Chapter

The Need for Basic, Translational, and Clinical Research in the Field of Hypertrophic Scars

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Abstract

Hypertrophic scar (HTS) is a fibrotic skin disorder that is marked by excessive inflammation and extracellular matrix deposition in response to cutaneous traumatic injuries such as burns, lacerations, incisions, and abrasions. HTS has various risk factors, available treatments, and treatment effectiveness. Research at the basic, translational, and clinical levels are in their infancy compared to fibrotic diseases in other organ systems. This chapter will review current in vitro and in vivo modeling, and highlight research needs to address gaps in the study of HTS. The following topics will be discussed in the chapter: a. Basic Science Research i. Seminal findings ii. Limitations to these models iii. Suggestions for topics of future research b. Translational Science Research i. Seminal findings ii. Limitations to these models iii. Suggestions for topics of future research c. Clinical Research i. Seminal findings ii. Limitations to these models iii. Suggestions for topics of future research.

Keywords: hypertrophic scar, basic and translational research, clinical research

1. Introduction

Hypertrophic scar (HTS) is a fibrotic skin disorder that is marked by excessive inflammation and extracellular matrix deposition in response to cutaneous traumatic injuries such as burns, lacerations, incisions, and abrasions. Additional fibrotic skin disorders such as keloid scars are often thought of as being the same pathophysiology existing along a continuum of severity with HTS, and hence are often studied as one scar type, despite their varied etiology. HTS is one possible outcome of wound healing and has various risk factors, available treatments, and treatment effectiveness. Research at the basic, translational, and clinical levels are in their infancy compared to fibrotic diseases in other organ systems and compared to the study of keloids. This chapter will review current *in vitro* and *in vivo* modeling, and highlight research needs to address gaps in the study of HTS.

The following topics will be discussed in the chapter:

Modeling of HTS

a. Basic Science Research

i. Seminal findings

ii. Limitations to these models

iii. Suggestions for topics of future research

b. Translational Science Research

i. Seminal findings

ii. Limitations to these models

iii. Suggestions for topics of future research

c. Clinical Research

i. Seminal findings

ii. Limitations to these models

iii. Suggestions for topics of future research

2 Basic science research

2.1 Seminal findings

Hypertrophic scar (HTS) can be defined through its hallmark clinical, histologic, cellular, and molecular features. Each of these categories are intertwined and contribute to the complex nature of HTS (**Figure 1**). HTS has been examined in the laboratory in studies of basic science for decades, and these findings have led to a number of discoveries about the features of HTS as described below. Despite this plethora of findings, there are some limitations to current knowledge and plentiful area for future research. HTS and keloid scar are often grouped together into one scar phenotype, and research focused on keloid scar is often thought to be applicable to HTS as well. Despite the similarities in these two fibrotic skin phenotypes, HTSs exhibit distinct histologic, cellular, and molecular features from keloids. The focus of this chapter is on HTS and not keloids, as the comparison of these two scar types would warrant its own review.

2.1.1 Histologic features

At a structural histologic level, there are several features of HTS including increased epidermal thickness, greater number of epidermal cell layers, and profound dermal thickness where HTS can reach up to several centimeters thick [1, 2]. HTS resulting from full thickness injuries lack dermal appendages such as hair follicles, eccrine glands, and apocrine glands. They also lack rete ridges and associated dermal papillae [1], and have increased cellularity and vascularity [3, 4]. Disorganized collagen with decreased inter-fibrillar spacing is a hallmark feature of HTS. Collagen changes its organization from the basket weave structure of normal skin to the nodular and whorl-like structure of HTS (**Figure 2**) [5–9].

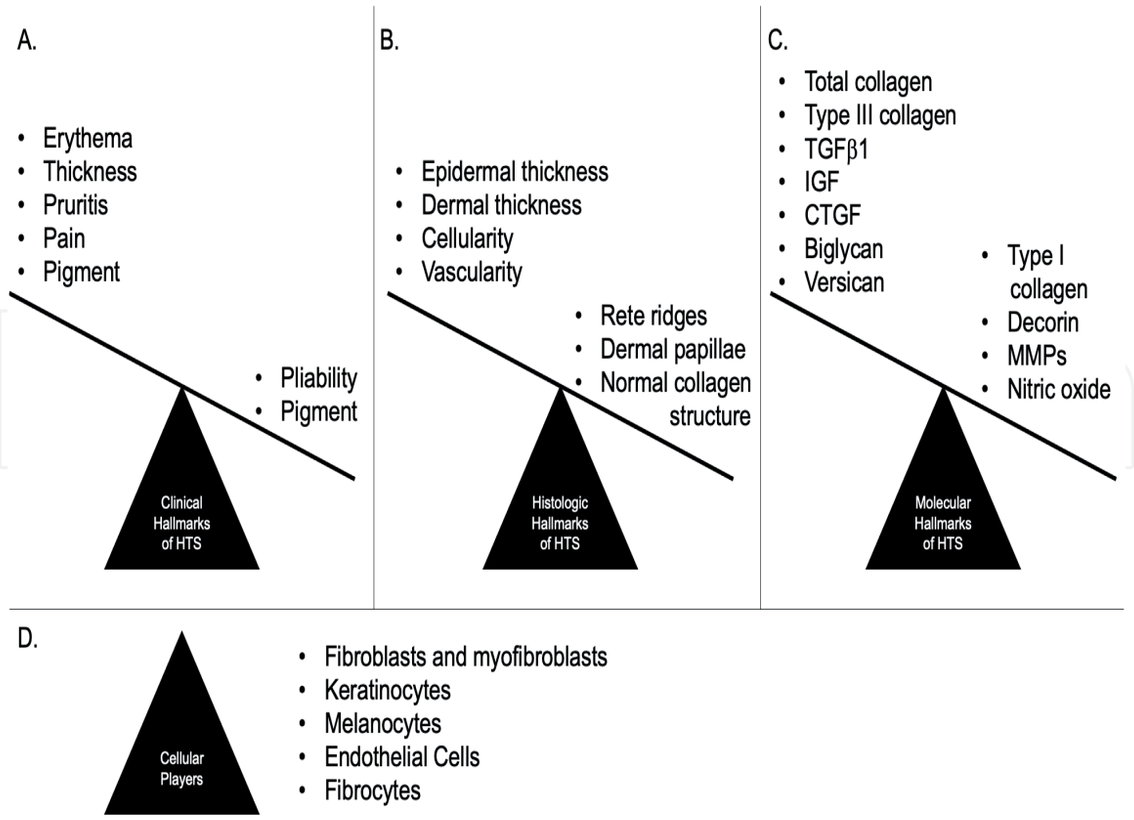

Figure 1.
There are four categories of hallmarks of HTS. These are clinical (A) histologic (B) molecular (C) and cellular (D).

2.1.2 Cellular features

2.1.2.1 Fibroblasts

Mesenchymal-derived fibroblasts are often thought of as the main contributors to the development of HTS due to the fact that they are the primary cell type which makes up the dermis. Since the dermis of HTS is thickened, and therefore comprises the large majority of the HTS volume, fibroblasts are the most populous cell type within HTSs. Fibroblast proliferation is upregulated in HTS and apoptotic processes are halted [10]. Fibroblasts are the cell type which deposit extracellular matrix (ECM). In normal skin, the processes of synthesis/deposition and remodeling of ECM exist in a delicate balance. In HTS, this balance is skewed towards synthesis with greatly reduced remodeling, resulting in scars with extreme elevation of ECM-related proteins (such as collagen types-1 and 2, tenascin, fibronectin, and tissue inhibitors of matrix metalloproteinase (TIMPS)) and downregulation of remodeling proteins (such as a multitude of matrix metalloproteinases (MMPs)) [11–13]. Fibroblasts participate in paracrine signaling with all of the other types of cells within HTS, and their production of proteins related to HTS is often regulated by these cells.

There is heterogeneity within fibroblasts with cells obtained from the papillary dermis having a different molecular signature compared to those derived from the reticular dermis [14]. Deep dermal fibroblasts are activated when a certain critical depth of injury is obtained [15]. Dunkin *et al.* used a graduated scratch model in 113 male and female healthy volunteers (ethnicity not reported) to determine this

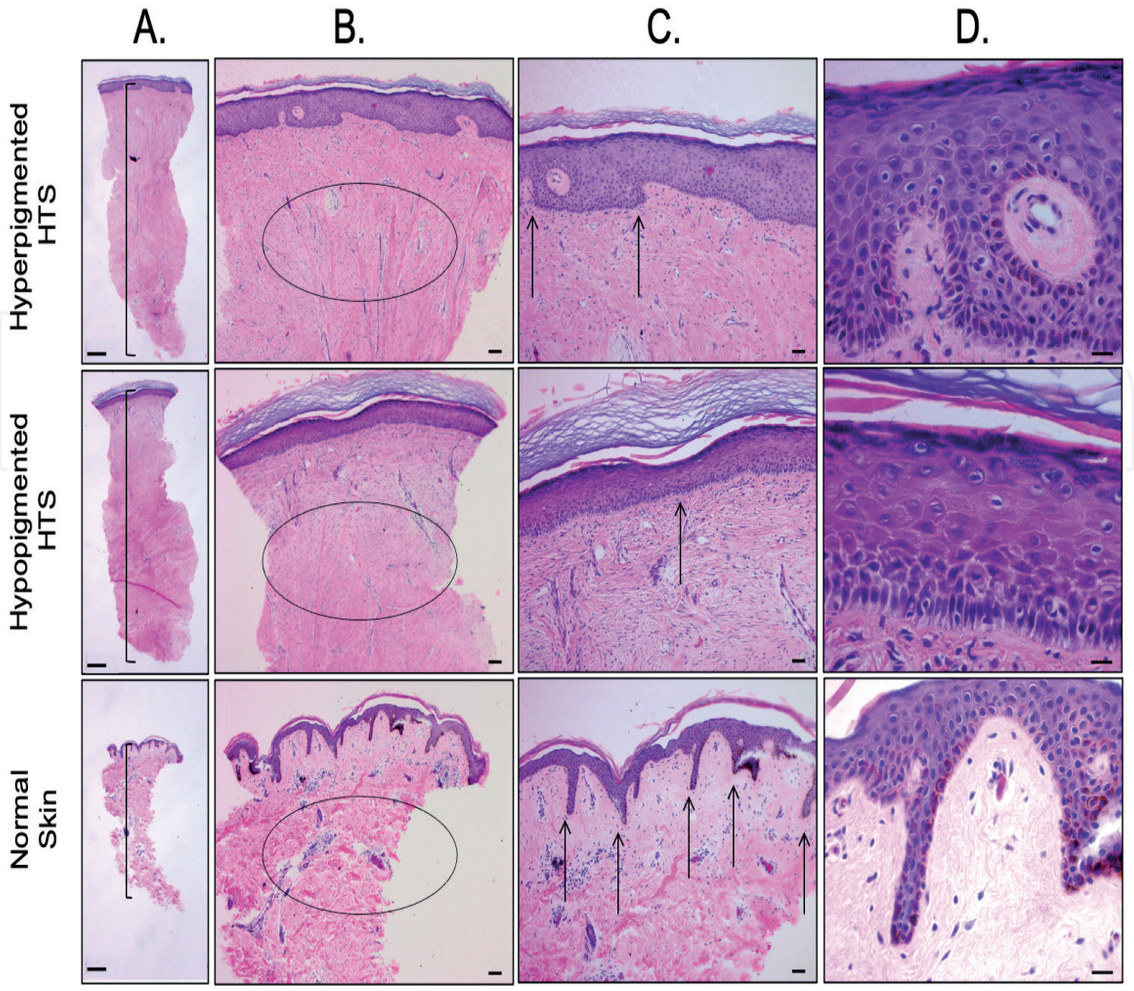

Figure 2.
Hematoxylin and &Eosin (H&E) showing histoarchitecture of HTS and skin. Regions of hyper- (top) and hypo (middle)-pigmentation share many of the hallmark characteristics of HTS compared to normal skin (bottom). Hyper- and hypo-pigmented scar and normal skin FFPE biopsies were stained with H&E. Scale bar= 500 μm at 1.25X (A) 100 μm at 5X (B) 50 μm at 10X (C) and 20 μm at 40X (D). Brackets indicate thickness (A) Circle indicates collagen organization (B) Arrows indicate rete ridges (C).

