POLYSACCHARIDES: HEALTH-ENVIRONMENT BINOMIAL

M.G. Tucci, °M. Belmonte Mattioli, G. Ricotti, °G. Biagini
Dipartimento Ricerche, I.N.R.C.A. Ancona
° Istituto di Morfologia Umana Normale, Università degli Studi di Ancona

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Summary

Polysaccharides are the main sources of biomolecules for the use as biomaterials in pharmacology and cosmetology: cellulose, alginate, pollulanes and chitin derivatives belong to this class of substances. The aim of the present study was to evaluate the properties of N-carboxybutil-chitosan as biomaterials to aid skin repair processes.
Morphological evaluations showed the ability of N-carboxybutil-chitosan to affect favourably collagen and extracellular matrix deposition, and confirmed the degradability of this molecule. Tissue repaired in presence of chitosan showed better histoarchiectural features with mature cell elements and orderly arrangement of collagen fibres than control areas.

Riassunto

I polisaccaridi sono la principale fonte di molecole per la realizzazione di biomateriali per l'industria farmaceutica e cosmetologica: cellulosa, alginati, pullulani e derivati della chitina sono i prodotti principalmente utilizzati. Lo scopo di questo studio è stata la valutazione delle proprietà del N-carboxybutil-chitosano quale biomateriale per la riparazione cutanea. Gli studi morfologici condotti hanno evidenziato come questa sostanza stimoli la deposizione di fibre collagene e di matrice extracellulare, e come tale molecola sia facilmente degradabile. Il tessuto ricostituito in presenza di N-carboxybutil-chitosan presenta caratteristiche istoarchietturali migliori rispetto al tessuto controllo con elementi cellulari più maturi e fibre collageniche ordinatamente disposte.

INTRODUCTION

Over the last few years the scope for the pharmacological and cosmetological utilisation of biomaterials - molecules of biological origin possessing specific functional and structural properties - has broadened considerably (1, 2).
Their principal characteristics are: absence or near-absence of antigenicity, inability to cause an inflammatory reaction, and a specific biological role in the organism (for instance, in the biomaterials employed in skin-tissue repair an important property is the ability to enhance stromal-cell colonisation and glycoprotein synthesis). They also need to be biodegradable and to produce no toxic metabolites. Last but not least, they must be easy to obtain and economically accessible since medicine increasingly needs to take into account cost-benefit criteria.
In fact, biomaterials possessing all these properties are still to be found, and considerable effort is being done to test new substances for the repair of lesioned skin tissue.
At present, polysaccharides are the main source of biomolecules for utilisation as biomaterials in pharmacology and cosmetology: cellulose, alginates, pollulanes and chitin derivatives belong to this class of substances. Their main structural-functional characteristic is the capacity, not shared by proteins, to form multi-ion complexes by modifying the activity of proteins (e.g. growth factors) (3). They are therefore valuable for their ability to react to, and modify, the micro-environment with which they are placed into contact.

Cellulose

This is the principal constituent of cell walls of superior plants. It is made up of glucose units condensed in linear, non-branching chains by means of beta bonds. It is insoluble to a large degree. In cell walls, cellulose molecules are arranged in parallel to form fibres. Cellulose has been used in biomedical applications for several years; in particular, it has been employed as a compound to make artificial saliva (4).

Chitin, chitosans

Chitin is a polysaccharide consisting of acetylglucosamine residues united by beta bonds and is an important component of mushroom cell walls. It has been employed as anti-cholesterol agent, in tissue-repair preparations, as anti-clotting, anti-thrombogenic and haemostatic material. and in cosmetic preparations (5). Its derivatives possess numerous biofunctional features which are listed in table I. Among chitin derivatives chitosans, which our group has been using for many years to stimulate skin cicatrisation, are especially interesting (6,7). Chitosan has been found to allow macrophage activation, to influence collagen deposition, to be easily biodegradable by the lysozyme and thus easily resorbable, and to possess bacteriostatic and bactericidal activities.
In patients with skin wound chitosan also allows healing without wound contraction and evident scar formation, it hinders secondary infections and, especially, facilitates cicatrisation.

