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STATE-OF-THE-ART REVIEW

Cutting into wound repair

Donovan Correa-Gallegos[1] and Yuval Rinkevich[2]

1 Institute of Lung Biology and Disease, Comprehensive Pneumology Center, Helmholtz Zentrum München, Munich, Germany
2 Institute of Regenerative Biology and Medicine, Helmholtz Zentrum München, Munich, Germany

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Correspondence
Y. Rinkevich, Institute of Lung Biology and Disease (iLBD), Helmholtz Zentrum München, Max-Lebsche-Platz 31, 81377 Munich, Germany
Tel: +49 (89) 3187 4685
E-mail: yuval.rinkevich@helmholtz-muenchen.de

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The skin is home to an assortment of fibroblastic lineages that shape the wound repair response toward scars or regeneration. In this review, we discuss the distinct embryonic origins, anatomic locations, and functions of fibroblastic lineages, and how these distinct lineages of fibroblasts dictate the skin's wound response across injury depths, anatomic locations, and embryonic development to promote either scarring or regeneration. We highlight the supportive role of the fascia in dictating scarring outcomes; we then discuss recent findings that indicate fascia mobilization by its resident fibroblasts supersede the classical de novo deposition program of wound matrix formation. These recent findings reconfigure our traditional view of wound repair and present exciting new therapeutic avenues to treat scarring and fibrosis across a range of medical settings.

Introduction

Tissue injury can lead to a spectrum of different outcomes from scarring to regeneration. Regeneration fully restores form and function. Scarring on the other hand is unsightly and compromises tissue function, although it often ensures survival of the organism. Inspired, by whole-body regeneration in flatworms and sea squirts [1,2], and full-limb regeneration of amphibians [3], medical scientists have tried for over a century to harness regeneration in man [4].

Mammalian skin regeneration is a rarity, and skin often repairs imperfectly by only restoring its main function as an external barrier without regaining its original architecture. Skin wounds that scar-over fail to restore secondary appendages, such as hair follicles and sebaceous glands, and a dense plug of scar tissue seals the wound instead [5]. Perturbations of this repair process can lead to even more severe skin pathologies.

The excessive production of scar tissue severely affects the normal physiology of numerous organs, and it often leads to a decreased life expectancy [6]. In the skin, pathological scars, such as keloids, hypertrophic scars, and skin contractures, impair normal movement and impact lifestyle and mental health [7]. On the other side, when wound repair and scarring are compromised, nonhealing chronic wounds expose patients to persistent infections, poor thermoregulation, and fluid loss [8]. Considering both pathological scars and nonhealing chronic wounds, the annual cost of wound care in the United States alone is likely between 50–100 billion dollars [9]. Thus, it is imperative to expand our knowledge on the skin's wound

Abbreviations
CDH2, N-cadherin; En1, engrailed-1; ENFs, En1-naïve fibroblasts; EPFs, En1-past fibroblasts; GJA1, gap junction alpha-1 protein/Cx43; LEF1, lymphoid enhancer-binding factor 1; NHS, *N*-hydroxysuccinimide; pEPF, postnatal EPFs.

repair mechanisms, which in turn would lead to innovative approaches to improve the skin repair process.

In this review, we summarize past achievements and the current world of skin scarring and regeneration to offer future perspectives for the field. Particularly, single-cell transcriptomics and genetic lineage-tracing methods have uncovered unexpected heterogeneity within cell types that participate in skin repair, for example, revealing many functionally different specialized populations of fibroblasts. Recently in our laboratory, we have shown that fibroblasts mediate massive extracellular matrix mobilization of soft connective tissue in response to injury. These cells thus control tissue architecture rather than merely producing extracellular wound matrix. While there have been many excellent reviews on wound repair [10–12], our angle here will be to reassess the field of injury repair in light of these two emerging concepts: fibroblast heterogeneity and soft matrix mobilization.

Wound repair depends on fibroblast diversity

Fibroblasts undertake multiple roles that move forward the repair process toward regenerative or scarring outcomes. Fibroblasts attract and regulate immune cells by secreting cytokines and chemokines in response to injury [13]. They also secrete various extracellular matrix proteins, such as fibrillar collagens, which form and mature a scar connective tissue at sites of injury [14]. Additionally, dermal fibroblasts differentiate into contractile myofibroblasts that actively modify their surrounding niche [15]. This functional versatility makes fibroblasts keystone components of the wound-healing processes. However, skin wounds do not necessarily end with scars. The skin exhibits a natural diversity of wound phenotypes and severities that include rare cases of regeneration, without scars.

Fibroblast heterogeneity in developing skin

More than six decades ago, Arthur Hess noticed that guinea pig fetuses repair scarlessly and thus have a 'greater growth (healing) potential than those of postnatal animals' [16]. In the following decades, these observations were replicated in other mammalian species including our own [17–25]. Indeed, regeneration potentially decreases sharply in later gestational stages. Injuries in 16-day murine embryos repair scarlessly while healing of injuries made just 2 days later resembles adult scarring [26–27]. The regeneration-to-scar phenotypic transition during fetal life was further documented in rats, marsupials, rabbits, pigs, and in nonhuman primates. A similar transition in wound response has also been documented in 2nd to 3rd trimester of human fetuses, revealing a universal trait in mammalian skin to transition from regeneration to scarring [24,28–30].

