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Review

Reflections on Endogenous Biological Drivers in Diabetic Lower Limb Wounds Recurrence

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Abstract: An impaired healing response underlies diabetic foot wounds chronicity, frequently translating in amputation, disability, and early mortality. In addition, diabetics frequently suffer of the underappreciated episode of post-epithelization ulcer recurrence. Recurrence epidemiological data are alarmingly high; so that the ulcer is considered in "remission" and not healed for the time it remains epithelialized. Recurrence may result from the conspiracy of behavioral and biological factors. Although the damaging role of behavioral, clinical predisposing factors is undebatable, the role of endogenous biological signalers that prime the residual scar tissue to recurrence has remained elusive. We propose that ulcer recurrence is deeply impinged by chronic hyperglycemia and its downstream biological effectors, which originate epigenetic drivers that enforce abnormal pathologic phenotypes to dermal fibroblasts, and keratinocytes as memory cells. Hyperglycemia-derived cytotoxic reactants accumulate and modify dermal proteins, reduce scar tissue mechanical tolerance, and disrupt fibroblasts-secretory activity. Accordingly, the combination of epigenetic and local and systemic cytotoxic signalers, induce the onset of "at-risk phenotypes" as premature skin cells aging, dysmetabolism, inflammatory, pro-degradative, and oxidative programs that may ultimately converge to scar cells demise. Unfortunately, post epithelialization recurrence rates data are missing in clinical studies of accepted ulcer healing therapies during follow-up periods. Intra-ulcer infiltration of epidermal growth factor exhibits the most consistent remission data with the lowest recurrences during 12-month follow-up. Recurrence data should be regarded as a valuable clinical endpoint during the investigational period for each emergent healing candidate.

Keywords: diabetic foot ulcer; ulcer remission; ulcer recurrence; ulcer relapse; diabetic complications; metabolic memory

Introduction

The prevalence of both type 1 and type 2 diabetes (T1DM and T2DM) and the associated complications are globally increasing (1-3). For T2-DM, its incidence has progressively escalated approaching to a pandemic condition that accounts for 90% to 95% of all the diabetic population (4).

Diabetes-affected patients have a significantly shorter life expectancy than nondiabetic individuals (5, 6). Accordingly, the seminal revolution brought about by insulin treatment, did not translate into a significant reduction of the chronic complications that preside morbidity and mortality (7).

Mounting evidences support the existence of a diabetes metabolic memory as a proximal trigger in the perpetuation of multi-organ complications (8), including the torpid healing response (9). Diabetic foot ulceration (DFU) is one of the most frightened diabetic complications, leading to amputation, disability, social exclusion, and early mortality (10). The lifetime incidence of foot ulcers has been estimated to reach up to 34% of diabetic subjects, contributing to 80% of all non-traumatic lower extremities amputations around the world (11, 12).

Although the onset of DFU is associated to predisposing factors as peripheral neuropathy, limb ischemia, cutaneous frailty; diabetic patients are affected by an intrinsic healing impairment that spans from the oral cavity mucosa, to lower extremities peripheral soft tissues (13-15). DFU chronicity phenotype (16) seems to be driven by precocious senescence, proliferative arrest, and apoptosis, as distal effectors of an abnormal hyperglycemia-associated epigenetic code (17, 18).

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Aside from the impaired healing response, a parallel conundrum in diabetic ulcer pathology is the high rate of recurrences after the primary reepithelialization (19, 20). In line with this fact, a classic report reveals that roughly 40% of diabetic patients have a recurrence within 1 year after ulcer healing (21, 22). Additionally, an underappreciated risk of DFU recidivism is its ability to "metastize" at anatomical niches away from the primary occurrence, frequently leading to a subsequent amputation (23).

Ulcer recurrence investigation has mostly focused on the identification and validation of predisposing clinical factors as poor glycemic control, previous ulcers, wound healing time, local skin damages, bone deformities, neuropathy, ischemia, diabetes duration, end-stage renal disease, toxic habits, and scar tissue mechanical stress (22, 24, 25). Although the deleterious role of behavioral factors is unquestionable (21, 26), we assume that there are endogenous biological signalers that prime the residual scar tissue to recurrence. Studies addressed to identify molecular pro-recurrence predictors are a contemporary need. We consequently examined potential molecular and cellular drivers, of local or systemic origin, that may concertedly cooperate behind ulcer recurrence. Having reviewed the literature, we may conclude that: (1) Endogenous deterrent factors underpinning the diabetic healing deficit are likely the same that cooperate for ulcer relapse. (2) These endogenous factors may be represented by soluble circulating signalers as by dysfunctional cells that secrete "pathological messages". (3) These factors are a consequence of hyperglycemia whereas some of them are glucose-derived chemicals. (4) Both wound chronification and recurrence are influenced by an abnormal cell physiology on the bases of an epigenetic code, resulting from the interaction of glucose and its derivatives with expression/transcription regulatory factors. (4) Numerous published clinical trials on innovative treatments for the healing of DFU, omit to mention recurrence rates during post epithelialization follow-up. Intervention with locally infiltrated epidermal growth factor (EGF) appears as a promising tool to achieve prolonged remission times.

