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van den Broek, L.J.

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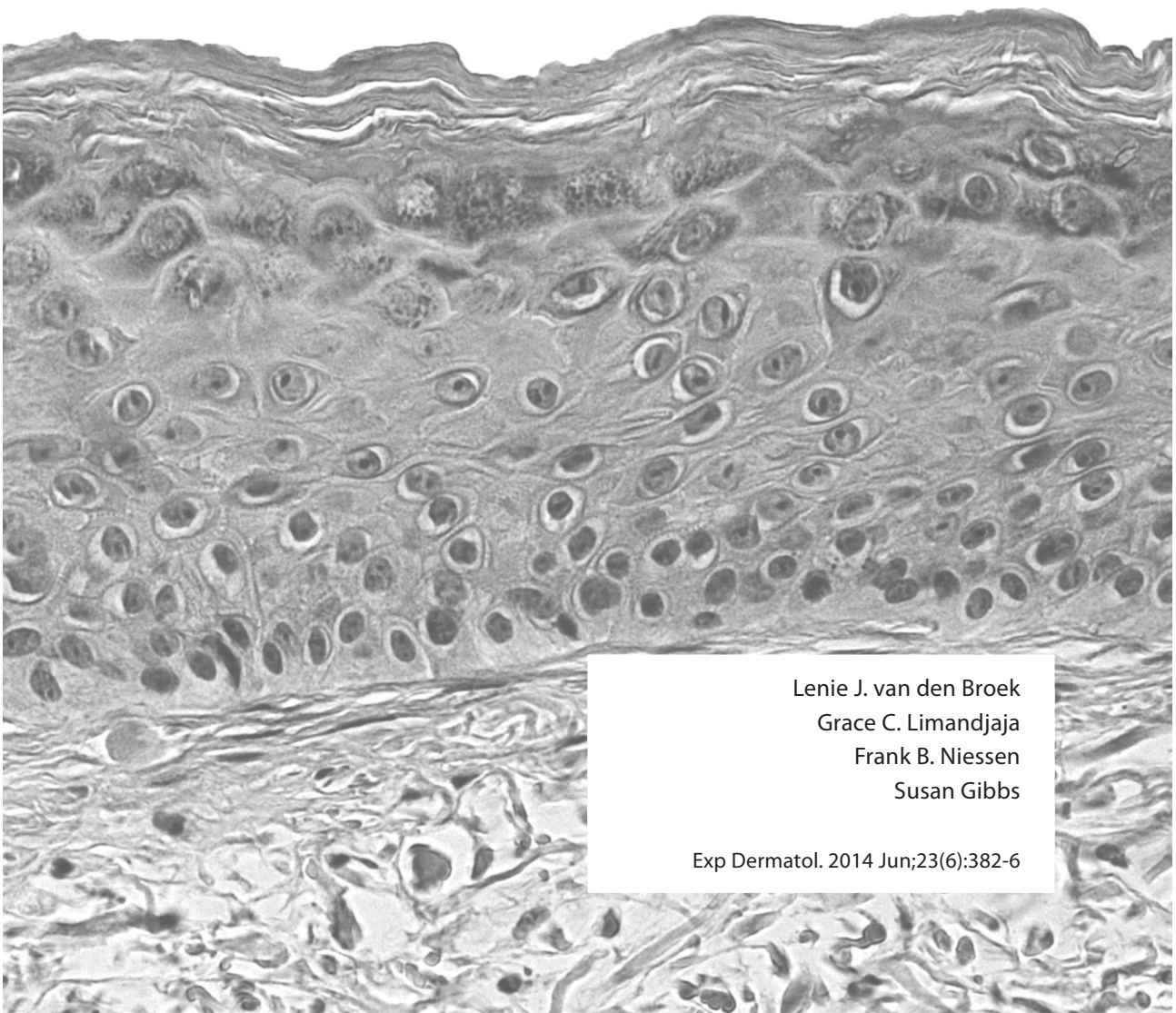
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# Chapter 8

## Human hypertrophic and keloid scar models: principles, limitations and future challenges from a tissue engineering perspective



