain information
7. Medication that will interfere with healing or scarring including anticoagulant medication (excluding low dose aspirin), chemotherapeutic agents, immunosuppressive drugs (*e.g.* systemic corticosteroids in the preceding 30 days) or immune modulating drugs (*e.g.* anti-TNF therapy for conditions such as rheumatoid arthritis)
8. Any prior or planned radiation treatment.
9. Any skin disorder that is chronic or currently active and which the investigator considers may adversely affect the healing of the acute wounds or involves the areas to be investigated in the trial (lateral hip)
10. Any history of significant hypersensitivity or allergies to any of the skin preparations (aqueous chlorhexidine solution, EMLA™), dressings (silicone sheeting, microporous tape, Opsite Post-Op™) or latex used in the study
11. Any past or planned surgery, including skin graft harvest, in the region of the putative scratch injury (lateral hip)

12. A personal or family history of methaemoglobinaemia (theoretical risk with topical EMLA™)
13. Any of the medications known to interact with EMLA™ (*vide infra*)
14. Any other significant disease or disorder which in the opinion of the Investigator, may either put the participants at risk because of participation in the trial, or may influence the result of the trial, or the participant's ability to participate in the trial
15. Participation in another clinical trial during the study or within 28 days prior to planned study entry
16. Patients at risk of keloid scar formation
17. Psychological, familial, sociological or geographical factors potentially hampering compliance with the study protocol and follow-up schedule
18. Any form of substance abuse, psychiatric disorder, or other condition that may invalidate communication with the study team
19. Pregnant women or women planning a pregnancy during the course of the trial

5.814 Identification of study recruits

Potential participants will be invited to contact the research team via advertisements in the form of posters, recruitment stands and social media posts. Subjects will be recruited from the setting of South Buckinghamshire NHS Trust (Stoke Mandeville Hospital) and surrounding universities. As with the study undertaken previously in this hospital, the trial will be advertised in the outpatient setting and by talks given by the researchers. The talks and accompanying information about the study will emphasise:

- the aims and potential benefits of the study
- the nature of pathological scarring

- how silicone sheeting has been suggested to work
- how silicone sheeting is used to treat scars clinically
- the current absence of strong evidence for clinical benefit
- the nature of the research study
- the nature of the scratch injury including photographs of the wound and 'late' images of the likely scarring
- that the small hip scars will be permanent
- the nature of the assessments at each follow up appointment
- the requirement for full follow up to 12 months in five separate appointments
- how to ask any further questions.

Potential recruits are asked to make their interest known and then, a week later, a formal meeting is arranged to further discuss the study. Only after confirmation of full understanding of the study is an individual recruited formally. Each subject is made fully aware that they can withdraw from the study at any time. The process of recruitment and consent is detailed in Figure 1.

5.815 Sample size

Our statistician Dr Paul White has estimated that 48 subjects will be required to demonstrate a statistical difference based on the results of similar studies (see attached reports – Appendix I). Interim analysis will be performed but only for the purposes of recruitment and safety monitoring.

The Primary Outcome measure is POSAS II is known to have good clinometric properties. Table 1 summarises power for sample sizes in the region $n = 36$ to $n = 50$ for the split-body design. Based on the given assumptions a sample size of $n = 38$ would have approximately 90% power to detect a medium effect size (alpha = 0.05, two-sided, paired samples t-test). Such a sample size would be sufficiently large for valid application of the paired sample t-test.

Table 1 Relationship between sample size and power for standardized effect sizes in the medium range (0.50, 0.55, 0.60, 0.65) in the split body design.

Sample Size (N)	Effect Sizes	Powers
36	.50, .55, .60, .65	.83, .89, **.94, .96**
38	.50, .55, .60, .65	.85, .**91, .95, .97**
40	.50, .55, .60, .65	.87, .**92, .96, .98**
42	.50, .55, .60, .65	.89, .**94, .97, .98**
44	.50, .55, .60, .65	**.90, .95, .97, .99**
46	.50, .55, .60, .65	**.91, .95, .98, .99**
48	.50, .55, .60, .65	**.92, .96, .98, .99**
50	.50, .55, .60, .65	**.93, .97, .99, .99**

A sample size of 40+ would have at least 80% power for demonstrating superiority over a weak effect (*d* = 0.1), but otherwise very large economically demanding sample sizes would be needed to demonstrate superiority by the lower bound margin.

5.816 Randomisation process

All patients will be treated with both topical silicone sheet and the control of a standard non-silicone-containing dressing. Since this is an intra-patient controlled trial involving two identical wounds on the same subject, the randomisation is simply as to which wound receives TSS and which receives no treatment.

After the creation of the identical scratch wounds on both hips, a non-adherent and absorptive dressing is applied (Opsite Post-Op, Smith and Nephew, Hull, UK). The outer dressing is removed three days later at the first follow up appointment. At two weeks, all the dressings are removed. After full assessment of the wound including subjective and objective outcome measures, the subject is given a sealed envelope, a supply of topical silicone sheeting (Cica-Care Gel Sheet, 12cm x 6cm, Smith and Nephew UK) and Opsite Post-Op, Smith and Nephew, Hull, UK . A full description of how the silicone is applied is detailed in Appendix VI and is a direct reproduction of the manufacturers' instructions. The application of silicone is demonstrated by review of a brief video demonstrating the process. The patient is to apply the Opsite Post-Op™ over the silicone dressing and the non-intervention site.

Using RedCAP® software, we will create a randomisation of participants on characteristics of age (18 to 70), biological gender (Male or Female) and Fitzpatrick skin type (I to VI) which will determine whether the silicone dressing is to be placed on the left or the right. A Stoke Mandeville Hospital plastic surgery department administrator who is not involved with the study will be able to see each participants name and which side they should put the dressing on. They will assemble 48 envelopes specific to each participant. Each envelope will have the name of the participant on it.

The envelope contains both the randomised allocation of treatment to either left or right-hand side hip (appendix ab) and detailed instructions on how to apply the TSS (appendix VI). It is opened when the subject has gone home on the day of review. All the documentation within the study emphasises the following:

- Subjects are not to reveal to anyone which side is being treated with TSS
- Subjects are asked not to treat either side with any other intervention during the study period.
- Subjects are mandated that, if they perceive one side as improving with either TSS or no treatment, that they should not divert from the study protocol, by, for example, treating both sides with the intervention that is perceived to be producing a better outcome

These three stipulations are emphasised to the subjects each time they return for follow up. If there is any suggestion that TSS deleteriously effects wound healing (such as delayed healing, skin reaction or infection), the Clinical Trial Coordinator will be informed immediately. The blind will be broken by the Clinical Trial Coordinator. This possibility is considered highly unlikely since TSS is widely used throughout the world and is not associated with these effects.

5.817 Process of obtaining informed written consent

All subjects meeting the inclusion criteria for the study, and not ruled out by the exclusion criteria, are eligible to take part.

Forty-eight subjects will be recruited over a 12 month period. Informed consent will be obtained after an explanation of the study and confirmation that each subject understands the protocol, the nature of follow up and the inevitable small scars present on the hip skin). The process from recruitment to consent is detailed below in Figure 1.

At each stage, the potential recruit to the study is provided with an information sheet and verbal explanation. Once recruitment is confirmed, the subject's GP will be informed that the patient has been enrolled. Informed consent will be obtained at least 24 hours before the procedure, to allow time for the patient to consider finally the implications of the study, ask further questions or withdraw their participation. Subjects are made aware that they can withdraw from the study at any time. A Study specific consent form will be completed as documented in Appendix bb with standardised description of the procedure, benefits and risks. A copy of the consent form will be included in the subject's trial notes. The subject will also keep a copy of the consent form.