The information analyzed for this work was retrieved from Pubmed and Google Scholar data bases, restricted to English language with no date limitation.

II.1- Putative epigenetic drivers in ulcer recurrence

Scar tissue is vulnerable to recurrence, an event that is theoretically propelled by an abnormal cellular physiology with an underlying epigenetic program, and a chemically-modified extracellular matrix by toxic products (17). Thus, ulcer relapses alike the impaired diabetic healing are clinical consequences of poor glycemic control, highlighting the pathogenic participation of endogenous cytotoxic-recurrence primers (20). Blood glucose levels and the hyperglycemia-derived products, constitute an environmental factor that impacts on the plasticity of epigenetic mechanisms, thereby modifying the whole transcriptome of skin cells (27). A primary multi-organ effect originated by deregulated glycemia consists in disrupting three major biological functions: DNA expression, RNA transcription, and protein translation. Studies have indicated that hyperglycemia may increase DNA mutations, DNA breaks, genomic instability, and particularly epigenetic dysregulation (28, 29). Accordingly, hyperglycemia and a constellation of downstream factors transform the cellular native epigenetic architecture, rendering a *de novo* code that largely alters cellular physiology (30). A clear association exists among hyperglycemia, the pattern of DNA methylation changes, diabetes complications unset and their clinical progression (31). Abnormal DNA methylation predisposes to diabetes susceptibility genes (32) and to perpetuate the diabetic phenotype in ulcer-derived fibroblasts, which is independent to the number of culture passages, and the presence of a normal glucose concentration in the culture medium. The discovery of this epigenotype, supports the role of epigenetic behind the persistent diabetic phenotypic behavior of ulcer's fibroblasts population (33). Another form of epigenetic modification, histone methylation, has been shown to correlate with glycemia levels and in turn with the formation of advanced glycation end products (AGEs) and the ensued hyperglycemia-related inflammation, oxidative stress, and apoptosis (34). It is known that AGEs as a biochemical hallmark of diabetes and a crucial ingredient within the damage cascade stick to histones, modifying chromatin structure and therefore its interaction capabilities, rendering an abnormal transcriptome (figure 1) (35, 36).

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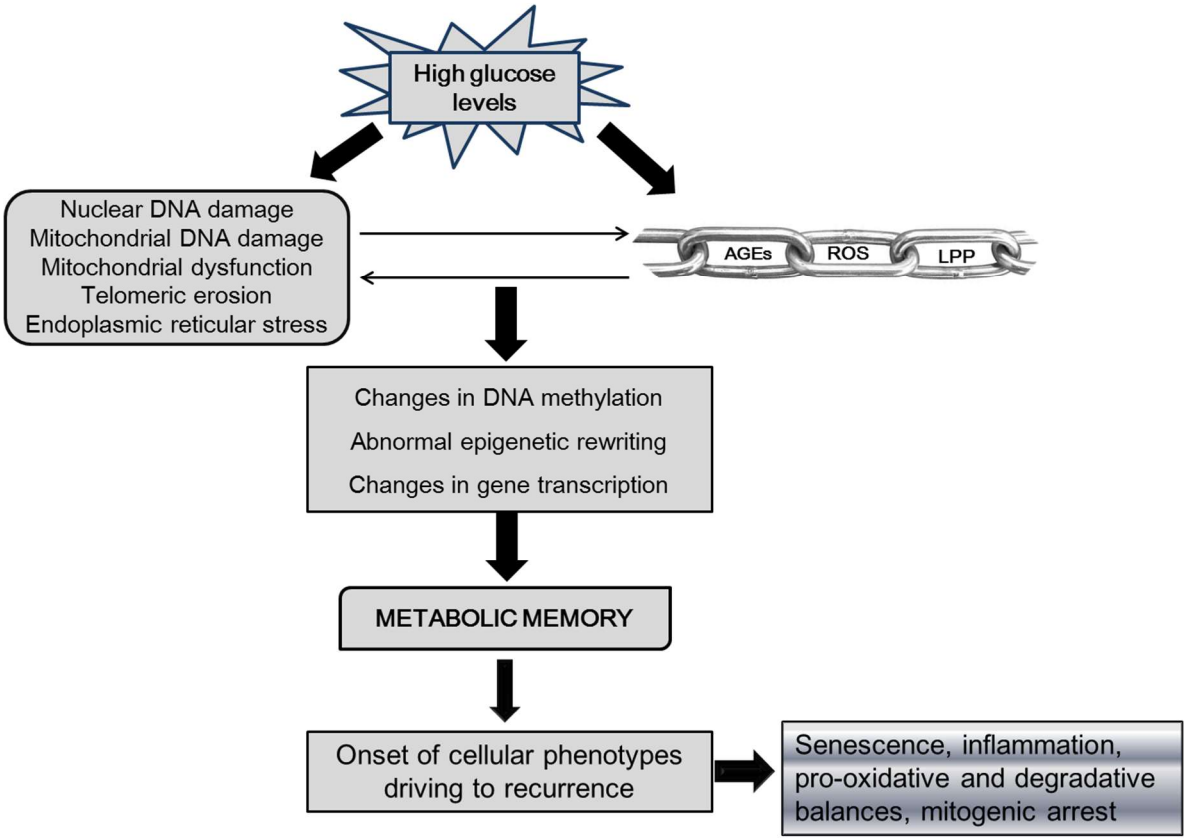

Figure 1. Theoretical epigenetic bases of recurrence.