critical depth of 0.56 mm, or 33% of normal skin thickness at the hip where the wounds were created. HTS thickness was measured with high-frequency ultrasound scanning and showed that when wounds were made down into the deep dermis, HTS resulted, while injury to a superficial depth did not. The mechanistic reasoning behind this finding was elucidated when studying fibroblast cells derived from 5 different skin layers. Skin was collected from reductive plastic surgery cases (without demographic data reported), and a dermatome was used to section the skin into ~0.5 mm pieces. This study showed that fibroblasts of the deep dermis have a similar molecular signature to HTS fibroblasts [16]. This signature included increased alpha smooth muscle actin (α-SMA)-positive cells that produced more collagen and less collagenase, increased versican, and decreased decorin. Hence, injury depth within the dermis is a critical driving factor for the development of HTS.

Hypertrophic scars demonstrate increased numbers of myofibroblasts, with greater quantities in scars of earlier phases of remodeling as compared to late-stage remodeling scars [8, 9]. Myofibroblasts contribute to the increased contractility of fibroblasts, and their differentiation occurs through a transforming growth factor beta (TGFß)-mediated signaling pathway [17]. Myofibroblasts (which express α-SMA in a similar manner to vascular smooth muscle cells) have increased rates of ECM synthesis. Further compounding ECM synthesis is the contribution of mechanical tension and its ability to contribute to the mechano-sensitive regulation

of differentiation processes of fibroblasts to myofibroblasts [18, 19]. This is why wounds that heal under tension, such as across joints, are at great risk for the development of scar-related contracture [20]. Many studies aimed at the development of potential treatments for HTS are intertwined with either the suppression of fibroblast to myofibroblast differentiation, or subsequent myofibroblast secretion of ECM [8, 21].

2.1.2.2 Keratinocytes

HTS keratinocytes are less well-studied in comparison to fibroblasts and in the past were thought to have a small role in HTS pathophysiology due to their small abundance compared to fibroblasts. However, they have been shown to be important drivers of HTS due to paracrine signaling with fibroblasts and melanocytes [22]. Their appearance in the healing wound often signals the transition from the proliferative phase to the remodeling phase, and HTS develops most often in wounds where there was delayed re-epithelialization, implying that these cells have a critical role to play.

Keratinocytes are found in the epidermis, which is the outermost layer of the skin and comprises only a small portion of its overall structure, less than 10% of normal skin volume [23]. Despite its limited volume, it is a complex structure that is composed of four layers of ectoderm-derived stratified epithelial cells that form a continuum of differentiated keratinocytes (basal, spinous, granular, and cornified layers). Epidermal cells are replenished from epidermal stem cells that reside in the basal layer and originate from the bulge of the hair follicle. Once an epidermal stem cell is a resident of the basal layer of the epidermis, it is unipotent and can only differentiate to produce daughter keratinocyte cells [24]. The main function of the epidermis is to act as a barrier from the external environment, and hence protect from damage. Epidermal integrity is maintained by cell-cell and cell-matrix connections made by desmosomes, adherens junctions, hemidesmosomes, and other structures comprised of a variety of integrins, glycoproteins, and proteoglycans [24]. Epidermal integrity is important for the maintenance of skin homeostasis, and yet damage can occur, especially during wounding or burn injury, due to bacteria, fungi, viruses, ultraviolet (UV) radiation, heat, or chemical exposure. The structure of the epidermis in normal skin is such that the basal layer of keratinocytes forms rete ridges in a predictable pattern which contribute to its attachment integrity.

It is known that keratinocyte-fibroblast (K-F) crosstalk is important during normal wound healing, and that keratinocytes can promote the development of fibroblast fibrotic processes. In an in-situ hybridization and immunohistochemical study of 22 Caucasian patients that had HTS resulting from partial thickness burns, expression of TGFß-1, TGFß-2, TGFß-3, basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF) were evaluated at 1-, 4-, and 7-months post-burn [25]. Keratinocyte expression of all proteins was up-regulated at one month compared to normal site-matched skin from the same patients. At 4 months, some protein expression had returned to normal, and at 7 months, all growth factors were as expressed in normal skin. This data shows that keratinocyte cells are highly dynamic post-burn, and their signaling with growth factors is likely to significantly affect fibroblast cell processes. In a tissue-engineered model of 3D skin cultures, when keratinocytes derived from normal skin were seeded onto HTS fibroblasts, dermal thickness decreased. Similarly, when HTS keratinocytes were seeded onto normal fibroblasts, increased dermal thickness was likewise observed. This increase in thickness was regulated by collagen, MMPs, and apoptotic-related processes [26]. Another paper displayed the importance of K-F crosstalk when studying fetal keratinocytes of differing gestational ages and the effect of

their co-culture with fibroblasts on pro-fibrotic proteins. The co-culture of HTS fibroblasts with fetal keratinocytes (fetuses are known to produce scarless healing phenotypes) decreased proliferation of fibroblasts and decreased expression of collagen-1, α-SMA, and fibronectin [27].

A number of different methods can be used when characterizing keratinocyte influence on fibroblast cells or vice versa. One simple method is growing each cell type in monoculture and collecting the conditioned media (CM) from these cultures and exposing the other cell type to the CM. Co-culturing of the two cell types can also be accomplished, however, differences in the optimum media properties for the two different cell types complicate this technique. Trans-well assays can be used to bypass this difficulty. 3D skin equivalents (either manufactured or lab-grown) can be used for a more complex system, and finally, organotypic cultures are also useful in the study of K-F crosstalk [22].

It has been suggested that HTS has altered expression of a number of keratin proteins and additional proteins related to proliferation and differentiation of keratinocytes. These alterations, which are contradictory in different reports, indicate a role for these processes in the pathophysiology of HTS development (**Table 1**) [2]. Keratin proteins make up a component of the cytoskeleton in the stratified epithelial cells of the skin epidermis [35]. They are an intermediate filament that are either classified as acidic (type I) or as neutral-basic (type II). Type I and type II keratins form heterodimers that interact with each other to contribute to mechanical resilience within keratinocytes. In addition, the basement membrane attachment proteins (such as collagen IV, laminin-5 and integrin ß4) of the epidermis to the dermis are known to be altered in HTS [2]. This alteration is evident in a lack of hemidesmosomes and focal adhesion proteins.

Lastly, keratinocytes are also critical as one component of the multicellular "epidermal melanin unit" [36]. They interact with melanocyte cells in the induction of constitutive pigmentation and acquired damage-associated pigmentation. Their response to thermal damage and secretion of protein signals to neighboring cells is altered in post-burn HTS as discussed below.

2.1.2.3 Melanocytes

Melanocytes are derived from the neural crest and ultimately reside in the skin, mucous membranes, and retinal pigmented epithelium [37]. Melanocytes distribute evenly along the dermal-epidermal junction and there is no difference in melanocyte cell number, size, or shape between light and dark skin [38]. There are, however, regional variations within individuals with the face and foreskin containing the largest number of melanocytes per area and the abdomen and lower extremities containing the fewest cells [36]. Melanocyte precursor cells, termed melanoblasts, reside in the hair bulb and can be mobilized upon injury to re-pigment healing skin. When dermal appendages are destroyed in full-thickness injuries, melanocytes repopulate healing wounds; however, the origin of these cells is not well studied and is currently unknown. Presumably, a pool of melanocytes migrates from the wound edges. It is also possible that blood-derived cells home to the wound bed, differentiate, and form a second pool of melanocytes. Melanocytes are characterized by the presence of two or more dendritic processes [36]. They are in contact with many keratinocytes and are able to interact with these cells through the extension of dendrites into a network of epidermal cells. One melanocyte can interacts with up to 30 keratinocytes.

The melanocyte and the keratinocytes with which it interacts form the multicellular "epidermal melanin unit" [36]. The two cell types work as a unit to determine the pigmentation phenotype of skin. Constitutive skin pigmentation is apparent

Protein	Type	Localization in epidermis of normal skin	Function	Role in HTS
Keratin-1	Neutral-basic (type II)	Spinous and granular layers	Interacts with K10 and desmoplakin.	Increased staining intensity towards the cornified layer compared to normal skin [28].
Keratin-2	Neutral-basic (type II)	Upper spinous layer	Associated with keratinocyte activation, proliferation, and keratinization.	Unknown
Keratin-5	Neutral-basic (type II)	Basal layer and suprabasal layer	Interacts with K14. Anchored to desmosomes via desmoplakin and plakophilin-1.	No different from normal skin [28]. Present In all layers of HTS with an increased number of epidermal cell layers [2]
Keratin-6	Neutral-basic (type II)	All layers	Interacts with K16 or K17. Activation of follicular keratinocytes during wound healing. Associated with hyper-proliferation. Induced by skin injury [29].	Up-regulated in HTS vs. normal skin throughout all suprabasal layers with strong staining [28]. Low or absent expression in normal skin [1].
Keratin-9	Acidic (type I)	Cornified layer	Expressed in palmo-plantar skin to relieve stress-bearing by increasing mechanical resilience.	Unknown
Keratin-10	Acidic (type I)	Suprabasal and cornified layers	Associated with differentiation.	Normal expression [1]. No different from normal skin [28]. More suprabasal staining compared to normal skin [2].
Keratin-14	Acidic (type I)	Basal layer and suprabasal, but not cornified layer	Interacts with K5	No different from normal skin [2, 28]. Present In all layers of HTS with an increased number of epidermal cell layers [2].
Keratin-15	Acidic (type I)	Basal layer	Interacts with K5	Present In all layers of HTS with an increased number of epidermal cell layers [2].
Keratin-16	Acidic (type I)	All layers	Interacts with K6. Associated with hyper-proliferation. Induced by skin injury [29].	Up-regulated in HTS vs. normal skin throughout all suprabasal layers [28, 30, 31]. Low or absent expression in normal skin [1].
Keratin-17	Acidic (type I)	All layers	Interacts with K6. Associated with hyper-proliferation and tumor growth [32].	Up-regulated in HTS vs. normal skin [28, 30]. Low or absent expression in normal skin [1].