Lenie J. van den Broek  
Grace C. Limandjaja  
Frank B. Niessen  
Susan Gibbs

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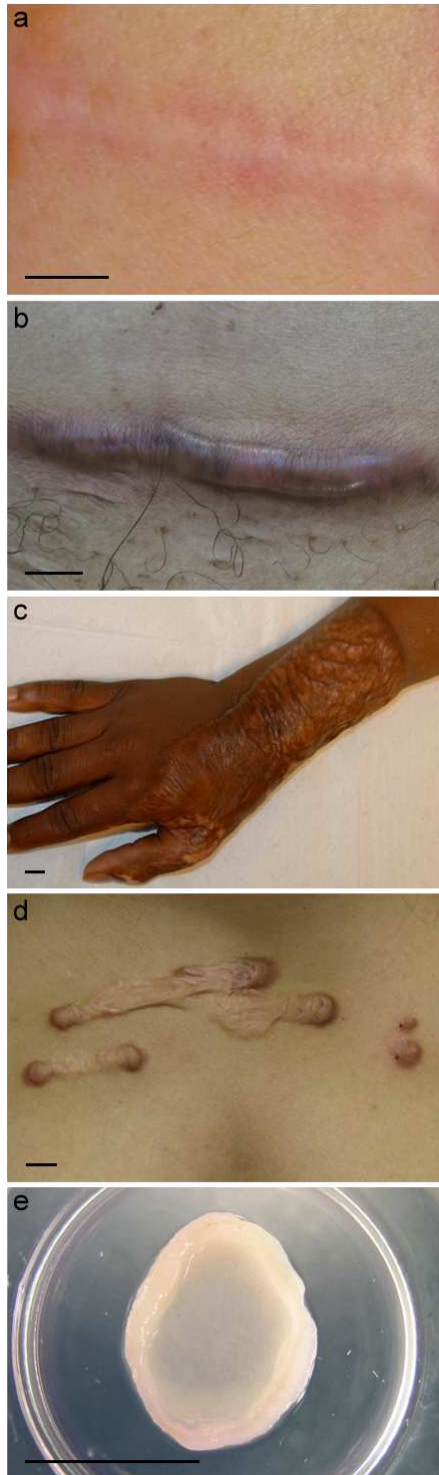
## **ABSTRACT**

Most cutaneous wounds heal with scar formation. Ideally, an inconspicuous normotrophic scar is formed, but an abnormal scar (hypertrophic scar or keloid) can also develop. A major challenge to scientists and physicians is to prevent adverse scar formation after severe trauma (e.g. burn injury) and understand why some individuals will form adverse scars even after relatively minor injury. Currently many different models exist to study scar formation, ranging from simple monolayer cell culture to 3D tissue engineered models even to humanized mouse models. Currently these high/ medium throughput test models avoid the main questions referring to why an adverse scar forms instead of a normotrophic scar and what causes a hypertrophic scar to form rather than a keloid scar. Also, how is the genetic pre-disposition of the individual and the immune system involved. This information is essential if we are to identify new drug targets and develop optimal strategies in the future to prevent adverse scar formation. This viewpoint review summarizes the progress on *in vitro* and animal scar models, stresses the limitations in the current models and identifies the future challenges if scar free healing is to be achieved in the future.

## INTRODUCTION

Wound healing starts directly at the time when the initial injury occurs. The healed skin always results in a scar and therefore for both the patient and the physician, a major outcome parameter in wound healing is the quality of the final scar (Figure 1). In general, after superficial injury the scar is barely, or may not even be visible to the naked eye. In the case of a deeper wound, the scar is often visible but is seen as a smooth, pale and flattened scar known as a normotrophic scar. However, in predisposed individuals and on some predilection sites on the body (e.g. sternum, ear-lobe), scar formation can result in increased fibrosis, which in turn can result in adverse scar formation (hypertrophic scar or keloid). A major challenge to scientists and physicians is to prevent abnormal scar formation and understand why some individuals will form abnormal scars even after relatively minor injury.

In order to develop optimal therapeutic strategies for the different types of scar it is essential to understand the pathology underlying these different scar types. Clinically the distinction between a hypertrophic scar and a keloid remains difficult<sup>1</sup>. Both hypertrophic scars and keloids can be firm, raised, itchy and painful. Both can have a significant physiological (limited joint mobility in particular with hypertrophic scars) and psychological (especially the face) impact on quality of life of the patient. The main clinical difference between the two adverse scars is that hypertrophic scars generally remain confined to the original wound borders, whereas keloids extend beyond the boundaries of the original lesion<sup>2</sup>. Keloids may also develop years after the initial injury, almost never regress, are more common among the darker pigmented skin (up to 6-10% in African populations) and may have a genetic background<sup>3</sup>. In contrast, hypertrophic scars occur within 4-8 weeks after injury, may diminish in time and are found in almost all patients when trauma is extensive (up to 91% following large deep burn injury)<sup>3,4</sup>. However, a significant group of patients (34-64%) undergoing standard surgical procedures will also develop a hypertrophic scar after closure of the incision wound<sup>5,6</sup>. All in all this indicates that, in addition to the standard response to extreme trauma, certain individuals are genetically predisposed to adverse scar formation. If this is indeed the case then this needs to be taken into account when developing physiologically relevant human scar models. Furthermore, it is important to maintain the clinical distinction between hypertrophic scars and keloids by developing distinct physiologically relevant models for each type of abnormal scar.



