BIOLOGICAL MECHANISMS OF CHRONIC WOUND AND DIABETIC FOOT HEALING: THE ROLE OF COLLAGEN

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BIOLOŠKI MEHANIZMI ZARASTANJA HRONIČNIH RANA I OZDRAVLJENJA DIABETIČNOG STOPALA: ULOGA KOLAGENA

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ABSTRACT

The treatment of chronic wounds is a continuously developing research focus. The problems of excessive mechanical forces, infection, inflammation, reduced production of growth factors, and lack of collagen will affect the results of treatment. The purpose of this study was to analysse the elements that lead to long-term non-healing of chronic wounds and trophic ulcers, including diabetic foot syndrome, by determining the optimal treatment algorithm. The paper presents an analysis of the world literature on the etiopathogenesis and principles of chronic wound treatment in diabetic foot syndrome. The epidemiology of chronic wounds of different genesis is presented. The issues of physiological and metabolic disorders in chronic ulcers affecting the process of wound healing are discussed. Particular attention is paid to collagen, which is a protein that forms the basis of connective tissue; collagen ensures the strength and elasticity of the skin, which confirms the importance of its role not only in aesthetics but also in the process of wound healing. Different types of collagen and their roles in the mechanisms of chronic wound healing in diabetic foot syndrome are described. The results of clinical studies evaluating the effectiveness of medical products and preparations, consisting of collagen with preserved (native collagen) and fractionated structures, in treating chronic wounds of diabetic foot syndrome are analysed. It has been shown that the use of native collagen preparations is a promising treatment for chronic ulcers and wounds, including diabetic foot syndrome, which makes it possible to increase the effectiveness of treatment and reduce the economic costs of managing these patients.

Keywords: *chronic wounds, diabetic foot syndrome, collagen.*

SAŽETAK

Protokoli lečenja hroničnih rana su u stalnom razvoju. Problemi prekomernih mehaničkih sila, infekcije, upale, smanjenja proizvodnje faktora rasta, nedostatka kolagena će uticati na rezultate tretmana. Svrha ovog rada je da analizira razloge koji su doveli do dugoročnog neizlecenja hroničnih rana, trofičkih ulkusa, uključujući sindrom dijabetičkog stopala, određivanjem optimalnog protokola tretmana. U radu je prikazana analiza dostupne literature o etiopatogenezi i principima lečenja hroničnih rana kod sindroma dijabetičkog stopala. Prikazana je epidemiologija hroničnih rana različite geneze. Razmatrana su pitanja fizioloških i metaboličkih poremećaja u hroničnim ulkusima koji utiču na proces zarastanja rana. Posebna pažnja se posvećuje kolagenu - proteinu koji čini osnovu vezivnog tkiva, osiguravajući snagu i elastičnost kože, što potvrđuje važnost njegove uloge ne samo u kozmetologiji, već posebno u procesu zarastanja rana. Opisani su različiti tipovi kolagena i njegova uloga u mehanizmima zarastanja hroničnih rana kod sindroma dijabetičkog stopala. Analizirani su rezultati kliničkih studija koje ocenjuju efikasnost medicinskih proizvoda i preparata na bazi kolagena sa očuvanom strukturom (nativni kolagen) i frakcionisanog kolagena u pogledu perspektive hroničnih rana kod dijabetičke terapije stopala. Pokazalo se da je upotreba nativnog kolagenskog preparata obećavajući pravac u lečenju hroničnih ulkusa i rana, uključujući i sindrom dijabetičkog stopala, što omogućava povećanje efikasnosti lečenja i smanjenje ekonomskih troškova tretmana ovim pacijentima.

Ključne reči: *hronične rane, sindrom dijabetičkog stopala, kolagen.*

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INTRODUCTION

The significant increase in the number of patients with chronic wounds, especially those with complications of type 2 diabetes mellitus and poor effectiveness of combination therapy, has created a challenge for practising surgeons, endocrinologists and other specialists to search for superior and more effective treatments for such patients. Long-term and continuous therapy often ends results in amputations, which produces a plethora of disabled individuals who are excluded from a productive social life; this outcome imposes an extra financial burden at all levels.

The pathophysiology of diabetic foot syndrome (DFS) includes many mutually potentiating components, such as neuropathies, vascular disorders, impaired immunity, and infections, creating a vicious cycle (1-3). Numerous studies have been dedicated to the treatment of patients with chronic wounds, which has helped to work out recommendations for evidence-based approaches that are now recognized by the world community (Table 1).