More recently, a series of heterochronic grafting experiments proved that embryonic skin repairs scarlessly even in the adult environment [29], and conversely, adult skin generates scars when transplanted in fetuses [31]. These results indicated that the uterine environment was not responsible for the scarless repair but rather that early and late embryos have different kinds of fibroblasts or fibroblasts in a different state of commitment and or activation.

Before, it was assumed that the changes in fibroblasts physiology accounting for the regeneration-to-scar transition may well be a matter of maturation or induction by the environment. However, exploring this hypothesis in mouse back skin, we found rather that fibroblast populations from regeneration stages of repair are replaced by clonal expansion of a different scar-forming fibroblast lineage [32]. The scar-forming fibroblast lineage is characterized by transient embryonic expression of the engrailed-1 (En1) transcription factor, also termed En1-past fibroblasts (EPFs), whereas the regenerative fibroblasts lack En1 expression, hence called En1-naïve fibroblasts (ENFs). We proved this using a cre-recombinase driver mouse of this gene crossed with fluorescent reporter mice where membrane-bound TdTomato (red fluorescent protein) is expressed in regenerative fibroblasts (ENFs) and replaced by membrane-bound green fluorescent protein in the scar-forming fibroblasts (EPFs) during back-skin development. In this system, regenerative and scarring fibroblasts are therefore separately colored by red and green fluorescence signal, respectively.

During the regeneration-to-scar transition, we observed the maturation of primitive dermis, characterized by the change from a fibronectin-based to a collagen-rich extracellular matrix architecture as well as cellular rearrangements in the dermis to form the mature layered reticulated pattern. Simultaneously to this maturation process, EPFs that originated from the somitic mesoderm migrated into the primitive dermis. ENFs are replaced by EPFs due to a much higher proliferation rate and low apoptosis in EPFs. This generates a gradual lineage replacement between the two ENF/EPF fibroblast lineages in the back skin.

This lineage replacement during the regeneration-to-scar transition led us to question whether EPFs were an intrinsically scar-forming population from the moment they populated the skin or if they undergo an additional maturation process. By generating

heterochronic transplants (i.e., where donor and host are of different ages) of sorted EPFs from regenerative or scarring stages into adult mice, we observed that the transplanted EPFs formed extracellular matrices comparable to adult scars, regardless of the age of the mice from which the EPFs came. These results indicated that EPFs are scar competent from the moment they arrive in the skin. Opposingly, heterochronic ENF transplants enhanced angiogenesis, suggesting these nonscarring fibroblasts are more regenerative. Overall, our experiments revealed that the regeneration-to-scar transition results from the replacement of primal ENFs by scar-forming EPFs. In this way, by using state-of-the-art fate mapping and transplantation techniques, we provided an explanation of Hess's observation of the enhanced healing potential of mammalian fetuses.

The physiological heterogeneity of ENFs has recently been explored in the adult back skin. Inducible genetic fate tracing shows that adult ENFs possess the capacity to turn on En1 transcription in tension-loaded incisional wound models, and in response to high skin tension become postnatal EPFs (pEPF) with scarring potential. Blocking the mechanotransducive activity of the transcriptional coactivator YAP1 prevents En1 transcriptional activation and ENF-to-pEPF conversion and promoted skin regeneration [33]. These findings indicate that En1 transcription may control fibrosis, serving as a central regulator of the scarring program. Further studies are needed to reveal the transcriptional link in fibroblasts between En1 and scarring.

Fibroblast heterogeneity in adult skin

The developmental switch from regeneration to scarring is a stark example of fibroblast heterogeneity, but there are also diverse kinds of fibroblasts in different compartments of the adult. While adult skin repairs by scarring, injuries in the oral mucosa regenerate [34,35]. Furthermore, within the oral cavity there are local differences in healing rate, which is faster in the soft than in the hard palate [36]. These observations suggest that the scarless repair is not restricted to embryos and offer further encouragement that regeneration could be harnessed in adult injuries.

Unlike the mesodermal origin of dermal fibroblasts, oral fibroblasts originate from the neural crest [37] and are multipotent, at least *in vitro* [38]. However, multipotency is unlikely to explain the regenerative potential, as oral and dermal fibroblasts have a similar expression of pluripotency markers [39].

The differences in healing between dermal and oral tissue have been thought to be a complex interplay resulting from the environmental milieu created within the oral cavity by the presence of saliva [40], a muted angiogenic response upon wounding [41], and a distinct fibroblast molecular profile and phenotype, including the presence of long telomeres and a resistance to fibrotic triggers such as transforming growth factor beta 1 [42,43]. Our heterochronic transplantations, however, indicate that fibroblasts dictate the scarless vs scarring outcome independently of their environment [32]. Importantly, EPFs can cause scarring when transplanted into oral cavity and, like ENFs, oral fibroblasts repair scarlessly when transplanted into skin [44].

More recently, we demonstrated that oral fibroblasts cannot migrate in a coordinated way like their scar-forming counterparts of the dorsal skin. This is important because cell–matrix interactions needed for migration are central to how new connective tissues are formulated in wounds to generate either a dense plug of connective tissue scar or a healthy reticulated lattice organization. Indeed, blocking coordinated migration of fibroblasts in the skin gave a similar regenerative phenotype to oral mucosa, suggesting that fibroblast migration dynamics plays a major role in repair outcome that diverges between dermal and oral tissue [45]. For now, the reason behind oral mucosal fibroblasts' regenerative prowess still remains a mystery and, furthermore, the full extent of their heterogeneity awaits to be elucidated.

