BIBLIO

KITO-KIT®

New biologically active dressing in the form of a sterile analgesic and healing gel, with no preservatives, no additives, no perfumes, indicated for the treatment of all types of wounds and burns.

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Summary

Scientific and bibliograhic summary

Chitosan is a subject of many scientific publications.

All of them highlighted and proved the multitude of chitosan proprieties. In this scientific and bibliographic summary, we have tried to present the most relevant publications to demonstrate that chitosan is unique and valuable for what it allows. In addition to its fantastic qualities, as biocompatibility and biodegradability, Chitosan has also an analgesic, healing and bacteriostatic power unlike any other.

A product that is benefic for the three stages of healing

No dressing works actively on healing phenomenon so far. Modern dressings are useful only on the last stage of healing. Therefore, they keep humidity, allow gas exchange, give a thermal and mechanic isolation and form a bacteriologic barrier.
Kito-kit® has all previous properties and the ones mentioned below:

1 | Hemostatic phase

• Hemostatis is done by an attraction of the blood cells negatively charged that, in contact with Chitosan charged positively, entails the formation of a clot and then the bleeding stops.

• Activity on the nerves endings by inducing a full sedation.

• Absorbs fluid associated with inflammation.

• Intrinsic antimicrobial and antifongal activity.

2 | Detersivo-Inflammatory phase

• Chitosan stimulates the migration of PMN as well as mononuclear, and accelerates the re- epithelialization and normal skin regeneration.

• Activates macrophages

• Chemo-attractors for the neutrophiles

• Activates platelets and inflammatory leucocytes

3 | Proliferative phase

• Helps to obtain an important granulation tissue formation by increasing the angiogenesis.

• Induces the formation of fibroblasts.

• Limits the secretion of collagen type 1 responsible of hypertrophic scars and Keloids while increasing the production of collagen 3 useful in the reconstruction of fabrics.

• Maintain properly the regulation of the water exchange and reduce amount of water loss.

• Forms a permeable film to air.

Chitosan properties

CHITOSAN PROPERTIES	STUDY PROTOCOL	RESULTS	MECHANISM	REFERENCE OF THE ARTICLE
Analgesic effect	Chitin/chitosan suspensions at concentrations of 1, 5, 10mg/ml were mixed with same volume of 0.5% acid acetic (AC). Each mixture was administered to the mice intraperitoneally at a volume of 25 ml/g body weight. For the control, the mixture of saline and 0.5% AC was injected.	The writhing time was decreased signicantly in the 5 and 10 mg chitin-AC groups, and in all of the chitosan-AC groups. The writhing depression rates of 5 and 10 mg chitin-AC were similar to those of 1 and 5 mg chitosan-AC, respectively.	Chitin and chitosan have been found to reduce the inflammatory pain due to intraperitoneal administration of acetic acid. This effect was also been shown to be dose dependent. In a preliminary study, AC was found to induce pain. Which indicate that the writhing is influenced greatly by the proton ions released from the AC, i.e. the pH of the AC. When the chitosan suspension was mixed with AC, the chitosan particles combined chemically with the proton ions in the AC solution, the amino group in the position of C2 changed into NH3+, and subsequently the particles resolved in the solution.	Analgesic effects of chitin and chitosan Y. Okamotoa,*, K. Kawakamia, K. Miyatakea, M. Morimotob, Y. Shigemasab, S. Minamia 680-8552, Japan Received 14 April 2001; revised 28 July 2001; accepted 1 August 2001
Hypertrophy scar fibroblast (HSF) and keloids fibroblast (KF) prevention	HSF and KF as well as normal dermal fibroblast (NDF) were cultured in the presence or absence of indicated dosage of chitosan, ranging from 20 to 320 µg/mL for 72 hours with MTT assay.	320µg/mL chitosan almost reduced the proliferation of HSF to 70% of the control. The proliferation of KF was reduced by almost 40% when treated with chitosan at 320 µg/mL.	HTS and KD result from increased of fibroblast density and an overabundance of dermal collagen. Scar Fibroblasts express TGF-β and b-FGF whose lead to significant alteration in composition of extracellular matrix. The concept that a transition of the normal fibroblast phenotype to KD and HTS phenotype is essential for the formation of these 2 type of scars. Consistently, our data further revealed that chitosan showed effective inhibitory capacity for the proliferation of HSF and KF and their production of TGF-β, b-FGF and type I and III procollagen.	The systematic effect of chitosan on fibroblasts derived from hypertrophic scars and keloids Lv C, Dai H, Xing X, Zhang J. Dermatol Venereol Leprol 2012;78:520 Received: Novembe r,2011Received:Nov ember,2011 Accepted: April, 2012.

Chitosan properties

CHITOSAN PROPERTIES	STUDY PROTOCOL	RESULTS	MECHANISM	REFERENCE OF THE ARTICLE
Antibacterial effect	Under an asepsis operation condition, the bacterium suspending liquid were evenly spread on LB solid state medium, then putting the chitosan on that medium after 30 min. Let all samples culture at 37 °C for 24 h. Finally the antibacterial circles were observed.	These results indicated that chitosan play an antibacterial role to the growth of on E. coli K88 and Streptococcus. Its antibacterial properties to Streptococcus by vacuum dryness for 10 min are better than that of cefradine and has better antibacterial effect on E. coli K88 than that of penicillin.	All the bacteria comprise negative charge under approximately neutral circumstance. that it is easy to combine with chitosan which are positive ion protonated. Then the electriferous state of protein in bacteria is transformed, the physiological function of the bacteria is disturbed, it is difficulty for bacteria to reproduce and the bacterial activity is finally restrained. E. coli K88 belongs to gram-negative bacterium, and Streptococcus belongs to gram-positive bacterium. Therefore CGSWD has good antibacterial effect on E. coli K88 and Streptococcus in the course of experiment,	Biological properties of the chitosan-gelatin sponge wound dressing. Chun-Mei Deng, Lan-Zhen He a, Ming Zhao b, Dan Yang a, Yi Liu a A Science Institute, Guangdong Ocean University, Zhanjiang 524088, PR China Affiliated Hospital of Guangdong Medical College, Zhanjiang 524001, PR China Received 9 October 2006; accepted 18 January 2007 Available online 27 January 2007
Promoting the wound healing	6 rabbits were divided into three groups. The first group of that were treated with ethacridine at a does, the second group coated with CGSWD, the third group treated with nothing coated with vaseline aseptic sterile gauze. Every wound was banded up with asepsis gauze and renewed medicines every other day as well as observed.	• Blank: Three twelfths wounds were infected. The scab skins naturally fell off, and scars formed were concave below the skin surface • Ethacridine: All of scabs cicatrized. The scab skins naturally fell off, the scars projected over the skin surface evidently • Chitosan: All of scabs cicatrized. The scab skins naturally fell off, the scars projected over the skin surface inconspicuously	From SEM ultra- micrographs, the collagen fasciculi of the new skin tissue treated with chitosan is arranged more naturally. The interspaces among collagen fasciculi are more even, and the thickness of its new skin is the same as that of the skin without wounding. chitosan has the same pharmacological function as ethacridine. All of the scabs also cicatrize. In addition, the new skin growth treated with the CGSWD is superior to that treated with ethacridine.	Idem