Unfortunately, even when a whole set of recommended treatments accompanied by continuous glucose monitoring and pharmacological support of adequate tissue perfusion is used, wound healing occurs within 12 weeks in only 24-50% of patients (4-7). The absence of an adequate understanding of the physiology of wound healing hampers the development of new wound care products for chronic wound treatment and, until now, did not allow for the development of evidence-based evaluation of the comparative effectiveness of different types of wound healing devices in patients with protracted, non-healing chronic ulcers.

Therefore, despite all the achievements in the health care system, chronic ulcers, especially in patients with diabetes mellitus, still remain the most topical medico-social issue, and the solution requires an in-depth investigation of common factors involved in the activation of physiological defence mechanisms and objectivization of the most promising methods of treatment.

EPIDEMIOLOGY OF CHRONIC WOUNDS

A wound is considered to be chronic (also known as protracted, non-healing wound or trophic) if it does not heal within 6 weeks of its existence or show any signs of healing despite appropriate standard wound care. The most common causes of chronic wounds include chronic lower extremity venous diseases (venous trophic ulcer), chronic arterial insufficiency (obliterating atherosclerosis) and diabetes mellitus (diabetic foot syndrome), as well as pressure ulcers (decubitus trophic ulcers), which occur as a result of pressure on weight-bearing areas for too long and/or disturbed innervation due to disorders of the nervous system. All these pathological conditions represent a very complex and most topical medico-social problem.

In this light, 1-2% of the population is suffering from chronic venous trophic ulcers of the lower extremities, which are the cause of 50-70% of all leg chronic wounds (8-9). Only half of venous trophic ulcers heal within 4 months, while 20-25% of those fail to undergo epithelialization even within 2 years (10-11). A quarter of leg ulcer cases are caused by atherosclerotic lesions of the lower limb arteries with haemodynamically significant reduction of tissue perfusion and disturbed oxygenation of the skin and soft tissues of the leg.

However, the two abovementioned conditions can be approached by fairly effective, primarily surgical treatments. The narrowing of the magistral blood vessels is eliminated using modern intravascular technologies. Unfortunately, diabetic foot syndrome (DFS) manifesting in the appearance of chronic ulcers on the weight-bearing areas of diabetic patients poses a far more serious problem with no effective solution to date. The occurrence of DFS is manifested clinically due to neural influences on trophic changes, and lesions in the microvascular bed occur due to glycation of the vessel wall proteins.

The global prevalence of diabetic foot syndrome is 6% (12). Every fourth patient with diabetes mellitus bears a heavy burden of DFS (13). That said, every year, 2-10% of diabetic patients develop DFS, and approximately 10% of affected lower limbs are amputated due to the occurrence of suppurative and gangrenous changes (14-16), making DFS a highly debilitating illness leading to global economic losses. According to the European Study Group on Diabetes and the Lower Extremity (Eurodiale), a high-level amputation surgery is performed in 5% of patients with DFS each year (17, 18). That said, a 5-year survival rate following amputation ranges between 30% and 70% (19). In patients with DFS, the arteries of the microcirculatory bed are affected in 50-80% of cases, and the severity of this injury correlates with the healing potential and determines the prognosis (20, 21), which translates into a colossal economic impact. The Eurodiale study has shown that the average cost of treatment of uninfected ulcers is 10 000

Table 1. Recommended approaches for the treatment of patients with diabetic foot syndrome (ranked by the level of evidence)

Treatment	Diabetic foot ulcer (primary)	Chronic diabetic foot ulcer
Plantar pressure redistribution	Recommended (1A)	Recommended (1A)
Surgical treatment of the wound	Recommended (2A)	Recommended (2A)
Antibiotics	Treatment of infection caused by aerobic Gram-positive bacteria (2A)	Broad-spectrum systemic antibiotics (2A)
Wound dressings using wound-healing agents	Recommended (2A)	No sufficient evidence available

euros versus 17 000 euros for infected ulcers with concurrent peripheral artery disease (18). In the USA,, the total treatment cost for protracted, non-healing diabetic ulcers with severe infection (complex surgical and conservative treatment and, due to its failure, below knee amputation) the is $190 000, according to a 2012 estimate (22).