**Figure 1. Macroscopic photographs of different scar tissues.** (a) Normotorphic scar developed after incision wound (breast). (b) Hypertrophic scar developed after incision wound (abdomen). (c) Hypertrophic scar developed after extreme 3<sup>rd</sup> degree burn injury (hand). (d) Keloid scar formed from pustule (sternum). (e) *In vitro* hypertrophic scar model: skin equivalent of reconstructed epidermis on adipose tissue-derived mesenchymal stem cells populated matrix. Bars = 1cm

## WOUND HEALING

Numerous reviews describe cutaneous wound healing as an interactive process involving not only skin residential cells and stem cells, but also infiltrating cells<sup>7,8</sup>. Upon tissue damage, inflammation is initiated by the release of cytokines and chemokines from the damaged tissue. Immune cells (granulocytes, monocytes, lymphocytes) are drawn into the wound bed, and neighbouring skin residential cells and regenerative stem cells start to proliferate, migrate and differentiate to close the wound<sup>7-10</sup>. Granulation tissue is deposited and extracellular matrix synthesized<sup>7,10,11</sup>. Therefore, the early immune response must be involved in the early development of the scar and must play a role in the final quality of the scar. Indeed adverse scars are thought to arise from an increased and prolonged inflammation. However, the type of immune response involving e.g. mast cells, neutrophils, macrophages, T-lymphocytes (especially T-helper2 cells) and Langerhans cells is also thought to be important<sup>3,12-16</sup>. Evidence also suggests that intrinsic aberrations in the immune system of those who form keloids exist. Peripheral blood mononuclear cells isolated from keloid-forming patients showed an altered secretion profile of growth factors and cytokines, an increased ability to induce fibroblast proliferation and were more inclined to differentiate into fibrocytes when compared to patients who form normotrophic scars<sup>17-19</sup>. Contradictory results suggest differences found between researchers could be due to the dynamics of wound healing and therefore the time of sample collection is very important<sup>16</sup>.

Taken together, literature suggests that the i) genetic predisposition of the individual and ii) the extent and type of the initial inflammatory response are key players in scar formation. Both of these are extremely difficult to investigate in current *in vitro* and animal models. In order to understand the mechanisms underlying scar formation, scientists have turned from conventional submerged monolayer culture models to tissue engineered models and even humanized mouse models (human skin is transplanted onto the animal). The progress made to date using these scar models is described below.

## CURRENT MODELS AND THE NEED FOR IMPROVEMENTS

Scar models are essential to investigate the pathogenesis of adverse scar formation, identify new drug targets and to test new therapeutics. Nowadays, animal models and *in vitro* cell culture and tissue engineered models are used with varying degrees of success to represent human scars. Examples are shown in tables 1 and 2 (see also extensive supplement tables 1 and 2 with references). Patient studies remain essential and shall always be necessary to validate potential novel anti-scar therapeutics identified in animal and *in vitro* scar models. Human individuals are rarely used to explore

**Table 1. Overview hypertrophic scar models**

	Dermal thickness	ECM synthesis	Contraction	No. of vessels	No. of cells	Epithelisation	Epidermal thickness	Rete ridges	Hair follicles	GF&C	Apoptosis	Fib. proliferation
<b><i>In vivo</i> human HTscar formation</b>	+	+	+	+	+	+	+	+	+	+	+	±
<b><i>In vivo</i> Animal Models</b>												
Grafting split-thickness human skin onto animal	+	+	-	+	+	-	+	+	+	+	+	-
Grafting HTscar to animal	+	+	-	-	-	-	-	-	-	±	-	-
Induction of HTscar: full-thickness wounds	+	+	±	+	+	-	+	+	+	±	+	±
Induction of HTscar mechanical stress to full-thickness wound	+	+	-	+	+	-	+	+	+	-	-	-
<b><i>In vitro</i> models</b>												
<u>Human healthy cells</u>												
Monolayer of fibroblasts (+/-scratch)	-	+	-	-	-	-	-	-	-	+	-	-
DE: FPL (+/-mechanical stress)	-	+	+	-	-	-	-	-	-	+	+	-
SE: reconstructed epidermis of KC on a dermal matrix containing ASC	-	+	+	-	-	+	+	-	-	+	+	-
<u>Human HTscar cells</u>												
Monolayer of fibroblasts	-	+	-	-	-	-	-	-	-	+	+	-
DE: FPL	-	+	+	-	-	-	-	-	-	+	+	+
SE: reconstructed epidermis of KC on a self-assembled matrix of fibroblasts	+	+	-	-	-	-	+	-	-	-	-	-
<b><i>Ex vivo</i></b>												
HTscar biopsies (+/-mechanical stress)	+	+	-	-	-	-	+	-	-	-	-	+