Fibroblast heterogeneity across skin depth

To understand fibroblast heterogeneity in the skin, and the spectrum of skin repair outcomes observed in the clinic they cause, it is appropriate at this point to give a short primer of skin anatomy. Human skin has three functionally discrete layers [46,47]: the outer layer, the epidermis, hosts keratinocytes and forms a stratified epithelium that varies from 0.075 to 0.6 mm thick (in areas like the palms and soles). The dermis below is a connective tissue layer between one and four millimeters thick across different anatomical regions. The dermis has extensive neurovascular and lymphatic networks and secondary skin appendices such as hair follicles and sebaceous glands. This layer has two sublayers: the upper papillary and the lower reticular dermis. The papillary dermis is just 0.3–0.4 mm thick and contains densely packed thin collagen fibers. In contrast, the reticular dermis forms interwoven bundles of thicker fibers. The deepest layer is the hypodermis that includes fat tissue sustained by two layers of soft connective tissue interconnecting the dermis with the musculoskeletal tissue. The hypodermis comprises the superficial fascia

with loosely arranged thin fibers that blend into the more densely packed deep fascia, which directly attaches to muscles [48].

A complex extracellular matrix architecture is produced and maintained by resident fibroblasts throughout the dermis and hypodermis, with functional differences between papillary, reticular, and fascia fibroblasts.

Papillary fibroblasts proliferate more, migrate faster, and are more resistant to apoptosis but are less contractile than reticular fibroblasts [49–57]. These fibroblasts also produce the basement membrane that sustains epidermis morphogenesis [56,58,59] and are more sensitive to deterioration with age [60,61].

Reticular fibroblasts express higher levels of collagens and profibrotic growth factors such as transforming growth factor beta 1 and connective tissue growth factor, suggesting that these deeper fibroblasts might be more capable of forming scars [62,63]. Fascia fibroblasts proliferate less than dermal fibroblasts, are less contractile, and failed to sustain epidermal morphogenesis *in vitro* [64].

Perhaps the most conclusive evidence of the functional divergence of the different fibroblast populations comes from skin reconstitution assays in mice. By cell sorting and transplanting back papillary, reticular, and hypodermal/fascia populations, Driskell and colleagues proved that each population has distinct intrinsic capabilities. While papillary fibroblasts reconstituted healthy skin architecture including hair follicles, reticular and hypodermal/fascia fibroblasts generated a scar-like tissue devoid of skin appendages [65]. This experiment conclusively proved that the layered architecture of the skin is the result of the action of functionally divergent fibroblast populations. Furthermore, this and previous comparative studies put the reticular and fascia fibroblasts in the spotlight as the direct agents of scarring.

It is imperative to fully characterize fibroblast diversity, and the advent of single-cell transcriptomics has revealed a more complex picture than expected before suggesting new fibroblast populations, although the functional significance of these new subtypes is yet to be demonstrated [66–72].

Likely these novel subpopulations are cellular states acquired by fibroblasts when undertaking specific functions. For example, the ratio of two murine papillary fibroblasts subpopulations changes during rest and growth phases of the hair cycle [73], suggesting that papillary fibroblasts change their transcriptomic profiles when supporting hair growth-related functions. Similarly, wound-healing studies annotated various fibroblast populations [74,75], which are likely states acquired by one, or by multiple, resident fibroblasts of the skin. Future, more extensive characterization of the nature, origins, and interconnections between these novel populations will provide a clearer and fuller picture of skin fibroblast functional heterogeneity.

The aforementioned heterogeneity of fibroblasts based on functionally distinct fibroblast compartments across skin depth provides an insight into the occurrence of clinically relevant scarring events.

Visible scarring results when injury depth is at least one third of the total epidermis–dermis thickness, while more superficial wounds heal scarlessly [76]. The occurrence of pathological skin scars, such as keloids, hypertrophic scars, and scar contractures, also directly correlates with deeper injuries [77–82]. These observations suggest the direct role of the deepest skin layers: reticular dermis and hypodermis—and their resident fibroblast populations—in producing scars.

Driskell and colleagues confirmed this hypothesis arising out of those clinical observations nearly a decade ago, via lineage-tracing studies in mice using a cre-recombinase driver mouse line, under the reticular and fascia marker: protein delta homolog 1. In this way, they observed that fibroblasts arrive in wounds from both of these compartments. By contrast, there were very few papillary fibroblasts in full-thickness wounds [65]. At that time, we did not know the relative contribution of both reticular and fascia fibroblasts and whether one population was more relevant for the scarring outcome.

To address this question, we developed a chimeric skin transplant model permitting the fate mapping of fascia vs reticular/papillary fibroblast [83]. In this transplantation assay, we manually generated chimeric dermis-fascia grafts from two reporter mice expressing different-colored fluorescent proteins. After reconstitution, an internal full-thickness wound was made in the middle of the graft and then transplanted into the back skin of mice. We observed that, after the repair response of the inner wound, fascia-derived cells thrived in the wound tissue making up to three-quarters of the cells on site.

We then combined the chimeric grafts with our EPF reporter system to inquire and prove that fascia resident EPFs could give rise to scarring-prone wound fibroblasts. We then reasoned that superficial injuries, which usually heal with minimal scarring, would repair without fascia fibroblast intervention. By performing chimeric grafts, in which the fascia section remained intact, we modeled superficial injuries and traced the contribution of fascia and dermal EPFs. As suspected, we observed that fascia EPFs were significantly reduced in superficial injuries and, more strikingly, this reduction of fascia EPFs directly correlated with

smaller scars. These results pointed to fascia fibroblasts as the principal agents of skin scarring.