Legend: AGEs-advanced glycation end-products, ROS-reactive oxygen species, LPP-lipoperoxidation products. Chronic high glucose level is the primary precursor of AGEs formation and accumulation in tissues, which is in pathophysiological link to ROS and LPP formation and accumulation. Hyperglycemia, AGEs, and ROS have a direct cytotoxic effect which includes damaging DNA and impairing mitochondrial function, which further amplifies ROS production and hypometabolism. High glucose burden and the above mentioned derivatives, introduce a novel epigenetic imprinting on the bases of a DNA aberrant methylation pattern, and abnormal transcription, and expression profiles. This foundation of metabolic memory perpetuates the diabetic phenotype in fibroblasts and keratinocytes, which includes inflammatory and senescence activators as proliferative arrest.

As above mentioned, hyperglycemia also modifies transcriptional profile by affecting mRNA, transcription factors, the production and extracellular release of microRNAs (miRNA), and long non-coding RNA (lncRNA) (37). El-Osta's findings inaugurated the contention that exposure to transient hyperglycemia, was sufficient to reprogram cells' native epigenetic program, and that these modifications largely persisted even beyond the normalization of glucose levels (38). In other words, the acute exposure of cells to high glucose stress, translates in chronic consequences that involves the onset of lasting pro-inflammatory and oxidative programs upon an altered epigenetic code. This concept was further validated in human cultured skin primary fibroblast (27) and vascular cells while showing that high glucose concentrations introduced significant transcriptomic modifications in genes controlling multiple pathways, all involved in wound healing events including angiogenesis (39).

MicroRNAs (miRNA) and non-coding RNAs in general are involved in an extensive array of cellular functions, representing an additional layer of epigenetic control in cell physiology (28). Deficiency of miRNA biogenesis has revealed their biological significance in the skin healing process (40). Interestingly, there is a substantial specificity and differentiation of miRNAs profiles for the specific type of diabetes and the evolving complications, which has encouraged its application as

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diagnosis and prediction biomarkers (40, 41). A large number of microRNAs have been implicated in pathological diabetic healing (40-45). A recent study identified the enhanced expression of miR-155 in peripheral blood of T2DM patients as a potential predictor for the onset of DFU (46), whereas the expression level of miR-203 in patients with DFU, positively correlated with the severity of the damage (47). High expression levels of miR-34c positively correlated with the amputation rates while negatively with the healing response, being also identified as an independent risk factor for ulceration and osteomyelitis (48). Likewise, long non-coding RNAs (lncRNAs) are an important epigenetic regulator at the level of histone methylation and gene transcription (37, 49). lncRNAs play a substantial role in diabetic wound healing, encompassing from infiltrating macrophages polarization control, to keratinocytes proliferation, and migration. Differential expression of lncRNAs in diabetic patients shows their involvement in impaired diabetic healing, and their putative role as biomarkers for diabetes-mediated damages (49-51). Although miRNAs and lncRNAs are included in the broad collection of hyperglycemic stress-related epigenetic derangements (52), it still remains elusive if some of the non-coding RNA forms are associated to scar tissue relapse, and could therefore be used as predictive biomarker.

Conclusively, genetic and epigenetic cell resources are impacted by chronic hyperglycemia establishing an abnormal epigenetic program that predisposes to the onset and perpetuation of diabetic traits as cellular senescence, proliferative quiescence, inflammation, oxidative imbalance, and apoptosis (39). Thus, scar tissue integrity is intrinsically jeopardized by these underlying silent primers, which may lead to tissue death as the ultimate event in recurrence, validating the concept of ulcer remission instead of ulcer healing.

II.2- Dermal matrix, fibroblasts, and keratinocytes in ulcer recurrence

Diabetes disrupts skin structure and physiology. Chronic and irregular courses of glucose and glucoxidation-derived products undermine skin cells physiology and progressively intoxicates the dermal matrix by the accumulation of AGEs, nitrosilation products, and free radicals byproducts (53, 54). Diabetics' intact skin shows reduced biomechanical resilience and stress tolerance, diminished elasticity, increased stiffness, and reduction of collagen and elastin contents, all together contributing to healing impairment (41, 55), and predisposing to scar tissue and epithelial coverage fractures. The above described dermal anomalies are largely associated to accumulation of AGEs crosslinked with long half-life proteins as collagen, which irreversibly and progressively affects skin matrix mechanics, induces premature aging, and impairs critical healing events as angiogenesis, fibroblasts attachment, and myofibroblasts-induced contraction (56, 57). Both AGEs and oxidative stress have direct cytotoxic effect on skin fibroblasts physiology, are instrumental ingredients of the ulcer cytopathic milieu, and ultimately contribute to mold the diabetes epigenetic map (figure 2) (58, 59). It is therefore inferable that scar tissue cells are born embedded within a milieu permeated by cytotoxic products.