PHYSIOLOGICAL AND METABOLIC DISTURBANCES IN PATIENTS WITH CHRONIC WOUNDS

The course of wound healing includes the following phases: inflammation (exudation), proliferation/regeneration (granulation tissue formation) and scar regeneration (epithelialization). Macrophages play a key role in wound healing. They phagocytize pathogenic organisms and tissue degradation products and stimulate formation of granulation tissue. Fibroblasts are required during the proliferation phase, as they produce the important structural elements including collagen, elastin and extracellular matrix proteins.

Wound healing depends on the particulars of the wound (aetiology, depth, and size) and the patient's general condition. In normal circumstances, the healing wound has a low level of bacterial contamination, inflammatory cytokines, proteases and active oxygen forms, and it also has a functionally active extracellular matrix and high mitotic activity of epithelial cells (23). However, to ensure the synthesis of granulation tissue, collagen and the ground substance of the extracellular matrix, energy is required in the form of adenosine triphosphate (ATP) molecules, which areis mainly produced in the mitochondria, provided a sufficient amount of oxygen is available. For that reason, any disturbance of the oxygen supply to cells leads to a several-fold reduction of ATP synthesis and a corresponding decrease of collagen synthesis. This is the main pathophysiological mechanism leading to the disturbance of normal tissue regeneration and formation of chronic wounds / trophic ulcers (1, 2).

Studies conducted in different countries demonstrated glycosylation of collagen in diabetes mellitus; therefore, despite the upregulation of type I collagen gene expression, there is an impaired synthesis of fully functional collagen molecules in the wound. Degraded tissue components and superactive matrix metalloproteinases (MMP) present in chronic non-healing wounds promote a protracted inflammatory response (24). Fibroblast physiology is changing as well (25). As a response to ischaemia/hypoxia fibroblast migration and proliferation decreasing, it reduced collagen production occurs (26, 27).

For this reason, along with basic methods of therapy, the administration of collagen is considered an important adjunct therapy in patients with various chronic wounds, protracted non-healing trophic ulcers and bed sores.

COLLAGEN-TYPES AND ROLES

Collagen (kolla - glue, genes - producing) is the most common mammalian protein representing 25-35% of all proteins in the body; i.e., approximately 6% of body weight (28, 29). Collagen forms the mechanical backbone of the connective tissue and ensures durability and resilience of the bones, ligaments, skin, vessels and other tissues. As with any other protein, even more so the ones with natural load-bearing function, collagen is constantly synthesized and catabolized. Allegedly, in young subjects, the amount of newly synthesized collagen is equal to 6 kg per year. In the second half of life, the rate of all synthesis reactions decreases, and the amount of newly synthesized collagen is reduced 2-fold. It is important to note that approximately 40% of the total collagen amount is present in the skin, which confirms its role in skin care and especially in the process of wound healing.

To date, scientists identified more than 40 genes that collectively encode 28 different types of collagen designated with roman numerals from I to XXVIII (28, 30). Such variability of collagen types is required to ensure different physiological functions in different tissues and organs. Characteristics of the most common types of collagen I – IV are presented in Table 2.

Table 2. The most common types of collagen I – IV.

Type	Genes	Tissues and organs	Composition	Associated pathology
I	COL1A1, COL1A2	Ubiquitously, especially in skin (dermis), ligaments, bones, fascias, dentin, cornea	1% - hydroxylysine, 33% - glycine, 13% - proline; low glycosylated, large-diameter fibrils	Rheumatism, dysplasias, Marfan syndrome, Ehlers-Danlos syndrome
II	COL2A1	Cartilages, vitreous body, cornea	>1% hydroxylysine, highly glycosylated fibrils thinner than in type I collagen	Collagenopathy, achondrogenesis, Stickler syndrome
III	COL3A1	Skin (mostly foetal skin), uterus, blood vessels, reticular fibres of haematopoietic organs	Excess of hydroxyproline, hydroxylysine deficiency, low-glycosylated	Aortic aneurism, dysplasia, Ehlers-Danlos syndrome
IV	COL4A1, COL4A2, COL4A3, COL4A4, COL4A5, COL4A6	Basal membranes	Excess of hydroxylysine and hydroxyproline, alanine deficiency, nearly completely glycosylated	Goodpasture syndrome

