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Epidermal Growth Factor Therapy Impact on Scar Tissue Resilience of Diabetic Lower Limbs Ulcers-An Enlightening Hypothesis

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Abstract

Diabetes patients experience an accelerated aging process that increases their predisposition to multiorgan complications and earlier death. Lower extremity ulceration and wound chronification are among the devastating complications of diabetes. Likewise, ulcer recurrence is a frequent and challenging event whose cellular and molecular driving forces have remained elusive. Thus far educative prevention and interventions on predisposing factors are the only tools to prolong ulcer remission. Cellular senescence plays a critical role in diabetics healing impairment. Hyperglycemia-associated glucooxidative stress and other biochemical derangements can foster this premature senescence. Since diabetic microenvironment is permissive to senescence, we have hypothesized that the increasing thresholds of scar's senescent fibroblasts may contribute to wound recurrence. Diabetic wounds healing therapies have not accounted for a consistent reduction in recurrences rates. Reviewing 18 clinical trials of representative therapeutic alternatives for the healing of diabetic lower extremity wounds; indicated that epidermal growth factor therapy-either topical or by infiltrative delivery, proved to be the only intervention associated to the lowest recurrences rates. According to literature evidences, we propose that epidermal growth factor exerts a local rejuvenating effect by replacing senescent cells, or by dismounting/reversing the fibroblasts' epigenetic senescence program. This growth factor may potentially act as a senolytic agent for diabetic wounds, promoting the neodermal resilience and tolerance to physical and mechanical stress.

Keywords: Diabetes; Epidermal growth factor; Diabetic foot ulcer; Cellular senescence; Ulcer recurrence; Ulcer remission; Chronic wound

Introduction

Healing chronic wounds demands prolonged medical attention and consumes a substantial amount of expenses. These lesions impose a dramatic social impact especially within the diabetic population which renders the largest figures of lower extremities amputations. Accordingly, enormous efforts are invested in developing innovative and efficient therapies to restore the physiological healing trajectory [1,2].

Aside from the diabetes-associated predisposing factors for foot ulceration and the ensued healing impairment, a common episode in diabetic patients is ulcer recurrence after the primary wound closure. Studies addressed to characterize this sensitive topic are relatively scarce and appear mostly circumscribed to predisposing factors such as bone deformities, neuropathy, ischemia, etc. Nonetheless, the role played by biological intrinsic factors related to scar-tissue cells' resilience, and neodermal matrix biochemical and physical properties, demand further investigation. A recently published review by Armstrong and co-workers reveals that roughly 40% of patients have a recurrence within 1 year after ulcer healing, almost 60% within 3 years, and 65% within 5 years. Thus, the authors judiciously consider that it may be more useful to think of patients who have achieved wound closure as being in remission rather than being healed [3].

Diabetic subjects are prone to orchestrate the wound chronification process which clinically translates in: (a) failure for triggering proliferative phase/granulation tissue outgrowth, (b) meager or histologically abnormal angiogenesis, (c) impaired wound contraction, and (d) stagnant re-epithelialization. In general terms, diabetic wounds-derived epidermal and dermal cells exhibit a poor spontaneous and growth factors-induced proliferative response potential [4]. Notoriously, all these traits are compatible with the repair process in senescencent tissues and organisms [5].

Diabetic wound fibroblasts are characterized by impaired migratory and secretory abilities which hinder matrix scaffolding and ultimately the creation of a granulation tissue plug. Few years ago we identified molecular markers [6] suggestive of premature senescence in biopsy-derived fibroblasts harvested from an ischemic, non-granulating diabetic lower limb wound. As derived from a diabetic donor, this population of senescent, arrested cells may be impinged by a variety of hyperglycemia-associated stressor factors including reactive oxygen species. Different pathogenic loops linked to diabetes progression and complications development, appear to be mechanistically related to the orchestration of a senescencent phenotype [7].

Simultaneous to the unparalleled increase in the incidence of diabetes, a constellation of therapeutic alternatives have emerged during the last 20 years to enhance the healing process of diabetic foot ulcers, ranging from the local administration of recombinant growth factors to *in vitro* engineered living skin equivalents [8]. Regardless of the primary healing clinical efficacy evidences described for different therapeutic modalities, recurrence rates remain disproportionately high [3,9-12].

The assembling of different and disperse pieces of knowledge regarding the role of EGF for the healing of diabetic lower limb wounds, have contributed to postulate that among the beneficial

biological effects of this growth factor could be the reversion of the senescent program within the scar environment. Thus, this article is both a hypothetical reflection and a revision of the biological foundations of the senescence process and its involvement in the pathogenic process of wound chronification and recurrence. We believe that EGF could be a useful antisenolytic therapy with an extraordinary therapeutic potential for chronic wounds and other age-related disorders.

Method Used Along Search Strategy

The search strategy involved Medline/Pubmed and Bioline International (www.bioline.org.br) data sources introducing key words as: chronic wounds+senescencent cells, diabetes+senescence, diabetic foot ulcers, EGF+cellular senescence, EGF+telomeres, replicative senescence+chronic wounds, fibroblasts+proliferative arrest. The clinical trials for the different pharmaceutical forms of EGF, PDGF/Regranex, Dermagraft, Apligraf, Vacuum Assisted Closure (VAC), etc., were studied. All the articles reviewed were restricted to English language.

Concise Views on the Molecular Basis of Cell Senescence

Senescence represents a terminal mitotic fate in which cells withdraw from the cell cycle and lose the capability to proliferate in response to growth factors or mitogens [13]. Two types of senescence have been described; telomere dependent and stress induced premature senescence (SIPS). Unlike replicative senescence (telomere dependent), cellular senescence does not necessarily involve telomeric attrition and can be induced by different stressor factors as ultraviolet light, reactive oxygen species, chemotherapeutics, ionizing radiation, and excessive mitogenic signaling [14,15]. Recent findings have established a causal relationship between senescence and aging: an in vivo selective clearance of p16-positive senescent cells ameliorates aging-related features in a mouse model of progeroid syndrome [16]. Mechanistically speaking, senescence relies on two main molecular pathways: p53-p21 and p16INK4A-Rb. Accordingly p16, a cyclin/cdk inhibitor, prevents phosphorylation of the retinoblastoma protein (Rb) by cyclin/cdk complexes. Under the hypophosphorylated state, Rb inhibits cell proliferation by preventing binding to E2Fs transcription factors, thus constraining them from stimulating transcription of genes involved in cellular proliferation and DNA replication. p16-Rb axis is pivotal to the establishment of cell-cycle arrest [16,17]. Recent findings indicate that p21 and/or p16Ink4a overexpression appears irreversible and predominantly orchestrated by epigenetic forces as the senescence-associated heterochromatic foci [18]. Genetically manipulated murine models that attained a robust constitutive p53 activity show a vast array of age-related features including reduced lifespan. Conclusively, unrestrained p53 activation acts as a crucial pro-aging factor [13].