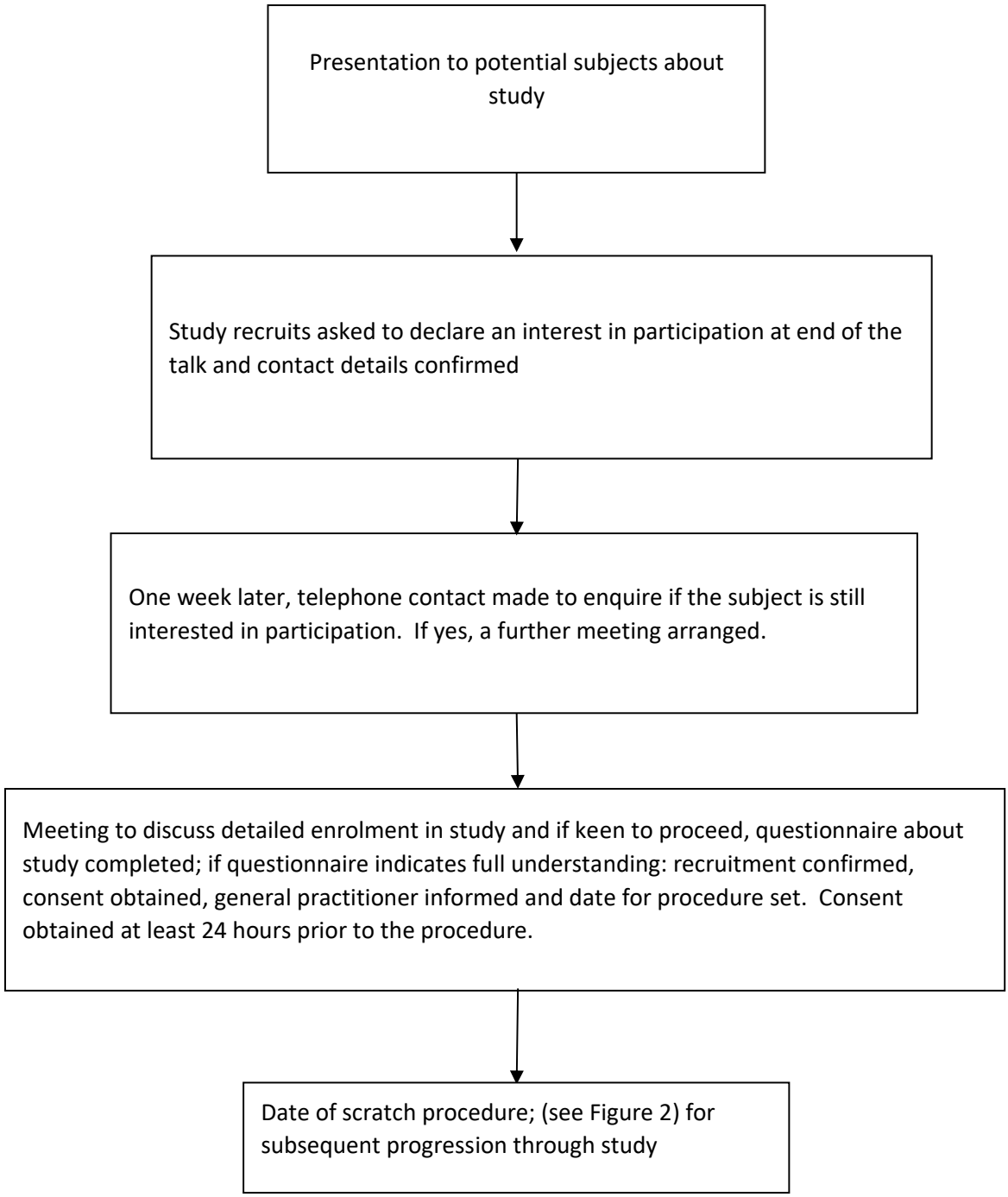


Figure 1. The process of recruitment. Most steps completed by the principal investigators or The *Clinical Trials Practitioner (experienced research nurse)* specifically employed for trial. Note, the subject is informed that they can withdraw from the study at any time point.

5.818 Procedure/treatment

All trial consent, data recording and analysis will be performed by the Clinical Trials Co-ordinator under the supervision of the Chief or co-investigator and the Data controller.

A flow diagram of the treatment schedule detailed below is given in Figure 2.

On the day of the procedure, the subject will be asked to devote two hours to prior assessment and the scratch procedure itself. If the subject takes any medication, this will be taken on the day of the procedure as per their normal routine.

On arrival, the patient will be asked for some baseline characteristics questionnaire to be completed by the Lead Investigator which will be kept with their subject notes (Appendix cc). This will confirm that there are no significant medical conditions which have otherwise not been declared and that, on the day of the procedure, the subject has no intercurrent illness.

The rest of the protocol follows that of the previous study carried out using the scratch model at Stoke Mandeville Hospital from 2000-2003.[10]

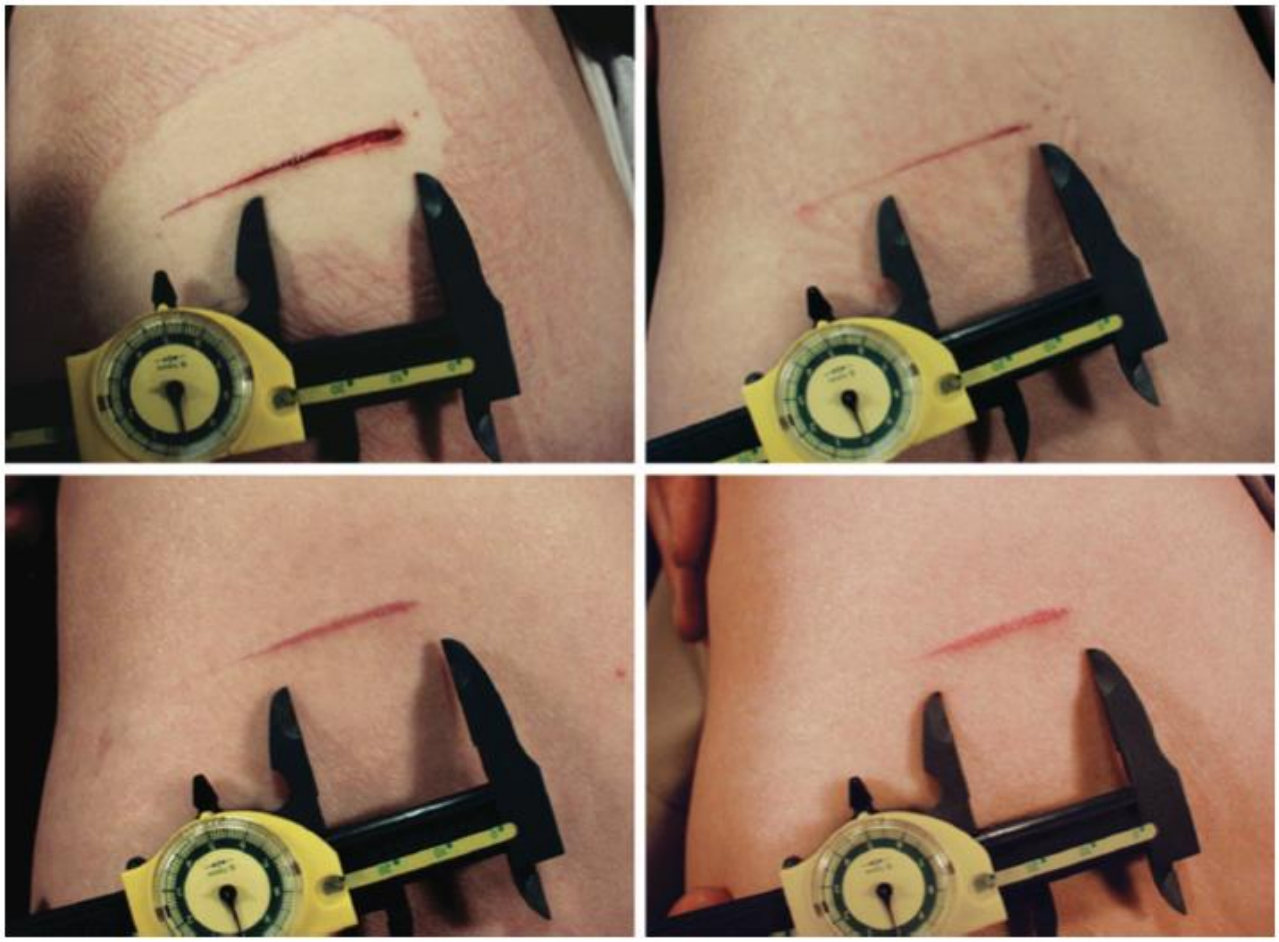


Figure 1a. Adapted from Dunkin et all 2007.[10] Series of stereotyped dermal wounds on the lateral hip in subject 1, showing the wound immediately after injury (*above*, *left*) and at week 1 (*above*, *right*), week 4 (*below*, *left*), and week 36 (*below*, *right*).