Overview of hypertrophic scar models and scar forming parameters which can be assessed. For more extensive information, limitations and references see supplement table 1. ASC, adipose tissue-derived mesenchymal cells; DE, dermal equivalent; Fib, fibroblast; FPL, fibroblast populated lattice; GF & C, growth factors & cytokines; HTscar, hypertrophic scar; KC, keratinocytes; SE, skin equivalent; +, marker can be assessed in model; -, marker is not yet studied or cannot be assessed in model; ±, contradictory results

the pathogenesis of adverse scar formation, probably due to ethical issues, logistical problems and also due to patient variation with regards to extent and duration of trauma. To overcome the problems confronted by patient studies, researchers have tried to extrapolate results from animal studies. Despite the large number of studies describing pigs, mice, rabbits, and other animals as models to investigate hypertrophic scarring or keloid formation, the basic skin physiology, immunology and therefore the wound healing process is markedly different with the result that animals do not develop scars which are comparable to adverse scars in humans<sup>20-23</sup>. In order to humanize the mouse more, a hypertrophic scar model has been described in which a healthy human

**Table 2. Overview Keloid scar models**

	Dermal thickness	ECM synthesis	Volume/weight	Contraction	No. of vessels	No. of cells	Epidermal thickness	GF&C	Proliferation	Apoptosis	Migration	Invasion
<b><i>In vivo</i> Human Keloid formation</b>	+	+	+	+	±	+	+	+	±	+	±	±
<b><i>In vivo</i> Animal Models</b>												
Grafting Kscar into animal	-	+	+	-	±	-	-	±	-	-	-	-
Induction of Kscar	-	-	-	-	-	-	-	-	-	-	-	-
<b><i>In vitro</i> Human Models</b>												
<u>Human healthy cells</u>												
NF co-cultured with CD14+ cells from keloid patients	-	-	-	-	-	-	-	+	+	-	-	-
<u>Human Kscar cells</u>												
Monolayer of keratinocytes	-	-	-	-	-	-	-	-	-	-	+	-
Monolayer of fibroblasts	-	+	-	-	-	-	-	±	+	+	+	+
DE: FPCL	-	+	-	+	-	-	-	+	-	-	-	-
Indirect co-culture of KK with KF	-	+	-	-	-	-	-	+	+	+	-	-
NK epidermis on KF-populated matrix	+	+	-	-	-	-	+	-	-	-	-	-
<u>Kscar explants</u>												
Air-exposed biopsy embedded in collagen gel (6 wk)	-	+	+	-	-	+	+	+	-	-	-	-

Overview keloid models and scar forming parameters which can be assessed. For more extensive information, limitations and references see supplement table 2. DE, dermal equivalent; FPCL, fibroblast populated collagen 1 lattice; GF & C, growth factors & cytokines; KF, keloid scar fibroblasts; KK: keloid scar keratinocytes; Kscar, keloid scar; NF, normal skin fibroblasts; NK, normal skin keratinocytes; -, marker can be assessed in model; +, marker is not yet studied or cannot be assessed in model; ±, contradictory results

split-thickness skin graft is transplanted onto the back of a nude mouse<sup>24,25</sup>. In a similar manner, in order to try to gain insight into the pathogenesis of keloid formation, keloid skin (full thickness or dermis only) has been directly transplanted onto nude mice<sup>26-30</sup>. The greatly reduced number of T cells in these mice reduces the chance of graft rejection. In this mouse model the immune component of wound healing and scar formation is severely compromised due to the immune deficient phenotype of the nude mouse. This is also supported by reports showing that mouse models in general poorly mimic human inflammatory events (e.g. burn wound trauma)<sup>23</sup>. The only human immune cells present are derived from the transplanted skin itself as human immune cells from the blood are absent<sup>31</sup>. The obvious solution would be a physiologically relevant and fully standardized *in vitro* human model in which different key cells types, thought to be responsible for excessive scar formation, can be added under controlled conditions.