Chitosan properties

CHITOSAN PROPERTIES	STUDY PROTOCOL	RESULTS	MECHANISM	REFERENCE OF THE ARTICLE
Toxicological evaluations	Every animal is observed in succession at 4, 24, 48 and 72 h after being injected.	No toxic symptom No pyrogen reaction No irritation on skin No Eye conjunctiva irritation Hemolysis rate of 1.23% is lesser than the criterion of 5%, which shows that chitosan does not have hemolysis function.	A series of toxicological evaluations of chitosan were performed. All of evaluation results conform to the demands in relative criterion. That proves chitosan is safe reliability as a surgical wound dressing.	Idem

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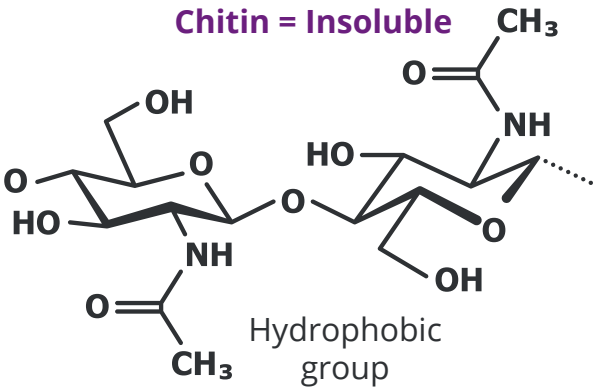
I. Chitosan raw material

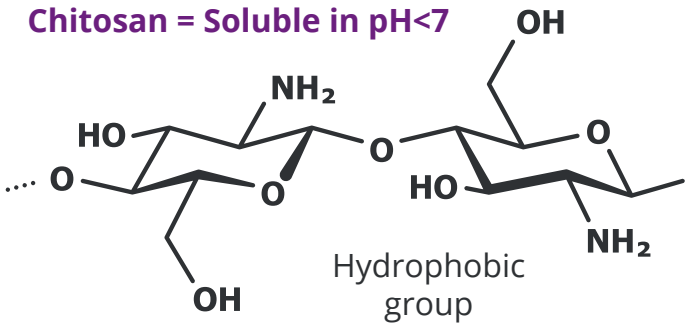
1 | Chemical knowledges on Chitin / Chitosan

Several works on this raw material has been performed such as those from Shahidi and Abuzaytoun from the departments of biochemistry and biology of the university of Newfoundland (Canada) : Chitin is derived from the greek word chiton, which means a coat of nail. It is the major component of the exosqueleton of invertebrates, crustaceans, insects, and the cell wall of fungi and yeast , in which chitin acts as a supportive and protective component. Chitin is the second most plentiful natural polymer on earth after cellulose. At least, 10 gigatons (1x1013 kg) of chitin is produced and hydrolyzed each year in the biosphere (N°92).

The journal of environmental science and health published in 2002 that the production of chitosan in nature has been estimated at a level of up to 109/1010 tones per year (N°10).

Chitin, poly-(164)-N-acetyl-D-glucosamine, is a cellulose like biopolymer found in a wide range of products in nature.





Chitosan, a copolymer of D-glucosamine and N-acetyl-D-glucosamine with beta-(164) linkage, is obtained by alkaline or enzymatic de-acetylation of chitin and is an abundant polymeric product in nature (N°92).
Chitosan is a polycationic substance that exhibits various promising biological activities which have made the polysaccharide increasingly important (N°10).

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French report from the CNRS assume that the chitosan chemical structure mimics the glycosaminoglycans constituting an important part of the extracellular matrixes of mammalian living tissues and then must be regarded as a decoy for biological media (N°105).

Chitosan has many useful applications in different fields mainly because of the presence of amino group: the presence of positively charged amino group repeatedly placed along the chitosan polymer chain, allows the molecule to bind the negatively charged surfaces via ionic or hydrogen bonding (Muzarelli 1973) (N°92).

The application research has largely been focused on chitosan because the 3 amino groups in this modified product contribute polycationic chelating and film forming properties (N°21).

A Japanese team from the department of veterinary evaluated chitin compared to chitosan. Those studies have been performed on wound healing with reference to chemical properties using a linear incisional wound model in rats. Wound break strength of the chitosan group was higher than the chitin group. In histological findings, many activated fibroblasts were observed around the wound in the chitosan group (N°14) Therefore, Chitosan has been selected.

2 | Formulation rational

A | Rational of the choice of chitosan:

Works from the department of chemistry, in Singapore, guess that Chitosan has been the better researched version of the biopolymer because of its ready solubility in dilute acids rendering chitosan more accessible for utilization and chemical reaction.
Thus, the production, chemistry and applications of chitosan are well known (N°5).

Studies of the effects of chitine/chitosan on wound healing with reference to chemical properties have been performed using a linear incisional wound model in rats ; it shows that when the chitin and chitosan groups were compared, the chitosan group was higher than the chitin group in each molecular size : in histological findings, more activated fibroblasts were observed around the wound in the chitosan group than in the chitin group, collagenase activity was also higher in the chitosan group (N°14).

B | Rational of the sterilization of the medical device:

Concerning the Sterilization issues: chitosan intended for parenteral administration have to be sterilized before use; common methods for their sterilization of pharmaceutical and medical include saturated steam (N°5), Rao and Sharma recommend steam autoclaving.

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C | Rational of the molecular weight chosen:
Studies from the department of pharmacy (Singapore), showed that the low molecular weight chitosan reacted more completely than chitosan of higher molecular weight (N°7).

The faculty of engineering, science and medicine (Thailand) studied in vitro culture of mouse connective tissue fibroblast and guess that Chitosan has the potential to produce cell scaffolds with biocompatible properties; they evidenced that low molecular-weight chitosan was more effective to promote and accelerate cell proliferation. The results elucidated that the blend with low-molecular-weight chitosan has a high potential to be applied as new materials for skin-tissue engineering (N°96).