- The subject rests on a couch for ten minutes
- Baseline measurements are made of the following in the region of interest (ROI) on the lateral hip (delimited by anterior superior iliac spine of pelvis, greater trochanter of femur, pubic area anteriorly and gluteal area posteriorly):
    - Laser Doppler imaging for blood flow using the Moor LDI; the instrument is calibrated at the start of each session and an average reading of blood flow flux is obtained across the whole ROI
    - Chromameter; an average of three readings are taken with the Minolta Chromameter in the middle of the ROI

    - Ultrasound scan; three readings are taken in the centre of the ROI and the images are used to calculate average thickness of dermis, epidermis and total skin thickness

- The site of the scratch is marked midway between anterior superior iliac spine and greater trochanter along the relaxed skin tension lines. The exact position is confirmed as symmetrical between the two sides by direct visualisation and measurement from bony landmarks. Two small dots are marked along the line but 2cm beyond the position of the future scratch with an indelible marker. This ensures that the scratch will not be made through skin that has been marked and yet will still lie at a symmetrical angle on both sides.
- 5% EMLA™ cream (Astra Pharmaceuticals, Kings Langley, United Kingdom) is applied to a thickness of 0.5cm along the line of the planned scratch in a rectangular shape of approximate size 8 x 4cm. The anaesthetic cream is covered by an occlusive dressing (Opsite Flexigrid 12cm x 12cm) that is stuck to skin beyond the limit of the cream so as to prevent it extruding until the skin has been anaesthetised fully.
- The subject then rests for 60 minutes until the skin is fully anaesthetised. This is confirmed by removal of the cream, sterilising the skin with aqueous chlorhexidine (STERETS® Unisept, Medlock Medical Ltd, Oldham, UK), and gently testing with a sterile needle tip.
- A fresh, sterile number 11 blade (Swann-Morton, Sheffield, UK) is mounted into the scratch jig
- The hip skin is stretched until taut by a first member of the research team. The base plate of the jig is then applied to the skin along the line of the previously marked dots and with the ‘deep’ end of the jig positioned laterally.
- Using several passes of the blade down the central recess of the base plate, a scratch is made in the top layers of the skin which, as detailed in previous work, extends down at the deep end to a maximal depth of 1.6mm and progresses to no injury at all at the superficial end of the scratch jig.

- The jig is removed and aqueous chlorhexidine is again applied to the site to ensure sterility
- A photograph is taken of the scratch using a standardised photographic set up to ensure constant angle, exposure and lighting:
    - A Canon EOS 1200D digital camera with 100mm f2.8 Macro USM EF lens and EM-140DG Macro flash
    - Measured and sited on a tripod perpendicular to, and at 50cm from, the scratch and illuminated from both sides at 45 degrees by two separate LP Micropro Litepanels
    - A colour wheel and metric scale (ruler) are placed within each image to allow later standardisation of colour and size
- Using sterile gloves an Opsite Post-Op™ dressing is applied over the scratch.
- The anaesthetic cream is removed from the opposite hip and the procedure is repeated to produce two scratches at exactly the same position on both hips
- Post-procedure, the subject is given standard advice both verbally and in written form (Appendix dd):
    - All the dressings stay intact for three days and until they are changed at the dedicated follow up clinic
    - The site of the scratches may give some mild discomfort once the anaesthetic cream wears off at approximately one hour; any discomfort is usually controlled with paracetamol tablets and experience from previous studies showed that it was rarely required
    - No bleeding is expected, but in the rare eventuality that dressings are soiled, the subject is to return to the research team for a change of dressings
    - Infection is highly unlikely but any symptoms such as new pain, malodourous discharge or shiny, red skin surrounding the dressing should prompt a review by the research team

- Any reaction to the dressings, as indicated by symptoms such as itch or swelling of the skin, should be reported to the research team
- The contact details for the research team including an out-of-hours contact number. Advice is given to contact the team if there are any queries.
- An appointment date and time are given for the first follow up review at 3 days

Subjects are reviewed at three days and two weeks after the procedure by The Clinical Research Nurse. At the first appointment, only the outer Opsite PostOp™ dressings are removed. At the second appointment at two weeks post-procedure, all dressings are removed and the scratch area is cleaned with aqueous chlorhexidine. This appointment is identical to all subsequent review appointments in terms of the assessment of the subject and it follows a standardised regimen:

1. The subject arrives and hip dressings are removed over the scratches. The subject rests while completing the POSAS 'patient' assessment scale. This allows time for the skin microcirculation to equilibrate.
2. The Clinical Researcher assesses both scars with the POSAS Observer Scale
3. The Clinical Nurse assesses both scars with the Vancouver Scar Scale
4. Laser Doppler imaging for blood flow is undertaken on both skin scars
5. Standardised photography is undertaken on both scars
6. Chromameter skin hue is assessed with the Minolota chromameter on both skin scars
7. High frequency ultrasound scanning of the scars is undertaken with the Episcan device

After the second follow up appointment at two weeks, the Research Nurse confirms that the initial scratch wound has healed. All such wounds had healed at two weeks across 113 subjects in our previous study.[10] At two weeks, if the wounds have not healed, the application of silicone is delayed a further week, when the participant is reviewed again by the research team to confirm healing. Once the wound has healed, the subject is then given the supply of Cica-Care TSS, Opsite Post-Op™ and the envelope detailing the random allocation of sides to treatment or control. Only when the patient has returned home is the envelope opened. Over subsequent weeks, the patient only treats the side for intervention (left or right) and places an Opsite Post-Op™ over the top. The other side is to have Opsite Post-Op™ only. The dressings are worn for a total of 50 weeks.

The patient returns for follow up at 3, 6 and 12 months post-scratch. At each of these appointments, the observers are blinded to which side has been exposed to silicone. It has been noted previously that the site of silicone application can appear as more suffused than normal skin at the moment the dressing is removed. To prevent this risk of skin change revealing the intervention side, the subject is asked to remove the silicone sheeting two hours before their clinical assessments. Unless the subject has had an issue with a reaction to the silicone, they are asked to wear the TSS for as long as possible through the study. If the subject stops using the TSS on the intervention side, they must agree to make the research team aware. Overall compliance with silicone application will be assessed at each appointment. The sequence of follow up appointments is detailed in Figure 2.

5.819 Financial Incentive

Participants of the study will be given a financial incentive for taking part. They will receive £100.00 in total. On their first visit where the scratches are made, they will receive £50.00. A further £50.00 will be given at their final 12 month follow up appointment.

The financial incentive will be given as cash or via bank transfer to the patients account.