Senescent cells also display distinctive morphological and biochemical phenotypes which define the senescent state from normal, post-mitotic, quiescent cell populations [19]. These cells appear of larger size, flattened morphology in culture, resistance to apoptosis, and altered gene expression including upregulation of SA-β-Gal. An abnormal secretory pattern of pro-inflammatory agents characterizes this population. The pro-inflammatory response known as senescence-associated secretory phenotype (SASP) is instrumented via the transcription factors nuclear factorκB (NFκB) and CCAAT/enhancer binding protein-β (CEBPβ). This SASP phenotype that includes the secretion of pro-inflammatory cytokines and proteases is believed to be activated upon DNA damage and/or p38MAPK signaling. At the end, these are metabolically active cells that release their inflammatory and senescence signals, thus spreading their phenotype to adjacent cells amplifying cellular arrest, senescence, and inflammation [20,21].

Of pathogenic relevance, senescent cells are also characterized by mitochondrial dysfunction which is an additional and concurrent source of free radicals and oxidative stress. Eventually, this vicious circle amplifies the senescent cell burden, consequently nurturing the 50 years old theory of oxidative stress and aging [22].

Diabetes, Cellular Senesce and Chronic Wounds

The definite pathogenic relevance of senescence for the onset of type-2 diabetes and the progression of its multiorgan complications, or whether senescence precedes or follows the onset of perpetual inflammation and insulin resistance are topics of intense debate [7]. Anyhow, diabetic patients experience an obvious accelerated aging process that increases their predisposition to morbidity and earlier death. This premature organismal aging, driven by an increased cellular senescence, is often expressed as an appreciable incompatibility between the perceived age and the actual chronological age [23]. Irrespective to the position of the senescent cells population within the pathogenic cascade as cause or consequence, the increasing burden of cutaneous senescent fibroblasts above a tolerable threshold, may preside skin aging and specifically the diabetic impaired healing response [24].

The high-level of oxidative stress induced by hyperglycemia and other derangements associated to diabetes have the potential to foster premature senescence. Diabetic microenvironment is therefore permissive to senescence which is not surprising since the two major molecular operators' of diabetic complications as, oxidative stress and endoplasmic reticulum stress have been recently associated to senescence, including its SASP phenotype [24]. In line with this, the "glucocentric theory" places hyperglycemia as the proximal trigger for the onset of a pro-inflammatory phenotype that may be defined as a diabetes- or hyperglycemia-associated secretory phenotype. A non-diabetic murine knockout model which somewhat mirrors the pathogenic inflammatory realm of diabetes, exhibit a heightened senescent fibroblasts population whose tissue regenerative potential is apparently reverted by anti-inflammatory or antioxidant treatments [25]. Aside from inflammation, the mechanisms of glucose-mediated senescence include mitochondrial dysfunction and a vicious circle of increased reactive oxygen species in a proximal position of the pathogenic cascade. From a pathobiologic point of view, it has been stated that low-level, chronic exposure to reactive oxygen species (ROS) accelerates telomere shortening, whereas high levels of ROS induce telomere-independent premature senescence [26]. The pathogenic role of oxidative stress for wound fibroblast senescence has been demonstrated in other non-diabetic chronic wounds types. Microarray and functional characterization of these premature senescencent fibroblasts confirm their decreased ability to withstand with oxidative stress [27]. The exaggerated processes of diabetic glucoxidation with the consequent formation and accumulation of advanced glycation end products (AGEs) and its agonistic binding to the receptor (RAGE) have a dramatic impact in inducing premature senescence [24]. Under the scenario of a continuous oxidative stress, inflammation, and glucotoxicity, senescent cells accumulate and spread their senescence messages to a systemic level [28].

The role of cellular senescence within the complex pathogenic realm of type-2 diabetes dates back to the 70's and 80's of last century. The theory is associated with the existence of a kind of a pro-senescencent state in fibroblast from pre-diabetic individuals which may predispose to wound healing failure [24,29]. Aside from the impact generated by the exposure to high glucose concentrations which impose pro-senescent and apoptogenic signature in most skin cells [30]; these studies allow to infer that a persistent heritable abnormality, related to a precocious cellular senescence is present in mesenchymal tissues of genetically predisposed individuals to suffer diabetes [29].

From those years it was known that cutaneous fibroblasts derived from insulin-dependent or insulin-independent diabetic subjects, not only exhibit abnormal replicative capacity *in vitro*, but that senescence appeared more precociously than in nondiabetic counterparts [4,31]. Rowe and co-workers pioneered the series of *in vitro* models, that described the reduced synthetic, proliferative and secreting capabilities of diabetics' cutaneous fibroblasts [32]. Further studies showed that the addition of conditioned media from type-2 diabetes mellitus fibroblasts induced a dose-dependent inhibition in normal fibroblast proliferation, thus raising the question of whether there is a "metabolic memory-soluble factor" that could be passively transferred from diabetic fibroblasts to non-diabetic counterparts, while the diabetic phenotype acts as the dominant one. This type of idiopathic replicative refractoriness found in fibroblasts from pre-diabetic and diabetic individuals has been unequivocally reproduced along the years [33,34] confirming the need for supplementary inputs to stimulate diabetic fibroblasts proliferation [35]. Since the early 80's it is known that hyperglycemia exposure to cultured cells directly or indirectly causes DNA damage and telomeres attrition. Both events are driving forces toward the onset of a senescent phenotype [24]. These *in vitro* models have enriched our knowledge on the molecular impact of high glucose exposure, and anticipated the thesis of "point of no return," beyond which hyperglycemia resulted in irreversible progression to premature senescence [36].