D | Rational of the chemical modification of chitosan:
The University of pharmaceutical science (Sao-Paulo, Brasil) studied the synthesis and physicochemical characterization of chemically modified chitosan by succinic anhydride; it showed that the succinic anhydride attached to the free amino groups presented along the chitosanÊs polymer chain imparts to the molecule different physicochemical properties not exhibited before the modification. Non-modified free chitosan is soluble only in acidic medium (pH<5,5). These chemical modifications enhance chitosanÊs solubility in slightly acid, neutral and alkaline media; these modifications made possible new applications of chitosan in biotechnological area since the solubility in neutral or slightly alkaline solutions is very important in a biological field (N°59).

E | Rational of the high degree of de-acetylation chosen:
The department of chemistry (India) published a review of chitin and chitosan applications in which they mentioned that Chitosan is the N-deacetylated derivative of chitine although this de-acetylation is almost never complete, chitosan is the universally accepted non-toxic N-deacetylated derivative of chitine; chitosan is the fully or partially N-deacetylated derivative of chitine with a typical degree of acetylation of less than 0,35 (N°4).

The University of science (Malaysia) reported the influence of the degree of de-acetylation of chitosan: Chitosan is commercially available from a number of suppliers in various grades of purity, molecular weight and degree of de-acetylation. It was reported that the degree of de-acetylation is one of the most important chemical characteristics, which could influence the performance of chitosan in many of its applications. The degree of de-acetylation determines the content of free amino groups in the polysaccharide (N°85).

In accordance with those results, the school of biochemistry (Oslo, Norway) founded that Higher de-acetylated chitosan inhibits contraction more strongly because of higher net positively charges; higher de-acetylated chitosan are more biologically active than chitine, it may be useful as potential anti-scarring agents and wound healing therapies

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(N°2).

It was found in a review of chitosan chemisty (University of Newfoundland, Canada) that chitosan with a high level of de-acetylation strongly motivated fibroblast proliferation, meanwhile samples with lower dergrees of deacetylation showed less activity; thus, as the degree of deacetylation increased, its antimicrobial effect on bacteria increase (N°92).

According to that, a study of chitosan as an antimicrobial agent, showed that Highly de-acetylated chitosans are more antimicrobial than those with a higher proportion of acetylated amino groups, due to increased solubility and higher charge density (N°8).

Studies of the effects of chitine/chitosan on wound healing performed in the faculty of engineering (Japan), refer to chemical properties using a linear incisional wound model in rats: the higher the de-acetylation degree becomes, the more the stronger the break strength becomes. Also, activated fibroblasts appeared more in the higher de-acetylation degree (N°14).

Thanks to an investigation at the institute of Technology (Taiwan) of the antimicrobial properties of chitosan, in the solid and liquid culture against bacteria, it has been proved that the higher de-acetylation degree and the higher concentration of chitosan generally have higher antibacterial activity (N°10).

F | Rational of the hydrogel form:

It's well known that the physicochemical properties of the hydrogel depend not only on the molecular structure and the degree of crosslinking, but also on the content and state of the water in the hydrogel (N°4).

We can find in a review of research and development works into medical dressings based on chitosan (Poland) that potential applications of chitosan in medecine can only be exploited if its usable forms are properly developed and prepared:

- In solution and gel it can be used as a bacteriostatic, fungistatic and coating agent
- Films and membranes are used in dialysis, contact lenses
- Chitosan sponges are used in dressings
- Chitosan fibers are used as resorbable sutures (N°56)

Hydrogels know a great interest for the diversity of their properties. Their capacity at absorbing water, make them a biocompatible decoy at a larger scale and opens new trends for the research on biomaterials (N°105).

In accordance with the evaluation of chitosan hydrogel for skin regeneration following third-degree burn (France), the physical hydrogel is adapted to the geometry of the wound: it perfectly covers the defect surface. We may consider that a physical hydrogel

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or a viscous solution of chitosan promoted the reconstruction of an elastic skin ,before the end of the first year after burning (N°62).

The study of a chitosan hydrogel, in the department of biomedical engineering (Japan) assume that chitosan, having a hydrogel forming property, is considered to be more advantageous in their application as adhesive material; the application of chitosan hydrogels may effectively interact with and protect the wound, ensuring a good moist healing environment (N°78).

g/ Rational of the vegetal source in addition to the animal source of chitosan :
Chitin is easily obtained from crab or shrimp shells and fungal mycelia. In the first case, chitin production is associated with food industries such as shrimp canning (N°4).

Studies were instituted to promote chitine from crab and shrimp shells as a marine resource and to alleviate a growing waste disposal problem in the shellfish industry (N°21). In the second case the production of chitosan-glucan complexes is associated with fermentation processes from aspergillus niger, mucor rouxxi and streptomyces, which involve alkali treatment yielding chitosan-glucan complexes. The alkali removes the protein and deacetylates chitin simultaneously. Chitin and chitosan are now produced commercially in India, Japan, Poland, Norway and Australia (N°4).

Fungal mycelial can become free and rich alternative source of chitin and chitosan beside the traditional industrial source (shellfish waste material) (N°100).

Furthermore, studies on chitosan obtained with fungal treatment show the same properties (N°90).

Advances in fermentation technology suggest that the cultivation of selected fungi can provide an alternative source of chitosan (N°79).

In addition, the chitin of fungi possesses principally the same structure as the chitin occurring in other organisms (from animal source) (N°80).

A United States patent relates to high yield production of chitosan or chitin from fungal cultures belonging to the family Mucoraceae and isolating chitosan or chitin from the culture; procedures for culturing fungi are well known in the art. Chitosan can be isolated and purified from fungal mycelia by standard methods (N°25).

About the production of chitosan by fungi: there are several attributes that make fungi a very amenable source for chitosan production. The extraction process from fungi is simple and cheap; in addition fungi can be grown easily on any simple medium or industrial (N°76).

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The faculty of chemistry (Osaka, Japan) is working on fungal chitosan and its application in medical sector enters to 12 years. In this research, fungal mycelia production and enzymatic chitosan extraction method have been developed to obtain high yield fungal chitosan in very easy way. The production of chitosan by fungus in a bioreactor at a technical scale offers additional opportunities to obtain identical material throughout the year and to obtain chitosans with a radioactive label, free of heavy metal contents such as nickel or copper. Fungal chitosan showed as an excellent growth stimulator and as an excellent scaffolding material to construct a biodegradable tissue regeneration template (N°51).

Studies of chitin content of cultivated mushrooms from the University of Veterinary Science (Hungary) showed that the chitin of cultivated mushrooms is a stable chemical component, and seems to be independent of the cultivars (varieties) (N°52).

An American patent published in 2001 is directed to chitosan recovered from microbial biomass, in particular fungal biomass, including yeast and filamentous fungi. The invention is also directed, in part, to improved methods of producing chitosan from chitin-containing biomass. The chitosan produced by the improved methods shows desirable properties which make the chitosan well suited for various applications. These properties include, in certain implementations, favorable levels of de-acetylation, viscosity, color and ash (N °83).