Protein	Type	Localization in epidermis of normal skin	Function	Role in HTS
Keratin-19	Acidic (type I)	Basal layer	Does not interact with a type II keratin. Marker of epidermal progenitor cells [2].	Not detectable in HTS [2].
Involucrin	N/A	Spinous and granular layers	Transglutaminase substrate protein. Is a precursor to cornification in keratinocytes	In normal skin, common staining in the granular layer, HTS showed increased spinous layers and some basal layers [1].
Loricin	N/A	Cornified layers	Expressed in terminally differentiated keratinocytes [33].	Normal expression [1]
Filaggrin	N/A	Granular and cornified layers	Interacts with keratin proteins. Important for epidermal barrier function and homeostasis.	Normal expression [1]. Differential gradient density in HTS vs. NS [28].
Ki67	N/A	Basal layer	Proliferation marker	In HTS, percent of positive cells in basal layer was the same as normal skin [1]. Ki67 positive cells were increased in HTS from breast reduction surgery at 3 months compared to normotrophic scars [31]. Increased expression in suprabasal layers in HTS [34].

Table 1.
Keratinocyte proliferation and differentiation proteins. Localization in normal skin, function, and role in HTS.

and can be observed in people of different races where, at baseline, without response to damage, there are different levels of pigmentation in people with different genetic backgrounds [39]. The induction of pigmentation can also occur when keratinocytes regulate exposure to the outside environment by processing and secretion of damage-associated environmental signals. Melanocytes then receive these protein signals and respond by upregulating pigmentation machinery within the cell. When pigment is produced, melanocytes package it into melanosomes, transfer it back to keratinocytes along their dendritic processes, and keratinocytes house melanin where it is used in a variety of functions [39]. It is clear that pigmentation develops due to an increased rate of melanogenesis, and not proliferation of melanocytes [36].

Keratinocytes and melanocytes form the basis of investigation when attempting to develop treatments for dyschromia in burn-related HTS. Most pigmentation disorders such as Hermansky-Pudlak Syndrome, Waardenburg Syndrome, Type I Occulocutaneous albinism (OCA), Piebaldim, and Temperature-Sensitive OCA are genetic in origin and are a result of mutations to genes involved in melanogenesis [40]. Because burn dyschromia is a result of trauma, and not genetics, it is possible that it can be treated and potentially reversed. The first suggested treatments were reported in the 1980s [41, 42]. Onur *et al.* used a technique whereby they employed dermabrasion in the hypopigmented HTS area to ablate the epidermis and grafted

this area with thin (0.2-0.3 mm) skin grafts. They were able to show "adequate repigmentation" in their case series of 18 patients. In 1991, investigators in the United States published a similar study where hypo-pigmented HTS area was also prepared using dermabrasion [43]. An "epidermal" sheet graft was then taken at a depth of 0.0006 inches (0.015 mm) using a dermatome. This method used a much thinner graft that contained only epidermis so as to limit donor site dyschromia. Eighty six percent of patients had a good result and 13% had an excellent result. The same group extended their findings in a 1996 publication of the same title where they added an additional 21 patients to their case series [44]. Their findings were similar and somewhat improved over their previous study. Similar studies continued to occur, one reported substituting dermabrasion with flash-scanned CO_2 laser to prepare the HTS area for thin split-thickness grafting (STSG) [45]. The papers described above report outcomes for patients treated exclusively for hypopigmented HTS. As time went on, this technique was also used to treat hyperpigmentation [46, 47]. In 1996 [48] and 1997 [49], concise reviews were published in *Burns* and the *Journal of Burn Care and Research*, respectively. They summarized the limited treatment options for postburn dyspigmentation. They described thin STSG after dermabrasion as the only surgical technique that was available at the time. The only other treatments that were mentioned were medical tattooing and temporary makeup [50–53]. Even as late as 2016, a paper was published showing the results of a retrospective chart review of patients who underwent dermabrasion and thin STSG from 1997-2007 [54]. Today, in many centers around the country, many patients are told that their only option for treatment of dyschromia is to use makeup or tattooing. Thin STSG treatment has benefits as discussed above; however, complications can occur. Some patients have experienced hyperpigmentation of the grafted site and donor site after sun exposure and cyst formation beneath grafts [42]. In addition, in patients with large TBSA injuries, who have extremely limited areas of normally pigmented skin, this sort of surgical correction is not an option. Lastly, there are only a few surgeons in the United States who perform this operation.

With this literature in mind, it is clear that none of these treatment methods are based on mechanistic reasoning. They rely on the transfer of cells or tissues from unaffected areas of the body to the HTS site. They can also lead to pigmentation abnormalities at the donor sites. As such, over the past many years, our lab has sought to investigate the mechanism of action of HTS dyschromia to develop treatments with a mechanistic basis that may be more efficacious, tissue sparing, and more widely applicable than prior methods.

Our group came upon the study of dyschromia while conducting a study on the effectiveness of pressure therapy in a Red Duroc pig model of HTS [55]. The scars for study also developed dyschromia with areas of hyper- and hypopigmentation. These scars closely resembled HTS that was observed in our patient population, and hence, samples were acquired for study. Grossly, the scars were hyperpigmented on the periphery with small islands of hyperpigmentation in the interior surrounded entirely by hypopigmentation. The inside of the scars were mostly hypopigmented. Unexpectedly and interestingly, due to the dogma that is currently reported in the literature, we discovered that melanocytes were present in equal amounts in regions of hyper- and hypo-pigmentation by immunofluorescent staining for a melanocyte marker, S100B [56]. This work also showed increases in staining for melanin by azure B and melanin activation proteins, alpha melanocyte stimulating hormone (α-MSH) and human melanoma black 45 (HMB45) in hyper- compared to hypopigmented scar. We next sought to further confirm the presence of melanocytes in regions of differential pigmentation, as well as look more in depth into the canonical pigmentation signaling cascade [57]. Melanocyte presence was confirmed by multiple assays including primary culture of these cells. In addition, a number of

target molecules were shown to be up-regulated at the mRNA and protein levels in hyper- vs. hypopigmented scar, including pro-opiomelanocortin (POMC), adrenocorticotropin hormone (ACTH), stem cell factor (SCF), melanocortin 1 (MC1R), stem cell factor ligand (cKIT), tyrosinase (TYR), tyrosinase-related protein-1 (TYRP1), and tyrosinase-related protein-2 (TYRP2) or dopachrome tautomerase (DCT). While this work was useful in elucidating the molecules of interest that are up-regulated in hyperpigmented scar, and downregulated in hypopigmented scar, a mechanistic reasoning behind the dysregulation in the first step in the pigmentation signaling cascade was not revealed in this work. Canonical signaling by a DNA damage and p53-associated pathway were ruled out, as these moieties were not differentially regulated in different pigment phenotypes. In subsequent work, POMC was further investigated as a potential root cause of hyper- and hypopigmentation. Methylation of POMC's promoter was studied; however, there were no differences to suggest that methylation is the cause of dyschromia [58]. In addition, we used full transcriptome microarray analysis to identify a number of pathways that were differentially regulated between the two pigmentation phenotypes. These pathways provided us with additional and future avenues of study for preventing and treating dyschromia [58]. Some of this work is summarized in **Figure 3**. These studies are currently underway in our laboratory, and include treating areas of hypopigmentation with pigmentation stimulators as reviewed in our recent paper [59]. A recent paper by Dutta *et al*. is one of the few other mechanistic papers investigating

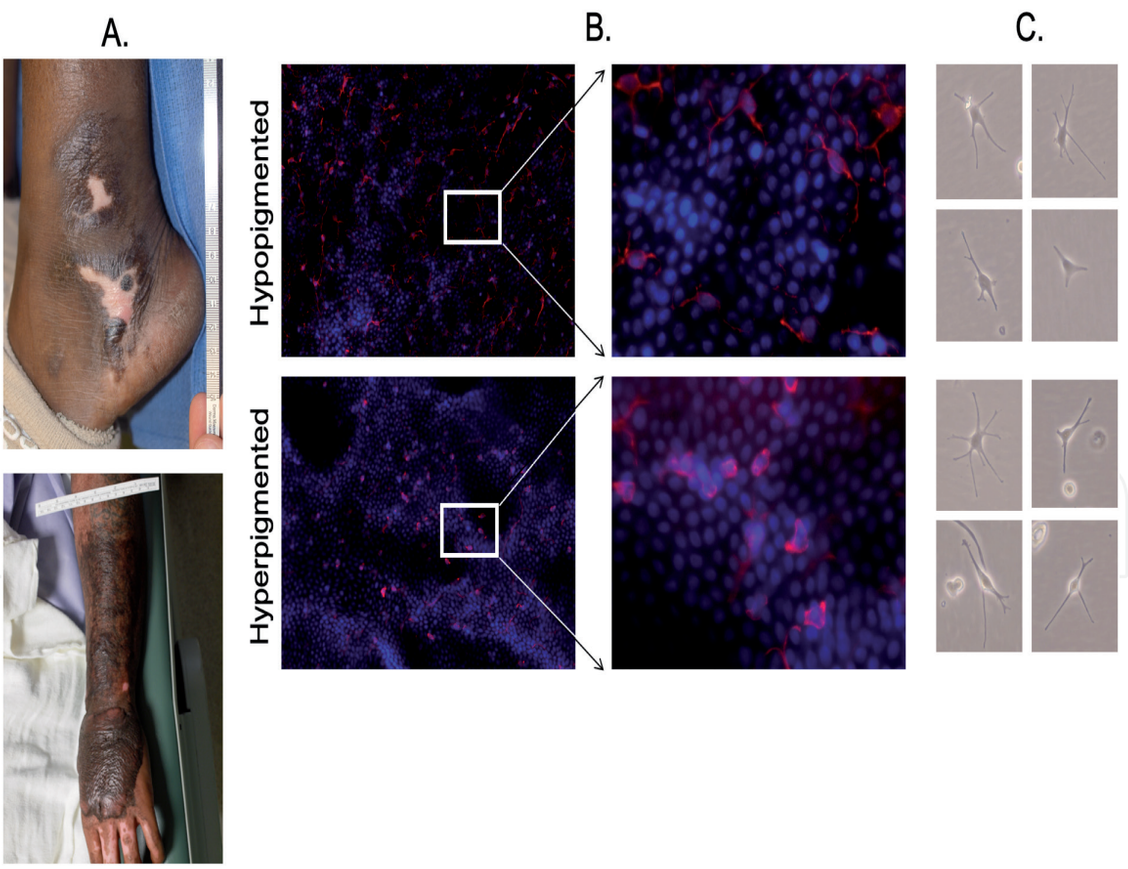

Figure 3.
Burn injury can result in dyspigmented scar that contains regions of hyper- and hypo-pigmentation with melanocytes in equal numbers. Examples of hyper- and hypo-pigmented burn HTS from two different patients (A) Epidermal sheets were stained for melanocyte marker S100β by en face staining. S100β (red), DAPI (blue). Scale Bar= 50μm at 10X (left) or 10μm at 40X (right) (B) Cells from regions of hyper- or hypo-pigmentation were isolated and imaged using phase contrast at 40X (B) Melanocytes and dendrite were counted in each region of pigmentation (C) Patient photographs were collected under an IRB-approved protocol and shared with patient consent.

hypopigmentation in burn patients [60]. This paper studied immunohistochemical staining for cytokeratin 5, MC1R, Ki67, loricrin, and TYRP1. They also showed that dendricity in melanocytes was altered in hypopigmented cells in cell culture. This is a valuable study of dyschromia in HTS, but also does not reveal a mechanistic cause of hypopigmentation as the work is mostly aimed at characterization. The study of HTS is still a relatively new area of research compared to some other organ systems where fibrosis has been studied extensively. Dyschromia in HTS is an even newer topic of study and should be a priority due to its psychosocial effects for patients with HTS.