Pollulane

Pollulane is a linear-chain regular homoglucan produced by some fungal microorganisms, mainly by Aureobasidium pullulans. Pollulane is considered a biocompatible, biodegradable "technological" polysaccharide with several possible industrial applications, included food manufacturing. Noxious effects on man and animals have not been reported despite the fact that pollulane is produced by mushrooms with phytotoxic actions. Finally, pollulane exerts an anti-oxidant action by regulating the inflammatory burst which frequently leads to the excess formation of pro-inflammatory molecules and noxious agents. The utilisation of these substances allows to obtain hydrogels which are still largely investigated.

ALGINATES

They are polysccharides characterised by the ability to transform into sol/gel phases independently of temperature based on the presence or absence of multivalent cations. This makes them ideal ingredients in preparations that promote tissue repair and, indeed, they have been used in medications for skin wounds (8). Alginates have been reported to possess haemostatic properties, but they do not particularly favour re-epithelialisation (9). They are also interesting for their marked anti-oxidising activity (10).

For many years, our group has been working on chitosans to assess their action in skin-repair processes. These studies have evidenced the remarkable properties of these substances (tables 1 and 2), which are used in odontostomatology, orthopaedics and dermatology.
The aim of the present study was to evaluate the properties of N-carboxybutyl chitosan as biomaterial to aid skin-repair processes.

PATIENTS AND METHODS

Medium-thickness dermo-epidermal grafts were collected from the front part of the left and right thighs of 5 patients, 2 women and 3 men. The donor site in the right thigh was dressed with a pad medicated with N-carboxybutyl chitosan whereas the left thigh served as control and was dressed with phytostimuline gauze.
Thighs were treated at 4-day intervals. On days 7, 10 and 30, a biopsy of both treated areas was collected from all patients. Bioptic fragments were divided into 2 portions, one for light microscopy (LM) transmission electron microscopy (TEM) study and the other for Scanning electron microscopy (SEM) observation only.

N-carboxybutyl chitosan

The material used in this study was in the form of small sterile pads measuring 10 x 20 x 0.5 cm obtained from an aqueous solution of N-carboxybutyl chitosan by dialysis and freeze-drying.
Analytical data of the material were: Mw 720,000 determined by laser light-scattering spectrometry, degree of acetylation 0.15, determined by spectrophotometry, degree of N-carboxybutylation, 0.27, determined by high-pressure chromatography, and pH of the 1% solution, 6.2.

Transmission electron microscopy

Specimens were be fixed in 2,5% glutaraldehyde, in 0,1% cacodylate buffer (pH 7.4), postfixed in 1% OsO4 in cacodylate buffer, dehydrated in increasing ethanol concentrations, and embedded in Araldite.
Semithin sections and ultrathin sections were be cut using a Reichert Ultracut E microtome, and were be stained with 2% toluidine blu. Ultrathin sections were counterstained with uranyl acetate and lead citrate and observed with a Philips CM 10 electron microscope.

Scanning electron microscopy

Specimens were fixed as described above, dehydrated in alchools, critical point dried in liquid CO2, mounted on metals stubs with a conductive glue and coated with a layer of gold to improve conductivity. The samples will be observed with a Philips 505 scanning electron microscope.

RESULTS

Control areas

At morphological analysis, the repair tissue on days 7 and 10 appeared rich in polygonal cell elements, with an evident nucleus and a fair number of inflammatory cells (figure 1). Cells (fibroblasts) and collagen fibres were irregularly

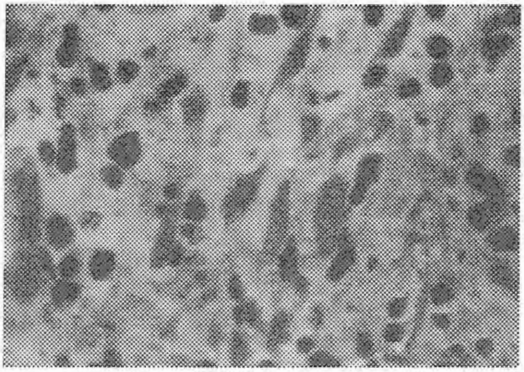

Fig.1 Control area: on day 10 the neoformed tissue exhibited a fair number of inflammatory cells (LM 40x, toluidine blue).