*In vitro* cell culture models have been used for many years to gain insight into different aspects of scar pathogenesis, but almost never to test potential scar treatments. Early models, using conventional monolayer cell cultures, compared normal and scar derived fibroblasts, or tried to induce a scar phenotype from healthy fibroblasts<sup>32-34</sup>. Although being simple, fast and inexpensive, skin comprises more than just the fibroblasts. Indirect co-cultures of keratinocytes (monolayer or differentiated epidermis) and fibroblast monolayers using transwell systems enabled the study of keratinocyte-fibroblast interactions and the evaluation of the effects on either cell type separately<sup>35-39</sup>. However, the lack of physiological relevance was obvious due to the absence of any resemblance with the 3D macroscopic fibrotic tissue structure typical of a scar. The expression of biomarkers derived from studies on gene and protein expression are most probably greatly influenced by the 3D structure present in a native scar. It was noticed that the introduction of a more physiologically relevant 3D environment (collagen or fibrin gel) and mechanical load positively influenced the behavior of fibroblasts towards the scar phenotype<sup>40</sup>. By enabling fibroblasts to produce their own matrix an even more *in vivo* like situation was created<sup>41</sup>. The realization that an extensive crosstalk between keratinocytes within the epidermis and fibroblasts within the dermis occurs to regulate the synthesis of extracellular dermal matrix<sup>42</sup> led to the introduction of organotypic skin equivalents being used to investigate scar pathogenesis. 3D skin equivalent models have been described using keloid fibroblasts in combination with normal skin derived keratinocytes<sup>35,43</sup>. This latter is considered a relevant limitation in the model since keloid keratinocytes have been described to be intrinsically different to normal skin derived keratinocytes<sup>36-38,44-47</sup>. Using a similar method, a fully differentiated epidermis constructed from keratinocytes isolated from hypertrophic scars on a fibroblast (healthy) populated dermal matrix was able to exhibit a few characteristics of an adverse scar (e.g. dermal thickness, epidermal thickness, collagen I) and illustrated the role of keratinocytes in hypertrophic scar formation<sup>48</sup>. Extensive implementation of these models for testing therapeutics is however limited by the lack of robust validated biomarkers and their dependence on excised scar tissue. This led to a recent development in our laboratory in which we were able to show that mesenchymal stromal (stem) cells derived from subcutaneous adipose (ASC) can be used to construct a tissue-engineered hypertrophic scar model. The model consists of a reconstructed epidermis derived from normal healthy human keratinocytes on a dermal matrix populated with ASC<sup>49</sup> (Figure 1). The hypertrophic scar model not only exhibits many characteristics of hypertrophic scars (e.g. increased collagen I secretion, contraction and epidermal thickness; decreased epithelisation, Il-6 and CXCL8 secretion), but also enabled relevant and quantifiable hypertrophic scar parameters to be identified and validated with anti-scar therapeutics (e.g. 5-fluorouracil, triamcinolon). Although this model is definitely a clear advancement, it is only representative of hypertrophic scar formation caused by severe trauma (e.g. burns) where the adipose tissue is exposed.

It is not representative of hypertrophic scar formation resulting after skin closure of an excision wound after routine surgery, nor of keloid formation, which can develop years after relatively minor injury.

Multipotent keloid-derived mesenchymal-like stem cells, found in the pathological niche of the scar, have also been implicated in the keloid formation<sup>50-54</sup>. Therefore, keloid explant models are interesting as they allow these cells to remain in their pathological niche. *Ex vivo* biopsies have been cultured at air-liquid interface, embedded in collagen gels<sup>55,56</sup>. The keloid phenotype persisted in culture as demonstrated by the maintenance of: collagen I and III expression, immune cell fraction (T-cells, B-cells, NK cells, mast cells, neutrophils, Langerhans cells), mesenchymal cells and endothelial cells. The functionality of this model was further confirmed by the reduced epidermal thickness and scar volume after treatment with the dexamethasone. While this model certainly shows promising potential, it is entirely dependent on a regular supply of scars that are both freshly excised and sufficiently large, which prevents widespread implementation.

## LIMITATIONS

From the above we can identify a number of clear limitations in the current available models.

*Animal models are not suitable for studying human adverse scar formation.* Apart from the ethical issues described in the 7<sup>th</sup> directive (3Rs - reduction, refinement, replacement), the physiology of animal skin and their immune system are so different from humans<sup>23</sup> that pivotal factors responsible for differences between normotrophic, hypertrophic or keloid scar formation are impossible to identify.