The studies by Vande Berg and Robson were seminal to define the protagonist character of senescencent fibroblast in torpid wound healing: (1) the wound healing process resumed in as much as the population of senescent fibroblasts decreased (decreasing number of p21 positive cells and an increasing portion of PCNA labeled cells). (2) Chronic wound fluid degrades exogenous growth factors, decreases the production of cyclin D1, phosphorylated Rb (pRb), and increases p21, which reflect the molecular fundamentals of cellular senescence [37,38]. Accordingly, in an attempt to explore the participation of prominent molecular mediators in fibroblasts proliferative arrest and premature senescence; cells from a clinically complex diabetic foot wound with ischemic ingredient and long evolution were examined. The fibroblasts exhibited a slow and progressive proliferative declining even under 'physiological' culture conditions. The arrested phenotype observed may be explained by the transcriptional upregulation of both p53 and p21Cip. The serine-15 p53 phosphorylated isoform revealed an intense nuclear expression which coincided with the substantial underexpression of cyclin D1. We proposed therefore that the dampened activity of the Akt/mTOR/cyclin D1 axis by the proximal activation of p53 and p21 somewhat contributed to impose the fibroblasts arrest/senescence program that translated in the clinical healing catastrophe [6]. Figure 1 summarizes the above described molecular events. Conclusively, fibroblasts senescence seems to clinically translate in wounds chronicity [39]. Skin epithelial cells are also involved in wound chronicity by orchestrating a senescence phenotype. Interestingly, regardless of the biological differences existing between a mesenchymal-derived (fibroblast) and an ectodermal counterpart as a skin keratinocytes, p53, p21, and p16 look as molecular operators in the senescencent landscape [40].

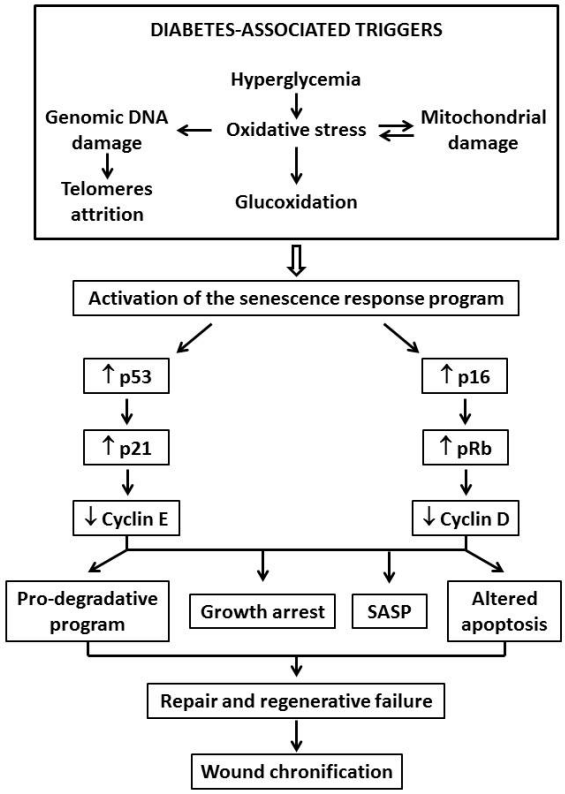

Figure 1: Putative mechanisms underlying diabetic wound chronification.

Fibroblasts exhibit abnormal behavioral and phenotypical traits in diabetic lower extremities wounds. One of the pillars of wounds chronification is fibroblasts senescence. The hyperglycemia downstream related biochemical derangements may activate the cellular senesce program, via telomeres attrition, and through the p53/p21, p16INK4A-Rb pathways. Consequently, cyclin E and cyclin D - are negatively regulated. In theory, the cellular senescent phenotype is compatible with behavioral markers of diabetic foot ulcers granulation tissue cells (fibroblasts and endothelial cells): proliferative arrest, over expression of degradative proteases, paracrine secretion of inflammatory mediators, as an exaggerated rate of apoptosis.

Epidermal Growth Factor (EGF) Therapy and Ulcer Recurrences

In the face of the advancements in wound care and the holistic approaches achieved during the last 20 years, chronic wounds remain as a major clinical problem [41]. Diabetic Foot Ulcer (DFU) therapies include the most advanced state-of-the-art knowledges and technologies [42]. Yet, a significant number of patients within the diabetic population are still bound to amputation, reulcerations, and short term survival [43-47].

Since the senescent phenotype depends on the downregulation of positive-acting cell cycle regulatory genes as c-fos proto-oncogene, Cdc2 and cyclins [48], which translates in inability to proliferate; the early idea that growth factors could reverse the arrest of the repair process appeared judiciously justified [17,49]. During those early days wound bed preconditioning was not a routine practice. This procedure ensures biofilm and debris removal, growth factors local bioavailability, signaling activation, and reduction of the senescent cells population burden [50]. EGF is perhaps the most widely studied growth factor. Since the 60's interpretation that its exogenous administration reprogrammed biological events chronologically established within specific temporary windows, this polypeptide has been used to repair multiple forms of wounds in both peripheral and internal tissues and organs [51]. Unquestionably, EGF is endowed with the sufficient biological competence for potentially reversing wounds' chronicity [52]. Today and after many years confronting peaks and troughs, EGF and platelet-derived growth factor (PDGF) stand as the sole growth factors within the clinical armamentarium to treat diabetic lower extremity wounds [53,54].

After reviewing 18 published clinical trials conducted during the last 20 years with representative therapeutic alternatives for the healing of diabetic lower extremity wounds; EGF therapy -either topical or by infiltrative delivery, targeting either low or high grade wounds, proved to be the only intervention associated with the lowest reulcerations rates (Table 1). The other interventions enlisted which showed a significant healing efficacy in the primary lesion, did not entail prevention of reulcerations. In some articles reulcerations rates are not reported. This observation somehow validates the notion that in addition to educative prevention programs, intrinsic biological factors may lie beneath the capability of the scar tissue to tolerate physical forces. If this notion is valid, the key question to answer is whether EGF exerts a local rejuvenating effect that may involve for instance the replacement of senescent cells. The integration of the evidences reviewed here from basic and clinical studies support the hypothesis that EGF may counter cellular senescence.