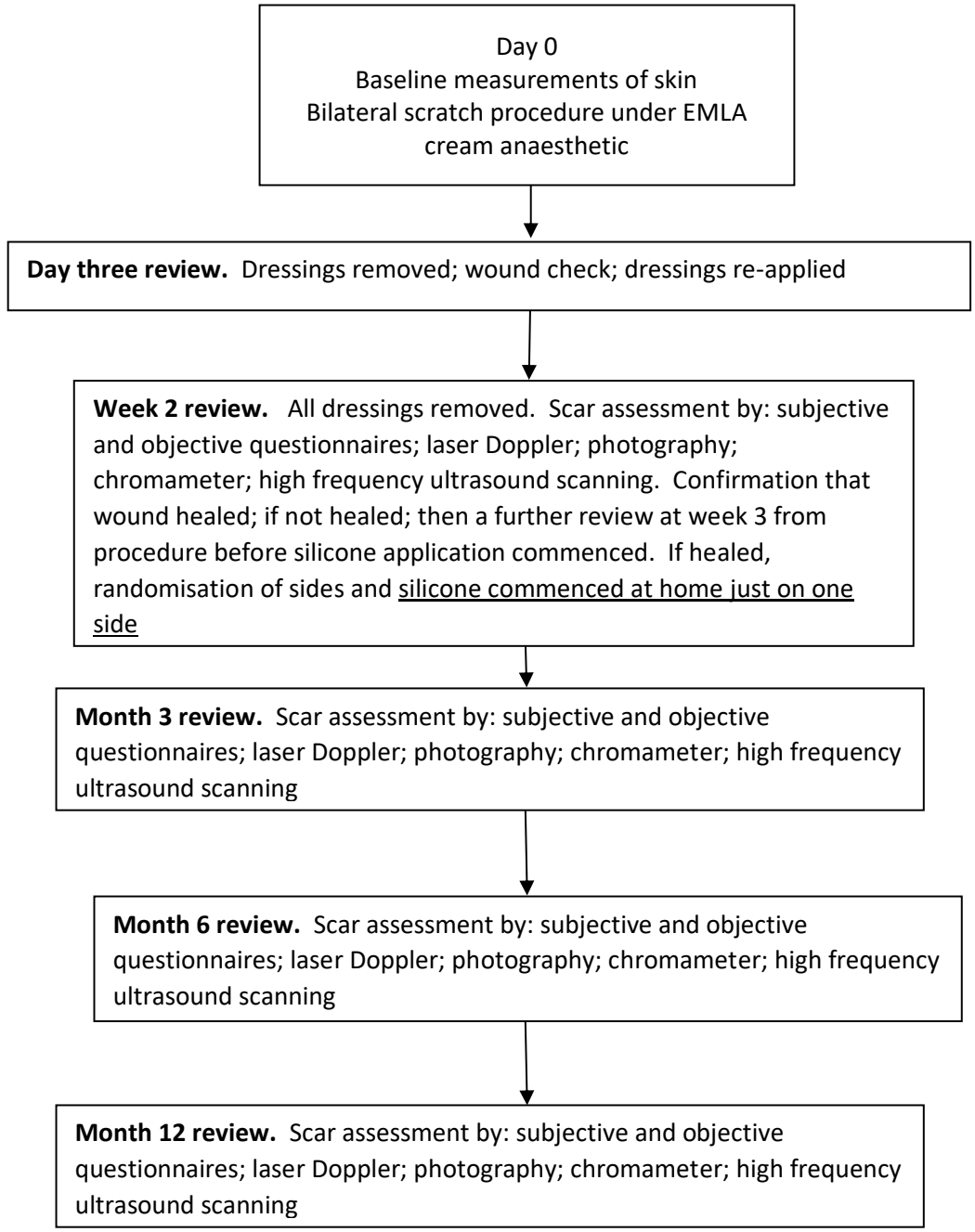


Figure 2. The process of scar analysis. Most steps completed by the principal investigators or The *Clinical Trials Practitioner (experienced research nurse)* specifically employed for trial. Note, the subject is informed that they can withdraw from the study at any time point.

5.82 Study Drug Supply

All serial numbers for EMLA™ and dressings will be entered into the clinical trial file. Accountability will be documented in the required fashion using a Pharmacy IMP information pack which will include an inventory log and dispensing log. All consumables will be acquired through NHS Logistics.

5.83 Concomitant Medication

For the area exposed to EMLA™, there is a theoretical risk of interaction with the following drugs. However, the overall concentration of EMLA™ is small and absorption is negligible on intact skin. Potential interactions include:

- Antiarrhythmics (e.g., amiodarone, dofetilide, mexiletine, tocainide), beta-blockers (e.g., propranolol), cimetidine, or other medicines containing lidocaine or prilocaine because the risk of side effects or toxic effects, including heart or nerve problems, may be increased
- Acetaminophen, acetanilid, aniline dyes (e.g., p-phenylenediamine), benzocaine, chloroquine, dapsone, naphthalene, nitrates (e.g., nitroglycerin, isosorbide), nitrites (e.g., sodium nitrite), nitrofurantoin, nitroprusside, pamaquine, para-aminosalicylic acid, phenacetin, phenobarbital, phenytoin, primaquine, quinine, or sulphonamides (e.g., sulfamethoxazole) because the risk of side effects, including blood problems, may be increased
- Succinylcholine because the risk of its side effects may be increased by EMLA® cream

All other agents involved in this study are not known to interact with any other medications. If the subject is on any of these drugs, this will be communicated to the PI, who will decide if the individual can participate.

5.84 End of study

All assessment of participants will be completed at 12 months following recruitment of the final volunteer. We anticipate that all follow-up of participants will be completed within 30 months of beginning the trial.

5.85 Assessment of Efficacy

The POSAS scar scale score will be the primary outcome measure. Scar assessment will consist of the following: blinded photographs will be assessed by a panel of four independent experts using an adapted version of the Manchester scale without the texture parameter (giving a maximum score of 24 – Appendix III) along with an intra-patient comparative scar scale (Appendix IV). Scars dimensions and colour will also be assessed using image analysis software provided by Zeiss. Once wound/scar assessment is complete, the data will be unblinded and will be analysed to see if there is a statistical difference in the grades of scarring between the TSS and no treatment (control) for all scars.

5.86 Assessment of Safety

5.861 Collection of toxicity data

The Surgical Research Nurse or Fellow will be responsible for collecting data on any potential adverse events that occur during the trial. All adverse events will be recorded in the Case Report Forms and will be reported and recorded in accordance with local and legislative requirements (as detailed in the section below on Recording and Reporting). The Surgical Research Nurse will report progress weekly to the Data controller and the Clinical Trial Coordinator including information on any adverse events that have occurred. All serious events will be communicated to the Clinical Trial Coordinator immediately (within 24 hours). This information will be disseminated immediately to the research team. Any clinical decisions relating to toxicity of any of the topical agents (EMLA®,

chlorhexidine, Cica-Care™, dressings) will be made by the Chief Investigator or in his/her absence, a co-investigator.

5.862 Definition of possible adverse events

5.863 Serious adverse events (SAEs)

Petechiae and purpuric skin reaction This is a very rare but mild side effect of EMLA® cream with several case reports in the literature.[19] The purpura typically resolves within two weeks.

Anaphylaxis has been reported as a rare complication of exposure to latex and chlorhexidine. A history of allergic reactions will be established in the first patient assessments to minimise this risk.

5.864 Adverse events (AEs)

Deleterious effects on wound healing

These are considered unlikely as topical silicone is already used widely for scarring and is available over-the-counter.

Skin irritation*:*

A transient skin irritation is sometimes noted with the application of topical aqueous chlorhexidine and with the dressings microporous tape, Opsite PostOp™ and Cica-Care™.