*Human cell culture models are still limited by their extreme simplicity.* A scar is generated by a complex cascade of cellular interactions starting at the initial time of injury. The numerous cell types which are involved such as fibroblasts, endothelial cells, keratinocytes, immune cells (e.g. mast cells, monocytes, macrophages, neutrophils, T cells, dendritic cells) to name but a few are not yet incorporated into relevant human culture models<sup>16,57-59</sup>. Furthermore mechanical loading is not taken into account in current models.

*The genetic predisposition factor is not taken into account.* With the exception of extreme burn trauma which nearly always results in hypertrophic scarring, an important pivotal factor which is not taken into account is the genetic predisposition of the individual. This predisposition will influence the entire process of scar formation from the inflammatory response to the tissue remodelling and final scar formation.

*Scar models have a limited duration of days/weeks whereas human scars develop over a period of months/years.* This means that while scar models will enable us to identify genes and proteins (biomarkers) reflecting the early stages of scar formation, the macroscopic

raised but at the same time contracted fibrotic structure of the scar is rarely pronounced to the extent that is characteristic of an adverse scar.

In our efforts to develop high/ medium throughput test models to study the beginnings of scar formation we have sacrificed the essence of the subject – why does an adverse scar form instead of a normotrophic scar and what causes a hypertrophic scar to form rather than a keloid scar. This information is essential if we are to develop optimal strategies in the future to prevent adverse scar formation.

### **CHALLENGES AND FUTURE PERSPECTIVES**

It is always easy to identify the limitations of a model. However, the solution to the limitations is more difficult and will be an extremely inspiring challenge to scientists. Advancements with constructing TERT immortalized cell lines should be exploited, making it possible to maintain cell strains representative of patients with different predispositions to normotrophic, hypertrophic scar and keloid as well as solving logistical and ethical limitations concerning freshly excised tissue. Recently an exciting new multidisciplinary scientific field, “organ-on-a-chip”, has been developing for organ and disease models which may also be suitable for *in vitro* scar models. Organ-on-a-chip involves engineered tissues which closely mimic their *in vivo* counterparts and consist of multiple different cell types adjacent to and interacting with each other under closely controlled conditions, and are importantly grown in a microfluidic chip. These controlled conditions will make it possible to mimic the environment of the skin (humidity, temperature, pH, oxygen levels), the elasticity of the skin, and the complex structures and cellular interactions within and between the different cell types of the skin. Importantly the microfluidics compartment, in addition to possibly prolonging the lifespan of the cultures, will mimic the blood and lymph vasculature enabling incorporation of immune cells into the model. Early examples are “lung-on-a-chip”, “intestine-on-a-chip”, “lymph node-on-a-chip” and “vasculature-on-a-chip”, used to study physiology, pathophysiology and to develop and discover drug targets<sup>60-63</sup>. Once a “scar-on-a-chip” model has been established it should be possible to generate abnormal scar models with different genetic predispositions to a normotrophic, hypertrophic and keloid scar using (e.g. TERT immortalized) skin and immune cells derived from patients. Such an approach will not only enable investigation of the general pathophysiology of scar formation, but will also allow research into effects of genetic influences on the disease process. Ultimately this will make it possible to develop a medium-throughput drug target discovery and development platform, comprising a library of different genetic backgrounds to be used as “*in vitro*” clinical trials.

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## SUPPLEMENT CHAPTER 8

Supplement table 1. Examples of published hypertrophic scar models and parameters