2.1.2.4 Endothelial cells

Endothelial dysfunction is a newer topic of study in the setting of trauma [61], sepsis [62], and burn injury [63]. It is known to play a role in acute burns where circulating levels of a proteoglycan component of the glycocalyx which is shed upon injury, syndecan-1 (SDC-1), is up-regulated in a dose-dependent manner in relation to injury severity. The glycocalyx is a complex meshwork of proteoglycans that line the luminal surface of blood vessels [64]. Circulating SDC-1 levels measured by ELISA can predict mortality, and the amelioration of the shedding of the endothelial glycocalyx is a topic of current research in illuminating best practices for burn shock resuscitation [65, 66]. Due to the link between acute burn care and the long-term systemic effects of burn injury, it is hypothesized that endothelial dysfunction likewise plays a role in the development of HTS, and if it can be ameliorated, then HTS may be able to be prevented or treated.

One characteristic of HTS that provides evidence for this link is the erythematous nature that can result in purple, red, or pink scars that tend to improve over time. This erythema results from hyper-vascularity from an increased presence of blood vessels within these scars [67, 68]. Laser doppler imaging has shown an increased microcirculation and perfusion in HTS compared to surrounding unburned skin [69]. This data is somewhat contradictory to reports that; although there is increased blood vessel presence, these vessels are often totally or partially occluded through unknown mechanisms.

HTS are sometimes thought of as benign tumors that are "fed" by this vasculature [70]. In comparison to fibroblasts and keratinocytes, endothelial cells in HTS are less well-studied [71]. They gained recognition when a link between hypertension and increased hypertrophic and keloid scar severity was reported [72]. Hypertension can directly affect vascular function and as such, endothelial dysfunction is thought to play a role in scar development.

Pressure therapy is thought to work in part by "starving" the scar by inducing hypoxia [4]. Multiple non-pressure-related studies that target VEGF or angiogenesis have been studied to alleviate scar [73–75]. One such drug is endostatin which is a potent endothelial cell proliferation inhibitor. Endostatin inhibits angiogenesis and has also been shown to have an effect on tumor growth and metastasis. Its effects have been studied in a rabbit ear model of HTS where it was injected intralesionally [76]. Treatment with endostatin resulted in decreased scar elevation index, decreased thickness, decreased microvessel density, and changes to collagen organization. Interestingly, mechanistic studies have focused on the effect of endostatin treatment on fibroblasts, and not endothelial cells [77, 78]. In addition, it has been shown in keloid scar that not only epithelial to mesenchymal transition (EMT), but endothelial to mesenchymal transition (EndoMT) may play a role in scar development [79]. Endothelial pericytes may undergo EndoMT to become myofibroblasts which secrete ECM [80]. This concept should be further studied in the context of HTS as well.

2.1.2.5 Fibrocytes

Fibrocyctes are a peripheral blood mononuclear cell population (PBMC) making up 0.5% of total leukocytes. These cells home to tissues during wound repair and play a role in fibrosis [81]. They are termed as such due to their "fibroblast-like" properties and spindle shape in adherent cell culture. They are an interesting cell type because prior to their discovery in 1994, it was thought that all of the cells that contribute to wound healing migrated from surrounding areas of un-injured epidermis or dermis; however, blood-borne cells are now known to be critical for wound healing as well. When these cells differentiate, they lose hematopoietic markers and gain mesenchymal markers such as collagen, vimentin, cluster of differentiation 34 (CD34), and α-SMA. These cells target wound sites during the initial stages of injury and contribute to the inflammatory phase of healing by secreting a distinct profile of cytokines and chemokines, hence chemo-attracting other inflammatory cells. In addition, fibrocytes are known to be involved in numerous fibrotic diseases such as pulmonary fibrosis, asthma, atherosclerosis, and renal fibrosis [82–84]. They are present in post-burn HTS [85] where they are known to contribute to the proliferative phase by secreting ECM and the remodeling phase by secreting MMPs. They also regulate fibroblast activity through signaling with TGFß-1 and connective tissue growth factor (CTGF) by increasing cell proliferation, and migration, and increasing α-SMA expression, hence increasing the contractility of collagen [86]. Fibrocytes have been identified as circulating cells in burn patients with a dose-dependent response in the number of circulating cells with increasing injury severity [87] and in a model of HTS in Red Duroc pigs [88]. Fibrocytes are a potential cell type to focus on when developing targeted therapies for systemic or local treatments for HTS. Indeed, when patients with HTS were treated with interferon-alpha (IFN-α) 2b, which was shown to stop fibrocyte differentiation in a dose-dependent fashion, the number of fibrocytes in HTS tissues was reduced, and the activity of the remaining cells was likewise reduced [89]. There may also be additional blood-derived cells of importance in regulating HTS [90].

2.1.3 Molecular features

There are also several molecular hallmarks of HTS including upregulation of overall collagen [91] with shifts in the ratio of type I and type III collagens [11, 12] in superficial and deep dermis. This upregulation generally results in higher levels of type III collagen in HTS [12]. TGFβ1 [92], insulin-like growth factor 1 (IGF1) [93], CTGF [94], platelet-derived growth factor (PDGF) [95], biglycan, pleiotrophin [96], and versican [97] are all upregulated, while decorin [97], MMPs, IFN-α 2b [98], interferon-gamma, and nitric oxide are downregulated compared to normal, uninjured skin [96, 99–103].

The complexity of HTS development is not only in mRNA transcriptomes that code for proteins, but in non-coding RNAs such as micro-RNA (mi-RNA), circular RNAs, and other long-non-coding RNAs. mi-RNAs bind to mRNA and most often lead to their post-transcriptional degradation prior to protein coding. Hence, mi-RNA can have a drastic effect on protein expression. In the last 6 years, a flood of papers describing the effect of a multitude on mi-RNAs on HTS fibroblasts have been published. Dahai and colleagues have led the way with their work on mi-RNAs 21 [104], 130a [105], 155 [106], 192 [107], and 494 [108]. mi-RNA-21 is one of the most extensively studied with 3 papers claiming that aberrant mi-RNA expression has a role on the fibroproliferative effects of HTS fibroblasts [104, 109, 110]. Additional mi-RNAs 145 and 200b have also been studied by more than one group and have been

connected to TGFß and Smad2/3 signaling pathways [111, 112]. Numerous others mi-RNAs have been studied such as mi-RNA 181b which regulates decorin production in fibroblasts [113], mi-RNA 22 which promotes fibroblast apoptosis [114], mi-143 which targets CTGF [115], mi-185 and mi-29 which both regulate TGFß and collagen-1 expression [116], and mi-137 which regulate pleiotrophin [117]. Circular RNA [118] and long-non-coding RNAs [119, 120] also seem to play a role in HTS.

2.2 Limitations to these models

2.2.1 Histologic

As shown above, a large number of studies use either freshly cut or formalin-fixed paraffin embedded tissue sections to study HTS at the histologic level. In patients, longitudinal biopsy studies are uncommon, though possible. As such, biopsies represent a snapshot in time and not the dynamic nature of HTS remodeling. Patients often have multiple hundreds of square centimeters worth of HTS. In addition, these areas of scar may look heterogeneous due to staged, variable acute burn interventions. Especially in large TBSA injuries, HTS phenotypes may differ regionally, and one small, often 3-mm tissue biopsy, is often not sufficient to display the heterogeneity (of color, thickness, elasticity) of scar within a particular patient.

2.2.2 Cellular

The co-culturing of keratinocyte and fibroblast cells is far more developed in the study of keloid scar compared to HTS where there are only a few papers which utilize these techniques [121, 122]. Due to the difficulty of obtaining HTS tissue from which to derive cells, and the lack of a universally agreed upon animal model to provide these cells, experiments attempting to understand HTS-related fibrotic processes often use skin cells from either immortalized lines (such as HaCat cells), or from normal skin donors from reductive plastic surgery cases [123]. These cells are inferior to using HTS-derived cells. Additionally, HTS resulting from cutaneous non-burn related trauma may have different mechanisms. Therefore, papers studying HTS from surgical incisions, such as in breast reduction surgery, may not apply to more severe HTS, such as that encountered post-burn injury [124]. Additionally, melanocytes are often not incorporated into *in vitro* models of HTS, under-emphasizing the importance of their role as photo-protectors of keratinocytes. Endothelial cells likewise are under-utilized in *in vitro* modeling of HTS, and should be added when attempting to model the full complexity of HTS. Fibrocytes and other blood-derived cells should also play a role in *in vitro* modeling for a more complete picture.

2.2.3 Molecular

After deriving primary cell cultures from HTS lesions and culturing cells *in vitro*, these cells often lose their molecular phenotype and don't secrete the same proteins as they do *in vivo*. With the addition of passaging of cells that further remove them from their *in* vivo environment, these cells become farther and farther from the pathology which researchers are attempting to study. Part of the loss of molecular signatures *in* vitro is most likely due to the paracrine signaling from a multitude of cell types that contribute to severe scar phenotypes. Non-coding RNA findings are very new, and should be validated and studied in the future and treatments related to these findings should be developed. Of note, all of these studies examine non-coding RNAs in fibroblast cells only.

2.3 Suggestions for topics of future research

2.3.1 Histologic

In studies utilizing patient tissue biopsies, time post-burn and post re-epithelialization, prior scar treatments, and demographic data including race and ethnicity should be judiciously reported. A large number of current studies report on Caucasian and Chinese populations, and future work should emphasize additional Asian, African, African American, Indigenous, and Hispanic patient populations. When one race or ethnicity makes up the large majority of samples, the title of the work should clearly indicate as such so as not to assert that all HTS contain these features. Blood collection should also be incorporated into prospectively-designed trials to evaluate the systemic responses to treatments or the natural systemic state that may contribute to HTS pathophysiology. Often, tissue biopsies are collected without companion blood samples. Details related to body location, and acute burn treatment of the particular biopsy site is likewise important for nuanced study of HTS at the histologic level. Longitudinal biopsies of scar can be performed in patients; however, large-scale studies should be reserved for animal modeling, and hint at the importance of both bench-to-bedside research, and bedside-(back)to-bench research.