The lidocaine/prilocaine eutectic mixture within EMLA® is occasionally associated with transient skin blanching, erythema, urticaria, allergic contact dermatitis, irritant contact dermatitis and hyperpigmentation. Only one case of erythema was observed in the 113 subjects undergoing scratch injuries in the previous study.[10]

5.865 Recording and reporting of AEs, SAEs, SARs and SUSARs

All AE's will be reported to the Trial Coordinator (non-serious - within 7 days, serious –immediately) and recorded in the study file and source documentation. If applicable the AEs will be followed up until resolved. Also, the main ethics committee will be informed if applicable. SAEs and SARs will be reported to the South Buckinghamshire NHS sponsor within 24 hrs on the appropriate SAE form. The SAE/SAR will be recorded in the study file and source documentation and will be followed up until resolved (if applicable). The SAE/SAR will also be reported to the ethics committee if appropriate. If the SAR is unexpected (a SUSAR) the SAE form will be completed and faxed to the SMH sponsor as soon as possible. The SMH sponsor will report the SUSAR to the MHRA within 7 days for life-threatening SUSARs and within 15 days for all other SUSARs. The ethics committee will be informed. The SUSAR will be followed up, information recorded in source documentation and an annual safety report compiled for the SMH sponsor.

Any occurrence of adverse events will be communicated to the Trial Coordinator and the Trial Steering Committee (TSC) on a monthly basis or as soon as possible if the event is a serious one. This information will then be disseminated to the research team. The Trial Coordinator will take responsibility for ensuring that this process is adhered to. Any clinical decisions relating to adverse events will be made by the Chief Investigator, or in his/her absence, the co-investigator.

5.87 Subject Withdrawal

Subjects are free to withdraw from the study at any time with no change to their subsequent treatment and care. Subjects who withdraw consent or drop out of the study within the recruitment period (first 12 months of the study) will be replaced. There will be no follow-up or data use upon patients who withdraw consent. For participants that drop-out, data collected to this point will be used and the patient will be contacted and asked if they will attend another follow-up appointment.

All patients will be asked to volunteer reasons for their withdrawal (although they are not obliged to do so) and any reasons given will be documented in the Case Report Form.

5.88 Definition of protocol deviations/violations

The main protocol deviations that might affect the correct performance and interpretation of the study are any changes in standardised treatment of the two treatment sites both between wounds in the same patient and between patients such as the TSS application procedure or procedure of wound closure. Patients will be advised to inform the Surgical Research Nurse on the number given in the pack and who will record the details on the case report form. The Trial Coordinator and Data Controller will be informed of all deviations and violations of protocol.

5.89 Recording of data

Data will be recorded on the Case Report Form by the Surgical Research Nurse on the day of surgical procedure and at each appointment with the patient thereafter. All data will be held in a password-protected Excel (or equivalent) spreadsheet in accordance with the requirements for data protection set out by the Information Commissioner (ICO). The PI will be registered with ICO. There will be a backup of all data after each session to a hard drive or equivalent.

5.90 Statistical considerations

An opinion has been obtained from our statistician Dr Paul White.

5.91 Statistical analysis

The randomised split-body design will be analysed using standard statistical techniques for dependent designs. The paired samples t-test will be used to compare POSAS data between silicone sheeting against control at each review point. If assumptions underpinning the test are violated to

such an extent that reliability cannot be placed in the comparison then the bootstrap version of the paired samples t-test will be used.

5.92 Source data/documents/confidentiality

Data will be stored on password-protected computers at Stoke Mandeville Hospital and will only be accessed by authorised personnel. The password protected laptop is sited in locked private offices. Information stored on the laptop will be anonymised data on password protected files. The dates of follow-up appointments will also be stored. Photographic record of the wounds will be taken and identified by date and a unique patient identifier number only. Recognition of the patient will not be possible from these photographic records and no personal data will be used to identify these records. These photographic records will be stored securely in locked offices in a building not accessed by the public. All data given to the independent professional statistician for analysis at the end of the trial will have coded identifiers for each patient and will not contain any personal information. The data handler will be registered with the Information Commissioner.

The research results will be disseminated in the usual fashion at national and international conferences and in peer-reviewed biomedical journals. Patient identification will not be possible from the publications.

5.93 Quality control/quality assurance

The day-to-day monitoring of the trial will be performed by the Surgical Research Nurse and/or Research Fellow. The Surgical Research Nurse will report monthly to the Trial Coordinator, who will ensure that the trial protocol is adhered to, that the Case Report Forms are being completed by authorised persons, and Adverse Events (reporting as appropriate): and the Data Controller who will

ensure that the quality of photographic records is sufficient and standardised and who will monitor recruitment rates/losses. On-site monitoring visits by the Trial Coordinator will be performed quarterly to ensure adherence to the protocol and verify correct recording of adverse events.

The Trial Coordinator will report quarterly to the Trial Steering Committee (TSC). The TSC will provide overall supervision of the trial and ensure that it is being conducted in accordance with the principles of Good Clinical Practice and the relevant regulations. The TSC will review monitoring reports from the Trial Coordinator and the Data Controller and will use the information provided to improve trial methods, which will then be disseminated to the research team. The TCS will monitor the progress of the trial, including recruitment, Adverse Events, data completeness, the standardised quality of photographic records, patient withdrawal/drop-out rates, and ensure that there are no major deviations from the trial protocol. The TCS will consist of the Trial Coordinator, the Chief Investigator, the co-investigator, the Data Controller, and be chaired by an experienced scientist (Professor Dominic Furniss) who is not a trial investigator.

A data monitoring Committee is not considered necessary since this intervention-controlled trial is of a well-characterised, widely available topical treatment that is most unlikely to cause any unexpected adverse effects and is being undertaken to assess long-term benefit (over the year following treatment) with recruitment taking place over 18 months. Also, although the data will be collected throughout the trial (in the form of questionnaires, photographs and scans) the data will not be quantified until all the patients have received treatment.

A level of quality assurance is implicit in the trial design since the trial results will be assessed blindly by an independent panel of experts.

Investigators will meet both before and during the trial for trial-specific training and monitoring process to review knowledge and understanding of trial procedures. Attendance at these meetings and their content will be recorded. For the utilisation of all scar scales, a learning package will be instituted as there is a clear learning curve associated with the subjective interpretation of parameters such as colour, pliability, etc.

5.94 Ethical considerations

This trial will be performed in accordance with the principals of the World Medical Association Declaration of Helsinki and ICH Good Clinical Practice (GCP).

Specific ethical considerations that relate to the trial are:

Visible Scarring

Inherent in the study design is that the participants will have two small scars on the hip region that are permanent. Amongst the many reasons for choosing the planar surface of the hip, a prominent scar at 18 months post-procedure (very rare in the pilot study of 2007) would be hidden by clothing at the site of the lateral hip.

Asymmetry

If TSS does prove highly effective, this trial may result in asymmetry of the scarring between treated and untreated hips. However any asymmetry is likely to be temporary, lasting only as long as the excessive scarring takes to resolve naturally for this particular patient population (usually at approximately 24 months post-surgery when most scars are flat and pale). In this event, the patients remain likely to consider any improvement of scars an advantage even if on one side only.

5.941 Possible adverse effects of treatment

Skin irritation

Mild and transient skin irritation is occasionally seen as a result of TSS application. rarer side effects include frank allergic reactions and desquamation.

Other adverse effects

The scratch procedure will be administered while the skin is anaesthetised and therefore no discomfort will be felt.

5.95 Publication policy

The results of this trial will be published in international biomedical journals of standing through a peer-review process and presented at relevant international specialized conferences. Publication and presentation of the trials results will take place in the 18-24 months following the end of the trial. All funding bodies will be acknowledged.

5.961 Appendix I – Statistical recommendations

O'Brien and Jones DJ (2013) report eight treatment studies comparing silicone gel sheeting with no treatment (average sample size $n = 27$).[3] They further report an overall average standardized effect size equivalent to Cohen's $d = 0.5$ or larger in favour of silicone gel sheeting. It is acknowledged that these studies suffer from methodological limits which may have induced an upward bias in the effect size, but equally, the meta-analysis suggests powering a methodologically sound study for a standardised effect sizes in the medium effect size region (Cohen's standardized effect size $d = 0.50$ to 0.65) would not be unreasonable.