	Histological features			Extra cellular matrix										GF & C	Others		Limitations / notes	Ref.											
	Dermal thickness	Contraction	No. of vessels	No. of cells	Epithelialisation	Epidermal thickness	Rete ridges	Hair follicles	Collagen 1	Collagen 3	Collagen content	Fibronectin	Decorin	Biglycan	Versican	$\alpha$ -sma			MMP-1 or MMP-9	TIMP-1	HSP46 or HSP47	TGF $\beta$ 1	CTGF	IL6/CXCR	Fibs proliferation	Apoptosis	No. of mast cells	No. of macrophages	No. of fibroblasts
<b><i>In vivo</i> human HTscar formation</b>	↑	↑	↑	↑	↓	↓	↓	↑	↑	↑	↑	↓	↑	↑	↑	↓	↑	↑	↑	↑	↑	?	?	↓	?	?	?	- difficult to distinguish normotrophic vs. hypertrophic scar formation	1-4
<b><i>In vivo</i> Animal models</b>																												- poor correlation human / animal	
<u>Grafting skin/scar onto animal</u>																													
Nude mice: grafted with split-thickness human skin	↑	↑	↑	↑	↓	↓	↑					↓	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↓	↑	↑	↑	- no clear distinction between different types of scars; rejection	5-7	
Transplanting HTscar to nude mice	↑									↑	↑								↑								- requires HTscar material	8,9	
Transplanting HTscar to nude rats									↑	↑																	- requires HTscar material	10,11	
<u>Induction of HTscar</u>																													
Rabbit ear model (excision wound)	↑	↑	↑	↑	↑			↑	↑	↑														↓				- specific to ears	12,13
Mice: mechanical stress to full-thickness excision wound	↑	↑	↑		↓	↓																	↑	↑					14
CXCR3 <sup>-/-</sup> mice: circular wound	↑				↑			↑	↑	↑		↑													↑	↑		- keloid characteristics	15
Rat: burn wounds by copper disk									↑	↑						↓													16
Duroc pig: wounds	↑	↑	↑										↓	↓										↑				- big animal	17,18
Guinea pig: circular wounds + coal tar	↑	↑			↑																				↑			- toxicity due to coal tar	19
<b><i>In vitro</i> models</b>																													
<u>Human healthy cells</u>																													
Monolayer of deep dermal fibroblasts												↓				↑				↑	↑	↑						- 2D culture; only fibroblasts	20,21

	Histological features				Extra cellular matrix										GF & C		Others		Limitations / notes	Ref.								
	Dermal thickness	Contraction	No. of vessels	No. of cells	Epithelialisation	Epidermal thickness	Rete ridges	Hair follicles	Collagen 1	Collagen 3	Collagen content	Fibronectin	Decorin	Biglycan	Versican	$\alpha$ -sma	MMP-1 or MMP-9	TIMP-1			HSP46 or HSP47	TGF $\beta$ 1	CTGF	Fibs proliferation	Apoptosis	No. of mast cells	No. of macrophages	No. of fibroblasts
Scratch assay monolayer fibroblasts																											- 2D culture /1 parameter; only fibroblasts	22
DE: deep dermal fibroblast in collagen-glycosaminoglycan matrix		↑								↑		↓		↑	↑	↓		↑			↑						- only fibroblasts	23
DE: mechanical stress to FPCL		↑							↑	↑						↓								↓			- only fibroblasts	24
SE: reconstructed epidermis of KC on a dermal matrix containing ASC		↑			↓	↑			↑						↑							↓					- no immune component	25
Co-culture: fibroblast & BM-MSCs		↑							↑	↑						↓											- no immune component	26
Co-culture: fibroblast & rat mast cell		↑								↑					↑							↑					- use of rat mast cell line	27
<b>Human HTscar cells</b>																											- requires HTscar material	
Monolayer of fibroblasts										↑	↑	↓									↑	↑		↓			- 2D culture; only fibroblasts	28-30
DE: FPCL		↑							↑	↑													↑	↓			- only fibroblasts	29,31,32
DE: fibrin gel containing fibroblast		↑							↑																		- only fibroblasts	33
SE: reconstructed epidermis of KC on a self-assembled matrix of fibroblasts		↑				↑			↑							↓	↑										- no immune component	34-36
<b>Ex vivo</b>																											- requires HTscar material	
Stretched HTscar biopsies						↑				↓					↑								↓				- limited manipulation	37,38
HTscar biopsies		↑																									- limited manipulation	39

**Examples of published hypertrophic scar models and parameters.** ASC, adipose tissue-derived mesenchymal cells; BM-MSC, bone marrow-derived mesenchymal stem cells; CXCL, chemokine (C-X-C motif) ligand; DE, dermal equivalent; Epid., epidermal; FPCL, fibroblast populated collagen 1 lattice; Fibs, fibroblasts; GF & C, growth factors & cytokines; HTscar, hypertrophic scar; IL, interleukin; KC, keratinocytes; Ref; references; SE, skin equivalent; ↓, decreased; ↑, increased; ?, contradictory results in literature/models or subtype/activation state may be more important than number of cells