In contrast to fascia fibroblasts' tendency to scar, their papillary counterparts lean toward scarless repair. A meta-analysis on single-cell transcriptomics from wounds of different sizes and, thus, different scarring/regenerating outcomes, showed that papillary fibroblasts are more prominent in smaller wounds that repair scarlessly. Not surprisingly, reticular and fascia populations were dominant in larger wounds that form scars [84].

The regenerative dexterity of papillary fibroblasts seems to be linked to their capacity to differentiate into mesenchymal populations that support hair follicle formation, such as dermal sheath, dermal papilla, and arrector pili cells [65,85,86]. The ability to reestablish all of these hair follicle-supportive cell types after injury depends on the action of lymphoid enhancer-binding factor 1 (LEF1), as knocking out this transcription factor in all dermal fibroblasts prevents hair follicle regeneration in small wounds in neonatal mice. Complementary, LEF1 knock-in into dermal fibroblasts promoted an improved outcome on large wounds by inducing hair follicle regeneration [87]. These results indicate that, potentially, the intrinsic capacity of fascia fibroblasts to form scars might be genetically and clinically reversible. Indeed, combined single-cell transcriptomics and lineage tracing of reticular and fascia fibroblasts suggest they can convert into hair follicle-supporting cells. These conversions occur in the rare events of hair follicle neogenesis in very large wounds and not during skin morphogenesis or homeostasis [86]. A revisited analysis of the single-cell data, however, revealed that the proportion of reticular and fascia fibroblasts that convert to these regenerative populations was significantly lower than in the reporter system [84]. Nevertheless, at any level, the plausible capacity of fibroblasts populations to interconvert, would have a huge clinical impact, as therapies aimed to reprogram scarring- into a pro-regenerative phenotype would improve repair and reduce the burden of pathological scars.

The direct influence of fascia, and to a lesser extent of reticular fibroblasts, on the scarring outcome and the regenerative capabilities of papillary fibroblasts—and oral fibroblasts—teach us again that fibroblast heterogeneity is a major driver of the contrasting repair outcomes (Fig. 1).

Classical *de novo* synthesis vs preexisting matrix models

The classical wound-healing process is divided into three distinct and overlapping stages [88]. (a) In the

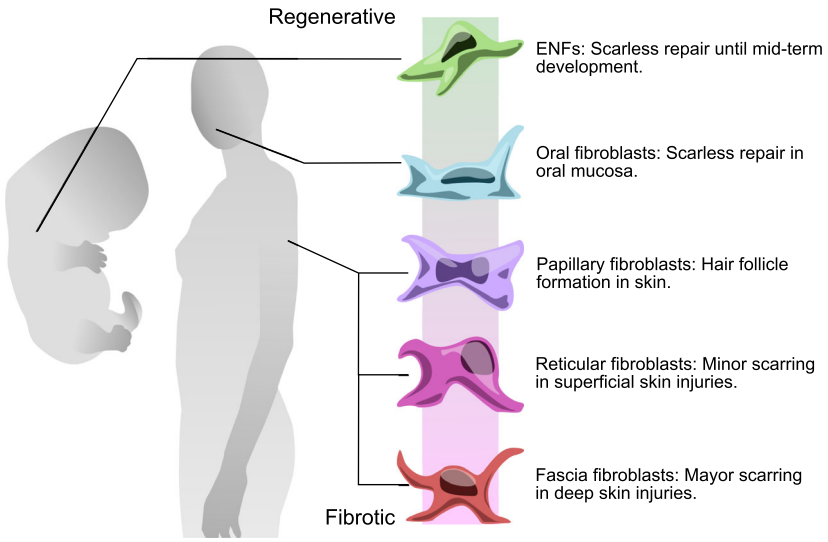

Fig. 1. Fibroblast heterogeneity dictates tissue repair outcomes. Fibroblast populations have functionally diverse regenerative vs fibrotic potential. Primal fibroblasts (ENFs) in the primitive dermis of fetuses confer full regeneration in intrauterine injuries. Clonal replacement of ENF with fibrotic EPF populations mediates the regeneration-to-scar transition observed between mid- and late-embryonic development. Oral fibroblasts promote scarless repair in oral mucosa injuries. Cutaneous papillary fibroblasts possess lower fibrotic capabilities and sustain hair follicle formation, thus restoring skin appendages. Reticular fibroblasts in the dermis have fibrotic potential and participate in the scar formation of superficial injuries but not in deep wounds. Fascia fibroblasts have the highest fibrotic prowess, generating large scars observed in deep injuries.

initial inflammatory stage, released platelets from damaged blood vessels promptly aggregate at the injury site. Platelet activation induces the maturation of circulating fibrinogen into a fibrin clot that works as a provisional matrix that plugs the open wound and prevents blood loss [89].

(b) In the proliferative stage, fibroblast numbers in the provisional matrix expand. Provisional matrix in wound bed is transformed into a granulation tissue that is rich in collagen III and fibronectin [90]. However, not all the provisional matrix becomes granulation tissue as the most superficial layer forms the scab. Is in between the scab and the granulation tissue that keratinocyte migrate to reestablish the barrier function of the skin [91].

(c) In the last remodeling stage, the granulation tissue further matures into scar tissue. Cellular density decreases while the extracellular matrix becomes arranged in a parallel pattern of thick collagen I fibers due to fibroblast-mediated contraction [92].