2.3.2 Cellular

It is as yet unclear what is the best model for studying HTS *in vitro*, though it is clear from the described studies above that using cells in monoculture is the most commonly used method. In addition, these cultures are often carried out in 2D. While using these simplified techniques may be useful for some research questions, it is important not to over-simplify scar modeling. By utilizing co-culture models of multiple cell types, these *in vitro* systems become more sophisticated and more similar to the *in vivo* environment. An example of a co-culture system that should be developed is endothelial cells with fibroblasts. The contribution of endothelial cells to HTS development is an area ripe for study. A method for isolating and culturing dermal microvascular endothelial cells has been published, and reports a relatively simple antibody-based cell sorting method that could be easily incorporated into cell isolation protocols from HTS tissue samples derived from patients or animals [125]. In addition, 3D cell cultures can be used to more closely mimic the organization of skin as a tissue structure. Such "scar in a jar" models are likely to be useful moving forward [126]. It has also been suggested that media components may be altered to create a pseudo "crowded" environment in culture. Crowders such as ficolls and dextrans of differing molecular weights are meant to take up a fractional volume of the media to mimic fibrosis where ECM crowds cells *in vivo*. Such techniques, termed macromolecular crowding, should be incorporated into *in vitro* models in future work [127, 128].

2.3.3 Molecular

Future work should examine additional cell types when studying non-coding RNAs. The transcriptional profile of messenger RNA seems no longer adequate to explain the complexity of HTS. Non-coding RNAs should be incorporated into studies at the molecular level. As the scientific community progresses towards more sophisticated assaying capability in additional molecular components of the cells, these should all be likewise incorporated into our models. One such example is the discovery in 2020 of a novel tRNA-derived small RNA that plays a role in HTS fibrosis [129].

Non-coding RNAs are an example of a mechanism that could be studied in samples or cells of dyschromic HTS lesions. The molecular and cellular mechanisms behind the development of dyschromia have not yet been elucidated, and may be related to epigenetic pathways such as non-coding RNAs. The reasoning behind this hypothesis is due to the fact that dyschromia often persists over many years, and does not improve over time like many other scar symptoms. This phenomenon seems to hint that there are epigenetic modifications that contribute to its long-lasting nature. In addition, when moving from *in vivo* to *in vitro* systems, dyschromia persists in cells, further providing evidence that epigenetic mechanisms may be at play. There are a multitude of areas that could be studied in relation to dyschromia including global or gene-specific methylation of melanogenesis or keratinocyte-secreted proteins, acetylation patterns, or histone modifications. In addition, an interesting phenomenon is that HTS dyschromia does not improve over time even with treatment of scars that improve additional symptoms such as scar thickness or pruritis. The link between fibroblasts and melanocytes are an interesting area of future study due to the fact that treatments targeting the dermal cells do not seem to have an effect on melanocytes. HTS dyschromia is one area of research that should be prioritized due to its importance to patient populations for whom dyschromia is a factor in their psychosocial health.

3. Translational science research

3.1 Seminal findings

3.1.1 Patient samples and the need for animal models

Patient samples and patient-derived cells are often used to study HTS at the translational level. While these techniques can be useful, longitudinal and large-scale studies likely need to occur in animal models. There is no perfect animal model for the study of HTS [130, 131]. Murine species are not acceptable models because of the "loose-skinned" nature of these animals (**Figure 4**) [132]. They also have a panniculus carnosus which allows them to heal by contraction instead of by granulation tissue deposition and re-epithelialization. As such, murine species do not form HTS and are not an acceptable model. Non-animal models for wound healing and scar formation have also been suggested [133]. These models include in vitro models utilizing co-cultures of HTS-derived cells and organotypic culturing of biopsies of HTS. *Ex vivo* models utilizing excised human skin have also been proposed. While these models are useful for certain research questions, the drawback of not having the full *in vivo* system is clear.

A number of animal models for HTS have been proposed, including nude mouse models of xenografted human normal skin or HTS, the rabbit ear model, the Yorkshire pig model, and the red Duroc pig model. Each of the models has its own inherent pros and cons and each can be useful depending on the details of the specific research question. In addition, some papers claim that there is no universally accepted animal model for HTS, and hence, there is discord in the literature about methods for HTS creation even within the same species.

3.1.2 Nude mouse models

Nude mouse models of xenografted normal human skin have been used to create human-like HTS [134–136]. Although the skin was normal, and not HTS when it was xenografted, the skin forms scar that has many of the morphologic

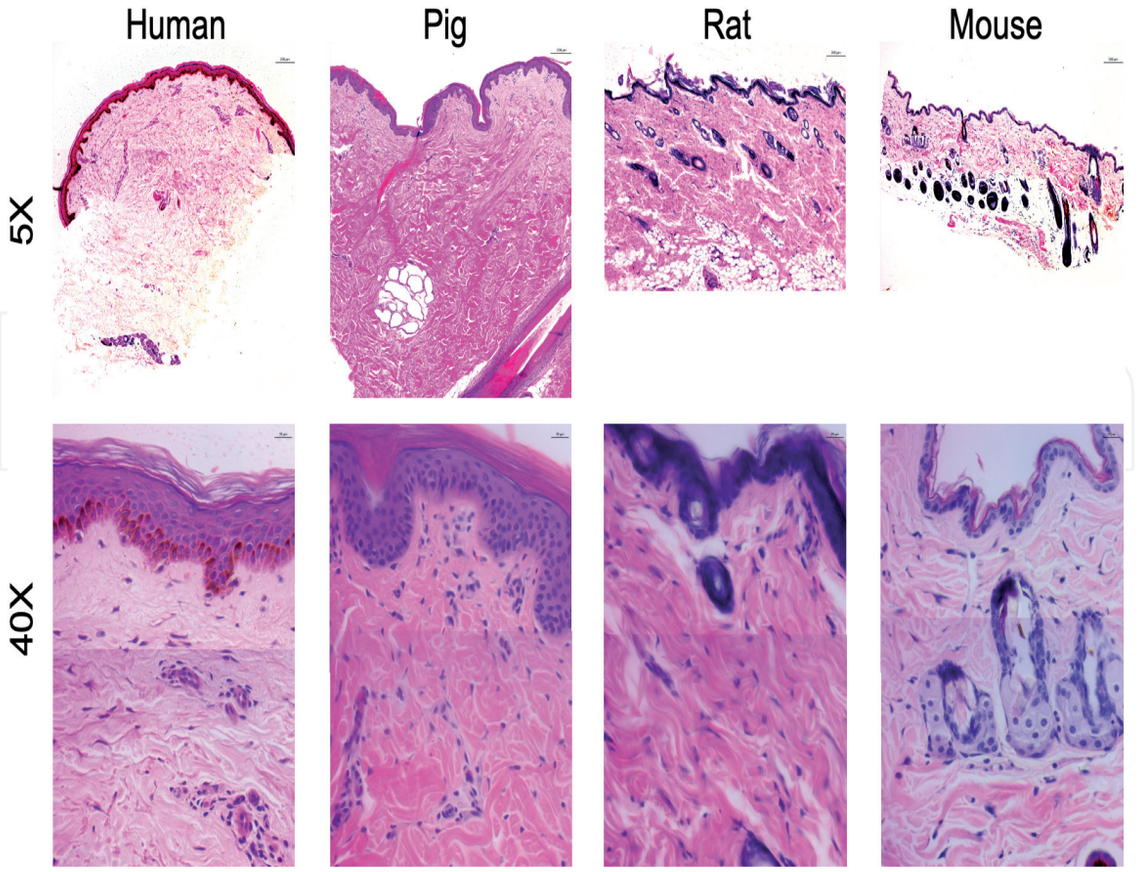

Figure 4.
Porcine skin most closely resembles human skin compared to rat and mouse skin. Epidermal and dermal thickness are similar and dermal appendages are present in similar densities. All animal work was conducted under IACUC-approved protocols.

and histologic criteria of HTS. Additional models have been developed over time where the normal skin is "scratched" to create a wound in the skin that then forms additional HTS [137].

3.1.3 Rabbit ear

The rabbit ear model likewise has its pros and cons [138]. It involves creating a small (6-8 mm) full thickness excisional wound in the ear of New Zealand white rabbits. Because the rabbit ear does not contain a panniculus carnosus, the small wounds heal with fibrosis and share some of the hallmarks of HTS including increased thickness, vascularity, and cellularity. In most papers, 6-8 scars are created on each ear and treatments are applied to each HTS as individual biologic replicates.

3.1.4 Yorkshire pig

The Yorkshire pig model is useful for modeling HTS similar in phenotype to scars from patients with baseline light skin pigmentation [139–144]. These scars are often described as "port-wine" scars due to their erythematous nature due to increased vascularity. They have increased thickness compared to normal skin (~2X), decreased tensile strength, different collagen architecture, increased vascularity, increased TGFβ expression, increased presence of myofibroblasts, and decreased presence of rete ridges [140]. This model is useful for certain research questions related to the prevention and treatment of scars that are present in patients with light skin pigmentation.

3.1.5 Duroc pig

In the 1970s, Silverstein *et al*. reported HTS development in red Duroc pigs after deep dermal wounding. They never published their model however, and it was not widely adapted. In 2003, Zhu *et al.* published an examination of a number of wound thicknesses and the resulting HTS that developed under different conditions in female red Duroc pigs [145]. They found that the creation of 8 cm by 8 cm (3.1 inches by 3.1 inches) deep partial thickness or full thickness wounds with a total dermatome setting of 0.06" to 0.09" led to the development of HTS that was thick, hypercontracted, and hyper-pigmented. In addition, there was disorganized collagen structure and many of the genes of interest known to be dysregulated in human HTS were appropriately dysregulated. The same group went on to publish a number of papers demonstrating the clinical, histologic, and molecular similarities of Duroc pig HTS to human HTS [68, 146–149]. Around the same time period, another group published similar findings in Duroc pigs [150, 151]. Gallant *et al.* used females or castrated males and created 2 cm by 2 cm (0.8 inches by 0.8 inches) full thickness wounds using a scalpel to remove the full thickness skin down to subcutaneous fat. Multiple small wounds were created in 2 rows of 10 wounds per flank. These HTSs ultimately progressed to become hyper-pigmented at day 70.

It was around the time of the publication of these novel Duroc pig HTS model papers that our lab became focused on developing a similar model for HTS. We began modeling with Duroc pigs to study partial thickness wounds in relation to donor site healing dynamics [152] and wound healing accelerating agents [153]. These wounds were created at a total dermatome setting of 0.06" and were 7.62 cm x 7.62 cm (3 inches x 3 inches) in size. These partial thickness wounds healed without the thick fibroproliferative nature of HTS; however, some hyper-pigmentation was observed. We then continued our investigation into the creation of full thickness wounds to generate HTS (**Figure 5**). During this project, where the primary goal was to study pressure delivery, HTSs were generated by full thickness wounding with a total dermatome setting of 0.09" and size of 10.16 cm by 10.16 cm (4 inches by 4 inches) [88, 154–158]. Throughout the course of this work, dyschromia, with regions of hyper- and hypo-pigmentation, was apparent [56].

3.2 Limitations to these models

3.2.1 Patient samples

Studying patient samples is most likely the best recapitulation of the disease process; however, this strategy has the drawback that it is a snapshot in time. These samples do not provide any information as to the natural history of HTS formation, as they must be taken after a scar is already formed. Additionally, HTS samples from patients are most likely at the severe end of the spectrum because the samples can most often only be collected during surgical HTS excisions.