**Supplement table 2. Examples of published keloid scar models and parameters**

	Histological features			Extracellular Matrix										GF & C		Others			Limitations / notes	Ref.												
	Dermal thickness	Contraction	No. of vessels	No. of cells	Epidermal thickness	Volume/weight	Collagen 1	Collagen 3	Keloidal collagen	Elastin	Fibronectin	Proteoglycans	GAGs	α-sma	MMP-2/MMP-9	TIMP-1/TIMP-2	PAI-2	HSP27 or HSP47			TBFB & related prot.	CTGF	VEGF	IL6/CXCL8/CCL2	Proliferation	Apoptosis	Migration	Invasion	Cell-spreading	Cell attachment		
<b>In vivo Human Keloid formation</b>	↑	↓	?	↑	↑	↑	↑	↑	↓	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	?	?	↑	?	?	?	?	?	3,40-52		
<b>In vivo Animal Models</b>																																
<u>Grafting Kscar into animal</u>																																
Nude mice: KF								↑		↑																					- poor correlation human / animal	
Nude mice: KF in 3D matrix								↑		↑																					- only fibroblasts	
Nude mice: dermal biopsy								↑		↑																					- only fibroblasts	
Nude mice: biopsy			#↓					↑		↑																					- requires Kscar material	
Hamster: biopsy								↑		↑																					- requires Kscar material	
								↑		↑																					- loss of epidermis; Kscar parameters not assessed	
<u>Induction of Kscar</u>																																
Horse: full thickness incisions																																- no development of Kscar-like tissue (EGT)
<b>In vitro Human Models</b>																																
<u>Human healthy cells</u>																																
NF co-cultured with CD14+ cells from keloid patients																																- only monocytes from Kscar patients
Indirect co-culture of HaCaT epidermis (overexpressing Activin A) with NF monolayer																																- transfected NK
NK epidermis on a fibroblast (Rspo2-transfected)-populated matrix								↑																								- transfected NF

	Histological features			Extracellular Matrix											GF & C			Others			Limitations / notes Ref.									
	Dermal thickness	Contraction	No. of vessels	No. of cells	Epidermal thickness	Volume/weight	Collagen 1	Collagen 3	Keloidal collagen	Elastin	Fibronectin	Proteoglycans	GAGs	α-sma	MMP-2/MMP-9	TIMP-1/TIMP-2	PAI-2	HSP27 or HSP47	TGFβ & related prot.	CTGF		VEGF	IL6/CXCL8/CCL2	Proliferation	Apoptosis	Migration	Invasion	Cell spreading	Cell attachment	
<b>Human Kscar cells</b>																														
Monolayer of keratinocytes																														- 2D culture; only keratinocytes
Monolayer of fibroblasts							↑	↑			↑	↑			↑	↑	↑	↑	?	?			↑	↑	↑	↑	↑	↑	- 2D culture; only fibroblasts	
DE: FPCL		↑					↑								↑														- only fibroblasts	
Indirect co-culture of KK monolayer with KF monolayer																							§↑	§↓					- no immune component	
Indirect co-culture of KK epidermis with KF monolayer*							↑	↑			↑																		- no immune component	
Direct co-culture of NK epidermis on a KF-populated matrix	↑	↑			↑									§↑															- no immune component	
<b>Kscar explants</b>																														
Submerged culture of dermal biopsy (1 wk)							↑	↑		↑	↑				↑														- limited manipulation; requires Kscar material	
Air-exposed culture of biopsy embedded in collagen gel (6 wk)				↑	↑	↑	↑	↑																					- limited manipulation; requires Kscar material; some ↓ epidermis viability &/or detachment	

**Supplement Table 2. Examples of published Keloid scar models and parameters.** CXCL, chemokine (C-X-C motif) ligand; DE, dermal equivalent; C, cytokines; Epid., epidermal; EGT, exuberant granulation tissue (equine Kscar equivalent); FPCL, fibroblast populated collagen 1 lattice; GAGs, glycosaminoglycans; GF & C, growth factors & cytokines; HTscar, hypertrophic scar; IL, interleukin; KF, keloid scar fibroblasts; KK: keloid scar keratinocytes; Kscar, keloid scar; NF, normal skin fibroblasts; NK, normal skin keratinocytes; SE, skin equivalent; TGFβ and related proteins: includes TGFβ1/2/3, TGFβ receptors, Smad 2/3/4; Ref., references; ↓, decreased; ↑, increased; ?, contradictory results in literature/models; §: organization of α-SMA expression; ‡: chondroitin; ¥: hyaluronic acid; #: occlusion of microvessels; ° no difference in MMP-9 expression; \$: applicable to fibroblasts; N.B. explant biopsies listed are full thickness (including the epidermis) unless stated otherwise; keloidal collagen includes the presence of thick collagen whorls as well as nodules.

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