EGF May Counteract Cellular Senescence

Beyond the effects of ageing on cellular migration and proliferation, a reduction in EGF receptor number and transduction responsiveness has been described in senescent fibroblasts, which may obviously suggest that EGF receptor (EGFR) manipulation could be of therapeutic benefit for problem wounds [55]. Experimental evidences derived from cancer basic science, support the hypothesis that EGFR aborts cellular senescence programs in response to certain forms of DNA damage [56]. Furthermore, EGF showed to activate the expression of human telomerase reverse transcriptase in cultured cells, via Ets-2, a cancer-specific transcription factor that appears to depend on EGFR-mediated Erk and Akt activation [57]. In line with this finding, stands our observation that granulation tissue cells from neuropathic diabetic wounds, exhibit a 308% increase of telomerase reverse transcriptase mRNA expression after four weeks of local infiltration with EGF (Figure 2). Whether this increase in telomerase expression translates or represents a renewal of proliferative competence, elimination of senescent cells population, or a reversion of the senescent phenotype is under current investigation (Unpublished observation). In a clinical language all these wounds were fully re-epithelialized in an average of 4 weeks and no patient recurred before month 25th. These evidences support the existing link between the EGF system and its potential senolytic repercussion. Experimental studies suggest that EGF is able to enhance cell survival and tissue replenishment before otherwise lethal scenarios, by controlling oxidative stress and mitigating cellular senescence [58]. Chronic wounds irrespective of their etiology are a rich source of pro-oxidant metabolites with a negative local and systemic impact including cellular senescence [59,60].

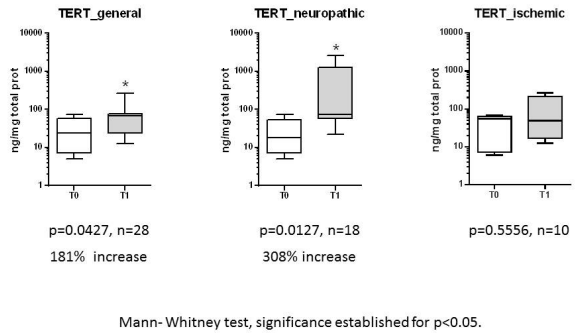

Figure 2: Telomerase reverse transcriptase (TERT) expression by diabetic foot ulcers.

TERT protein expression appears to significantly increase (p=0.01) in granulation tissue cells, derived from neuropathic lesions following 9 to 12 interventions with locally infiltrated EGF (Time 1, T1). The comparison is done with same patient (paired samples) samples, collected when the wound bed was properly conditioned to begin with EGF infiltration treatments. No effect was observed for refractory ischemic ulcers-derived samples. Mann-Whitney test, significance established for $p<0.05$.

In the context of free radicals overproduction as proximal detonators of events leading to ulceration and wound chronification, the pharmacologic manipulation of the EGFR system proved its benefits in a specific clinical process. The study by Garcia-Ojalvo and co-workers shows that diabetic ulcerated subjects behave as a unique pathological group as they exhibit an exacerbation of the oxidative stress arm along with a concomitant deterioration of the antioxidant reserve as compared to non-ulcerated diabetic individuals. EGF intra-lesional infiltrations for 3-4 weeks contributed to restore circulating levels of several redox status markers, up to values close to those of non-ulcerated diabetic patients and non-diabetic subjects. The authors consider that EGF is a key factor in restoring redox balance and indirectly attenuating premature senescence, apoptosis and proliferative arrest [61]. Furthermore, the EGF intra-ulcer infiltration also tended to restore the systemic balance between MMP-9/TIMP-1, suggesting the recovery between pro-degradative and pro-synthetic forces, which may denote an attenuation of the SASP-associated phenotype [62]. This study contributes to validate the notion that relieving oxidative stress in chronic wounds would ameliorate fibroblast dysfunction.

Finally, although EGF has been regarded as merely a mitogen for many years, a 2015 groundbreaking study revealed for the first time that EGF exerts a potent anti-senescence activity in certain stem and

differentiated cells upon the receptor stimulation. The study showed that cell cultures depleted of EGF orchestrated a senescencent phenotype with enlarged morphology, elevated SA-β-gal activity, decreased proliferation, reduced Rb phosphorylation and elevated p21 expression. These results suggest that cells cultured require EGF as a mechanism to escape from senescence and ensure proliferation, thus placing EGF as a central driver in preserving mitogenic competence and suppressing senescence [63]. Consistently, the anti-senescent role of EGF extended to adipose stem cells, promoting its proliferation and differentiation potency as markedly delaying their senescence [64].

Clinical Interventions with Epidermal Growth Factor (EGF). Includes both topical and intralesional infiltrations			
Reference	**Study population**	**Conclusions**	**Reulceration**
Tsang, et al. [65].	61 neuropathic patients with grades I or II, by the Wagner Classification.	application of EGF-containing cream, in addition to good foot significantly enhances diabetic foot ulcer wound healing and reduces the healing time	Not reported.
Le Tuyet, et al. [66].	28 neuropathic subjects with foot ulcers on Wagner's grades I and II were recruited.	EGF has positive effects on healing of moderate-to-severe foot ulcers and demonstrated being safe to diabetic patients.	Not reported.
Hong, et al. [67].	89 patients enrolled for the prospective, open-label trial, crossover study. EGF 0.005% twice-a-day treatment.	This study suggests that topical treatment with EGF combined with advanced dressing may have positive effects in promoting healing of chronic diabetic foot wounds	Recurrence was not noted during the 6-month observation. 5 patients showed new lesions different from the prior site.
Berlanga-Acosta, et al. [68].	Twenty-nine in-hospital patients with diabetic neuropathic or ischemic lesions treated in a non-controlled study. Lesions, classified as Wagner's grade 3 or 4. EGF 25μg/ infiltration/48 hours.	Preliminary evidences suggest that EGF intralesional infiltrations may be effective in reducing diabetic lower limb amputation	Wound recurrence after 1 year of follow-up appeared in only one patient.
Fernandez-Montequin et al. [69].	Two EGF dose levels were used in ischemic or neuropathic patients with Wagner's grade 3 or 4 ulcers. EGF was given through intralesional injections, three times per week for 5–8 weeks	rhEGF local injection enhances advanced DFU healing and reduces the risk of major amputation.	After 1-year follow-up, only one patient relapsed.
Fernandez-Montequin et al. [70].	149 patients were randomized to receive EGF 75 or 25 μg or placebo, three times per week for 8 weeks via intralesional infiltration	75 μg dose enhanced granulation and recombinant human EGF (rhEGF) local injections offer a favorable risk–benefit balance in patients with advanced DFU closure.	After 1-year follow-up, no EGF-treated patient relapsed.
Dumantepe, et al. [71].	Seventeen diabetic patients with full-thickness lower extremity ulcers of more than 4 weeks. Intralesional injections of EGF 75 μg three times per week.	Intralesional EGF administration up to complete closure can be safe, effective and suitable to improve healing of diabetic foot ulcers.	After 1-year follow-up, only one patient relapsed.
Yera-Alos I, et al. [72].	Pharmacovigilance study with 1788 patients with 1835 DFU treated from May 2007 to April 2010 with hrEGF, 25 or 75 μg, intralesionally 3 times/week	The favorable benefit/risk balance, confirms the beneficial clinical profile of intralesional hrEGF in the treatment of DFUs.	Relapses occurred in 5% person-years, regardless of the ulcer etiology or other characteristics
Lopez-Saura P, et al. [73].	Post marketing experiences with intralesional EGF, in more than 2000 subjects, with 75% probability of complete granulation response, 61% healing, and a 16% absolute and 71% relative reduction of the risk of amputation. Benefit includes ischemics.	The benefit-risk balance seems thus quite favorable. The post marketing information ratifies the results of the clinical trials in terms of efficacy, safety and impact.	The frequency of relapses at any moment was significantly lower ($p<0.001$) in patients that received rhEGF (2.0% and 1.3% person-years of follow-up for the 75 μg and 25 μg doses, respectively) as compared to the control group (7.9% person-years). No effect was seen on the appearance of new DFU on other locations.
Clinical Interventions with Platelet Derived Growth Factor (PDGF/Regranex, Becaplermin)			
Reference	**Study population**	**Conclusions**	**Reulceration**
Steed, et al. [74].	118 patients with neuropathic ulcers were randomized to either topical rh-PDGF-BB (2.2 μg/cm2 of ulcer area) or placebo until the ulcer was completely	Once-daily topical application of rhPDGF-BB is safe and effective in stimulating the healing of chronic, full-thickness, lower-extremity diabetic neurotrophic ulcers.	In the placebo group, 46% of the originally healed ulcers recurred at a mean time of 8.5 weeks, in the rhPDGF-BB group 26% of target ulcers