In this classical view, the different matrices of the wound bed at each stage, including provisional matrix, granulation tissue matrix, scab, and scar matrix, are all generated anew—or *de novo*—and built upon by platelets and wound fibroblasts [93]. However, revisiting historical observations puts this axiom in doubt.

A series of studies of the role of granulation tissue, from the 1950s, are inconsistent with the *de novo* matrix deposition as the sole method for wound repair.

Taking advantage of the need for ascorbic acid for collagen biosynthesis [94], Abercrombie and colleagues investigated the influence of *de novo* collagen production in the wound contraction of guinea pigs under an ascorbic acid-deficient diet [95]. They observed that scorbutic animals, which were deficient for *de novo* collagen biosynthesis, repaired skin injuries at similar rates to animals fed with normal food. More surprisingly, the levels of hydroxyproline in the scabs of scorbutic animals were also equivalent to control animals. Ascorbic acid is essential for the intracellular hydroxylation of prolines in newly synthetized procollagens, being hydroxyproline the most abundant modified amino acid in mature collagens [94]. Therefore, significant amounts of hydroxyproline in scabs in the absence of collagen biosynthesis argue against the notion that scabs are fully synthetized from scratch and suggested that other sources of premade collagen were likely forming the provisional matrix and scab.

Two years later, a series of publications, led by Jerome Gross, sought to test the influence of the granulation tissue in the wound contraction. Granulation tissue of wounds on guinea pigs, and later replicated on swine, was manually excised showing a negligible effect on the normal wound contraction rate [96–98]. These experiments were designed to prove that wound contraction was mediated by the surrounding tissue and not the central granulation tissue itself. The observations made, however, sat uncomfortably with the *de novo* formation of the granulation tissue. In a panel of granulation tissue 'knockout' experiments, the researchers excised the granulation tissue repeatedly every time it became visible. The re-appearance of new granulation tissue occurred on average every third day in guinea pigs and every day in the case of swine. Measurements, however, indicated that significant collagen production only started 1 week after injury [96], making it improbable that fibroblasts could generate, *de novo*, such a bulk of granulation tissue matrix in just a few days after each excision.

The authors disregarded the apparent unending capacity to reform the granulation tissue in short time frames, yet speculated, almost prophetically, on the fascia source of the wound fibroblasts: 'Thus, there is little evidence that wound fibroblasts are derived from the adjacent dermis. They may prove to be a population different from the dermal cells, probably hypodermal (fascia) fibroblasts, lacking the potential for regenerating the normal dermal architecture or inducing epidermal differentiation'. [98].

It would take more than 60 years, after these seminal observations, for definitive proof to arise, that preassembled matrices can be mobilized in response to injury. In order to fate-map the premade extracellular matrix, our group developed a simple, yet robust, method to label all extracellular matrix proteins *in vivo* based on the use of the amine-reactive cross-linker chemistry. Amine-reactive chemical groups, like *N*-hydroxysuccinimide (NHS) esters, create covalent amide bonds with the primary amines that are in all proteins. These chemical groups have been traditionally used to tag proteins, such as antibodies, *in vitro* [99]. Taking advantage of this capacity, we used NHS esters carrying fluorochromes to conjugate the preassembled matrix of the mouse back-skin and fate-map their movements in response to injury [83].

Subcutaneous injection of the NHS esters before injury specifically labeled the extracellular matrix of the superficial and deep fascia compartments, which remained stable for weeks. Only 3 days after injury, and to our surprise, three-quarters of the total collagen in the wound matrix derived from the preassembled fascia matrix. This indicates that the fascia matrix was mobilized to the injury site from an external repository of preassembled material. Importantly, we observed that activated platelets invaded the premade fascia

matrix near the wound surface, indicating that the provisional matrix originated from preassembled material coming from the fascia in conjunction with the action of platelets.

Using live imaging of skin-fascia explants, we observed a natural contraction behavior of the fascia matrix at a rate of 11.4 μm per hour. Translating similar dynamics *in vivo*, at this rate the fascia matrix could be mobilized across the average thickness of the mouse back skin of 200 μm [100] in just 17 h, accounting for Gross' group observations that granulation tissue was quickly restored after excision.

At the proliferation stage, we observed that both the scab and the granulation tissue below the regenerated epidermis were also derived from the premade fascia matrix. Physically preventing the mobilization of the fascia tissue completely prevented scar formation and a phenotype resembling nonhealing chronic wounds. Altogether, these observations implicate that premade fascia matrix gives rise to the other wound matrices: provisional, granulation tissue, scab, and scar matrices.

Only the soft fascia matrix could be mobilized, as the more rigid dermal matrix failed to be mobilized into the injury site. We then noticed that fascia matrix mobilization was restricted to full-thickness wounds while more superficial wounds closed without fascia matrix action, and instead, new deposited collagens were formed the scar matrix. These observations lead us to envision two different repair mechanisms depending on the injury depth.

On the one hand, superficial injuries that only penetrate the dermis would rely on the classical *de novo* synthesis of extracellular matrix components at the injury site as dermal matrix is too rigid to be mobilized (Fig. 2A). On the other hand, full-thickness injuries that penetrate deep into the fascia trigger the mobilization of the soft matrix reservoir into open wounds. The mobilized fascia matrix then generates the provisional matrix that later matures into the other wound matrices: scab, granulation tissue, and scar matrices (Fig. 2B).

