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NEW PROSPECTS FOR CUTANEOUS WOUND HEALING AND KELOID TREATMENT

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Summary

The early steps of skin tissue repair are sustained by fibroblast proliferation, collagen deposition, and re-epithelization. On the contrary, injury or surgery in predisposed individual can result in hypertrophic scar.

Keloid is characterized by an increased number of fibroblasts, mast cells and a greater collagen synthesis with irregular bundles conformation which contributes to the formation of a disordered repair tissue.

However some medical-grade chitosans seem to have a reparative action on wounded tissues, inducing the formation of ordered repair tissue where collagen bundles had a regular direction. In this way, wounds did met slow evident scar formation or the wound contraction proper of the keloid.

The aim of this study was to control the activity of a gel based on a new patented hydrosoluble chitosan derivative of medical grade to repair and improve the cutaneous microenvironment on wounded tissues.

Dermo-epidermal grafts were performed with the aid of a dermotome on the frontal part of the thighs of 10 patients aged 30 to 50 years. In every patient a donor site was dressed with N-carboxybutylchitosan pads, the other was treated with phytostimuline gauze.

On day 10 and 30, when the surgical wounds were clinically healed, bioptie fragments were obtained from both areas.

Ultrastructural analysis of the areas treated by our chitosan derivative, showed a regular distribution of the collagen network and the general aspect of the derma gave an overall impression of histoarchitecture order which was more evident than in controls.

Overall, the epithelium was organized and citologically normal.

The studied chitosan derivative seems to favour the cell proliferation, the rapid re-epitheliazation of the tissue and optimizing the cutaneous repair, limitating the process of wound contraction.

It seems this gel could be considered as an innovative cosmeceutical or medical device able to speed up the tissue repair and to avoid the keloid formation

Riassunto

Il processo di riparazione della cute □ caratterizzato da alcune fasi principali: proliferazione di fibroblasti, deposizione di collagene e riepitelizzazione. In soggetti predisposti, tuttavia, insulti e/o interventi chirurgici possono dare origine a cicatrici ipertrofiche (cheloidi). Il cheloide □ caratterizzato sia da un aumento del numero di fibroblasti e mastociti, sia da una maggiore sintesi di fibre collagene i cui fasci tendono ad assumere una conformazione irregolare determinando la comparsa di un tessuto di riparazione disordinato.
Alcuni "medical-devices" a base di chitosano sembrano avere, tuttavia, un'azione riparativo/ricostitutiva inducendo una riparazione tissutale ordinata, con fasci di collagene disposti in maniera regolare, riducendo le possibilit□ di formazione di cicatrici ipertrofiche e/o di cheloidi.
Scopo del presente studio □ stato la valutazione della capacit□ di un nuovo chitosano idrosolubile, applicato quale gel, migliorare il microambiente cutaneo di un tessuto lesionato.
Lo studio □ stato condotto su 10 pazienti, aventi un'et□ compresa tra 30 e 50 anni, sottoposti ad innesti dermo epidermici a medio spessore. Le zone donatrici di innesti sono state realizzate sulla parte anteriore di entrambe la cosce in tutti i pazienti; un□area □ stata medicata con il prodotto in studio, l□altra con fitostimolina come area di controllo. Ø stata eseguita una biopsia delle aree trattate e di quelle di controllo al 10¡ e 30¡ giorno.
Le analisi ultrastrutturali condotte sulle aree trattate con il gel di chitosano, hanno mostrato una distribuzione dei fasci di collagene regolare e un derma con un ordine istoarchitetturale pi□ evidente rispetto alle zone di controllo. Inoltre l'epitelio si mostrava organizzato e citologicamente normale.
Questo derivato del chitosano sembra pertanto favorire la proliferazione cellulare e la rapida riepitelizzazione del tessuto, ottimizzando cos□ la riparazione cutanea e limitando il processo di contrazione della ferita. Tale gel potrebbe quindi essere utilizzato in medicina o in cosmetologia come mezzo per accelerare la riparazione tissutale e per evitare la formazione di cheloidi.