	resurfaced or for a maximum of 20 weeks		recurred at a mean of 8.6 weeks.
Embil, et al. [75].	134 patients with diabetics lower extremity ulcers with Wagner's grades I and II	Results of this study further confirm the efficacy and safety of becaplermin gel for the treatment of lower extremity diabetic ulcers.	Complete healing of ulcers was achieved in 57.5% of patients, with a mean time to closure of 63 days and a recurrence rate of 21% at 6 months.
Smiel, et al. [76].	Included 922 patients with non-healing lower extremity diabetic ulcers randomized to receive a standardized good ulcer care, or good ulcer care plus becaplermin gel-30 µg/g, or becaplermin gel-100 µg/g, in the four studies	Becaplermin gel at a dose of 100 µg/g once daily, in conjunction with good ulcer care, is effective and well tolerated in patients with full thickness lower extremity diabetic ulcers.	Not reported
Wieman, et al. [77].	382 patients with type 1 or type 2 diabetes and chronic ulcers of at least 8 weeks' duration. patients were randomized to receive becaplermin gel 30 µg/g, becaplermin gel 100 µg/g, or placebo gel.	Becaplermin gel 100 µg/g, significantly increased the incidence of complete wound closure and significantly reduced the time to complete closure of chronic diabetic neuropathic ulcers.	The incidence of ulcer recurrence was 30% in all treatment groups, the durability of ulcer closure was comparable in all treatments
Clinical Interventions with Devices			
Reference	**Study population**	**Conclusions**	**Reulceration**
Marston, et al. [78].	314 patients to evaluate complete wound closure by 12 weeks in a randomized, controlled, study at 35 centers throughout the U.S. Patients were randomized to either the Dermagraft treatment group or control (conventional therapy)	The data from this study show that Dermagraft is a safe and effective treatment for chronic diabetic foot ulcers.	Not reported
Veves A, et al. [79].	276 patients from 11 centers were enrolled. Neuropathic ulcers, Wagner grade 1 to 2, and an area of at least 1 cm2 (greatest length × greatest width) were studied.	Promogran, a wound dressing consisting of collagen and oxidized regenerated cellulose, was as effective as moistened gauze in promoting wound healing in diabetic foot ulcers.	Not reported
Blume P, et al. [80].	342 patients were enrolled at one Canadian and 28 U.S. sites. Neuropathic lesions, stages 2 or 3 by Wagner's scale. calcaneal, dorsal, or plantar foot ulcer ≥ 2 cm2 in area after debridement	Negative pressure therapy appears to be as safe as and more efficacious than advanced moist therapy for the treatment of diabetic foot ulcers.	Not reported
Armstrong D, et al. [81].	162 patients into a 16-week, 18-centre, randomized clinical trial in the USA. Patients who were randomly assigned to NPWT (n=77) received treatment with dressing changes every 48 h. Control patients (n=85) received standard moist wound care according to consensus guidelines.	More patients healed in the NPWT group than in the control group (43 [56%] vs. 33 [39%], p=0·040). The rate of wound healing, based on the time to complete closure, was faster in the NPWT group than in controls (p=0·005)	Not reported
Veves, et al. [10].	208 patients with neuropathic ulcers were assigned Graftskin (112 patients) or saline-moistened gauze (96 patients, control group).	Graftskin for 4 weeks results in a higher healing rate when compared with state-of-the-art currently available.	At 6 months, the incidence of ulcer recurrence was similar in the Graftskin and control groups.

Table 1: Diabetic foot ulcers clinical interventions.

Concluding Remarks

Here we have reviewed the main mechanisms and roads connecting the pathogenic role of hyperglycemia, oxidative stress, and cellular senesce for the inception of the process of wound chronification that afflicts diabetic patients. We were inspired by mounting contemporary evidences which suggest that EGF therapy is privileged with the ability to reduce and/or delay ulcer recurrence. We consequently hypothesized that in addition to the well-identified predisposing external factors which may certainly abort ulcers remission, amalgamated systemic and local biological factors can impact on scar tissue tolerance thus leading to ulcer recurrence. Among these "resilience or vulnerability factors", the scar tissue burden of senescent cells may play a definitive role. One solid truth is that diabetes

complications are largely impinged by cellular senescence and that hyperglycemia acts as a permissive factor. Sidewise, tissue repair decline with age given that senescent cells increase. Therefore, we have addressed our discussion to the contention that EGF intervention includes the pharmacological competence to prevent or reduce the accumulation of senescencent cells within the scar area. EGF discovery was presided by its ability to accelerate epithelial growth and differentiation upon its injection to newborn mice which was subsequently interpreted as that, EGF exogenous administration proved to impact on the chronological setting of evolutionarily programmed events. In other words, this growth factor proved to retune the temporary window of biological events.