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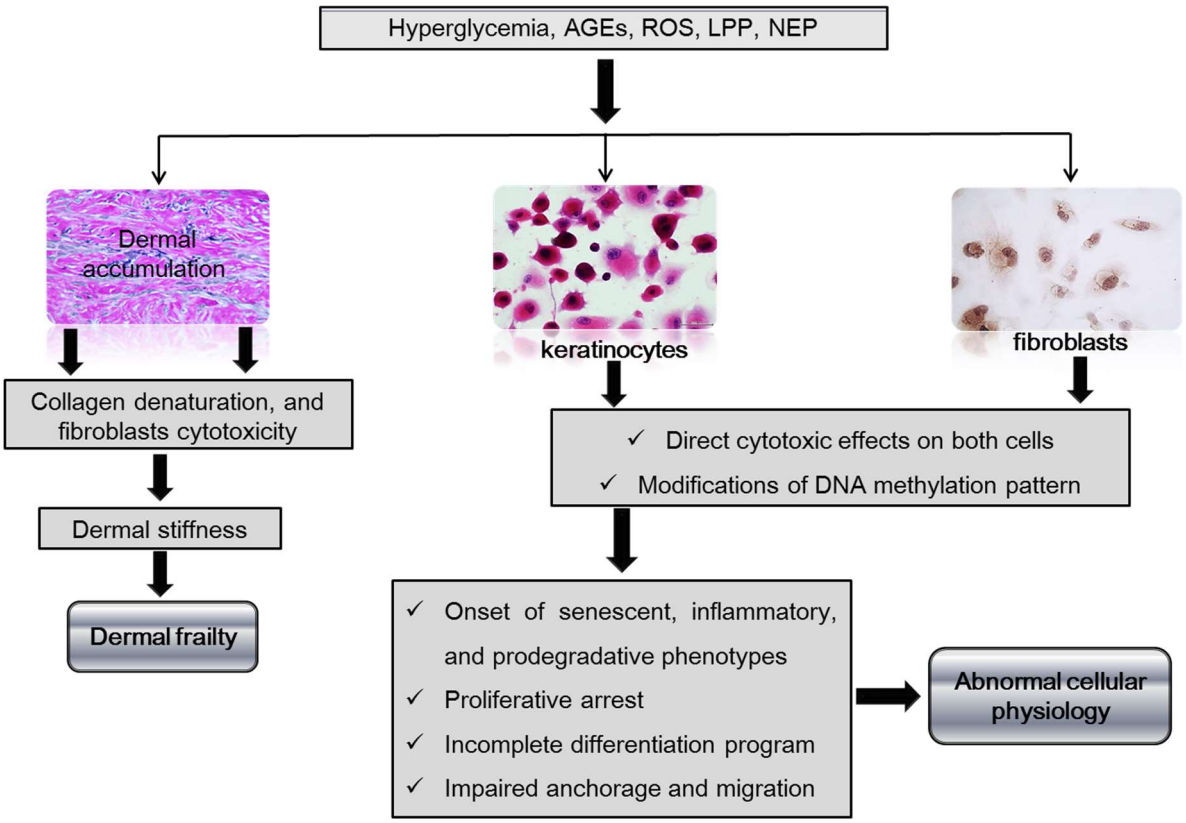

Figure 2. Impact of hyperglycemia and derived cytotoxic products on the dermal matrix and skin cells.

Legend: AGEs-advanced glycation end-products, ROS-reactive oxygen species, LPP-lipoperoxidation products, NEP-nitrosylation end-products. Hyperglycemia and its metabolic associated derivatives as AGEs and ROS accumulate in the dermal collagen, and alter the chemical and physical properties of the skin, becoming vulnerable and frail. In addition these are cytotoxic products that jeopardize dermal fibroblast survival and secretory activity. Hyperglycemia and the downstream cytotoxic products damage the native DNA methylation pattern, subsequently modifying gene transcription. The onset of a diabetic phenotype with an abnormal behavior of keratinocytes and fibroblasts is driven by a de novo re-written epigenetic code.