In superficial lesions, the rebuilding of a normal tissue is made possible by the basal cells of the epidermis, which proliferate and cause the tissue to regenerate. This process becomes more difficult for lesions, which penetrate deeply into the dermis, because the layer of basal cells whose proliferation would create a new epidermis is lost, and its formation is entrusted only to cells bordering the injured area. This process, however, is very slow and frequently interrupted by infections that occur in this type of lesions. When the lost of tissue is severe this is replaced by healing tissue, as it is impossible to obtain a perfect "restitutio ad integrum". The scar tissue is a particular connective tissue, frequently poor in elastic fibers, which can not be compared to the dermis because its has no normal elasticity and yields easily, with pathological stretching of its matrix structure. Another evident characteristic of scar tissue is tendency to retract. Wound healing alterations, i.e. excessive cicatrization, leads to cheloids.

Aim of the present study was the evaluation of the following materials abilities: a) favour formation of physiologically and histologically valid extracellular matrix; b) enhance vascularization and ordered collagen fibre formation; c) favour basal cell migration and proliferation; d) protect against infections; e) accelerate healing in slowly healing wounds (1-4).

The use of glycosaminoglycans in wound treatment is not new, and was justified by the occurrence of hyaluronic acid, chondroitin-6-sulfate, and dermatan sulphate, among other compounds, in the extracellular matrix. Nevertheless chitosans were found to be biocompatible and degradable in the human body, and their unique basically made them more promising for tissue regeneration and microbial growth depression than other polysaccharides. In this study, 5-8 high molecular weight N-carboxybutyl chitosan (700,000 Da) was used either as a transparent membrane or as a freeze-dried material having a sponge-like appearance, the diameter of pores being in the range 1 - 500 microns.

The pads were obtained sterile after the chemical manipulation and lyophilization; thus the freeze-dried dressing can be packaged under sterile conditions with no further treatment. In any case, however, sterilisation was done by irradiating with 60-cobalt gamma rays (1.4 Mrad) the freeze-dried N-carboxybutyl chitosan in sealed plastic envelopes: this treatment did not affect the chemical and physical characteristics of N-carboxybutyl chitosan.

The chemical treatment of chitosan leading to water-soluble N-carboxybutyl chitosan included ultrafiltration and dialysis (membrane cut-off 100,000 Da) which permitted removal of foreign matter, contaminants, pyrogens and salts from the N-carboxybutyl chitosan solution. Such treatments also permitted to refine the average molecular weight by eliminating small polysaccharide fragments. Thus, the resulting freeze-dried wound dressing was found to be well suited for applications in vivo and, thanks to its sponge-like and expanded form, it interacted in an active manner with cells migrating from adjacent tissues into the implant, while the chemical structure of N-carboxybutyl chitosan acted as a template for the extracellular matrix reconstruction.

The macromolecules present in the extracellular matrix probably exert two main functions in the histoarchitectural organisation: they provide a medium where cells can migrate, and mediate adhesion between cells and substrate. Specific binding sites confer to those molecules the ability to interact with each other; for instance fibronectin binds to hyaluronic acid, collagen and heparin.

Substances released by platelets and macrophages stimulate the proliferation of fibroblasts, which characterises wound repair morphologically. Fibroblasts then secrete collagen that initially is immature but able to provide a structural support to fragile newly formed capillaries.

The morphological role of glycosaminoglycans

is known to lead to cellular proliferation, stromal collagen network and functional characteristics close to normal. The ordered deposition of collagen and the histoarchitectural reconstruction of cutaneous wound tissues can be modulated by N-acetylglucosamine polymers (chitin derivatives) supplied to the wound. Similarities between some modified chitins and hyaluronic acid have been underlined.

CUTANEOUS REPAIR IN PATIENTS

Patients undergoing plastic surgery were subjected to medium thickness dermo-epidermal explant. The donor site (ca. 50 cm2) was dressed with freeze-dried sterile pads of N-carboxybutyl chitosan; the control donor site on the same patient was treated with phytostimuline gauze (5).
During the healing period, the square shape of the wound was preosserved while its size decreased progressively, as a point of difference from controls where the square shape was lost soon. In no case infections were reported and complete healing occurred after 8 days (7 for controls).
Ultrastructural analysis identified fibroblasts of elongated shape, arranged according to precisely oriented parallel lines. Vascular structures were largely present, while the inflammatory cellular component was occasional. The rather loose collagen network showed a regular distribution. The general aspect of the derma had an overall histoarchitectural order more evident than in controls. The epidermis showed in general a linear junction with derma, deprived of marked offshoots. The epithelium was in general organised and cytologically normal, even though a Malpighian layer appeared less extended than in controls. The skin reached its final differentiation with superficial aspects of normal keratinisation.