Cell–cell interactions drive stored matrix mobilization

Our more recent findings indicate that cell–cell interactions and migration dynamics play a fundamental role on stored matrix mobilization in response to injury (Fig. 2B).

Employing intravital and explant live imaging microscopy we observed that fascia EPFs aggregate and coordinately migrate in swarms from the fascia upwards into wounds in a funnel motion [45]. This particular migration behavior formed a spiral-patterned granulation tissue matrix. Chemical blocking or genetic deletion of N-cadherin (CDH2) prevented fascia EPF swarming and resulted in smaller wounds with an irregular granulation tissue matrix pattern. These observations suggested that the matrix mobilization is intimately linked to fascia fibroblast swarm migration, which in turn is facilitated by CDH2-mediated cell adhesions.

Interestingly, swarm migration of fascia fibroblasts was also presciently suggested by Gross' group more than half a century ago when speculating on the origins and movements of the granulation tissue: 'One might speculate that the movement is produced by directional mass migration of connective tissue cells' [97].

CDH2-induced swarming occurred only in response to injury as no expression of this protein is detected in healthy skin. Yet, CDH2 expression was persistent in human hypertrophic scars [45]. We speculate that persistent swarming would continue to relocate soft matrix into the scar tissue, promoting its maturation into further scar matrix. Thus, an unregulated swarming and its ensuing mobilization of matrix could account for the scar tissue growth beyond the original injury site observed in hypertrophic scars and keloids. Indeed, human keloid fibroblasts shared a marker expression profile with fascia fibroblasts [83], indirectly suggesting the constant allocation of fascia fibroblasts into keloid lesions. Supporting this idea, a humanized-mouse model for keloid formation, using the skin reconstitution method with keloid-derived human cells reproduced most of the histological traits of keloid lesions with the notable exception of the 'invasiveness' or growth beyond the original injury site [101]. The authors suggest that the limited number of pathological cells or the absence of human microenvironment/supporting cells might account for the lack of this hallmark phenotype. Alternatively, a constant supply of cells and motile matrix from the fascia, not reproduced in this model, could push the original scarring tissue beyond their original boundaries and account for keloid overgrowth.

Besides CDH2-mediated cell adhesions, gap junctions also appear to regulate swarming of matrix-carrying fibroblasts. Gap junction alpha-1 protein/Cx43 (GJA1) is expressed in fascia EPFs during injury repair and forms gap junctions between fibroblasts that actively transfer calcium signals. Similar to CDH2 inhibition experiments, chemical blockage of these gap junctions altered fascia EPF swarm migration, prevented fascia matrix allocation into injury sites, and resulted in significantly smaller scars [102].