Another pathogenic ingredient predisposing to ulcer recurrence is the unusual premature skin cell aging brought by hyperglycemia and its derivatives (60-62). Diabetes is a mitochondrial-related disease, and not surprisingly, mitochondrial dysfunction is considered a primary trigger of skin aging and other phenotypic manifestations as impaired healing (62). A variety of mitochondrial and other diabetes disorders as hyperinflammation, high proteolytic activity, defective oxidative phosphorylation, local hypometabolism, excessive ROS generation, and accumulation of AGEs are found in skin fibroblasts and keratinocytes (63, 64) which translate in healing impairment (65). We deem that the persistence of these factors and/or their epigenetic signature may contribute to ulcer recurrence. In line with this notion, magnetic resonance studies confirmed that edema and hypometabolism of lipids and aminoacids, persist during remission time rendering scar tissue vulnerability (66). Decisively, diabetes biochemical derangements are a major and direct skin-aging driving factor, causing cellular dysfunction, and dermal proteins denaturation and decay (67, 68).

Skin fibroblasts are a functionally heterogeneous mesenchymal cell population with a central role in wound repair. These are sensitive cells with a large reserve of plasticity and reprogramming before external clues, which may alter their biological behavior and ultimately the wound healing fate (69). Most importantly, fibroblasts are cells endowed with the ability to retain a memory from their positional location, mechanical, and inflammatory environments, and specially a metabolic memory. Accordingly, fibroblasts can sense intracellular and extracellular metabolic changes in their

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microenvironment and consequently orchestrate a long-lasting phenotypic response (70). Thus, the society of wound fibroblasts may modify the course of the healing process as the long term fate of the residual scar (33, 59). Short term exposure of cultured, healthy, non-diabetic donor dermal fibroblasts to high glucose burden hampers proliferation, anabolism, and migration signaling pathways and orchestrates senescence (71), thus mirroring the phenotypic pattern detected in diabetic fibroblasts explanted from foot ulcers (59, 72). Hyperglycemia causes apoptosis of dermal fibroblasts, reduces collagen expression, and upregulates RELA/p65 expression, which implicates the onset of pro-inflammatory and pro-degradative profiles (53, 73, 74). Increased repertoire of inflammatory biomarkers is associated with the course of non-healing DFU whereas elevated figures of neutrophil-to-lymphocyte ratio (NLR) show a positive correlation with increased risk of amputation, and ulcer septic complications (75). The presence of endogenous skin-damage predisposing markers was identified by single-cell transcriptome studies of DFU specimens, in which multiple fibroblast cell clusters showed an increased inflammation pattern, changes that were likewise detected in areas of intact skin of diabetic subjects (76). Thus, scar tissue aftermath may depend on fibroblast metabolism, its secretory capability of extracellular proteins, and its control over the quality and duration of the inflammatory reaction (65).

Aside from its critical physiology, keratinocyte is the veteran sentinel cell that initiates the healing cascade after the epidermal integrity is disrupted (77). Like dermal fibroblasts, keratinocytes are also memory cells (78, 79) whose "response to wounding" is seriously affected by high-glucose stress (80). Hyperglycemia introduces alterations of keratinocytes metabolism, adhesion, migration, proliferation, and differentiation (81, 82), all having an epigenetic fundamental through abnormal changes in DNA methylation (83-85). Similarly, the onset of a senescent phenotype by epidermal cells may be a major molecular gear for ulcer recurrence. Hyperglycemia and its chemical derivatives shape an epigenetic landscape in which upregulation and post-translational modifications of p53, p21, and p16 contribute to keratinocytes senescence (17). Hypothetically, significant for ulcer recurrence could be the active expression of matrix metalloproteinase-9 (MMP-9), a type IV collagenase expressed by keratinocytes at the wound's leading edge, which may hinder re-epithelialization when up-regulated by AGEs. MMP-9 levels are elevated by hyperglycemia and glycation products via the upregulation of ten-eleven translocation enzyme 2 (TET2) gene expression. TET2 expression is higher in epidermal cells of diabetic patients than in normal skin, which appeared to be consequent of high levels of α-ketoglutarate. Of note, the levels of α-ketoglutarate correlate with local hypoxia, ischemia, and with poor glycemic control, exemplifying how the local environment and metabolism impacts on wound cells physiology via epigenetic mechanisms (9, 86).

An intrinsic fragility of the epidermal layer may be a predisposing factor for ulcer recurrence. One of the hallmarks of diabetic wounds is the high rate of keratinocytes proliferation versus an unsuccessful differentiation platform. Studies in diabetic mice have demonstrated an abnormal skin differentiation program due to an underlying keratinocyte dysfunction. Human non-diabetic keratinocytes exposed to hyperglycemic stress, and diabetic subjects-derived epidermal cells, exhibit in common a differentiation dysfunction mediated by an overexpressed c-Myc, which blunts differentiation by activating the WNT/β-catenin pathway (87). Previous observations had already indicated that activation of the β -catenin pathway, and an enhanced expression of c-Myc, were implicated in the diabetic torpid re-epithelialization response by disrupting keratinocyte migration and differentiation (88).