3.2.2 Nude mice

While these models are useful, and they have shown retention of the HTS and pigmentation phenotype even 1-year post-xenografting, the etiology of HTS is not the same as the etiology of full thickness wounding or burn injury. Lastly, this model relies on the availability of large amounts of normal human skin, a resource that is not universally available.

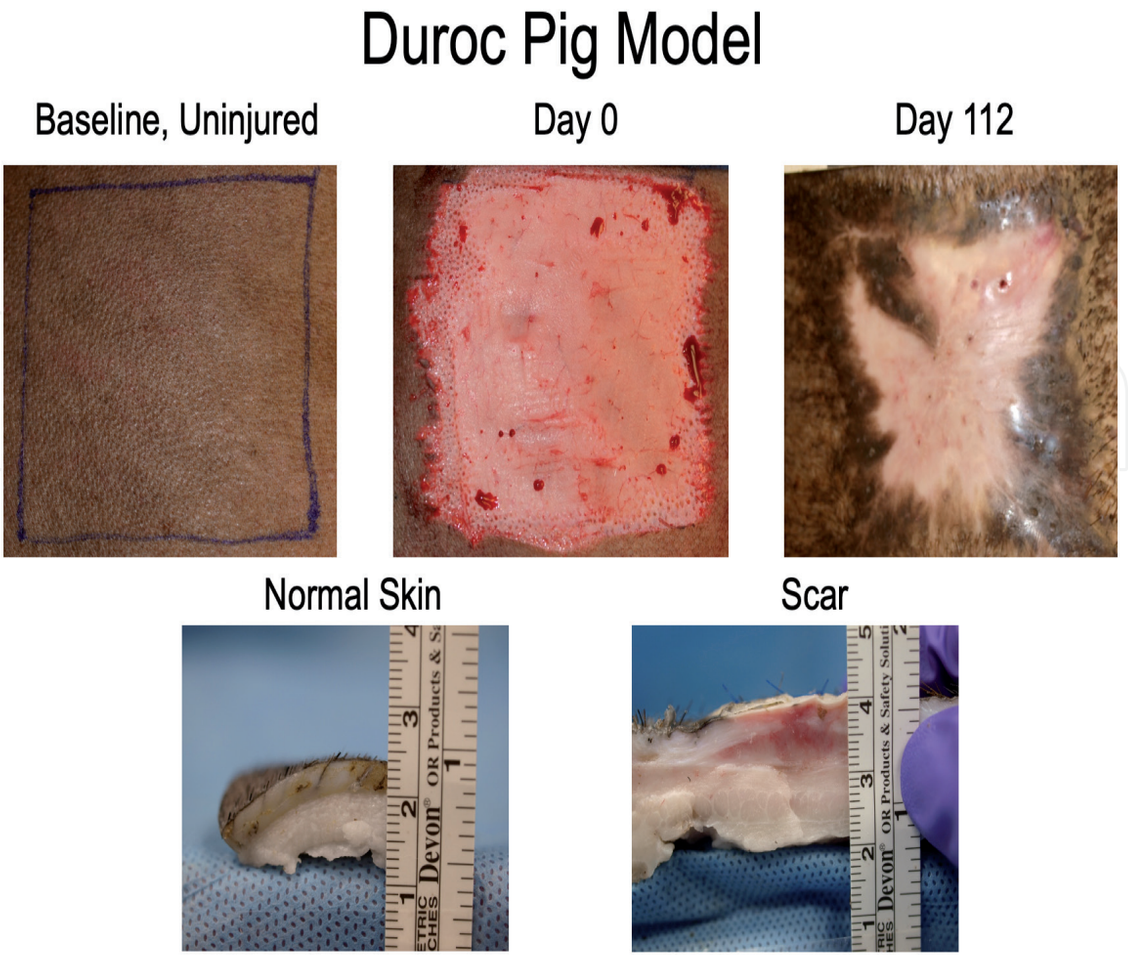

Figure 5.
Our lab's model of full-thickness excisional wounding to generate HTS in Red Duroc pigs. Baseline, un-injured Duroc skin has a red-brown phenotype. Full-thickness excisional wounds created by dermatome down to subcutaneous fat with no residual dermal appendages are created. Dyschromic, rigid, thick, HTS is generated 112 days post-wounding. This scar is thick compared to normal skin.

3.2.3 Rabbit ear

The model is affordable and has been used successfully to investigate a number of drug treatments; however, not many of these have made the leap to clinical treatments. This model does not acknowledge the potential local-regional effects of drug treatments on HTSs. Lastly, the overall skin structure of the rabbit ear is not similar to human skin because it sits upon a bed of avascular cartilage [138]. The model's main limitation is the size of the scars for study, which are small. In addition, dyschromia is not evident in these scars.

3.2.4 Yorkshire pig

In Yorkshire models of HTS, the resultant scars are not as thick as human HTS and they are not raised above the surrounding tissue. This phenomenon is most likely because fibroblasts derived from Yorkshire skin have an inherently less fibroproliferative phenotype compared to Duroc pig fibroblasts [159]. Fibroproliferation was demonstrated by Duroc pig fibroblasts to show increased actin stress fiber formation and adhesion complexes, decreased cell migration, increased cell contraction, and differential expression of HTS-related genes of interest (α-SMA, type I collagen, decorin, and TGFβ) compared to Yorkshire pig fibroblasts. Lastly, the

Yorkshire pig model for HTS is insufficient for certain research questions because there is no melanin-related dyschromia in this model.

3.2.5 Duroc pig

From 2012, when our lab began with this model, to present day, multiple labs have adopted the Duroc pig as a model for HTS formation. There is still no universal method for the creation of scars as some labs use excisional techniques at different depths and with different instruments [160–162], burn techniques [163–166], and burn, excision, and auto-grafting techniques [167–170]. Many scar models use small wound sizes that are not a significant wound burden on the animal. The full thickness nature of the injuries created in our model, as well as the large size, helps to closely mimic the HTS features that were observed in patients during the parallel timeline that experiments were taking place. Finally, the use of pigs, and specifically Duroc pigs, which are not a common animal breed, is expensive due to housing considerations and operative room time. This fact is often a limiting factor for their use.

3.3 Suggestions for topics of future research

3.3.1 Patient samples

Basic and translational scientists should work closely with physician-scientists to ensure that the questions they are trying to answer in basic or translational models are important for patients. As a community, we need to determine how improvements can be made to the implementation of translational work to the bedside. Too often, prevention or treatment techniques for HTS are developed in animal models only to stall out and never reach patient care settings despite their effectiveness.

3.3.2 All models

Often times, basic and translational science research lag behind current clinical findings such that patients receive treatments based on anecdotal evidence. This was the situation when fractional CO_2 ablative lasers were first used to treat HTS in patients. The cellular and molecular mechanisms behind this treatment had not been revealed even though patients continued to receive and benefit from this treatment. When questions arise in the clinical setting, it is useful to return back to the bench in order to test hypotheses. It is difficult, expensive, requires a large number of patients, and extensive regulatory review processes to conduct a clinical trial with patients. As an alternative, hypotheses that are early on in their development would benefit to be studied in animal models. The optimization of prevention or treatment effectiveness by testing different application techniques (systemic, injections, topical, laser channel drug delivery), dosing, and time-courses can all be studied in animals prior to testing in patients. Safety profiles are also critical to complete in translational work.

3.3.3 Pig models

Researchers using pig models for studying HTS debate which species produces the best model for study. Even when "definitive" papers are published declaring a model to be the most similar to human HTS, debate still follows. This was the case for the development of the Red Duroc pig model. Although the authors of

the multiple papers characterizing this model never claimed that it was a perfect animal model, its development was still novel and important. Even so, researchers have been altering and optimizing this model ever since and rarely, if ever, use their original experimental methods to create HTS. The Duroc pig model, even in our laboratory, is admittedly not perfect. The resultant scars are not as raised above the surrounding skin compared to human scar. As such, they never truly become as thick as the most severe human scars. In addition, it is difficult to create a homogenous scar phenotype, specifically with regards to dyschromia, as the development of this symptom is still unclear in its etiology. In addition, full-thickness burning without excision and grafting, or the use of excisional wounding alone without burn is a controversial topic. Some researchers believe that including the burn, only to excise it after 2 days, may not be a judicious use of resources, and sometimes exclude the burn if the goal is to develop HTS for study. Some researchers emphasize the importance of including burn if the research question is mechanistic in nature. Models which incorporate healing by secondary intention or delayed excision and grafting more closely mimic burn-wound healing in low-income countries or in patients with very large TBSA injuries, two situations which are associated with some of the most severe HTS. In high income countries, early excision and autografting of full thickness or deep partial thickness burns is more common and accessible due to available resources. There are a few models that comprehensively include burn, excision, and autografting techniques in pigs, however, they often result in nicely healed wounds without extensive HTS. Patient factors that contribute to severe scarring even at autograft sites are not fully recapitulated in animal models. In addition, it is experimentally difficult to incorporate scarring across joints in animals. Frequently, wounds in pig models are situated on the flank, a site relatively shielded from tension with normal movement. Tension in healing wounds is an important topic for future study. Lastly, the use of pigs for scar research is extremely expensive. Low numbers of replicates can be incorporated onto each pig if systemic and local-regional contributions are being taken into account. The use of nude mouse models of scar or humanized mouse models, are thus a very intriguing concept. These models, which are substantially less expensive, will allow for large numbers of experimental replicates without systemic confounders. While current nude mouse models are useful, we should continue to report new techniques to create scars in these animals. For questions concerning the etiology of HTS, current models are inadequate.

4. Clinical research

4.1 Seminal findings

4.1.1 The development of hypertrophic scar

Incisional wound healing is well-described and typically marked by minimal inflammation and non-pathologic scar [171, 172]. Delayed wound closure or that which heals by secondary intention or contraction is, by contrast, marked by prolonged inflammatory events followed in many cases by degrees of fibrosis and ultimately HTS. Severity of scar after wounding of the skin is related to wound size, depth, anatomical location, tension, nature of injury, infection, environmental factors, and genetic predisposition [173]. HTSs have been succinctly summarized as an over-abundant synthesis of collagen and under-abundant or absent remodeling process. Despite this oversimplification, over the past several decades, there has been a large amount of research investigating the mechanism behind HTS