Based on its supramolecular organization, collagen can be divided into two categories; i.e., fibrillar and non-fibrillar collagen (29-32). The main fibrillar types of collagen are types I, II and III. The most common in adults is type I collagen, which possesses the greatest tensile mechanical strength due, among other things, to fibrils of the largest diameter. The non-fibrillar collagens may form networks with various topological properties (e.g., type IV collagens (basal membranes), type VIII collagens (cornea, blood vessels), type VI collagens (beads-on-string-type structures; cartilage, blood vessels, skin, uterus, lungs, kidneys); types XXVI and XXVIII (many tissues and organs), and type VII collagens (anchor filaments; skin, oaoesophagus, cornea, chorion). Many non-fibrillar collagens are bound to the surface of collagen fibrils (types IX, XII, XIV, XIX-XXII), and some of these are transmembrane proteins (types XIII, XVII, XXIII, XXV).

Therefore, collagen fibrils are the macromolecular compositions containing several types of collagen and their bound proteins (33). The composition of different fibrils depends on their stage of development and type of tissue, which is why the definition of specific structures, such as "type I collagen fibrils" or "type II collagen fibrils", is an oversimplification. In this regard, adult skin mostly contains type I collagen (80-85%). That said, up to 20% of all collagens belong to type III collagen (which dominates during embryogenesis but in adulthood makes up 5-10%), including types V, VI, VII, XII, XIV and other collagen types. Collagen fibrils in ligaments mostly comprise types I and III collagen; cartilage comprises types II and XI and the cornea comprises types I and V collagen (29, 32).

The direction of collagen fibrils is important for providing tensile strength and resilience to tissues and organs. Considering the direction of collagen, in the central part of human skeleton bones (pipe bones and flat bones), collagen fibrils appear to run parallel in the longitudinal direction, whereas in the peripheral part they run in a transverse direction. The parallel fibrils in the ligaments secure resistance to mechanical loading, whereas the collagen fibril network does so in the cartilage. In the dermis, collagen fibrils form the network and the level of development is proportional to the applied load or pressure; hence, the most developed network is present in the skin of the heel. In the skin of the healing wound, the collagen fibril network shows a peculiar kind of randomness.

Collagen is synthesized and secreted mostly by fibroblasts inat different degrees of maturity and by cells producing the intercellular substances that enhance wound healing. Fibrillar collagens are primarily synthesized in the form of a soluble precursor, procollagen (25, 34). Procollagen molecules are converted to collagen by proteinases during or after their secretion (35). Mature collagen molecules pack together to form fibrils, and the process is regulated by superficial and extracellular proteins such as other collagens, integrins, and fibronectin (33, 36-38).

It is important to emphasize that collagen is not merely the passive structural component of the molecular backbone. The biological function of collagen is to mediate interaction with different cell surface receptors and other extracellular matrix proteins. Interaction of collagen with specific cell receptors triggers signal events that regulate cell migration, adhesion or proliferations. There are three main families of collagen receptors: collagen-binding integrins, collagen-binding immune receptors and discoidin domain receptors. The latter are kinase receptors that bind the main fibrillar collagens of types I-III and regulate cell proliferation, differentiation and matrix modulation (39-41), thereby ensuring the wound/ulcer healing process.

COLLAGEN-BASED MEDICAL DEVICES AND PRODUCTS

The above-mentioned studies convincingly demonstrate the enormous roles of collagen, collagen-based products and medical devices in the stimulation of the regenerative and proliferative phase of the wound healing process. Collagen-based products mainly differ from each other by the degree of purity and cross-linking, relative percentage of different collagen types, source and form of collagen presentation. Sources of collagen may include skin, intestine and other organs. After collection of source material, the manufacturers prepare each product according to specific individual processes by removing cell components and retaining the natural matrix. The degree and methods of purification of collagen products are different, which directly affects the preservation of the collagen structure. In some cases, the collagen structure is preserved (native collagen), and in other cases it is not (fractionated collagen). It is supposed that cleaved collagen with a degraded collagen matrix still maintains properties inherent to a three-dimensional spiral molecule (42). To increase the tensile strength of fibrillar protein, some companies use collagen cross-linking or multiple lamination methods. Each approach has its own potential advantages and drawbacks.

Manufacturing of biomaterials based on ***native collagen*** allows complete preservation of its natural structure and removal of cell elements carrying specific cellular markers (melanocytes, macrophages, and lymphocytes) as well as portions of blood vessels and hair follicles that ensure low antigenicity of collagen devices (43).