EGF is a well-reputed cytoprotective growth factor, and its activity is driven by the agonistic stimulation of the phosphatidylinositol 3-kinase (PI3K)-Akt axis upon the receptor phosphorylation. Accordingly, a variety of *in vitro* and *in vivo* experimental models have documented the ability of EGF to enhance cells, tissues and animals' survival following otherwise lethal insults, including cytotoxic oxidants. Whether EGF certainly perpetuates wound closure by downregulating cellular senescence operators, may presuppose its therapeutic relevance for pressure ulcers in elderly or disable patients. These are empty niches and contemporary clinical challenges to be afforded and in which cellular senescence may play a critical role. In summary, EGF may potentially be acting as senolytic agent for diabetic foot wounds thus promoting the tissue resilience.

References

1. Jupiter DC, Thorud JC, Buckley CJ, Shibuya N (2016) The impact of foot ulceration and amputation on mortality in diabetic patients. I: From ulceration to death, a systematic review. Int Wound J 13: 892-903.
2. Shin JY, Roh SG, Sharaf B, Lee NH (2017) Risk of major limb amputation in diabetic foot ulcer and accompanying disease: A meta-analysis. J Plast Reconstr Aesthet Surg 70: 1681-1688.
3. Armstrong DG, Boulton AJM, Bus SA (2017) Diabetic Foot Ulcers and Their Recurrence. N Engl J Med 376: 2367-2375.
4. Berlanga-Acosta J, Schultz GS, Lopez-Mola E, Guillen-Nieto G, Garcia-Siverio M, et al. (2013) Glucose toxic effects on granulation tissue productive cells: the diabetics' impaired healing. Biomed Res Int 2013: 256043.
5. Telgenhoff D, Shroot B (2005) Cellular senescence mechanisms in chronic wound healing. Cell Death Differ 12: 695-698.
6. Berlanga-Acosta J, Mendoza-Mari Y, Martinez MD, Valdes-Perez C, Ojalvo AG, et al. (2013) Expression of cell proliferation cycle negative regulators in fibroblasts of an ischemic diabetic foot ulcer. A clinical case report. Int Wound J 10: 232-236.
7. Prattichizzo F, De Nigris V, La Sala L, Procopio AD, Olivieri F, et al. (2016) "Inflammaging" as a Druggable Target: A Senescence-Associated Secretory Phenotype - Centered View of Type 2 Diabetes. Oxidative Medicine and Cellular Longevity 2016: 1810327.
8. Marti-Carvajal AJ, Gluud C, Nicola S, Simancas-Racines D, Reveiz L, et al. (2015) Growth factors for treating diabetic foot ulcers. Cochrane Database Syst Rev 10: CD008548.
9. Embil JM, Papp K, Sibbald G, Tousignant J, Smiell JM, et al. (2000) Recombinant human platelet-derived growth factor-BB (becaplermin) for healing chronic lower extremity diabetic ulcers: an open-label clinical evaluation of efficacy. Wound Repair Regen 8: 162-168.
10. Veves A, Falanga V, Armstrong DG, Sabolinski ML, Apligraf Diabetic Foot Ulcer S (2001) Graftskin, a human skin equivalent, is effective in the management of noninfected neuropathic diabetic foot ulcers: a prospective randomized multicenter clinical trial. Diabetes Care 24: 290-295.
11. Yazdanpanah L, Nasiri M, Adarvishi S (2015) Literature review on the management of diabetic foot ulcer. World J Diabetes 6: 37-53.
12. Braun LR, Fisk WA, Lev-Tov H, Kirsner RS, Isseroff RR (2014) Diabetic foot ulcer: an evidence-based treatment update. Am J Clin Dermatol 15: 267-281.
13. Rufini A, Tucci P, Celardo I, Melino G (2013) Senescence and aging: the critical roles of p53. Oncogene 32: 5129-5143.
14. Kordinas V, Ioannidis A, Chatzipanagiotou S (2016) The Telomere/Telomerase System in Chronic Inflammatory Diseases. Cause or Effect? Genes (Basel) 7.
15. Naylor RM, Baker DJ, van Deursen JM (2013) Senescent cells: a novel therapeutic target for aging and age-related diseases. Clin Pharmacol Ther 93: 105-116.
16. Baker DJ, Wijshake T, Tchkonia T, LeBrasseur NK, Childs BG, et al. (2011) Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. Nature 479: 232-236.
17. Munoz-Espin D, Serrano M (2014) Cellular senescence: from physiology to pathology. Nat Rev Mol Cell Biol 15: 482-496.
18. Nacarelli T, Liu P, Zhang R (2017) Epigenetic Basis of Cellular Senescence and Its Implications in Aging. Genes (Basel) 8: E243.
19. Wang C, Jurk D, Maddick M, Nelson G, Martin-Ruiz C, et al. (2009) DNA damage response and cellular senescence in tissues of aging mice. Aging Cell 8: 311-323.
20. Biran A, Krizhanovsky V (2015) Senescent cells talk frankly with their neighbors. Cell Cycle 14: 2181-2182.
21. Nelson G, Wordsworth J, Wang C, Jurk D, Lawless C, et al. (2012) A senescent cell bystander effect: senescence-induced senescence. Aging Cell 11: 345-349.
22. Pole A, Dimri M, Dimri G (2016) Oxidative stress, cellular senescence and ageing. AIMS Molecular Science 3: 300-324.
23. Behm B, Schreml S, Landthaler M, Babilas P (2012) Skin signs in diabetes mellitus. Journal of the European Academy of Dermatology and Venereology 26: 1203-1211.
24. Palmer AK, Tchkonia T, LeBrasseur NK, Chini EN, Xu M, et al. (2015) Cellular Senescence in Type 2 Diabetes: A Therapeutic Opportunity. Diabetes 64: 2289-2298.
25. Jurk D, Wilson C, Passos JF, Oakley F, Correia-Melo C, et al. (2014) Chronic inflammation induces telomere dysfunction and accelerates ageing in mice. Nat Commun 2: 4172.
26. Clark RA (2008) Oxidative stress and "senescent" fibroblasts in non-healing wounds as potential therapeutic targets. J Invest Dermatol 128: 2361-2364.
27. Wall IB, Moseley R, Baird DM, Kipling D, Giles P, et al. (2008) Fibroblast dysfunction is a key factor in the non-healing of chronic venous leg ulcers. J Invest Dermatol 128: 2526-25240.
28. Esposito K, Nappo F, Marfella R, Giugliano G, Giugliano F, et al. (2002) Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. Circulation 106: 2067-2072.
29. Goldstein S, Moerman EJ, Soeldner JS, Gleason RE, Barnett DM (1979) Diabetes mellitus and genetic prediabetes. Decreased replicative capacity of cultured skin fibroblasts. J Clin Invest 63: 358-370.
30. Berlanga-Acosta J, Armstrong D, Schultz G, Herrera-Martinez L (2014) Chronic Wounds with Emphasis in Diabetic Foot Ulcers. BioMed Research International 2014: 890352.
31. Goldstein S, Moerman EJ, Soeldner JS, Gleason RE, Barnett DM (1978) Chronologic and physiologic age affect replicative life-span of fibroblasts from diabetic, prediabetic, and normal donors. Science 199: 781-782.
32. Rowe DW, Starman BJ, Fujimoto WY, Williams RH (1977) Abnormalities in proliferation and protein synthesis in skin fibroblast cultures from patients with diabetes mellitus. Diabetes 26: 284-290.
33. Lerman OZ, Galiano RD, Armour M, Levine JP, Gurtner GC (2003) Cellular dysfunction in the diabetic fibroblast: impairment in migration, vascular endothelial growth factor production, and response to hypoxia. Am J Pathol 162: 303-312.