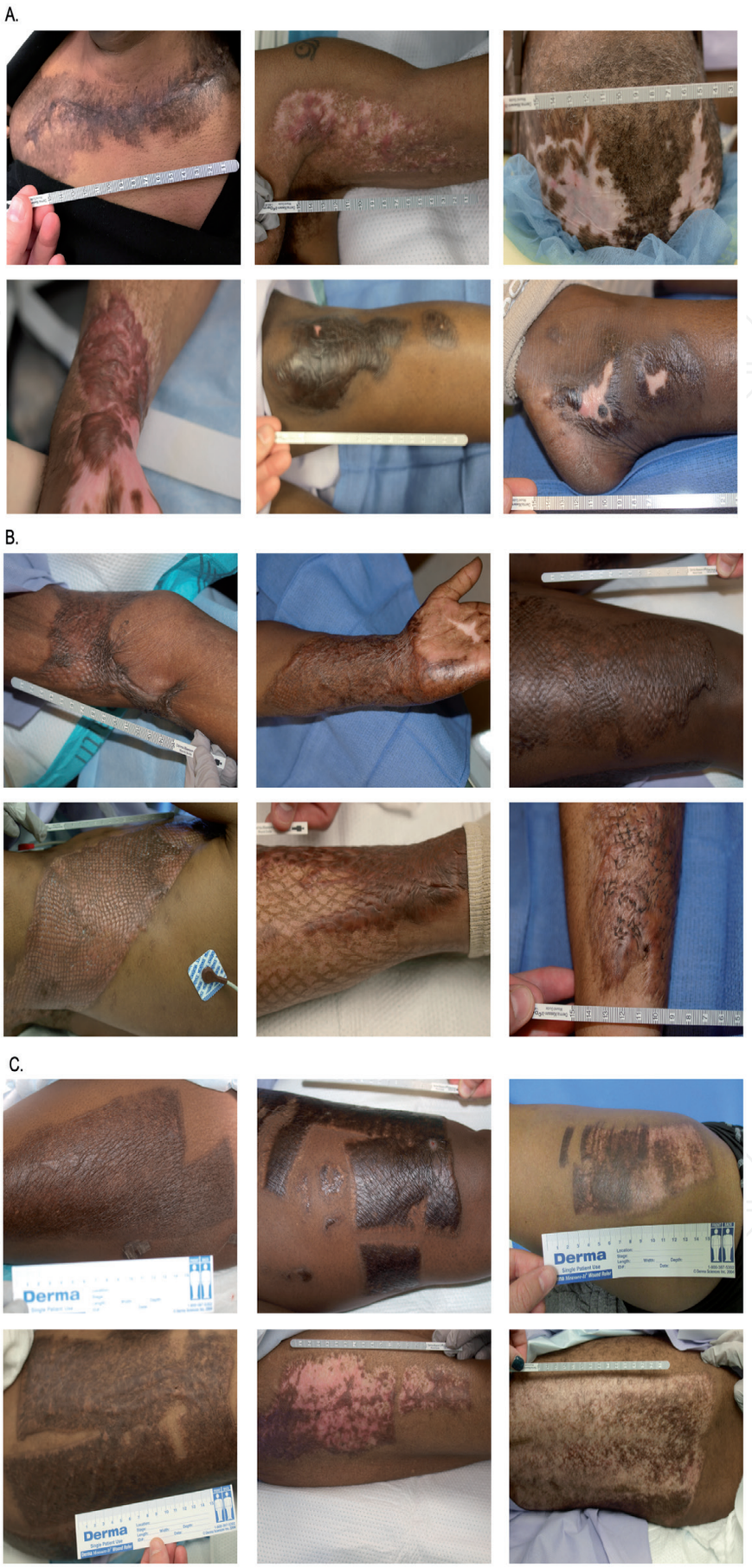

Figure 6.
HTS can result from a variety of injuries. HTS can result from wounds that are not treated with autologous skin grafting (A) treated with mSTSG (B) from donor sites (C). One of the main symptoms of HTS that can be observed is dyschromia. Patient photographs were collected under an IRB-approved protocol and shared with patient consent.

formation [100, 174]. The known severe inflammatory response that occurs after thermal injury has been suggested as an explanation for the greater incidence of HTS after burn injury than after other sources of injury [175]. A longer time to healing is associated with a greater risk of HTS, and in the case of burn injury, the most important prognostic indicators for HTS are the depth of original burn and corresponding time to wound closure [176, 177]. HTS can occur in wounds that were allowed to heal without mSTSG (**Figure 6A**), in wounds treated with mSTSG (**Figure 6B**), and in healed donor sites (**Figure 6C**) [178, 179]. Reliably, open wounds and full thickness burns that do not receive timely interventions to facilitate soft tissue coverage result in thickened, inflexible, pathologic HTS, though even wounds that do receive timely intervention may be susceptible.

Frequently, two to three weeks is used as a rule of thumb for time to wound closure for minimization of the development of HTS, with an understanding that a shorter time to healing is typically associated with a decrease in scar severity. It follows then that impairments to expedient wound healing are likely to contribute to the likelihood of HTS. Such impairments may include nutritional deficiencies (vitamin C, vitamin D, protein-calorie malnutrition), vasculopathy (peripheral arterial disease, diabetic microcirculatory disfunction, venous insufficiency), cigarette smoking, and infection among others [172]. It still occurs that patients with otherwise similar injury qualities and wound healing contributors develop varied severities of HTS, and this finding has been inadequately explained. There is evidence that certain genetic pre-dispositions put patients at risk for the development of HTS [180, 181]. Accordingly, patients of Asian, Hispanic, African American, or African descent are more likely to develop scar and exhibit increased scar severity [177, 181, 182].

The natural history of HTS generally predicts that a scar will be hypervascular, hypersensitive, dyschromic, and pruritic for the first few years after injury, typically accompanied by thickness and impaired flexibility. Some improvement in these qualities is to be expected over the following years, and some authors even suggest that HTS will revert to flat, asymptomatic scar [172]. In our own practice, however, patients with burn-related HTS may present decades after injury with persistent pain, itch, thickness, and range of motion limitations at sites of scar, suggesting that in at least some patients, these never truly resolve without targeted intervention. Due to the highly pervasive nature of HTS, and the fact that large total body surface area burn injuries are now survivable, a shift in focus has put HTS rehabilitation and recovery at the forefront of current research efforts by burn providers.

4.1.2 The evaluation of hypertrophic scar

Currently available scar evaluation tools include both subjective scales and objective mechanisms. In 2012, Tyack, *et al.* published a systematic review of eighteen different burn scar rating scales used in clinical and research settings [183]. The Vancouver Scar Scale (VSS) is most frequently cited and is a widely used, clinician-reported scale composed of four metrics: vascularity, pigmentation, pliability, and height. Scars with lower scores are characterized as "better" and more similar to normal skin [183, 184]. A number of modifications to the VSS have been made and applied in differing circumstances [183].

The Patient and Observer Scar Assessment Scale (POSAS) is also commonly used and is similar in its evaluation of the above metrics, but it provides additional levels of evaluation that quantify a patient's experience of pain and itch [185–191]. This scale was originally created for the evaluation of linear scars, but has been used in the evaluation of a much wider variety of scars [190]. Some criticism of the use of this scale in the context of HTS is that patients are asked to compare an area of

scar to that of normal skin. This question prompts dissatisfying responses despite clinical interventions and improvements, as we know that there is no currently available method by which to convert HTS back to normal skin.

Other scar assessment scales noted in a 2010 systematic review by Brusselaers, *et al.* include the Seattle Scar Scale, Manchester Scar Scale, Hamilton Scar Scale, Inventory of Potential Reconstructive Needs, and the Stoney Brook Scar Evaluation [191]. The four original components of the VSS are commonly evaluated in most scar rating scales, but additional parameters beyond these do not share clear consensus in systematic reviews [183, 191].

A wide variety of objective scar measures have been developed and used to some degree, though without consistent or widespread adoption. A scar's height may be simply measured with a standard ruler, though this is an incomplete technique because it represents only scar thickness above the level of the surrounding skin and does not take into account variability in height within a given scar [192]. Planimetry improves upon this method by measuring the surface area of a scar, accounting for its variations in height, but still does not account for scar area deep to the visible surface [193]. Various three-dimensional imaging techniques are available, but these are not widely used due to their high costs [194, 195]. High frequency ultrasound may be used to more completely gauge scar thickness and is gaining some popularity for evaluation of the effects of HTS interventions [192, 196]. Elasticity or stiffness of scar can be measured by various methods including suction, pressure, torsion, and extension, all typically with a non-invasive probe [197]. Acoustic methods for scar assessment use sound waves to detect heterogeneity in scar tissue [198]. Transcutaneous oxygen tension can be measured with skin electrodes, as HTS have been noted to have a lower partial pressure of oxygen than healthy skin, however this technique has not been described frequently in the literature over the past 3 decades [199, 200]. Trans-epidermal water loss estimates the barrier function of scar related to the moisture content of skin [201]. Range of motion can be measured with a standard goniometer to estimate disability of movement due to scars in proximity to joints [197].

4.1.3 The treatment of hypertrophic scar

There are currently limited treatment options for HTS, and as such, prevention based on appropriate acute management of cutaneous injury is of paramount importance. When HTS develops despite good acute management, there are several treatment strategies that are employed. In decades past the main approach to the treatment of HTS was to "wait and see" and allow HTSs to regress over a period of years. While HTSs are known to get better over time, in contrast to their counter parts, keloid scars, which remain stagnant or worsen over time, HTSs almost never regress back to the structure or functionality of normal skin. This "wait and see" strategy often leaves patients with moribund scars that would benefit from additional treatments. Additionally, were interventions offered earlier on, patients have the potential to prevent multiple years of suffering. Early prevention strategies using our current multi-modal approach are critical to preventing severe scar. Common, longstanding treatments include compression, massage and stretch, silicone gel and sheeting, drug injection, and surgical tissue rearrangement, all of which have a role in prevention and treatment of HTS symptoms, but have drawbacks and can result in suboptimal outcomes when used in isolation [202–206].

Compression therapy is a widely used technique that has been considered standard of care for burn-related HTS for over 50 years. It is most commonly provided via elastic compression garments or plastic molded face masks for the first year following a burn injury. These are worn 23 hours per day and have been shown

to improve scar height and erythema over time when custom fitted to a pressure of 20-30 mmHg [207]. Acceptance of this treatment modality is based mainly on anecdotal experience as the mechanism of action is not fully understood [208]. In a recent evidenced-based practice review by Sharp *et al.*, pressure therapy is recommended as a successful scar treatment which results in improved aesthetic outcomes by reducing scar height and erythema [203, 208–212]. Patient compliance and duration of treatment are important factors in the success of pressure therapy [213]. Our lab has studied the effect of compression on HTS extensively [55, 214] and revealed its mechanism of action to be primarily related to the induction of changes in collagen levels and types [157], elastin levels [156], and MMP levels [154]. Additionally, we have demonstrated that pressure therapy not only acts through mechanical forces, but induces changes in the HTS at the cellular and transcriptomic level that induce remodeling [155]. Despite pressure therapy's success in treating some symptoms of scar, patients often have sub-optimal functional and cosmetic outcomes even after treatment, which lead them to seek additional care.

Massage and stretch are routinely suggested for treatment of HTS despite mixed and limited evidence of their effectiveness [175, 215–218]. These techniques have minimal cost and can in many instances be self-administered with little to no risk, thus their use persists in the absence of convincing studies. A 2006 Cochrane review of silicone-based interventions for the prevention or treatment of HTS determined that there was only poor quality weak evidence of its benefit [219]. Since that time, more promising evidence for the benefit of silicone-based treatments have emerged. Proposed mechanisms of action include increased temperature, increased hydration, and bestowing a polarized charge to tissues, though none of these mechanisms have conclusively been shown [175]. It seems that the combination of silicone with compression therapy is advantageous compared to silicone or pressure therapy alone and provides benefits pertaining to pigmentation, vascularity, pliability, and itch [175, 220]. Intralesional injection of HTS may be with glucocorticoid (commonly triamcinolone, TAC) 5-fluorouracil (5-FU), or verapamil. Suggested mechanisms of action are suppression of the inflammatory response with TAC injection, reduction in the synthesis of ECM with verapamil injection, and inhibition of cell growth and induction of apoptosis with 5-FU injection [175]. The combination of 5-FU and TAC appears to have the greatest effect according to a recent systematic review [175, 221]. Surgical management of scar is a large topic in its own right and is not reviewed here; however, absent large scar excision with accompanied closure by grafts or flaps, zig-zag patterned incision, rearrangement, and suture approaches (z-plasty, w-plasty, and others) benefit linear HTS by a decrease in focal tension with accompanied histologic changes [222, 223].