When introduced into the wound, native collagen plays the role of an exogenous matrix, which stimulates fibroblast/macrophage chemotaxis, providing the basis for directed migration of cell components of the wound bed. As a result, the collagen implant activates fibroblast proliferation and secretion, which secures angiogenesis and stimulates activity of immune cells (lymphocytes and macrophages) that take part in the regulation of regeneration. Finally, the native exogenous collagen gradually resorbs and is substituted by the recipient's own connective tissue. Therefore, native collagen is a kind of "stencil" for the formation of the recipient's own tissue, which makes it far more superior compared to products containing cleaved collagen (29, 43, 44).

Examples of products containing native collagen include the following: (1) Integra (Integra Life Sciences Corp, Plainsboro, New Jersey, USA) – pigskin collagen matrix; (2) Collost biomaterial (BioPHARMAHOLDING LLC) – cattle dermis collagen containing mostly type I collagen; and (3) Primatrix (TEI Biosciences Inc, Boston, Massachusetts, USA) – acellular collagen derived from bovine foetal dermis containing mostly type III collagen.

Type I collagen is the most studied species. In the *in vitro* experiments, it accelerated formation of the intercellular matrix by dermal fibroblasts (45). The native type I collagen binds a number of proteases and inflammatory cytokines (including neutrophil elastase, MMP-2, interleukins (IL) IL-6, IL-8 and IL-1, superoxide-anion, and peroxynitrate), which are abundant in the wound exudate from a chronic wound/ulcer (46, 47). In an *in vivo* study on epithelial cells derived from dermal microvessels, type I collagen was shown to activate angiogenesis (48). These studies demonstrated that topical application of type I collagen modulates the milieu of the chronic wound showing the effect as soon as 2 weeks after collagen application (47).

Type III collagen is a homotrimeric fibrillar collagen that dominates at the first step of wound healing, ensuring the initial cell migration and differentiation. Studies with a COL3-negative mouse model have shown that type III collagen modulates scar formation through the effect on migration and differentiation of myofibroblasts. The absence of type III collagen resulted in disruption of structure, and its relative deficit led to an increased scar size and faster wound closure compared to normal COL3-positive mice (49). The results of these studies call into question the higher effectiveness of type III collagen in the healing of chronic wounds. However, there are data attesting to the possible transformation of type III collagen to type I collagen (50). In addition, the experimental study using the pig model and recombinant type III collagen gel demonstrated its wound healing effectiveness (51). However, the gel was used as a delivery agent for cultured autologous skin cells (keratinocytes and fibroblasts), which promoted the formation of wound granulation tissue.

Fractionated collagen acts as a bioactivator that employs collagen molecule fragments to recruit macrophages and fibroblasts for wound healing. Fractionated collagen functions as a pseudosubstrate for MMPs; hence, fractionated collagen binds and inactivates MMPs, thereby suppressing proteolysis of the intercellular matrix proteins.

An example of fractionated collagen is Cellerate Rx (Wound Care Innovations, Ft. Lauderdale, FL, USA), within which collagen fibrils are cleaved to approximately 1/100 the length of the original fibrils, which results in disruption of virtually all internal bonding and an increased rate of collagen integration. However, the structural advantages of collagen are lost. Fractionated collagen is supposed to act as a bioactivator that employs collagen fragments to recruit macrophages and fibroblasts. The other examples are the so called "foamed" collagens, which manufacturers claim have a production method that is based on collagen degradation with subsequent blending of collagen with cellulose. This enables preservation of a classical triple-helical collagen structure that promotes binding of elastase, activating the MMP action (52, 53). Products of this kind include Prisma and Promogran (Systagenix, North Yorkshire, United Kingdom), which contain approximately 55% collagen and 44% cellulose and, in the case of Prisma, an additional 1% of silver ions bound to the cellulose matrix. Biostep and Biostep Ag (Smith & Nephew, Largo, FL, USA) are also products of this kind. They contain collagen, carboxymethyl cellulose and sodium alginate, which facilitate cell migration and tissue regeneration. Biostep additionally contains ethylenediaminetetraacetic acid, which inhibits proteolytic enzymes in chronic wounds thereby improving wound healing. Puracol Plus and Puracol Plus AG (Medline Industries, Mundeline, IL, USA) contain pure collagen without additives.