Study of the biocompatibility of chitosan obtained by fungal and their effects have been performed in the school of Pharmacy of Manchester (United Kingdom) and It has been reported that chitosan from microfungal culture could affect the rate of proliferation of human fibroblasts in culture; in the context of its possible use in the promotion of wound healing, during which appropriately controlled fibroblast proliferation is essential, fungal chitosan has been investigated. The results of this study may aid the selection of fungal chitosan as a potential wound management material (N°89).

II. Well known chitosan: safety and tolerance

The safety of chitosan for oral and topical application has been established in a number of animal and human studies (N°65).

The department of experimental surgery and biomaterial investigation of the Wrooclaw Medical academy (Poland), presented examples of well-known uses of chitosan in medical dressings ; those examples showed no cytotoxic nor intracutaneous irritating action detected and indicate it stimulates fibroblast division and accelerates wound healing (N°56).

A review of chitosan applications in the department of chemistry, University of Roorkee

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(India), reported that its immunogenicity is exceptionally low. Chitin and chitosan have excellent properties such as biocompatibility, biodegradability, non-toxicity, adsorption properties, etc. (N°4).

Included in the implantable applications of chitosan reviewed at the National University of Singapore, is the following question: why research on chitosan as an implantable material?

• First because such „natural‰ materials have been shown to better promote healing at a faster rate and exhibit greater compatibility with humans
• Second : new concepts in implantable medical require „temporal‰ features dictating the biomaterial to be biodegradable into non toxic by-products (N°5)

In the study of the new facets of research regarding chitine, the scientific team assumes that Chitosan promotes wound healing activity and is non allergenic and absorbable (N°21). Moreover, the study of the therapeutic potential of chitosan, in the University of Chongking (China), evidences that chitosan is found to be a natural-based, non toxic, biocompatible and biodegradable polymer with antimicrobial activity (N°23).

Studies in vitro, investigating the effects of chitosan (Korea University), assume that Chitosan has many useful biological properties such as biocompatibility, biodegradability , non toxicity and bioactivity (N°39). Chitosan has been used as a safe excipient in drug formulations over the last 2 decades; clinical tests do not report any inflammatory or allergenic reactions following implantation, injection, topical application or ingestion in the human body (N°72).

Furthermore, bandages made of chitosan , approved by the FDA in the USA, have been widely used during the Iraqi war on soldiers severe injuries; they call it „shrimp‰ bandages that contain chitosan. There was no sign of allergenicity in soldiers who were allergic to schrimp (Mientka, 2003) . Thus, chitosan received the „generally recognized as safe‰ (GRAS) by the FDA in the USA. Rao and Sharma (1997) reported no toxicity for 2% chitosan solution in acetic acid, when applied on punctured bleeding capillaries in several types of animal models (N°92).

An ocular irritation test on rabbits (school of Pharmacy, Geneva) clearly demonstrated the excellent tolerance of chitosan after topical administration onto the corneal surface (N°3).

III. Chitosan: Evidences of the bioactivity

1 | Request of a wound

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In the year 2001 the global wound care market had an annual growth rate of 15%. Restoration of tissue continuity after injury is a natural phenomenon, infection, quality of healing, fluid loss and other complications that enhance the healing time represents a major clinical challenge (N°67).

Thus, there is an increasing need to develop new biodegradable materials to be used in tissue engineering and a high demand for skin repair treatment (N°63).

Wound healing is a complex process that can be compromised by a number of factors. Although with proper care, some wounds fail to heal in an appropriate fashion and may become chronic. From the different studies reported in literature, chitosan seems to be an excellent candidate dressing material for the wound healing applications (N° 67).

In addition to the wound healing acceleration, the complementary request of a wound are mainly :
• Maintain a moist environment at the wound interface
• Provide bacterial protection (N°63): Infections that develop in traumatic and surgical wounds remain a major problem despite decades of advances in antibiotics and antiseptics, there is therefore an increasing need for topical antimicrobial products that can be applied to potentially contaminated wounds (N°24)
• Allow gaseous and fluid exchange
• Be non toxic, non allergenic and non sensibilizing
• Sterile (N°63)

The review of chitosan dressings currently used, from the Institute of Medical science (India), reports that Chitosan possesses biological activities and affects macrophage function that helps in faster wound healing. The biological properties including bacteriostatic and fungistic properties are particularly useful for wound treatment (N°67). Furthermore, several US patents on chitosan dressings describe the ability of chitosan to promote the healing of wounds, and that it produces a significant improvement in wound healing strength (N°6 ,N°93).

2 | Tissue engineering and wound healing evidence

Numerous in vitro studies, animal studies, as well as clinical studies evidence the bioactivity of chitosan in the field of wound healing and tissue engineering. According to in vitro studies from the polymer department (Egypt), chitosan is a biopolymer that has been well known as being able to accelerate the wound healing (N°63).

A | In vitro demonstration and understanding of the mechanism:
Pletora studies in vitro have been performed the last 3 decades, all of them proved the

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fantastic bioactivity of chitosan, non exhaustive examples are showed below.

In vitro culture of mouse connective tissue, performed in the department of chemical engineering (Thailand and Japan), one of the most interesting effect of chitosan on wound healing is the formation of granulation tissue with angiogenesis. It was reported that chitosan induces fibroblasts to release interleukine, which is involved in migration and proliferation of fibroblasts. In experimental animal models, chitosan was shown to influence all stages of wound repair.

Indeed, wound healing may be divided into 4 continuous phases:
• Haemostasis
• Inflammation
• Proliferation and maturation
• Remodeling

In the inflammatory phase chitosan has unique hemostatic properties; in addition, chitosan can be served as GAG analog in stimulating cellular processes of dermal tissue regeneration. Those studies provide some evidence that chitosan can be used to accelerate wound healing (N°96). Studies of the Institute of biopolymers (Poland), report that thanks to their specific biological properties like haemostatic, granulation, etc. chitosan based dressings are suitable for wounds during the various healing phases (N°61).

The school of biochemistry and molecular biology evidences that the effect of chitin and partially de-acetylated derivatives chitosan on the human dermal fibroblast mediated contraction of collagen lattice examinated in vitro as a model for the contraction of cutaneous wounds in vivo. This work indicates that highly de-acetylated chitosan inhibits fibroblast-mediated contraction of collagen lattices and may therefore be useful as a therapeutic agent to reduce contraction and therefore scarring in wound healing in vivo. Chitosan has shown considerable potential as a therapeutic agent for wound Healing. Chitosan has also been shown to bind collagen (forming a ionic complex) and to protect it from digestion by collagenase ; therefore chitosan could reduce scarring following wounding (N°2). The increasing interest in chitosan is caused by its biological activity resulting from its susceptibility to degradation under the influence of enzymes present in body fluids such as lysozyme and N-acetylglucosaminedases. The degradation products, being chito-oligomeres, are able to stimulate macrophages and positively influence collagen sedimentation, thus accelerating the wound healing process (N°56).