Over the past 20 years, laser and light-based therapies have gained increasing popularity amongst clinicians who work to treat burn HTS [224–226]. Initially, the pulsed dye laser was most commonly used, and has now been supplemented in many centers by fractional ablative laser platforms [227–230]. Fractional photothermolysis was first described in 2004 [231]. This variation of laser treatment is based on the concept of creating multiple individual microscopic channels in a targeted treatment area. The laser causes small, limited zones of photothermolysis within tissue due to focal energy deposition at a wavelength absorbed by tissue water [232, 233]. The microinjuries are small enough that skin barrier function is preserved and healing is achieved without new scar formation [231, 234]. In addition to clinical improvements, histological and molecular evaluations of treated HTS reveal changes in inflammatory responses, matrix remodeling, and overall scar structure [168, 235]. This technology was initially created with the goal of aesthetic treatment of photo-damaged skin, but has shown increasing applicability for the treatment of various traumatic scars over the past decade [236].

4.2 Limitations to current clinical knowledge

4.2.1 The development of hypertrophic scar

Patients of similar age, health, and nutritional statuses with injury types and clinical managements in common may still develop different scar phenotypes. A trend toward worsened scar hypertrophy in skin of color has been observed [182, 237]. To further elucidate these basic genetic predispositions, various investigators have attempted to focus on genotypic variations associated with HTS. Carriers of some specific major histocompatibility complex alleles (HLA-DRB1*15, HLA-DQA1*0104, DQB1*0501, and DQB1*0503) show a genetic susceptibility to keloid disease, but these are not as clearly implicated in HTS [173]. The melanocortin 1 receptor single nucleotide polymorphism R163Q was associated with severe HTS in a study of 425 subjects in 2015 [182]. In 2016, the same group found that a missense variant of a mitogen-activated protein kinase pathway inhibitor PTPN5 was protective against HTS in a study of over 538 subjects [238]. Investigational studies such as that by Tsou, *et al.* have use cDNA microarray analysis to suggest that greater than 100 genes are differentially expressed between HTS and normal skin or non-pathologic scar. Implicated genes appear to be involved in collagen expression, growth factors, and MMPs and their inhibitors, however there was great variation between individual scars, and the total number of scars was small [239]. Given the expected high variability between individuals, large numbers of scars that will generate "big data" sets still need to be evaluated to discover reliable trends with respect to gene expression in HTS.

A less well-studied HTS feature is the dyspigmentation or dyschromia that occurs in the cells of the epidermis, namely the keratinocytes and melanocytes. In addition to the functional limitations of HTS, the aesthetic symptoms of HTS can also have severe psychosocial effects on patients which contribute to challenges with social reintegration and lead to decreased quality of life, and are, therefore, of importance to study [240–245]. In addition, dyspigmentation is difficult to predict, heterogeneous with regions of hyper- and hypo-pigmentation, can persist without improvement over time, and is pervasive amongst patients with baseline dark skin pigmentation [246].

4.2.2 The evaluation of hypertrophic scar

The various objective and subjective measures of hypertrophic scar have unique strengths and weaknesses [183, 191, 200]. The optimal combination of measures to evaluate scar severity and response to treatment has not been outlined. In a 2015 evaluation of the VSS, there was no consensus amongst 130 burn care providers as to what value on the scale constituted clinically significant HTS [247]. Lee *et al.* have recently attempted to delineate an optimal global scar evaluation protocol with the combination of a modified VSS and a panel of objective scar measurement tools [247]. While not yet validated or accepted widely, the intent of the work is valuable and a collaborative effort to this end will benefit both clinicians and researchers working with HTS.

4.2.3 The treatment of hypertrophic scar

As previously described, many current clinical interventions to prevent and treat HTS are used based on clinical experience with variable evidence. Proposed mechanisms exist for the use of silicone, compression, intralesional injection, and local tissue rearrangement, though these have not been explicitly outlined

[175]. The current treatments described above have varied effectiveness for relieving symptoms of HTS such as thickness, pliability, and pain, but are ineffective for the treatment of dyschromia. In fact, most research into HTS focuses on methods of targeting dermal remodeling, and does not focus on the epidermis. This focus is due to the fact that HTSs are often characterized by fibroblast cells and ECM that make up the dermis [99, 159]. These cells are the general focus of most research because they contribute to the thick, non-pliable, and contracted symptoms of HTS [248].

A consensus statement on laser treatment of burn scars was created in 2014 highlighting currently demonstrable benefits of f**ractional ablative CO_2 (FCO_2) laser scar revision** (LSR). These include a small immediate increase in range of motion (ROM) as a result of photomechanical scar release, followed later by improvements in pliability, durability, texture, dyschromia, and further range of motion, all of which have been attributed to a collagen remodeling response [188]. Experience with FCO_2 LSR at our institution reflects these improvements in the evaluation of patients with burn HTS. The improvement in HTS after treatment with FCO_2 is well-documented [249, 250]. How and when these improvements occur has not been clearly defined. Existing studies frequently demonstrate changes in HTS in a bimodal fashion—only prior to and after multiple treatments in a course of LSR [225, 229, 230]. In practice, rarely does a patient undergo a single FCO_2 treatment for symptomatic HTS, however an adequate number of treatments, frequency of treatments, preferred settings, timing, and expected time course of outcomes is still undefined.

4.3 Suggestions for topics of future research

4.3.1 The development of hypertrophic scar

The incomplete understanding of genetic predisposition to scar is an area wide open for investigation. The patient-specific contributing factors leading to the development of HTS, as well as the degree to which a given individual responds to any clinical intervention, has not been elucidated with any practical clarity. If patient-specific information were to become available, targeted interventions for both the prevention and treatment of HTS could be developed for best outcomes. The work begun by Tsou, *et al.* as well as Sood, *et al.* could be continued, as the number of scar and skin samples needed to establish clear gene expression trends in HTS is overwhelming [182, 238, 239]. That said, banking results from skin and scar of varied patient ethnicities, ages, injury types, clinical interventions, and health backgrounds could yield enormous information pertaining to the prevention, development, and treatment of HTS. Once a genetic blueprint associated with or protective from HTS is more clearly outlined, the interplay of a specific patient's age, injury-specifics, and medical comorbidities will add further layers to the ability to develop targeted approaches to the prevention and treatment of severe HTS.

4.3.2 The evaluation of hypertrophic scar

Scar assessment tools have been evaluated largely in the context of burn HTS [200]. No clear standard exists and a clearly defined pathway to scar evaluation is yet to be determined. The standardization of these measures is needed in order to reliably evaluate the effectiveness of the clinical treatments for HTS. Many available tools are useful and promising; however, scales have not been established to define normal ranges nor expected values which represent clinically significant

improvement. Likewise, devices have not been adopted similarly across multiple centers offering similar clinical managements. Work aimed at a standard set of metrics with accessible tools would benefit HTS-focused clinicians, researchers, and their patients. These metrics should incorporate patient-centered measures with a focus on symptoms affecting quality of life in the subjective realm; objectively, goals include a standard method to quantify scar size, thickness, vascularity, pigment, patterning, and flexibility with reproducibility and accessibility across healthcare delivery systems.

4.3.3 The treatment of hypertrophic scar

Many of the approaches for treatment of HTS has come from anecdotal use and experience. Future study of HTS may include validating any of these techniques. In any study of therapies for HTS intervention or prevention, there exists the challenge of developing and incorporation of appropriate controls. There is some expected improvement and remodeling of scars over time even without interventions, but the degree to which these occur is varied amongst different people and even within the same person. A valuable approach to this issue has often been through the design of interpatient controls by randomizing multiple distinct scars in a single patient or through split-scar studies, comparing treated and untreated portions of the same scar [251–253]. Separate scar interpatient controls are unable to account for the variability of scar physiology based on patient genetics, lesion location, or differences in original wound depth. Large scale trials, potentially with the assistance of multiple-center enrollment, would be most effective at minimizing these naturally-occurring confounding factors. In split-scar studies, the effect on an untreated portion of scar in direct proximity to a treated portion of scar is also not clear, and the systemic effect on areas untreated, however distant, deserves attention moving forward as various treatment approaches are studied.

Of course, the testing and validation of any scar treatment technique would require reliable, reproducible evaluation of treated and untreated HTS to speak to true efficacy. As noted above, current evaluation techniques carry wide potential, but have not been standardized in many cases with respect to normal ranges, multi-institutional adoption, timing of use, and expected values for scars of different ages, injury types, and locations. Clearly defining a suggested set of evaluation metrics for scars would contribute to the field by allowing researchers and clinicians to communicate consistently when testing various scar prevention and treatment strategies moving forward.

Laser scar revision is the most promising development for the treatment of HTS in the past decade. Variability in technology, frequency of treatment, power and density settings, and concomitant laser-assisted drug delivery all present potential targets of study to optimize this approach. The ideal laser depth of penetration has been suggested to be 50-75% of the thickness of a scar by Isler-Fischer, *et al.*, with depths of penetration outside this range offering little benefit [254]. Whether a difference exists between 50 and 75% depth of penetration is not known, nor whether these ranges are altered by various scar attributes, which may include scar age, patient age, scar vascularity, scar location, prior treatments, and more. Waibel, *et al.* introduced the use of optical coherence tomography (OCT) immediately prior to laser intervention to gauge the real time thickness of a given scar and optimization of power settings based on these results [255]. This approach holds promise for patient- and scar-specific interventions, though clarity is lacking on whether optimal power based on scar thickness changes in a linear or non-linear way. Laser-assisted drug delivery frequently includes the application of TAC or 5-FU to a scar treated with fractional ablative laser resurfacing. A 2019 study did not show

a clear advantage of one medication over the other, and continuing to optimize this technique is an area still available for future study [256]. The inherent variability in the administration of laser and light based technologies with and without the concomitant application of medications is a target for study in a clinical trial proposed out of UNC in 2018 [257]. The authors offer a flowsheet with proposed combinations of the above with the goal of highlighting efficacy of different treatments and combinations thereof. Study designs such as these are sophisticated and are likely to assist in defining treatment guidelines from a large pool of differing approaches to laser scar revision.

5. Conclusion

The development of HTS is widespread after cutaneous traumatic injury and has profound effects on the quality of life of the patients who suffer from it. The variability between patients' injury etiologies, wound locations, acute and long-term treatment approaches, medical comorbidities, and ethnicities makes research into the pathophysiology and treatment of HTS complex and multilayered. The combination of optimal modeling and broad patient representation in research is likely to afford improved translatability of future scientific discovery related to HTS.

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