Tarlton J.F. et al. in 2013 showed that fractionated collagen Promogran can decrease the MMP activity in all types of chronic wounds. However, the effectiveness of this compound depends on the acidity of the milieu as any modulation of proteolytic activity is lost at a neutral pH (54).

It was suggested that cleavage of collagen increases the rate of its integration. However, during collagen fractionation, the structural advantages of the collagen matrix are lost. In addition, the benefits of an increased rate of collagen integration are arguable as it entails the necessity of frequent repeated use of the compound. In contrast, the intact collagen is resistant to lymphocyte action and is retained longer in the wound bed acting as an exogenous matrix (55). Another shortcoming of fractionated and subsequently cross-linked collagen is its densely packed structure that prevents penetration of fibroblasts and their interaction with the collagen matrix (56).

In 2016, Wiegand C. et al. conducted a study to evaluate the physiological effect of the native and fractionated collagen on a chronic wound healing process (57). It was shown that fibroblasts seeded onto the native collagen matrix demonstrated exponential growth, whereas the proliferation rate of fibroblasts on the fractionated collagen matrix was very low. The use of native collagen is accompanied by a more effective and significant MMP sequestration. In addition, native collagen causes a marked *in vitro* stabilization of the platelet-derived growth factor BB (PDGF-BB).

Considering the results of the above-mentioned studies, one can surmise that native but not fractionated collagen, mostly of type I, is preferable for the treatment of trophic ulcers and protracted non-healing wounds.

USE OF NATIVE COLLAGEN IN THE TREATMENT OF DIABETIC FOOT SYNDROME

It was shown that the use of native collagen products promotes chronic wound area reduction (58). Native collagen products (Collost) promote faster wound pro-

cess transition to an active regeneration stage and lower wound contamination by microorganisms (59, 60). In a study by Ivanus S.Ya., by day 12 of treatment with Collost, the wound's level of contamination did not exceed 10^5 CFU/g (61). Use of Collost led to a 1.8-fold reduction in time of wound preparation for autodermoplastic closure (32.0±4.6 days versus 56.8±8.7 days in the comparator group) (62). Preliminary results of a multicenter, randomized prospective clinical trial encompassing 71 patients with diabetic foot syndrome Wagner II – III showed that native collagen (Collost) effectively decreased the length, width, area and volume of diabetic ulcers so that epithelialization could occur faster (63, 64). After 4 weeks of treatment, complete epithelialization was observed in 22.2% of patients from the main group (Collost) and in 8.6% of patients from the comparator group (standard therapy). In the main group, they managed to achieve reduction of the wound area by 67% versus 39% in the comparator group, compared to corresponding values obtained on day 1 (65).

The other multicenter, randomized, controlled clinical trial that enrolled 307 patients also demonstrated a shorter time to complete diabetic ulcer closure in patients receiving native collagen product (Integra) (66). Use of native collagen reduces the pain syndrome in patients with chronic wounds including those with DFS (58, 59, 67).

It is important to emphasize that the use of native collagen products does not produce side effects (58, 59, 63, 64, 68), which attests to high safety of this treatment.

Inclusion of native collagen-based biomaterials in the treatment regimen for DFS also demonstrated its cost-effectiveness. For instance, it was shown that Collost brings down the resulting necessary cost to the treatment effectiveness ratio (69).

Comparative evaluation of native collagen products versus other up-to-date medical products used for topical treatment of DFS demonstrated high relative effectiveness and safety of the former. In a comparative clinical study, the use of Collost membrane and epidermal growth factor-based formulation Heberprot-P in patients with DFS showed the superiority of the collagen-based biomaterial (70). There was a higher rate of positive dynamics in the course of the wound process, a higher incidence of complete epithelialization (complete healing or positive dynamics in 89% of patients in the Collost group and 19% in the comparator group), fewer amputations (0% and 32%, respectively) and fewer cases of individual intolerance to native collagen products (Collost biomaterial) (70).

A comparative study of Apligraf living cell therapy and PriMatrix native collagen in the treatment of diabetic (n=40) and venous (n=28) foot ulcers revealed high effectiveness of both methods. It was found that ulcers in patients receiving PriMatrix healed faster than in patients treated with Apligraf despite a larger wound area in the former group (56).