The polymer material research department (India) reported that chitosan stimulates the migration of PMN as well as mononuclear, and accelerate the re-epithelialization and normal skin regeneration (N°63).

In addition, the chemistry department (India) reviewing applications of chitosan, reported that chitosan may be used to inhibite fibroplasia in wound healing and promote

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tissue growth and differentiation in tissue culture (N°4).

Effect of chitosan on proliferation secretion of normal and keloid skin fibroblasts has been performed in different universities (Korea, China) and they concluded that Chitosan can accelerate wound Healing and reduce scar formation; it inhibits the proliferation of keloid fibroblasts and promoted the proliferation of normal skin fibroblasts . Chitosan has no influence on the collagen secretion in normal skin fibroblasts, but inhibits the secretion of type I collagen of keloid fibroblasts in the cell culture. Keloid fibroblasts selectively increase the biosynthesis of type I collagen. Chitosan has an activity on the regulation of the ratio of collagen I/IV in the metabolism of keloid fibroblasts by inhibiting the secretion of collagen I.
Definitely, chitosan promotes the proliferation of normal skin fibroblast and inhibites the proliferation of keloid fibroblast so it has a great potential to the used in functional wound Healing Accelerator (N°39).

The department of biomedical engineering (USA), reviewed applications of chitosan-based biomaterial in cartilage tissue engineering, it shows that chitosan could accelerate wound Healing by enhancing the functions of inflammation cells and repairing cells ; they assume that chitosan is a novel scaffold material for tissue engineering (N°11).

Moreover, researches of the potential use of chitosan as a cell scaffold for cartilage tissue engineering, performed and presented at the 47th annual meeting of the orthopedic society (USA) describe that the incorporation of Chitosan into a collagen scaffold is known to increase a mechanical strength; Chitosan has been attempted as a scaffold for cartilage repair because it facilitates the formation of cartilaginous tissue by creating an appropriate environment for the generation of an engineered cartilage construction (N°38). Adjoining those studies, a Japan team form the department of orthopedic surgery, reports that Chitosan has a great potential as a desirable biomaterial in cartilage tissue scaffolds (N°45).

B | In vivo evidence and mechanism

The animal model has been widely used in order to investigate all the potential of chitosan, some of them are shown below.

From the results of the department of clinical veterinary medicine (Japan) clinical and in vivo studies, each stage of the wound healing is enhanced by administration of chitin and its derivatives to a wound; furthermore, effects of chitin and its derivatives were clearly demonstrated, namely, they activate platelets, inflammatory leucocytes, fibroblasts and vascular endothelium. They demonstrate direct effect of chitin and its derivatives on proliferating of normal human keratinocytes.

It is well known that chitosan quickly regenerates granulation tissues in the wound, however, excessive granulating tissue prevents skin regeneration. From the present

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results, clinical evidence on granulating tissue and skin regeneration by chitosan or chitin administration on wounded surface was clearly recognized (N°101).

A large review of chitosan applications and health effects, written by the department of biochemistry (Canada), report that chitosan has been tested to have both material and biological properties that might be beneficial to enhance wound repair; in addition, Chitosan has a great influence on the different stages of wound healing in experimental animal models; it interacts with and modulates the migration behavior of neutrophils and macrophagus, modifying subsequent repair process such as fibroplastica and re-epithelialization. The procedure for promoting wound healing by chitosan has been studied, and it was reported that the wound recovering material composed of chitosan speeded up wound healing and afforded a good-looking skin surface (N° 92).

Potential application of chitosan in veterinary medicine has been reviewed by, notably, the department of Pharmaceutical technology (Turkey) they report that mechanisms of acceleration of wound healing have been investigated in vitro and in vivo by several groups, they found these polymers to accelerate wound healing, decrease treatment frequency, and give comfortable and painless wound surface protection.. In addition to accelerating wound healing, chitosan is also capable of activating host defenses to prevent infection (N°72).

The department of veterinary medicine (Japan) studied the effects of chitine/chitosan on wound healing, using a linear incisional wound model in rats. They measured break strength of the wound and collagenase activity in the tissue as an indicator of wound healing. The results indicated that chitosan enhanced and increased wound healing acceleration. Chitosan was found to enhance collagenase activity which is related to the remodeling in the wound healing process, this enzyme is produced mainly by fibroblasts and inflammatory cells; moreover, many clinical studies proved that scar formation does not occur at the wound site in the presence of chitosan (N°14).

The study of the preparation and in vivo evaluation of the wound healing properties of chitosan microsphere, indicate that Chitosan is a biopolymer which can be considered for its potentially useful properties in the pharmaceutical field. The results obtained showed Chitosan particules present good wound healing properties, thus leading to rapid cicatrization (N°70).

The International Islamic University (Malaysia) investigated chitosan films as dressing for punch biopsy wounds in rats, the results suggested that chitosan film treatment might have beneficial influence on the various phases of wound healing such as fibroplasia, collagen synthesis and contraction resulting in faster healing, in conclusion, chitosan is able to promote wound healing with minimal scar formation (N°17).

The research institute, division of biomedical Engineering (Japan), studied the action of

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a chitosan hydrogel as a wound dressing. They reviewed many developments that have enabled the use of chitosan in the medical and veterinary fields especially in the field of skin wound management.

Chitosan has many useful and advantageous biological properties in their application as a wound dressing namely:

- Biocompatibility
- Biodegradability
- Haemostatic activity
- Anti-infectional activity
- And the property to accelerate wound healing

They have been many studies which have demonstrated that chitosan accelerated wound healing and remedies using chitosan for treatment of wounds have already been marketed. Their in vitro studies demonstrated that the chitosan hydrogel has a chemo-attractive ability and stimulates the migration and proliferation of dermal fibroblasts cells. Those studies demonstrated that the chitosan hydrogel has a chemo attractive ability and stimulates the migration and proliferation of dermal fibroblast cells.

The positively charged chitosan molecules adsorb some substances involved in cell proliferation and migration, such as growth factors and cytokines from blood plasma or exudate in the wound; and that the adsorbed substances stimulate the cell proliferation and migration.

As a result, the chitosan hydrogel may be a promising new dressing for wounds occlusion and as a tissue adhesive, especially suitable in situations requiring urgent hemostasis (N°78).