The Primary Outcome measure is POSAS II is known to have good clinometric properties. Table 1 summarises power for sample sizes in the region $n = 36$ to $n = 50$ for the split-body design. Based on the given assumptions a sample size of $n = 38$ would have approximately 90% power to detect a medium effect size (alpha = 0.05, two-sided, paired samples t-test). Such a sample size would be sufficiently large for valid application of the paired sample t-test.

The power in Table 1 is for superiority. It is further worth considering powering for superiority by a margin. Table 2 provides power to demonstrate superiority over a weak to small effect (Cohen's $d = 0.1$ and 0.2 respectively) against a medium sized effect (Cohen's $d = 0.5$, 0.55, 0.60, 0.65) translating to a clinically relevant difference of 0.3 or larger. These stronger considerations suggest a sample size of 40+ would have at least 80% power for demonstrating superiority over a weak effect ($d = 0.1$), but otherwise very large economically demanding sample sizes would be needed to demonstrate superiority by the lower bound margin.

Table 1. Relationship between sample size and power for standardized effect sizes in the medium range (0.50, 0.55, 0.60, 0.65) in the split body design.

Sample Size (N)	Effect Sizes	Powers
36	.50, .55, .60, .65	.83, .89, **.94, .96**
38	.50, .55, .60, .65	.85, **.91, .95, .97**
40	.50, .55, .60, .65	.87, **.92, .96, .98**
42	.50, .55, .60, .65	.89, **.94, .97, .98**
44	.50, .55, .60, .65	**.90, .95, .97, .99**
46	.50, .55, .60, .65	**.91, .95, .98, .99**
48	.50, .55, .60, .65	**.92, .96, .98, .99**
50	.50, .55, .60, .65	**.93, .97, .99, .99**

Table 2. Relationship between sample size and power for exceeding a margin of superiority (standardized effects 0.1 and 0.2) for true effects in the medium range (0.50, 0.55, 0.60, 0.65)

Sample Size N	Margin	Effect Sizes	Powers	Margin	Effect Sizes	Powers
36	0.1	.50, .55, .60, .65	.76, .84, .90, .94	0.2	.50, .55, .60, .65	.55, .66, .76, .84
38	0.1	.50, .55, .60, .65	.78, .86, .92, .95	0.2	.50, .55, .60, .65	.57, .68, .78, .86
40	0.1	.50, .55, .60, .65	**.80, .88, .93, .96**	0.2	.50, .55, .60, .65	.59, .70, .80, .88
42	0.1	.50, .55, .60, .65	**.81, .89, .94, .97**	0.2	.50, .55, .60, .65	.61, .72, .82, .89
44	0.1	.50, .55, .60, .65	**.83, .90, .95, .97**	0.2	.50, .55, .60, .65	.62, .74, .83, .90
46	0.1	.50, .55, .60, .65	**.85, .91, .95, .98**	0.2	.50, .55, .60, .65	.64, .76, .85, .91
48	0.1	.50, .55, .60, .65	**.86, .92, .96, .98**	0.2	.50, .55, .60, .65	.66, .77, .86, .92
50	0.1	.50, .55, .60, .65	**.87, .93, .97, .99**	0.2	.50, .55, .60, .65	.67, .79, .87, .93

5.962 Appendix II – Modified Vancouver Scar Scale

Figure 1. Modified Vancouver Scar Scale (5, 6)

Burn Scar Assessment:

Pigmentation (M):
0 Normal
1 Hypopigmented
2 Mixed
3 Hyperpigmented

Pliability (P):
0 Normal
1 Supple – flexible with minimal resistance
2 Yielding – giving way to pressure
3 Firm – inflexible, not easily moved, resistant to manual pressure
4 Banding – rope-like tissue that blanches with extension of the scar
5 Contracture – permanent shortening of scar, producing deformity or distortion

Height (H):
0 Flat
1 <2mm
2 2–5mm
3 >5mm

Vascularity (V):
0 Normal
1 Pink
2 Red
3 Purple

***Pain:**
0 Non
1 Occasional
2 Requiring medication

***Pruritus:**
0 Non
1 Occasional
2 Requiring medication

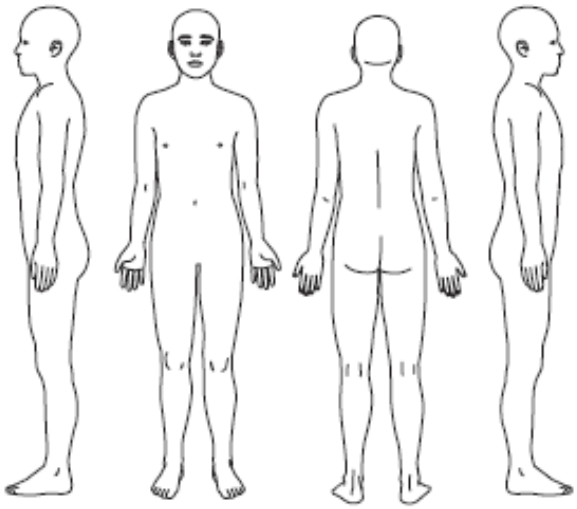
*not included in phase one

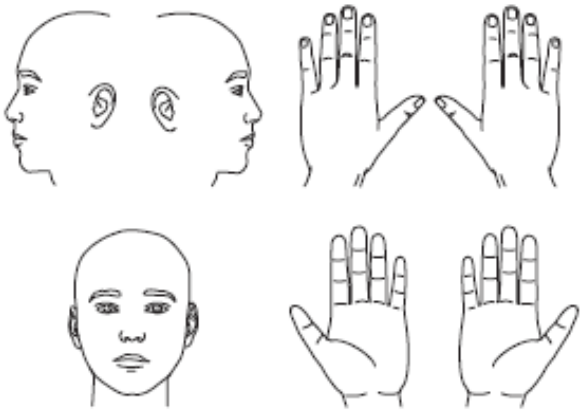
5.963 Appendix III – The Patient and Observer Scar Assessment Scale (POSAS) 2.0 Observer Scale

POSAS Observer scale

The Patient and Observer Scar Assessment Scale v 2.0 / EN

Date of examination:	**Name of patient:**
Observer:	
Location:	**Date of birth:**
Research / study:	**Identification number:**





1 = normal skin — worst scar imaginable = 10

PARAMETER	1	2	3	4	5	6	7	8	9	10	CATEGORY
VASCULARITY	○	○	○	○	○	○	○	○	○	○	PALE | PINK | RED | PURPLE | MIX
PIGMENTATION	○	○	○	○	○	○	○	○	○	○	HYPO | HYPER | MIX
THICKNESS	○	○	○	○	○	○	○	○	○	○	THICKER | THINNER
RELIEF	○	○	○	○	○	○	○	○	○	○	MORE | LESS | MIX
PLIABILITY	○	○	○	○	○	○	○	○	○	○	SUPPLE | STIFF | MIX
SURFACE AREA	○	○	○	○	○	○	○	○	○	○	EXPANSION | CONTRACTION | MIX
OVERALL OPINION	○	○	○	○	○	○	○	○	○	○	

Explanation

The observer scale of the POSAS consists of six items (vascularity, pigmentation, thickness, relief, pliability and surface area).
All items are scored on a scale ranging from 1 ('like normal skin') to 10 ('worst scar imaginable').
The sum of the six items results in a total score of the POSAS observer scale. Categories boxes are added for each item. Furthermore, an overall opinion is scored on a scale ranging from 1 to 10.
All parameters should preferably be compared to normal skin on a comparable anatomic location.