We also deem that a successful reciprocal and dynamic communication between epidermal keratinocytes and dermal fibroblasts, is mandatory to ensure scar tissue health and accordingly prevent wound recurrence. This notion is founded on the evidences of signaling crosstalk between these two major cells lineages. Keratinocytes nurture fibroblasts and myofibroblasts activity via the paracrine secretion of vascular endothelial growth factor-A (VEGF-A), transforming growth factor-β1 (TGF-β1), and connective tissue growth factor (CTGF), whereas reciprocally fibroblast-derived TGF-β1 expression, is essential for keratinocytes physiology including migration (89). High glucose levels and AGEs are known to disrupt this dermo-epidermal cells homeostatic circuit by reducing forkhead box O1 transcription factor (FOXO1) expression in keratinocytes, thus hindering

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keratinocytes ability to produce TGF-β1 (figure 3) (89). All together these findings converge to highlight: (1) the impact of glycemic control in keratinocytes physiology, (2) the significance of keratinocytes epigenetic imprinting in scar tissue integrity, and (3) the biological significance of a healthful dermo-epidermal axis.

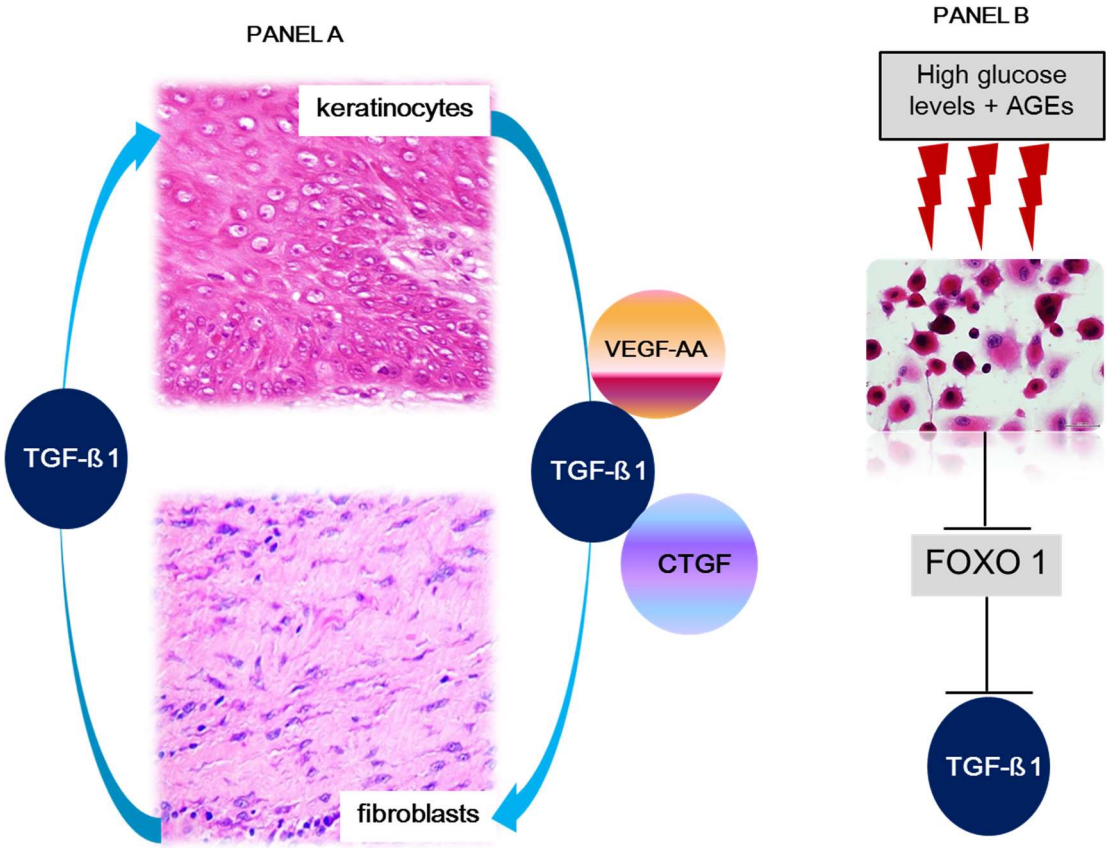

Figure 3. Remission duration may depend on dermo-epidermal axis homeostasis.

Legend: VEGF-A., vascular endothelial growth factor-A, TGF-β1., transforming growth factor-β1, and CTGF., connective tissue growth factor. FOXO1., forkhead box 1. Panel A: Dermal fibroblasts and epidermal keratinocytes conserve a dynamic and reciprocal crosstalk with the exchange of signalers for both cell populations homeostasis. Under physiological conditions epidermal keratinocytes secrete growth factors involved in fibroblasts physiology, including the synthesis, secretion, and turnover of dermal matrix proteins. The circuit includes the secretion by fibroblasts and utilization by keratinocytes compartment of TGF-β1. In this context, this growth factor appears to participate in keratinocytes stability, migration, turnover, and differentiation. Panel B. In diabetic subjects, and hyperglycemia experimental settings, high glucose levels and AGEs results in significantly reduced TGF-β1 expression by keratinocytes. This event is due to FOXO1 molecular changes which consequently reduces FOXO1 ability to promote TGF-β1 expression.