The use of chitosan hydrogels for skin regeneration following third degree burns on mini pigs has been investigated by the CNRS (France), the aim of the study was to assess whether this material was totally accepted by the host organism and allowed in vivo skin reconstruction of limited area third-degree burns. All the results showed that chitosan materials were well tolerated and promoted a good tissue regeneration. From the beginning of the granulation chitosan favours inflammatory cells migration and angiogenesis. With the physical hydrogel the precious presence of type I and IV collagen fibers suggests a great maturity of the dermis and thus, an advanced healing with the dermal-epidermal junction reconstruction occurring from the edges of the wound.

This reconstruction occurs uniformly from the healthy tissue, perpendicular to the surface and on the whole area of the wound. The physical hydrogel and the viscous solution allow the rapid remodeling of the extracellular matrix becoming similar to the native dermis, especially by its aesthetic aspect and its great flexibility (N°62).

Bibliographic argumentation

In accordance with a review on chitosan dressings written by the Institute for Medical sciences and technology (India), treatment with chitosan demonstrated a substantial decrease in therapeutic time with minimum scar formation on various animals.

It showed that chitosan facilitated rapid wound re-epithelialization and the regeneration of nerves within a vascular dermis. Chitosan provides a non protein matrix for three dimension tissue growth and activates macrophagus for tumoricidal activity.

It stimulates cell proliferation and histo-architectural tissue organization.

Chitosan is a hemostat which helps in natural blood clotting and blocks nerve endings reducing pain.

In brief, chitosan wound dressing:
• Minimize scarring
• Provide scaffold for cell growth
• Forms barrier against infection
• Encourage natural blood clotting
• Block nerve endings to reduce pain
• Absorbs fluids from inflammation
• Strengthen new tissue (N°67)

The accelerating effects of chitosan for healing have been experimented in open wound in dogs in the laboratory of veterinary surgery (Japan) The efficacy of chitosan for wound healing were concluded as follows:

• Acceleration of the infiltration of the polymorphonuclear leucocytes into the wound area
• Activates the migration of the fibroblasts into the wound area
• Stimulation of the migration of the macrophagus
• Stimulation of proliferation of fibrablasts and production of type III collagen (N°9)

The study of the effects of chitosan on collagen synthesis in wound healing on rats in the department of veterinary surgery (Japan) put in evidence that chitosan accelerates wound healing. Chitosan most notable property is its propensity to induce abundant granulation tissue formation with angiogenesis. Chitosan induces stable fine collagen fibers synthesis in the early wound healing process (N°13).

The laboratory of veterinary surgery (Japan) researched the mechanism of wound healing with chitosan in vivo and in vitro and had interesting findings: Chitosan activates immunocytes and inflammatory cells such as:

• Polymorphonuclear

Bibliographic argumentation

• Macrophagus
• Fibroblasts
• Angioendothelial cells

Chitosan:
• Accelerates infiltration of inflammatory cells
• Pronounces more granulation
• Activates the macrophagus
• Accelerates the migration of PMN
• Accelerates the production of osteopontin by PMN (osteopontin OPN plays a role in granulomatous inflammation like other inflammatory cytokines) (N°41)

In addition, a study of the control of wound infections using a chitosan-based wound dressing has been performed in the division of applied chemistry (China) the publication reflects that chitosan is well known for accelerating the healing of wounds in humans.

Chitosan is done to stimulate the migration of PMN and mononuclear cells and accelerate the re- epithelialization and regeneration of normal skin.

It has been observed that after treatment with chitosan, wounds heal without excessive granulation tissue and scar formation. Chitosan was found to promote the attachment and growth of fibroblasts (N°33).

Otherwise, a collective review on carboxymethyl chitosan and its application (India, Japan and China) shows that chitosan used in tissue engineering serves as a synthetic extracellular matrix that provides signals to the cells and guides new tissue growth.

Topical application of chitosan markedly diminishes post operative fibrosis and adhesion formation without untoward cardiac side effects.

Chitosan may mediate the inflammatory response to tissue injury.

The direct use of chitosan in vitro promoted wound healing. The actions involved are the significant enhancement in proliferation of the normal skin fibroblast but inhibition of proliferation of keloid fibroblast and decrease in the secretion of collagen type I (N°54).

The mechanism has been evidenced by the department of pharmaceutical technology (Turkey): Once placed on the wound, chitosan adhere to fibroblasts and favor the proliferation of keratinocytes and thereby epidermal regeneration.

The chitosan films are very good biomaterial for superficial wound healing.

Bibliographic argumentation

Mechanism of acceleration of wound healing have been investigated in vitro and in vivo by several groups; they found chitosan to:
• Accelerate wound healing
• Decrease therapeutic frequency
• Give comfortable and painless
• Give wound surface protection

Chitosan:
• Enhances the functions of inflammatory cells such as
• PMN (phagocytosis, production of osteopontin and leukotriene B4)
• Macrophagus (phagocytosis, production of interleukine IL-1, etc)

As a result chitosan promotes granulation and organization, indeed, one of the most interesting effect on wound healing is formation of granulation tissue with angiogenesis.

Chitosan induce fibroblasts to release interleukine 8 which is involved in migration and proliferation of fibroblasts and vascular endothelial cells.

Application of the gel onto an open wound induced significant wound contraction and accelerated wound closure and healing (N°72).

C | Clinical studies demonstration:
A complete review of Dr Khor from the department of chemistry (Singapore) describes the favorable characteristics of Chitosan for promoting rapid dermal regeneration and accelerated wound healing, suitable for applications extending from simple wound recovering to sophisticated artifical skin matrixes; the study reflects that Chitosan is a good wound healing material.

Report of the 8th International chitine and chitosan conference by Dung and al. on the chitosan membrane shows clearly good results reported from a 3 years clinical trial study that treated more than 300 patients for:
• Deep burns
• Orthopedic
• Trauma
• And ulcer conditions

Evidence is done that chitosan material is suitable as wound healing material (N°5).
In another special study, applications of chitosan films have been performed in sulcoplasty operations: 21patients referred for sulcoplasty operations were included.

In this peri-oral area, an ideal tissue-conditioning agent promotes healing, causes minimal pain to the patient, prevents infection, results in minimal scarring, and easy to use. The process of re-epithelialization that leads to healing of oral mucosal wounds results

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from a carefully orchestred proliferation and migration of epithelial cells. The wound surface should be kept damp enough to obtain the benefits of accelerated healing, but there should be no bacterial invasion that may cause delayed wound healing. In addition to the wound healing function, chitosan, with its bioadhesive property and its biocompatibility, and its microbial property, which is well demonstrated in this study, chitosan is an excellent candidate for tissue conditioning (N°102).

Different clinical investigations reveal that chitosan shows an enormous haemostatic potential and antibacterial effect, that it promotes intercellular matrix growth and that, finally, it induces regular collagen deposition avoiding scar formation in tissue.