Explanatory notes on the items:

- **VASCULARITY** Presence of vessels in scar tissue assessed by the amount of redness, tested by the amount of blood return after blanching with a piece of Plexiglas
- **PIGMENTATION** Brownish coloration of the scar by pigment (melanin); apply Plexiglas to the skin with moderate pressure to eliminate the effect of vascularity
- **THICKNESS** Average distance between the subcutical-dermal border and the epidermal surface of the scar
- **RELIEF** The extent to which surface irregularities are present (preferably compared with adjacent normal skin)
- **PLIABILITY** Suppleness of the scar tested by wrinkling the scar between the thumb and index finger
- **SURFACE AREA** Surface area of the scar in relation to the original wound area

5.964 Appendix IV – The Patient and Observer Scar Assessment Scale (POSAS) 2.0 Patient Scale

POSAS Patient scale

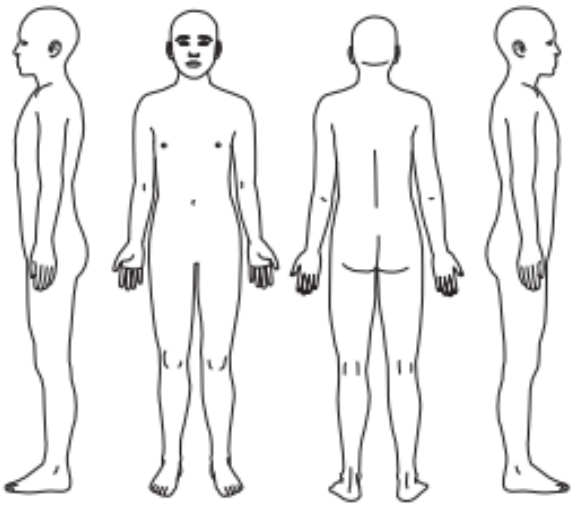
The Patient and Observer Scar Assessment Scale v2.0 / EN

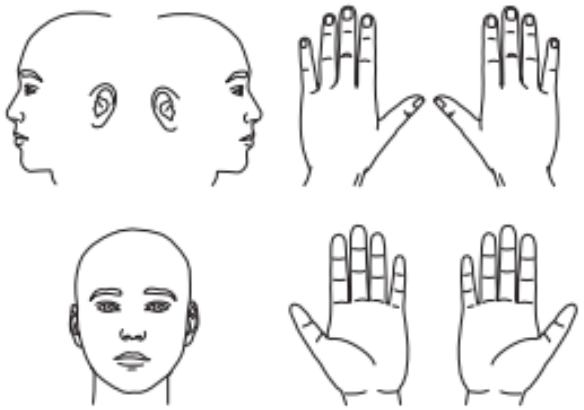
Date of examination: _____ **Name of patient:** _____

Observer: _____

Location: _____ **Date of birth:** _____

Research / study: _____ **Identification number:** _____





	1 = no, not at all									yes, very much = 10
	1	2	3	4	5	6	7	8	9	10
HAS THE SCAR BEEN PAINFUL THE PAST FEW WEEKS?	○	○	○	○	○	○	○	○	○	○
HAS THE SCAR BEEN ITCHING THE PAST FEW WEEKS?	○	○	○	○	○	○	○	○	○	○

	1 = no, as normal skin									yes, very different = 10
	1	2	3	4	5	6	7	8	9	10
IS THE SCAR COLOR DIFFERENT FROM THE COLOR OF YOUR NORMAL SKIN AT PRESENT?	○	○	○	○	○	○	○	○	○	○
IS THE STIFFNESS OF THE SCAR DIFFERENT FROM YOUR NORMAL SKIN AT PRESENT?	○	○	○	○	○	○	○	○	○	○
IS THE THICKNESS OF THE SCAR DIFFERENT FROM YOUR NORMAL SKIN AT PRESENT?	○	○	○	○	○	○	○	○	○	○
IS THE SCAR MORE IRREGULAR THAN YOUR NORMAL SKIN AT PRESENT?	○	○	○	○	○	○	○	○	○	○

	1 = as normal skin									very different = 10
	1	2	3	4	5	6	7	8	9	10
WHAT IS YOUR OVERALL OPINION OF THE SCAR COMPARED TO NORMAL SKIN?	○	○	○	○	○	○	○	○	○	○

5.965 Appendix V – The Manchester Scar Scale

Manchester Scale (Visual Scale) Parameters:

(1) visual analogue scale (VAS)

(2) colour

(3) surface

(4) contour

(5) distortion

(6) texture

The visual analogue scale is anchored at excellent and poor at the extremes of a fixed length scale. The position chosen along the analogue scale will be given a score by measurement between 0 and 10 at a later stage.

Color (compared to the surrounding skin)

1 Perfect

2 Slightly mismatched

3 Obviously mismatched

4 Gross mismatch

Appearance of skin over the scarred area

1 Matte

2 Shiny

Contour

1 Flush with surrounding skin

2 Slightly proud/indented

3 Hypertrophic

4 Keloid

Distortion

1 None

2 Mild

3 Moderate

4 Severe

Texture **(Note this section will be removed for grading of scars from photographs)**

1 Normal

2 Just palpable

3 Firm

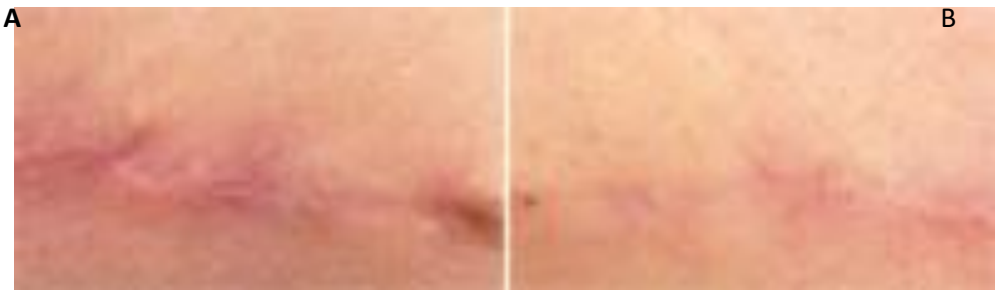
4 Hard

Total score for graded items = SUM (points for the 5 graded parameters)

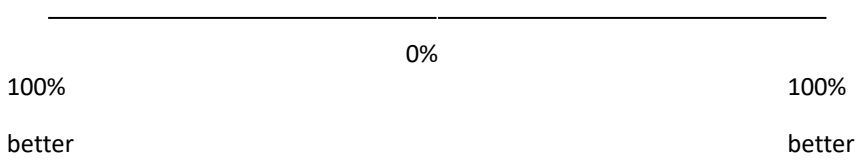
	Original scale	Adapted for grading of photographs
Interpretation:		
• minimum score:	5	4
• maximum score:	18	14
Plus points from the analogue scale		
• minimum score:	5	4
• maximum score:	28	24

• The higher the score the more abnormal the scar.

Appendix Vb Manchester Scar Scale Intra-patient comparative photographic scar scale
Example of utilisation of scale grading



A **B**



Combines a visual analogue scale and ranking in one scale

Self-anchoring scale

- 0 is a true measure of no difference
- score by first choosing the best scar
- use the worse scar as an anchor for determining the level of improvement of the other scar (marked as a percentage)

Extreme scores

- 0% = no perceptible differences between the two scars
- 100% = one of the scars is undetectable

5.966 Appendix VI - Advice on using Cica-Care ™

Cica-Care™ Silicone sheeting taken from the Smith and Nephew website (URL: http://www.smith-nephew.com/professional/products/advanced-wound-management/cicacare/; accessed 12th February 2017).

How to Use CICA-CARE

Take a look at the following 8 simple steps to effective treatment, and see just how easy it is to gently soften, flatten and fade red, dark or raised scars with CICA-CARE.

Wash your hands before use and then gently clean the scar and surrounding skin. Thoroughly dry the area before applying CICA-CARE.

Peel the lid from the tray containing CICA-CARE and remove the gel sheet.

With a pair of scissors, cut the sheet to the size of the scar - allowing a little overlap all the way round. Store the remaining gel sheet in a dry place, like the tray the gel came in.

Remove the printed plastic sheet to reveal the adhesive.

Apply CICA-CARE, adhesive side down, to the scarred area without stretching the strip. If your scar is in an awkward position you may wish to use a light bandage or tape to keep Cica-Care in position. Do not hold the gel sheet too tightly to the skin as this may cause irritation of the scar and surrounding area.