34. Hehenberger K, Kratz G, Hansson A, Brismar K(1998) Fibroblasts derived from human chronic diabetic wounds have a decreased proliferation rate, which is recovered by the addition of heparin. J Dermatol Sci 16: 144-151.
35. Loot MA, Kenter SB, Au FL, van Galen WJ, Middelkoop E, et al. (2002) Fibroblasts derived from chronic diabetic ulcers differ in their response to stimulation with EGF, IGF-I, bFGF and PDGF-AB compared to controls. Eur J Cell Biol 81: 153-160.
36. Blazer S, Khankin E, Segev Y, Ofir R, Yalon-Hacohen M, et al. (2002) High glucose-induced replicative senescence: point of no return and effect of telomerase. Biochem Biophys Res Commun 296: 93-101.
37. Vande Berg JS, Rose MA, Haywood-Reid PL, Rudolph R, Payne WG, et al. (2005) Cultured pressure ulcer fibroblasts show replicative senescence with elevated production of plasmin, plasminogen activator inhibitor-1, and transforming growth factor-beta1. Wound Repair Regen 13: 76-83.
38. Vande Berg JS, Robson MC (2003) Arresting cell cycles and the effect on wound healing. Surg Clin North Am 83: 509-520.
39. Stephens P, Cook H, Hilton J, Jones CJ, Haughton MF, et al. (2003) An analysis of replicative senescence in dermal fibroblasts derived from chronic leg wounds predicts that telomerase therapy would fail to reverse their disease-specific cellular and proteolytic phenotype. Exp Cell Res 283: 22-35.
40. Min BM, Oh JE, Choi CM (2004) Retinoic acid delays keratinocyte senescence by suppression of betaig-h3 and p16 expression and induction of telomerase activity. Int J Mol Med 13: 25-31.
41. Demidova-Rice TN, Hamblin MR, Herman IM (2012) Acute and impaired wound healing: pathophysiology and current methods for drug delivery, part 1: normal and chronic wounds: biology, causes, and approaches to care. Adv Skin Wound Care 25: 304-314.
42. Futrega K, King M, Lott WB, Doran MR (2014) Treating the whole not the hole: necessary coupling of technologies for diabetic foot ulcer treatment. Trends Mol Med 20: 137-142.
43. Frykberg RG, Banks J (2015) Challenges in the Treatment of Chronic Wounds. Adv Wound Care (New Rochelle) 4: 560-582.
44. Moura LI, Dias AM, Carvalho E, de Sousa HC (2013) Recent advances on the development of wound dressings for diabetic foot ulcer treatment--a review. Acta Biomater 9: 7093-7114.
45. Amin N, Doupis J (2016) Diabetic foot disease: From the evaluation of the "foot at risk" to the novel diabetic ulcer treatment modalities. World J Diabetes 7: 153-164.
46. Andrews KL, Houdek MT, Kiemele LJ (2015) Wound management of chronic diabetic foot ulcers: from the basics to regenerative medicine. Prosthet Orthot Int 39: 29-39.
47. Alavi A, Sibbald RG, Mayer D, Goodman L, Botros M, Armstrong DG, et al. (2014) Diabetic foot ulcers: Part II. Management. J Am Acad Dermatol 70: 21.e1-21.e24.
48. Esposito F, Ammendola R, Faraonio R, Russo T, Cimino F (2004) Redox control of signal transduction, gene expression and cellular senescence. Neurochem Res 29: 617-628.
49. Papanas N, Maltezos E (2008) Becaplermin gel in the treatment of diabetic neuropathic foot ulcers. Clin Interv Aging 3: 233-240.
50. Leaper DJ, Schultz G, Carville K, Fletcher J, Swanson T, et al. (2012) Extending the TIME concept: what have we learned in the past 10 years? (*). Int Wound J 9: 1-19.
51. Berlanga-Acosta J, Gavilondo-Cowley J, Lopez-Saura P, Gonzalez-Lopez T, Castro-Santana MD, et al. (2009) Epidermal growth factor in clinical practice - a review of its biological actions, clinical indications and safety implications. Int Wound J 6: 331-346.
52. Tiaka EK, Papanas N, Manolakis AC, Georgiadis GS (2012) Epidermal growth factor in the treatment of diabetic foot ulcers: an update. Perspect Vasc Surg Endovasc Ther 24: 37-44.
53. Berlanga-Acosta J, Fernandez-Montequin J, Valdes-Perez C, Savigne-Gutierrez W, Mendoza-Mari Y, et al. (2017) Diabetic Foot Ulcers and Epidermal Growth Factor: Revisiting the Local Delivery Route for a Successful Outcome. Biomed Res Int 2017: 2923759.
54. Berlanga-Acosta J (2011) Diabetic lower extremity wounds: the rationale for growth factors-based infiltration treatment. Int Wound J 8: 612-620.
55. Ashcroft GS, Mills SJ, Ashworth JJ (2002) Ageing and wound healing. Biogerontology 3: 337-345.
56. Wang M, Morsbach F, Sander D, Gheorghiu L, Nanda A, et al. (2011) EGF receptor inhibition radiosensitizes NSCLC cells by inducing senescence in cells sustaining DNA double-strand breaks. Cancer Res 71: 6261-6269.
57. Hsu CP, Lee LW, Tang SC, Hsin IL, Lin YW, et al. (2015) Epidermal growth factor activates telomerase activity by direct binding of Ets-2 to hTERT promoter in lung cancer cells. Tumour Biol 36: 5389-5398.
58. Hu ZX, Chen CL, Yang JS, Zhou ZL, Song ZM, et al. (2014) PI3K-mediated glioprotective effect of epidermal growth factor under oxidative stress conditions. Int J Ophthalmol 7: 413-420.
59. Soneja A, Drews M, Malinski T (2005) Role of nitric oxide, nitroxidative and oxidative stress in wound healing. Pharmacol Rep 57: 108-119.
60. Gordillo GM, Sen CK (2003) Revisiting the essential role of oxygen in wound healing. Am J Surg 186: 259-263.
61. Ojalvo AG, Acosta JB, Mari YM, Mayola MF, Perez CV, et al. (2017) Healing enhancement of diabetic wounds by locally infiltrated epidermal growth factor is associated with systemic oxidative stress reduction. Int Wound J 14: 214-225.
62. Demaria M, Desprez PY, Campisi J, Velarde MC (2015) Cell Autonomous and Non-Autonomous Effects of Senescent Cells in the Skin. J Invest Dermatol 135: 1722-1726.
63. Alexander PB, Yuan L, Yang P, Sun T, Chen R, et al. (2015) EGF promotes mammalian cell growth by suppressing cellular senescence. Cell Res 25: 135-138.
64. Ai G, Shao X, Meng M, Song L, Qiu J, et al. (2017) Epidermal growth factor promotes proliferation and maintains multipotency of continuous cultured adipose stem cells via activating STAT signal pathway in vitro. Medicine (Baltimore) 96: e7607.
65. Tsang MW, Wong WK, Hung CS, Lai KM, Tang W, et al. (2003) Human Epidermal Growth Factor Enhances Healing of Diabetic Foot Ulcers. Diabetes Care 26:1856–1861.
66. Tuyet HL, Nguyen Quynh TT, Vo Hoang Minh H, Thi Bich DN, Do Dinh T, et al. (2009) The efficacy and safety of epidermal growth factor in treatment of diabetic foot ulcers: the preliminary results. Int Wound J 6: 159–166.
67. Hong JP, Jung HD, Kim YW (2006) Recombinant human epidermal growth factor (EGF) to enhance healing for diabetic foot ulcers. Ann Plast Surg 56: 394-398.
68. Acosta JB, Savigne W, Valdez C, Franco N, Alba JS, et al. (2006) Epidermal growth factor intralesional infiltrations can prevent amputation in patients with advanced diabetic foot wounds. IWJ 3: 232-239.
69. Fernández-Montequín JI, Infante-Cristiá E, Valenzuela-Silva C, Franco-Pérez N, Savigne-Gutierrez W, et al. (2007) Intralesional injections of Citoprot-P (recombinant human epidermal growth factor) in advanced diabetic foot ulcers with risk of amputation. Int Wound J 4: 333-343.
70. Fernández-Montequín JI, Valenzuela-Silva CM, Díaz OG, Savigne W, Sancho-Soutelo N, et al. (2009) Intra-lesional injections of recombinant human epidermal growth factor promote granulation and healing in advanced diabetic foot ulcers: multicenter, randomized, placebo-controlled, double-blind study. Int Wound J 6: 432-443.
71. Dumantepe M, Fazliogullari O, Seren M, Uyar I, Basar F. (2015) Efficacy of intralesional recombinant human epidermal growth factor in chronic diabetic foot ulcers. Growth Factors 33: 128-132.
72. Yera-Alos IB, Alonso-Carbonell L, Valenzuela-Silva CM, Tuero-Iglesias AD, Moreira-Martínez M, et al. (2013) Active post-marketing surveillance of the intralesional administration of human recombinant epidermal growth factor in diabetic foot ulcers. BMC Pharmacol Toxicol 14: 44.
73. López-Saura PA, Yera-Alos IB, Valenzuela-Silva C, González-Díaz O, Río-Martín Ad, et al. (2013) Medical Practice Confirms Clinical Trial Results of the Use of Intralesional Human Recombinant Epidermal Growth