These properties make chitosan effective in all 4 phases of wound healing (haomostasis, inflammation, proliferation, differentiation).

Thus, chitosan is a universal and bioregulatory wound dressing (N°58).

Regarding tissue engineering application: advanced studies promote the use of chitosan for cell transplantation to regenerate tissue:
• The utility of chitosan scaffolds to support cells growth and proliferation
• Suitable as immuno-isolator material
• Possibility to support respiratory epithelial cells for a possible tissue engineering trachea
• Chitosan exerts a strong influence on nerve cells attachment and proliferation.

In summary, the many examples reported of chitosan neat or in combination with other polymers have demonstrated the significant promise of these polymers to tissue engineering that can be fine tuned to suit the increasing focus that this field requires (N°5).

D | Clinical evidence on severe wounds: burns

Burns are among the most common injuries encountered by mankind (N°105). The main requirements in burn wound management is an economical, easy to apply, readily available dressing or method of coverage that will provide good pain relief, protect the wound from infection, promote healing, prevent heat and fluid loss (be elastic and non antigenic and adhere well to the wound (N°50).

When applied to extended burns and chronic wounds, classical treatments, are insufficient in preventing the scar formation and promoting healing. Thanks to chitosan hydrogels, there is a new route to treat burn injuries. Studies on the healing on extended third-level burns have been performed on pigs (CNRS, France); chitosan hydrogels showed very good results on third-level burns healing; this healing was faster than with a classical treatment and the neo-formed skin was very flexible as for a native skin (N°105).

Bibliographic argumentation

Skin repair is an important field of the tissue engineering, especially in the case of extended third degree burns, where the current treatments are still insufficient in promoting satisfying skin regeneration. Hydrogels only constituated of chitosan and water were processed and applied to the treatment of full-thickness burn injuries. The aim of the study (CNRS, France) was to assess whether this material was totally accepted by the host organism and allowed in vivo skin reconstruction of limited area third-degree burns.

All the results showed that chitosan materials were well tolerated and promoted a good tissue regeneration. They induced inflammatory cells migration and angiogenetic activity favouring a high vascularization of the neo tissue. At day 22, type I and IV collagens were synthesized under the granulation tissue and the formation of the dermal-epidermal junction was observed. After 100 days, the new tissue was quite similar to a native skin, especially by its aesthetic aspect and its great flexibility. From the results we may consider chitosan materials as interesting for burn wound healing (N°62). According to a review of the institute of chemical fibers (Poland), the University of Washington Medical Center (USA) is the producer of a chitosan dressing material to regenerate skin after serious second and third-degree burns (N°56).

In Japan, chitosan is specially renown since a three years old Russian boy whose skin was burnt over 80% in total area, dramatically recovered thanks to the chitin/chitosan dressing Beschitin-W® in august 1990 (N°104).

Comparative clinical studies with a collagen dressing in burns (39 cases) and in diabetic chronic wound (68 cases), show in favor for the chitosan treatment, that the adhesion was better, the frequency of necessary redressing was lower by 50%, that the granulation was two times quicker, and that the epithelialization was accelerated by 30%. These studies also demonstrate a substantial improvement of compatibility (pain, burning, hitching) and of side effects (allergic dermatitis) (N°58).

IV. Chitosan other crucial properties

1 | Haemostatic activity

Hemorrhage is a leading cause of death from trauma. Effects of a chitosan dressing on blood loss, survival and fluid use after severe hepatic injury in swine has been studied and published. Those studies evidence that the chitosan dressing reduced hemorrhage and improved survival after severe liver injury (N°65). A review of chitine and chitosan applications of the department of chemistry (India), shows that chitosan fibers were found to be thrombogenic and haemostatic and therefore can be used as haemostatic material (N°4).

Bibliographic argumentation

A review of chitosan-based wound dressings of the division of biosurface technology (India) highlights that chitosan is known in the wound management field for its haemostatic properties, and helps in natural blood clotting and blocks nerve endings reducing pain (N°67).

The testing results of different chitosan medical dressings already marketed (Institute of chemical fibers, Poland) indicate the high hemostatic potential of the chitosan dressing as well as its ability to absorb wound blood. This dressing was honoured with a gold medal at the Eureka 2004 innovation exhibition in Brussels and Genius'2002 in Budapest (N°56).

From the large review of the department of biochemistry (Canada), report is done on bandages made of chitosan, they were investigated in the field of military in the new war in Iraq; those bandages are used immediately after injury to control bleeding and were found to save numerous lives. Those bandages can stop capillary bleeding and stanch severe arterial hemorrhaging (N°92). It's about the Hemcon chitosan-based hemostatic dressing which is approved by the FDA for hemorrhage control; those bandages after a military use (prehospital combat casualties in Iraq) are still used in civilian emergency medical services for moderate to severe hemorrhage (N°26).

2 | Antimicrobial evidence

A | General points

The excessive use of antibacterial agents is causing a high impact on environment and all existing life, mainly human being health. There is a worldwide trend to explore new alternative that control postharvest pathogenic diseases, giving priority to methods to reduce disease incidence and avoid negative and side effects on human health.

The chitosan, a polycationic polymer, is currently receiving a great deal of attention. It became a valuable compound undoubtedly due to its appealing intrinsic physicochemical and biological properties (such as biocompatibility, biodegradability, bioactivity, in addition to its lack of toxicity, its allergenicity and antimicrobial effects (N°46). It's important to control any infection of a wound under dressing, the treatment of wounds requires the suppression of bacterial growth. Chitosan has been shown to provide inhibition of bacterial proliferation in the treatment of infected wound. Chitosan is capable of activating host defenses to prevent infection, thereby, offering an alternative to the use of antibiotics (N°72). Chitosan has attracted considerable interest due to its antimicrobial and anti-tumor activities, and its immune enhancing effects, chitosan has a wide inhibition spectrum not only gram+ and gram- but also yeasts and moulds (N°95, 92, 71, 39, 23, 8), furthermore, Chitosan has minimal foreign body reaction and an intrinsic antibacterial nature (N°39).

Bibliographic argumentation

B | Studies reports

At the school of dentistry (Turkey) a study of the Application of chitosan films in sulcoplasty operations has been performed including 21 patients referred for sulcoplasty operations. During the re-epithelialization period, infectious reactions may occur due to the presence of activated oral micro-flora that lead delayed wound healing. Microbiological evaluations were carried out on the samples taken from the surface of the healing tissue and the surgical splints on postoperative. There was a significant decrease in colonization of candida species, staphylococcus species, streptococcus species, difteroid basilies, ascherichia coli, neisseria species, on both the healing tissue and the surgical splint after application of the chitosan formulation. With its bioadhesive property and its biocompatibility, and its microbial property, which is well demonstrated in this study, chitosan is an excellent candidate for tissue conditioning (N°102).