HEALING OF ULCERS IN AGED PATIENTS

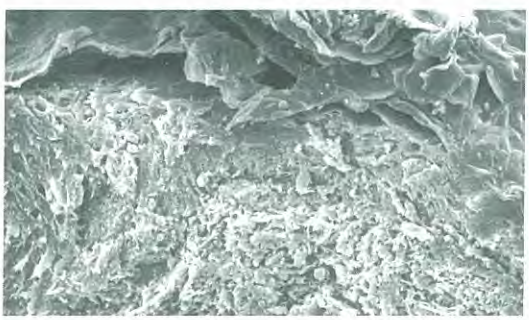
In aged skin, collagen undergoes cross-linking reactions and physico-chemical alterations; proteoglycans decrease in general and their percent ratios are altered. Vascular walls undergo thickness increase and a reduced quantity of oxygen reaches the cutaneous tissues with unfavourable consequences in case of ulcers and burns. Hypertension, oedema, atherosclerosis and diabetes further reduce the quantity of oxygen, nutrients and cells having defensive action (leukocytes and macrophages) reaching the cutaneous tissue.
10 Patients (average age 62) affected by ischemico-ulcerative lesion of the legs were studied; every patient had altmost two ulcerative lesions: the bigger one was treatedwith N-carboxybutyl-chitosan, the smaller with phytostimuline gauze. If the lesions had the same size, the one in the lower site was treated with N-carboxy butyl-chitosan, the upper one with phytostimuline gauze. Bioptic specimens were kept far to morphological study on 30° days when the lesions were all healed.
Compared to controls, a more rapid epithelialisation was remarked (7 days instead of 15-20 days). In no case infections occurred and good haemostasis was observed. From a morphological point of view, controls showed the usual disordered deposition of collagen fibers, whilst in patients treated with N-carboxybutyl chitosan a correct histoarchitecture of the regenerated skin was observed with, in particular, an ordered organisation and vascularization of the derma. In these patients, N-carboxybutyl chitosan exerted favourable effects on the partially altered metabolism presented by senescent cutaneous tissues and favoured functional recovery.

CONCLUSIONS

Sterile, pyrogen-free and pure N-carboxybutyl

chitosan is affordable via a chemical treatment of chitosan, which introduces carboxybutyl groups on some of the polymer glucosamine units. Water-solubility is achieved while preserving the cationic character that is important from several standpoints, including antimicrobial action (6).

The novel group artificially introduced into chitosan was expected to be non toxic and proved to be so. Experimental evidence indicated that a major part of the carboxybutyl groups is in the methyl pyrrolidinone form which introduces an aspect of similarity with synthetic polymers and monomers widely accepted in the pharmaceutical, medical and cosmetic field, such as poly(vinyl pirrolydone) (7,8) and pyroglutamate (9). In practice, in our study N-carboxybutyl chitosan was well tolerated by all patients.



Regenerative Aspects of Human Skin

Chitosan is an insoluble, partly crystalline substance in the form of irregular and abrasive grains. Even though it can be put into an amorpbous form, its hydrogen-bonded structure lives little chance for effective interactions with wound tissues. On tbe other hand, chitin/chitosan dressings recently developed as non-woven tissues can be used on wounds but there is no evidence of any biological action (10-14). N-Carboxybutyl chitosan, on the contrary, combines in itself desirable chemical characteristics and biological significance. By acting as a template for the extracellular matrix regeneration, it heals wounds and ulcers with little or no scar formation, leading to better functionality and histoarchitectural organisation than with other dressings.

N-carboxybutyl chitosan favoured an orderly reconstruction of dermal architecture, a rapid reconstitntion of the stromal network. This study demonstrates that it is possible to modulate the wound healing processes with the aid of biomaterials to produce replacement-like processes, rather than scar tissue formation. The biomaterial here examined took an active part in the repair processes. The biomaterial exhibited particular biological significance, suggesting different therapeutical application in relation with specific morpho-structural properties: it conld be used, as structural modulators in wound dressing.

Finally, as the application of chitin and chitosan to wounded human tissues has been experimented only in recent years, different kind of molecular modification of this polysaccharide must he performed.

Regenerative Aspects of Human Skin

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