III- Ulcer recurrences in the clinical arena

Although recurrence rates reports of diabetic foot ulcers appear to broadly differ in the current literature (90), statistical data are alarmingly high despite the variety of healing interventions, and improved multidisciplinary management of the condition (19, 91). The literature on ulcer recurrence quite often misses data on the most specific recurrence time points, and anatomical sites (19); whereas unfortunately, not all the clinical trials examining the healing efficacy of drugs, devices, or management approaches, include information about recurrence rates during a reasonable follow-up period (92). Table 1, summarizes the recurrence data of relevant clinical investigations (clinical trials and meta-analysis) comprising three major groups of recently developed products: recombinant proteins (EGF and PDGF-BB), cellular and/or tissue-based products, and devices (vacuum assisted

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closure/negative pressure wound therapy). Of a total of 20 articles reviewed, recurrence data were reported in 10 (50%).

Table 1. Post-healing recurrence rates reported in major clinical studies of biologics, cell and/or tissue-based products, and VAC.

Reference	Major outcome	Administration route	Follow up period	Recurrences
Recombinant Epidermal Growth Factor (EGF)				
Tsang MW et al. 2003 (93)	20 of 21 diabetic foot ulcers healed with daily application of 0.04% (wt/wt) hEGF for 12 weeks	Topical	6 months	ND
Hong JP et al. 2006 (94)	The study suggests that topical treatment with EGF combined with advanced dressing may have positive effects in promoting healing	Topical	6 months	No recurrences were observed in EGF group
Park KH et al. 2018 (95)	The phase III study supports the efficacy and safety of spray-applied EGF treatment for DFUs, by significantly increasing healing velocity and decreasing time to complete healing	Topical	ND	ND
Viswanathan V et al. 2006 (96)	It took 13 weeks for ulcers to heal in the control group versus 9 weeks in the test group. In the test group, 90% of ulcers healed in 15 weeks compared with 22 weeks in the control group	Topical	ND	ND
Tuyet HL et al. 2009 (97)	Easyef (topical EGF spray) has positive effects on healing of moderate-to-severe foot ulcers and demonstrated being safe to diabetic patients	Topical	ND	ND
Fernández-Montequin J et al. 2009 (98)	Locally infiltrated EGF at 75 μg enhanced granulation tissue growth and wound closure	Intralesional injection	12 months	No recurrences reported for EGF groups
Gomez-Villa R et al. 2014 (99)	Patients with DFU who received intralesional rhEGF application resulted in complete healing, larger epithelialization of the wound bed, and reduction of ulcer area	Intralesional injection	ND	ND
Bartın M & Okut G 2022 (100)	The study shows that intralesional administration of EGF in T2DM can prevent amputations in DFU and also accelerate wound healing	Intralesional injection	6 months	Two cases in the group receiving EGF

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Yera-Alos IB et al. 2013 (101)	Post-marketing study including 1788 patients treated with intraulcer injected EGF. 1835 DFU (81% Wagner's grades 3 or 4; 43% ischemic) were treated. Re-epithelization was documented in 61% of the 1659 followed-up cases	Intralesional injection	14 months	5% / year
López-Saura PA et al., 2013 (102)	A review summarizing the clinical information about intralesional use of EGF for high grade DFU, in more than 2000 subjects. It confirms the results of the clinical trials, with 75% probability of complete granulation response, 61% healing, and a 16% absolute, and 71% relative reduction of amputation risk	Intralesional injection	12 months	The frequency of relapses at any moment was significantly lower ($p<0.001$) in patients that received rhEGF: 2.0% person-years of follow-up
Kahraman M et al. 2019 (103)	Study aimed to investigate the long-term outcomes after intralesional epidermal growth factor injections in the treatment of 34 diabetic patients with foot ulcers.	Intralesional injection	60 months	Of 29 patients involved in the 5-years follow up, 27 were ulcer free
Regranex or Becaplermin (rh-PDGF-BB)				
Embil JM et al. 2000 (104)	Results of the study further confirm the efficacy and safety of becaplermin gel for the treatment of lower extremity diabetic ulcers	Topical	6 months	21% of recurrence in Becaplermin-treated patients
Smiell JM et al. 1999 (105)	Treatment with becaplermin gel at a dose of 100 μg/g once daily, in conjunction with good ulcer care, is effective in patients with full thickness lower extremity diabetic ulcers	Topical	3 months	ND
Wieman TJ et al. 1998 (106)	Becaplermin gel 100 μg/g significantly increased the incidence of complete wound closure by 43% (50 vs. 35%, P=0.007) and decreased the time to achieve complete wound closure by 32%	Topical	3 months	The incidence of ulcer recurrence was ≈30% in all treatment groups
Ma C et al. 2015 (107)	Topical platelet derived growth factor does not appear to significantly improve healing of Wagner grade I diabetic foot ulcers	Topical	6 months	No difference was observed between groups in recurrence followed by amputation
Cellular and tissue-based products				