Russian scientists have developed a patented method for DFS therapy that comprises the sequential use of conservative therapy, which includes surgery combined with an ultrasound hydrosurgery procedure and application of a native collagen-based product; this method allowed the authors to reduce the incidence of organ removal surgery and complications by 15-33%, shorten duration of the inpatient stay by 20% and decrease the number of return visits by 13.4-18.5%, which has been demonstrated in a large study in 1195 patients (60, 71, 72).

USE OF FRACTIONATED COLLAGEN IN PATIENTS WITH DIABETIC FOOT SYNDROME

Lobmann R. et al. (2006) assessed the effects of Promogran in 33 patients with DFS and found no statistically significant differences in the levels of MMP mRNA, IL-1β and TNF-α compared to those in the control group. In addition, the MMP levels in the wound measured by an ELISA also did not differ significantly between the groups. However, the IL-1β level was elevated on day 8 only in the Promogran group (p=0.01), and a significant reduction in the MMP-9/TIMP-2 ratio was found in the collagen treatment group. Nonetheless, the latter group demonstrated a higher rate of wound healing (73).

Similar results were obtained by Motzkau M. et al. (2011) in a randomized study in 19 patients with DFS of whom 13 patients received fractionated collagen (74). A 26-day observation did not reveal any differences in the expression of MMP mRNA, TNF-alpha and other MMPs in the wound tissue. At the same time, the authors' statements regarding statistically significant reduction of the wound area in the group receiving collagen (p = 0.003) werewas more than doubtful considering the small sample size.

The effectiveness of fractionated collagen products can be improved. In this regard, Kakagia D.D. et al. showed that the efficacy of Promogran alone is lower than that of Promogran combined with growth factors (75).

The largest trial on the effectiveness of fractionated collagen in patients with DFS is the study by Veves A. et al. (76), which was carried out in 276 patients aged between 23 and 85 years who had been randomly assigned to the Promogran group (n=138) and the mock-treated group (wet dressing, n=138). After 12 weeks of treatment, 37% of patients from the Promogran group demonstrated complete wound closure versus 28% in the mock-treated group (p=0.12). More pronounced differences were found for patients with ulcers of < 6 months duration (45% and 33%, respectively, p=0.056); however, the difference did not reach statistical significance. Therefore, this large multicenter study failed to demonstrate the significance of effectiveness of fractionated collagen in the treatment of patients with DFS.

Therefore, the analysis of results obtained with products of fractionated collagen did not reveal conclusive benefits of its use in patients with DFS compared to studies employing native collagen products.

CONCLUSION

Treatment of chronic wounds is in a continuously advancing direction. The issues of excessive mechanical forces, wound infection and inflammation, decreased production of growth factors and, of course, collagen deficits, will have an impact on treatment outcomes. Numerous studies have shown that collagen-based products are bioactivators and promote regeneration of the recipient's own tissues through integration with the surrounding tissues. Their major benefits are the regulation of the biochemical milieu of the wound and the stimulation of chemotaxis and angiogenesis. These products resemble a thin layer of the natural skin but are free from the shortcomings inherent in foreign cell elements associated with skin graft rejection.

However, not all collagen-based products and devices are the same. They differ in composition and degree of preservation of the natural collagen matrix. Native (non-cleaved) collagen serves as a matrix for directed migration of macrophages and fibroblasts into the wound bed, and it activates chemotaxis, proliferation and secretory activity of cell elements. During the course of wound healing, the collagen is dissolved and replaced by the recipient's own connective tissue. The native collagen-based products modulate protease activity and stimulates production of the recipient's own collagen.

Unlike native collagen, fractionated (cleaved) collagen interacts less actively with fibroblasts because of its densely packed components; accordingly, it has a less pronounced influence on the rate of cell migration and proliferation. However, the effectiveness of collagen products depends on the acidity of the milieu and it goes down at a neutral pH.

The advantages of physiological action of native collagen-based products are confirmed by their clinical effectiveness. Native collagen-based products promote accelerated transition of the wound process to the regeneration stage, shorten the chronic wound healing time, help to decrease the chronic wound area, reduce the extent of bacterial contamination, alleviate the pain syndrome and reduce the recurrence rate. The administration of native collagen-based products enables ingthe reduction of the resulting necessary cost tothe treatment effectiveness ratio.

Therefore, administration of native collagen-based products is a fairly promising direction in the treatment of chronic ulcers and wounds, including those in patients with diabetic foot syndrome. This enables improvement ofin the effectiveness of treatment and reduces the cost of providing medical care for such patients.

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