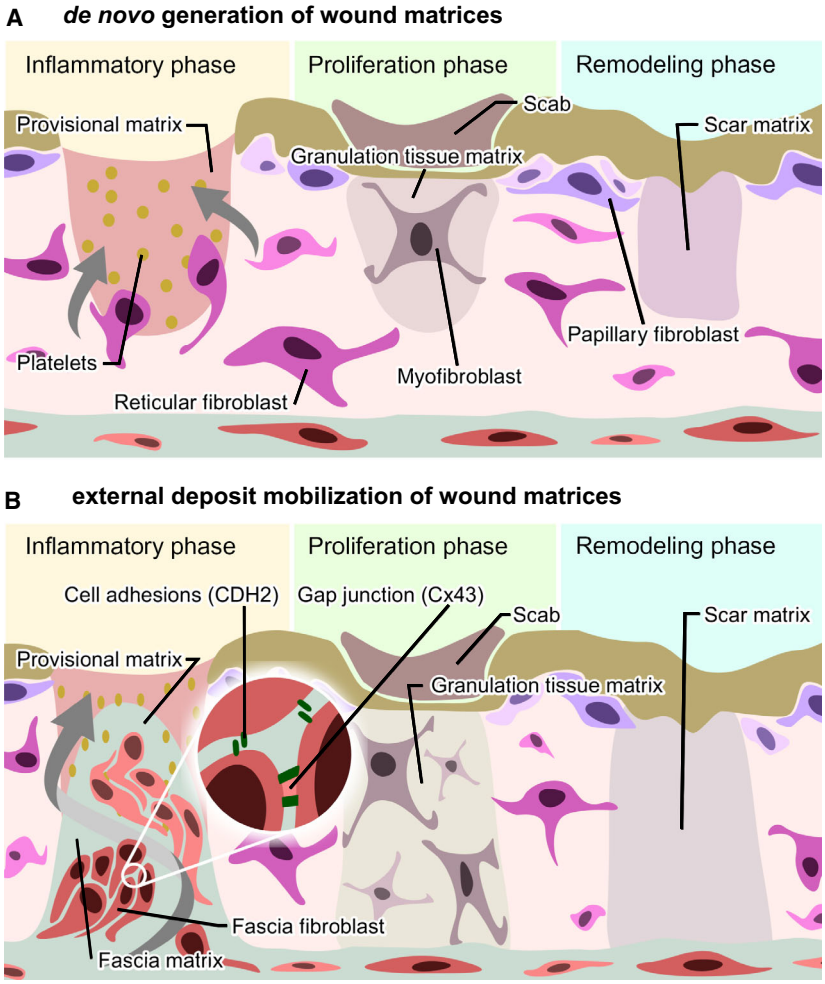

Fig. 2. Models of wound matrix formation. Specialized matrices arise at each stage of the wound-healing process. During the inflammatory phase, a provisional matrix seals the open injury. This provisional matrix matures during the proliferation phase to form the granulation tissue and scab matrices that seal breached open wounds. The granulation tissue matrix further matures during the remodeling phase to generate the scar matrix via myofibroblast activity. (A) In the classical *de novo* model of wound matrix formation, the primordial provisional matrix is generated anew by activated platelets, which mature a fibrin and fibronectin clot from circulating plasma. Reticular fibroblasts then migrate into the provisional matrix to deposit collagen III fibrils to form the granulation tissue matrix. Further deposition by myofibroblasts forms a collagen I-rich mature scar matrix. (B) Deep injuries that breach into the superficial connective tissue in the hypodermis trigger the mobilization of fascia matrix to seal the wound. The mobilization is mediated by swarm-type migrations of fascia fibroblasts in a funnel motion upwards through the dermis. Coordinated swarming behavior results from CDH2-mediated cell adhesion and Cx43-formed gap junctions between fascia fibroblasts. The mobilized fascia matrix, together with platelet activity, forms the provisional matrix of deep injuries. Fascia, and in lower extent reticular fibroblasts, helps mature the provisional matrix into the granulation tissue, scab, and resulting mature scar matrix.

Previous reports have also shown that chemokine-induced influx of cytosolic calcium preceded migration in human dermal fibroblasts [103]. Similar activation waves via juxtacrine interactions, between scar-forming cells, have also been observed during surgery-induced abdominal adhesions, which are postsurgical fibrous scars that develop in the abdomen [104].

Currently, it remains unclear whether intercellular communication, based on the intracellular calcium waves, is needed for the coordinated swarming. However, it is tempting to speculate that signal amplification through calcium waves, traveling via gap junctions on fascia fibroblasts, mediates the coordinated swarming behavior that leads to massive mobilization of soft matrix into wounds.

Altogether, these observations point toward the clinical potential of therapies regulating the matrix mobilization to prevent and alleviate the effects of

pathological scars. Blocking the mobilization, either physically, by inhibition of CDH2- or GJA1-mediated swarming, or by ablating fascia fibroblasts, failed to promote full skin regeneration but resulting scars were smaller, suggesting that blocking matrix mobilization allows reactivation of the classical *de novo* process for shallow wound matrix formation.

Most probably, these two mechanisms work simultaneously as a safety policy to ensure repair of skin injuries, even in the absence or malfunction of one of these mechanisms. Therefore, holistic approaches that take into account both imported and newly synthesized matrix will be necessary in future research into improving human skin repair.

Fascial system in fibrosis and repair

The novel external repository mechanism and the participation of the superficial fascia invite the investigation of the possible generalized role of this, and other soft connective tissues, in repair and fibrotic events (Fig. 3).

Superficial fascia is highly homogeneous across the body and, just like the skin, its thickness changes dramatically depending on the anatomical location. Studies on cadavers showed mean thickness ranges from only 42 μm in the chest to 189 μm in the legs [105,106]. Interestingly, superficial fascia is constantly thicker in women compared to men in an array of anatomical sites [105], whereas male skin is consistently thicker in the same locations [107]. Men back skin is 55% thicker than in women (2.3 vs 1.5 mm) [107] but female back superficial fascia is 15% thicker than in men (0.16 vs 0.14 mm) [105]. The combination of thinner skin with thicker superficial fascia would imply that injury on women would more easily breach the fascia compartment. This would mobilize matrix more often in women and inflict bigger and pathological scars more easily. This is indeed the case with hypertrophic scars and contractures in burn victims; although more men are treated for burns, pathological scars are more prevalent in women [108].

Age also influences the occurrence of pathological scars in burn victims. Older patients have lower risk of extreme scarring [108] and, consistently, the superficial fascia becomes thinner with age [109]. In contrast, human fetuses at stages where the regeneration-to-scar transition occurs show a progressive thickening of the superficial fascia tissue [110], further supporting its role in the acquisition of the scarring ability.

Dupuytren's disease provides additional evidence that the superficial fascia generates fibroses. In this disease, collagen III-rich fibrotic nodules appear in the superficial layer of the fascia at the joints of the fingers. Dupuytren's contractures are where the nodes evolve into contractile collagen I-rich nodule cords that often permanently bend the ring and pinkie fingers. Besides environmental and hereditary risk factors, the etiology of the disease likely involves trauma. The disease evolution closely mimics the wound-healing process, including an inflammatory process, collagen III-to-I extracellular matrix deposits, and myofibroblast differentiation [111]. Recognizing the inherent scarring capacity of fascia fibroblast in the back skin, it is reasonable to imagine that the abnormal activation of similar fibroblast repositories into