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Veves A et al. 2001 (108)	At the 12-week follow-up visit, 63 (56%) Graftskin-treated patients achieved complete wound healing compared with 36 (38%) in the control group (P=0.0042).	Topical – bioengineered skin substitutes	6 months	The incidence of ulcer recurrence was similar for Graftskin and control groups
Marston WA et al. 2003 (109)	Patients with chronic diabetic foot ulcers of >6 weeks duration experienced a significant clinical benefit when treated with Dermagraft versus patients treated with conventional therapy alone.	Topical- bioengineered skin substitutes	ND	ND
Zelen CM et al. 2016 (110)	EpiFix® (dehydrated human amnion/chorion membrane) is superior to standard wound care SWC and Apligraf®, in achieving complete wound closure within 4–6 weeks.	Topical – bioengineered skin substitutes	ND	ND
Zelen CM et al. 2014 (111)	Study addressed to evaluate recurrence rates of DFU healed with use of dehydrated human amnion/chorion membrane (EpiFix) in 18 available subjects with healed DFU. Wound median size of 1.7 cm^2.	Topical – bioengineered skin substitutes	9-12 months	17 wounds remained healed
Vacuum Assisted Closure (VAC)/ Negative Pressure Wound Therapy (NPWT)				
Blume P et al. 2008 (112)	A greater proportion of foot ulcers achieved complete ulcer closure with NPWT (73 of 169, 43.2%) than with advanced moist therapy within the 112-day active treatment	Topical- sub atmospheric pressure over the wound area	ND	ND
Armstrong DG et al. 2005 (113)	More patients healed in the NPWT group than in the control group (43 [56%] vs 33 [39%], p=0·040)	Topical- sub atmospheric pressure over the wound area	ND	ND

ND- not defined.

According to the data collected, post epithelialization recurrence rates are high, especially those observed in the nation-wide phase III clinical trial in the USA for Regranex/Becaplermin (106). Inversely, EGF treatment based on the intralesional infiltration delivery appears to provide the longest remission times, with the lowest recurrence rates (Table 1). A recent systematic review of randomized controlled trials, investigating different recombinant GFs for the purpose of wound healing, concludes that EGF is the most effective GF to enhance DFU healing (92). Similar conclusion is drawn from another meta-analysis stratified by the types of administration route (intralesional injection and topical administration) in which six studies involving 530 patients were eligible for reviewing (114). It is likely that EGF is the most broadly studied GF in wound healing (115), and interestingly, its healing effects are far more notorious as much the wounds are bigger (96), suggesting that EGF is endowed with a broad therapeutic window. Aside from that, it is likely that the success of the infiltrated EGF in prolonging scar tissue integrity, may reside on its ability to positively impact on the skin cells abnormal epigenetic program, and/or reduce the society of

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senescent fibroblasts (17). Definitively, durability of ulcer-free time should be regarded as a primary endpoint of major benefit in clinical trials for developing products/treatments (116).

IV- Concluding remarks and future directions

Diabetes is likely the most convincing scenario to illustrate how a trivial exogenous factor such as the level of blood glucose may reshape the native epigenetic program and ultimately build a disease-pathologic memory. The existence of this hyperglycemia-mediated metabolic stress memory explains why prior hyperglycemic exposure is not "forgotten" with time and successive cell generations. On the other hand, diabetes is an exemplary disease in terms of the generation and the progressive accumulation of hyperglycemia-derived cytotoxic products, some of them being cumulative in tissues like the skin. Accordingly, the conjunction of epigenetic and the consequent abnormal cellular behavior, along with the chronic cytotoxicity exerted by the spillover of AGEs and free radicals are biological factors that impair the healing response, and continuously jeopardize scar tissue homeostasis, stability, and viability. It is not surprising therefore that under this environment, societies of senescent and mitosis-refractory cells are found entrenched within the residual scar and the skin of diabetics in general. The fact as stated by Armstrong and co-workers, that recurrence may globally affect up to 40% of the patients on the first year after re-epithelialization, in addition to be distressing may indicate that: (1) the alert sense implicit in the ulcer remission concept has not sensitized enough to patients and wound care providers, (2) glycemia control may remain insufficient in the post-healing period, (3) endogenous, biological drivers remain silently active deteriorating the homeostasis of scar tissue and cells, (4) not all the innovative treatments contemporarily accepted to enhance acute ulcer healing, translate in prolonged remissions and far less in definitive healing, (5) treatments to be considered as effective are called to promote scar tissue resilience and offer a reasonable remission time.

We hold the argument that future innovations for chronic wounds and accordingly prevent ulcer relapses, must ideally entail the ability to target the cell epigenetic core which could erase the chronic hyperglycemic stress memory, reduce the burden of senescent cells, and impose "healthy" re-differentiation programs. Manipulating the diabetic metabolic epigenetic code may indefectibly assist in the control of the all its chronic complications. Of note, however, this therapeutic dream line must be anticipated by the well-deserved glycemic control.

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