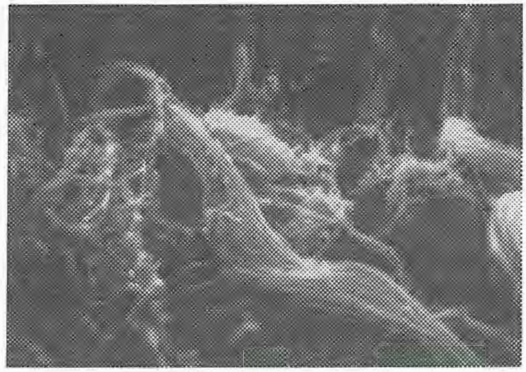

Fig.1 Control area: on day 10 bundles of irregularly-arranged collagen fibres were observed (SEM).

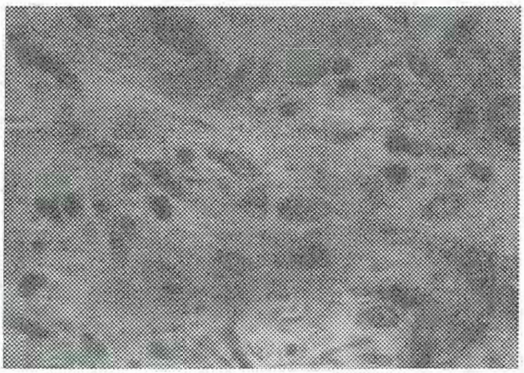

Fig.3 Repaired area treated with N-carboxybutyl chitosan. Inflammatory cells are less numerous than in the control area on 10 day (LM 40x, toluidine blue).

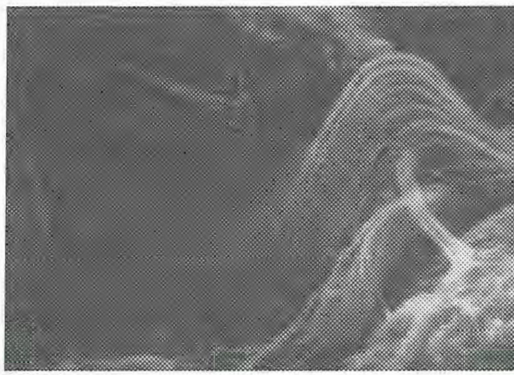

Fig.4 Repaired area treated with N-carboxybutyl chitosan. Collagen fibres intertwine to form orderly-oriented bundles (SEM).

interacting with the surrounding environment may favour the release of free radicals which damage the host tissue. By contrast, chitosans have anti-oxidising properties: their utilisation allows to regulate the inflammatory burst, which often results in excess formation of pro-inflammatory molecules and noxious agents (9), with negative consequences on the tissutal micro-environment.

The present analysis of skin-tissue repair in the presence of N-carboxybutyl chitosan confirms the properties reported for this substance (11, 12): its ability to affect favourably collagen deposition, its degradability by the lysozyme and therefore its resorbable nature, and the supply of N-acetylglucosamine to the reconstructing extracellular matrix are all reflected in the better histoarchitectural features of this tissue, with mature cell elements and an orderly arrangement of collagen fibres.

When discussing the development of new technologies or substances, it is necessary to consider their consequences not only on human Health but also on the Environment. Evaluating their impact on the latter is crucial in the light of the fundamental role attributed to the

Environment in human health.
Cellulose, which is the most common, and probably the most easily accessible and low-cost molecule, may be difficult to dispose of after some types of industrial processing. By contrast, chitosan may be recovered from the reprocessing of waste material of pharmaceutical manufactures by utilising the bacterial walls of the prokaryotes employed for antibiotic synthesis, or be obtained from mushroom cultivations.
Also in the pharmaceutical and the cosmetological fields, the Health-Environment binomial is fundamentally important. The utilisation of new or innovative biomaterials in products should be conditioned by the availability of materials and mode of disposal, avoiding the substances whose use or degradation produce adverse environmental effects.
Polysaccharides possess all the necessary features to be considered first-choice materials in this sense. Their ability to be utilised to make other biopolymers, the capacity to modulate cell responses to tissue-environmental stimulation and their potential for environment-friendly conversion make polysaccharides valuable molecules also in terms of the preservation of Health and the Environment.

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Author Address:
Prof. Graziella Biagini
Istituto di Morfologia Umana Normale
Facoltà di Medicina e Chirurgia
Via Tronto n.10/A.
Torrette, 60020 Ancona