CICA-CARE should be applied to the scar for 4 hours on the first two days of use. The application time should then be increased by 2 hours a day to enable your skin to get used to the gel strip.

Clean CICA-CARE twice daily with a mild soap. Once you've cleaned the gel strip, rinse it well.

Dry the CICA-CARE strip with a non-fluffy towel before re-applying. Ensure the scar and surrounding area is washed too.

Treatment Times

Treatment times vary from person to person and depends on the nature of the scarring. However, on average and if used correctly you can expect best results after 2-4 months.

At first, CICA-CARE should be applied to the scar for 2 hours a day, building up by 2 hours a day to allow your skin to get used to the gel strip.

CICA-CARE can be washed and re-used. Each strip lasts between 14 and 28 days, making it a very cost-effective scar treatment.

Cleaning CICA-CARE

To remain fully effective, we recommend CICA-CARE is cleaned twice daily. During warm weather or during periods of increased physical activity, CICA-CARE should be cleaned more frequently to maintain maximum contact between the gel strip and the treatment area.

Cleaning CICA-CARE is straightforward. Simply wash the strip with a mild soap. Avoid using any other household cleaning products.

To dry CICA-CARE after cleaning, use a non-fluffy (lint free) cloth and, before reapplying, clean your scar area too.

For your comfort and convenience, CICA-CARE can be removed for you to wash and bathe as normal.

5.967 Appendix ab – Patient allocation form

Buckinghamshire Healthcare NHS
NHS Trust

A single blinded, randomised trial into the efficacy of silicone sheeting for cutaneous scars.

Dear Patient,

Thank you for taking part in our study.

You have been allocated to group X; this means you need to apply the silicone sheet to the scratch on your Y hip.

During your visits, please do not tell any of the research team on which side you are using the silicone.

Within this envelope, there is a page detailing how to apply the Cica-Care™ silicone sheeting. This will give you instructions on how to use your silicone sheet during the study. Now that the scar is forming, we would like you to apply the silicone sheeting as often as you can and ideally, all the time.

If you have any questions, please do not hesitate to contact a member of the research team on the following number …… .

Passive sentences 60%

Flesch Reading ease 72

Fiesch Kincaid Grave 7.1

5.968 Appendix bb – Patient Consent Form

Buckinghamshire Healthcare NHS
NHS Trust

IRAS ID: 237100

Centre Number:

Study Number:

Patient Identification Number for this trial:

PATIENT CONSENT FORM

Project: Trial into the effect of silicone on scar tissue in the skin of healthy people – The B.E.S.T Study
A single blinded, randomised trial into the efficacy of silicone sheeting for cutaneous scars. A Best Evidence Scar Treatment (BEST) Group Trial
IRAS ID Number: 237100

Lead Researcher – Dr Riyam Mistry
Chief Investigator – Miss Alexandra Murray

Please Initial

- I have read the Participant Information Sheet dated 25/11/19 version 2.6. The nature and purpose of the research project has been explained to me. I understand and agree to take part. ☐
- I understand the purpose of the research project, my involvement in it and have had the opportunity to consider the information and have questions answered satisfactorily. ☐
- I understand I will have a scar on both of my hips that will be permanent. I understand the possible risks and complications that can occur including small risks of infection and bleeding. In a minority of patients, scars can be temporarily itchy, red and raised. ☐
- I understand that I may withdraw from the research project at any stage and that this will not affect my care at Buckinghamshire Healthcare NHS Trust now or in the future. ☐
- I understand that while information gained during the study may be published, I will not be identified and my personal results will remain anonymous. ☐

- I understand that data will be stored on site at Buckinghamshire Healthcare NHS Trust and for the purposes of statistical analysis, The University of the West of England. ☐
- I agree to my General Practitioner being informed of my participation in the study including any necessary exchange of information between my GP and the research team. ☐
- I understand that information stored held and maintained by Buckinghamshire Healthcare NHS Trust may be used to help contact me or provide information about my health status. ☐
- I understand that I may contact the lead researcher (Riyam Mistry) or supervisor if I require further information about the project. Also, if I wish to make a complaint relating to my involvement in the research, I may contact the Chief Investigator at Stoke Mandeville Hospital (Alex Murray). ☐
- NHS Trust R&D staff may require access to the patient medical notes in the case of audit. ☐
- I agree to take part in the above study. ☐

Signed …… (research participant)

Print name …… **Date** ……

Signed …… (Person taking consent)

Print name …… **Date** ……

Contact details

Lead Researcher: *Riyam Mistry MBChB BSc(Hons) (Plastic Surgery Junior Research Fellow)*

riyammistry@nhs.net

Research Nurse: *Judith Abrams RN (Specialist Research Nurse)*

judithabrams@nhs.net

Chief Investigator: *Alexandra Murray MBChB, MD, FRCS (Plast) (Plastic Surgery Consultant) Alexandra.murray@nhs.net*

5.969 Appendix cc – BEST Study Patient Form

Patient Identification Number for this trial:

BEST Study Patient Form

1. Date of birth - -/- -/- - - -

   Aged between 18 and 60? Yes ☐ No ☐

2. Biological Gender

   Male ☐ or Female ☐

3. Fitzpatrick Skin Type

   I ☐ II ☐ III ☐ IV ☐ V ☐ VI☐

4. Aware that there will be scars on either hip?

   Yes ☐ No ☐

5. Aware of follow up commitments?

   Yes ☐ No ☐

6. Any history of psychiatric illness including body dysmorphia, depression or personality disorder?

   No ☐

   Yes ☐ give details

7. Any significant past medical history that could affect wound healing?

   No ☐

   Yes ☐ give details

8. Any use of anti-rheumatoid drugs, chemotherapy agents or steroids?

   No ☐

   Yes ☐ give details

9. Any planned radiotherapy?

   No ☐

   Yes ☐ give details

10. Any skin condition/disease that could affect the area under investigation?

    No ☐

    Yes ☐ give details

11. Any past or planned surgery to the research site?

    No ☐

    Yes ☐ give details

12. Any history of reaction/allergy to EMLA cream, aqueous chlorhexadine and dressings?

    No ☐

    Yes ☐ give details

13. Any medications that could react to EMLA cream?

    No ☐

Yes ☐ give details

14. Any personal or family history of keloid or hypertrophic scarring?

    No ☐

    Yes ☐ give details

15. Any pregnancy, or planned pregnancy during the course of the trial?

    No ☐

    Yes ☐ give details

16. Any issue that could interfere with communication or getting to the hospital for follow up clinics?

    No ☐

    Yes ☐ give details

Baseline Observations

- Pulse _____BPM
- Blood Pressure _____/_____ mmHg
- Temperature _____°C
- Oxygen saturations _____%

5.97 Appendix dd - Post-op Patient Information

Post-op Patient Information Leaflet

BEST Study

Thank you for taking part in our research study, below is some information about how to look after your operation site over the next 3 days.

Dressings

Please keep the dressings we have put on today in place until we see you at your follow up appointment in 3 days' time. The dressings are waterproof so should be okay in the shower but we do not recommend submerging them in water (such as a bath or swimming pool).

Pain/discomfort

As you leave today, the anaesthetic cream we put on will wear off in about an hour. You may experience some mild discomfort or pain at the site we have performed the procedure. This can be controlled with a bit of paracetamol; our experience in previous studies has shown that this is rarely required.

Bleeding

No bleeding is expected from the procedure site, however if you notice that the dressings seem to be soiled with blood, please contact us for advice on what to do next.

Things to look out for

If you notice any **redness, itch** or **swelling** around the site of the dressing please contact us. In the very rare event that the operation site gets infected, there may be signs of **smelly discharge** from the wound site and the **pain** may get significantly worst.

If you notice any of these, please contact us.

Your next appointment is on ____/____/_____ at ____:_____

We look forward to seeing you then.

(Insert allocated phone number for research unit in and out of hours)

Chapter 5 References

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