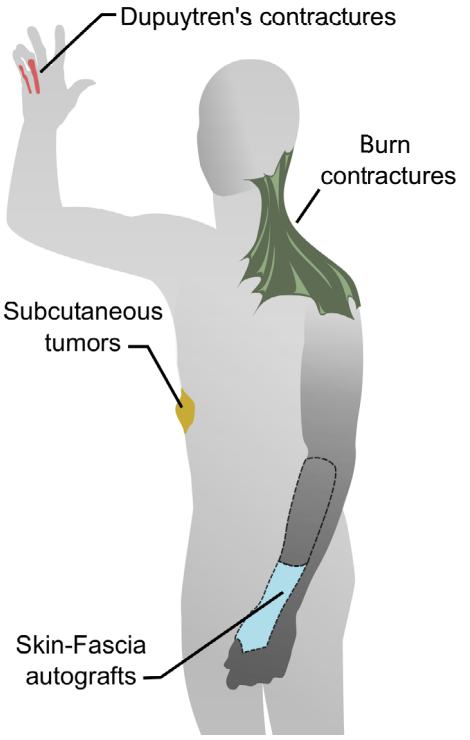

Fig. 3. Superficial fascia dictates fibrosis and repair. Several fibrotic conditions are closely linked to the superficial fascia. Skin contractures in burn victims are more prevalent in deep skin burns, suggesting the involvement of the superficial fascia in thermal injury scars. Contractures are also more common in women than men and correlated with thinner skin and thicker fascia in women. Dupuytren's contractures result from fibrotic nodules that form in the superficial fascia of the joints in the digits. Subcutaneous tumors that form in the superficial fascia are often more aggressive than dermal tumors. The tumor–fascia relation has been used in the clinic for more accurate cancer prognosis. Fascia autografts have been used for decades in the clinic for surgical corrections due to its natural faster patching qualities compared to dermal allografts.

maturing nodes could account for Dupuytren's contractures.

The superficial fascia might also contribute to the tumor stroma formation. Malignant subcutaneous tumors often develop near or within the superficial fascia, while more superficially located tumors are usually benign [112–114]. Just as with Dupuytren's nodes, tumor stroma formation mirrors the wound-healing process. Tumor progression is closely linked to the appearance of an immature provisional matrix, composed of fibrin and fibronectin, that develops into a collagen-rich fibrous matrix. In contrast to the normal wound healing, the cancerous cell-induced microenvironment instructs the conversion of recruited fibroblasts into persistently activated cancer-associated fibroblasts. The activity of these specialized fibroblasts molds the tumor stroma, which regulates tumor immunity and metastasis [115]. Interestingly, cancer-associated fibroblasts, recruited after transplanting melanoma cells into the back skin of mice, were derived from the scarring-prone lineage of EPFs [44]. Bearing in mind that fascia fibroblasts also belong to this lineage, it would be informative to test whether this population is an important source of cancer-associated fibroblasts. This would account for the preponderance of aggressive tumors that form in the superficial fascia. Thus, therapies designed to block mobilization of external matrix reservoirs could potentially prevent the formation of the tumor stroma and halt cancer progression.

The superficial fascia beneath the skin is part of an anatomical continuum of soft connective tissues termed the 'fascial system'. The fascia research society defines the fascial system morphologically to '...consists of the three-dimensional continuum of soft-, collagen-containing, loose and dense fibrous connective tissues that permeate the body'. And functionally, it '... interpenetrates and surrounds all organs, muscles, bones and nerve fibers, endowing the body with a functional structure, and providing an environment that enables all body systems to operate in an integrated manner' [116]. The ubiquity of soft connective tissue, combined with its mobility potential, suggests it will be highly fruitful to explore its prospective role on repair process in different organs. Taking into account the recently described role of superficial fascia fibroblasts in forming skin scars, it is plausible that resident fibroblasts of other soft connective tissues from the fascia system also share the same scar-forming potential.

In light of the role played by the superficial fascia in injury repair, it is not surprising in retrospect to realize that grafts including soft connective tissue from the fascia system have been used in the clinic for decades. These autologous graft techniques vary, in the source and treatment of the graft tissue, and in respect to the defect to correct. The common factor, however, is the inclusion of soft connective tissues such as the superficial and deep fascia [117].

There are many innervations and vascular networks in fascia tissue, so fascia grafts improve proprioception and blood supply restoration and quickly repair large injuries preventing infection. For example, fasciocutaneous grafts from the forearm can be used to effectively correct various hand injuries without severely limiting their function after repair [118].

Furthermore, grafting fascia-only, without the overlying dermis, is sufficient to promote repair in surgical reconstructions. Grafts from the deep fascia from the thighs, called *fascia lata* [119], are commonly used by surgeons with better clinical outcomes than dermal allografts [120].

Future clinical and blue skies research will provide the biological basis explaining the benefits and risks of using diverse fascia system tissues in reconstructive surgery.

Concluding remarks

Findings in recent years have been shaping two emerging concepts in the field of wound repair. The duo of fibroblast heterogeneity and soft tissue matrix mobilization have helped reconcile classical observations that could not be accounted for in the traditional wound-healing model.

Regenerative fibroblast replacement by scarring fibroblasts explains the regeneration-to-scar repair transition observed in maturing mammalian fetuses. Meanwhile, functional divergence of fibroblasts populations and their compartmentalization in adult tissues helps us predict (scarring vs scarless) repair outcomes. The characterization of regenerative (papillary and oral fibroblasts) and scarring populations (reticular and fascia fibroblasts) paves the road toward innovative approaches to boost regenerative agents while limiting the action of scarring ones during wound repair. Furthermore, as we elucidate the interconnections between those populations, direct reprogramming of scarring populations toward a regenerative phenotype could help reverting the negative effects of unsightly and clinically debilitating scars.

Lastly, the involvement of distant matrix recruitment to injury sites begs for holistic approaches when studying and treating wounds. Indeed, the continuum nature of the superficial fascia fibrillar networks means that the whole fascia system should be considered.

This new orientation will be crucial to prevent fibrosis and improve our regenerative repair capabilities in several organs.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

DC-G performed the literature research and figure preparation. YR coordinated the review's narrative.

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