Factor in Advanced Diabetic Foot Ulcers. Adv Pharmacoepidem Drug Safety 2:128.

74. Steed DL (1995) Clinical evaluation of recombinant human platelet-derived growth factor for the treatment of lower extremity diabetic ulcers. Diabetic Ulcer Study Group. J Vasc Surg 21:71-81.

75. Embil JM, Papp K, Sibbald G, Tousignant J, Smiell JM, et al. (2000) Recombinant human platelet-derived growth factor-BB (becaplermin) for healing chronic lower extremity diabetic ulcers: an open-label clinical evaluation of efficacy. Wound Repair Regen 8: 162–168.

76. Smiell JM, Wieman TJ, Steed DL, Perry BH, Sampson AR, et al. (1999) Efficacy and safety of becaplermin (recombinant human platelet-derived growth factor-BB) in patients with nonhealing, lower extremity diabetic ulcers: a combined analysis of four randomized studies Wound Repair Regen 7: 335-346.

77. Wieman TF, Smiell JM, Su Y (1998) Efficacy and Safely of a Topical Gel Formulation of Recombinant Human Platelet-Derived Growth Factor-BB (Becaplermin) in Patients With Chronic Neuropathic Diabetic Ulcers: A phase III randomized placebo-controlled double-blind study. Diabetes Care 21: 822-827.

78. Marston WA, Hanft J, Norwood P, Pollak R, Dermagraft Diabetic Foot Ulcer Study Group (2003) The efficacy and safety of Dermagraft in improving the healing of chronic diabetic foot ulcers: results of a prospective randomized trial. Diabetes Care 26: 1701-1705.

79. Veves A, Sheehan P, Pham HT (2002) A randomized, controlled trial of Promogran (a collagen/oxidized regenerated cellulose dressing) vs standard treatment in the management of diabetic foot ulcers. Arch Surg 137: 822-827.

80. Blume PA, Walters J, Payne W, Ayala J, Lantis J (2008) Comparison of Negative Pressure Wound Therapy Using Vacuum-Assisted Closure With Advanced Moist Wound Therapy in the Treatment of Diabetic Foot Ulcers. Diabetes Care 31: 631–636.

81. Armstrong DG, Lavery LA, Diabetic Foot Study Consortium (2005) Negative pressure wound therapy after partial diabetic foot amputation: a multicentre, randomised controlled trial. The Lancet Volume 366: 1704-1710.