The antimicrobial properties of chitosan were also investigated in vitro, in the solid and liquid culture against bacteria in order to assess the potential for using chitosan, results indicated that chitosan is potential as a natural disinfectant, it has antibacterial and antifungal characteristics (N°10). Moreover, studies from the Korea University have shown that chitosan can reduce the infection rate of experimentally induced osteomyelitis by staphylococcus aureus in rabbits (N°39).

Example extracted from a US Patent on wound filling composition indicate the microbiostatic and microbiocidal effectiveness of increasing chitosan concentration: chitosan provides antimicrobial activity (N°93).

A Study with hemcon bandages on rats has shown that chitosan is highly active in killing bacteria. This fast bactericidal action prevents the bacteria from proliferating in the wound and subsequently invading the tissue (N°24). A concentration of 1 to 1,5 % has been evidenced to complete inactivation of staphylococcus aureus (after 2 days incubation). However, Darmadji and Izumimoto (1994) reported that growth inhibtion of Escherichia coli required a 0,1% chitosan concentration (N°92).

Therefore a concentration of 2 % has been selected.

C | Mechanism

The antimicrobial activity of chitosan is well known against a variety of bacteria and fungi due to its polycationic nature (N°46). In accordance with studies performed by the institute of applied chemist (China), the antibacterial activity is currently hypothetized as surface interference and permeable inhibition (N°10).

A review on applications of chitosan (department of chemistry, India) reports that the growth of Escherichia coli was inhibited in the presence of more than 0,025 % chitosan. The cationic amino groups of chitosan probably bind to anionic groups of these microorganisms, resulting in growth inhibition (N°4).

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A study of the effects of chitosan (korea University) explains the mechanism as well: Its cationic amino groups associates with anions on the bacterial cell wall, suppressing biosynthesis, plus, disrupts the mass transport across the cell wall accelerating the death of bacterias (N°39) A positive charge on the NH3 group of the glucosamine monomer allows interaction with negative charged microbial cell membrane that lead to the leakage of intracellular constituent (N°95).

Finally the mechanism of the antimicrobial effects of chitosan has been fairly well established: a leakage of proteinous and intracellular component occurs due to the interaction between the positively charged chitosan molecules and the negatively charged microbial cell membrane. Being a chelating agent, chitosan has the ability to selectively bind trace metals, which prevents production of toxins and microbial growth.

Thus, chitosan has the ability to produce phytoalexins and cell wall phenol and callose. Chitosan is also an activator for several defense processes in the host tissue (N°92, 24)

3 | Various other properties

Because chitine and chitosan possess many beneficially biological properties such as:
• Antimicrobial activity
• Biocompatibility
• Biodegradability
• Haemostatic activity
• Wound healing properties

Much attention has been paid to its biomedical applications; furthermore, antioxydant properties of chitosan have been studied in vitro; the results showed that chitosan was good in antioxydant properties, especially antioxydant activity, scavenging ability on hydroxyl radicals and chelating ability on ferrous ions (N°90).

V. Chitosan numerous applications

Using chitosan in medical applications has attracted huge interest because of having a lot of advantages as:
• Being natural renewable source
• Being the most abundant polymeric material in the earth
• Biocompatibility
• Biodegradability
• Easy availability
• Non toxicity

Biofunctionality including:

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• Antithrombogenic
• Haemostatic
• Immunity enhancing
• Antitumor activity
• Immunoadjuvant activity
• Acceleration of wound healing
• Antimicrobial activity
• Blood compatible
• Anti-tumor: Suzuki (1996) reported that chitosan can act as inhibitor of growth tumor cells via their immuno-enhancing effects
• Anti-ulcer agent: chitosan has ulcer-healing action, repeated oral administration accelerate the gastric ulcer healing
• Analgesic effect
• Mucoadhesive that can adhere to hard and soft tissue (N°74, N°90, N°72, N°92, N°67)

Thanks to the wide variety of biofunctionality mentioned previously together with the very safe toxicity profile, chitosan has logically various potential application fields ranging from:
• Drug delivery
• Eye contact lense
• Digestible sutures
• Wound healing dressing material
• Artificial skin
• Antimicrobial agent
• Various medical suppliers:
• Separation membrane
• Adsorbant for affinity chromatography
• Adsorbant for metal cations
• Matrix for immobilization of biomolecule
• Antitumor activity
• Applications in controlled drug release and bioseparation
• Immunological application
• Support for biosensors

A wide variety of medical applications for chitosan and its derivatives have been reported and often marketed over the last three decades:
• Dentistery, orthopedic, ophthalmology and in surgical procedures
• Artifical skin : it appears that chitosan, having a structural characteristics similar to glycosaminoglucanes, could be considered for developing substratum for skin replacement
• Dressings, Bandages, film and sponges (numerous patents and trade marks)
• ophthalmology: chitosan possesses all the characteristics required for making an ideal contact lense / optical clarity, mechanical stability, permeability, particularly toward

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oxygen, wettability and immunological compatibility. The antimicrobal and wound healing properties of chitosan along with an excellent film capability, make chitosan suitable for development of ocular bandage lenses
• Drug delivery: all the interesting properties of chitosan make this natural polymer an ideal candidate for controlled released formulations
• Cell carrier: it was confirmed that the porous surfaces of the chitosan beads form a good cell carrier in various fields (enzymatic immobilization, chromatographic support, adsorbant of metal ions or lipoproteins and cell culture)
• Cell stimulating materials in plants and animals
• Fat trapper: chitosan fibers differ from other fibers in that it possesses a positive ionic charge, which
gives it the ability to bond chemically with the negatively charged lipids, fats and bile acids
• Antibacterial agent
• Blood anticoagulant : sulfate derivative might be useble as heparinoids for artificial blood dialysis
• Antithrombogenic and haemostatic material
• Treating major burns
• Blood dialysis membranes
• Artificial blood vesicle
• Encapsulation of neutraceuticals
• Chromatography
• Analytical reagent
• Surgical sutures (N°4, N°74, N°90, N°72, N°92, N°48 ,N°91)

The upcoming applications are numerous and various as well:

A study of chitosan for orthopaedic tissue-engineering in the department of Orthopaedic surgery (USA) concuded that at present, chitosan is one of the most promising biopolymers for tissue engineering and possible orthopaedic applications (N°91).

Orthopedic and periodontal applications : several studies have focused on the use of chitosan as a component in calcium based cements in the development of bone substitutes.

Drug delivery applications: is a very active area for chitosan as a carrier for various active agents including drugs and biologics (N°5).

Animal experiments and clinical studies were carried out to reveal the reaction of chitin/chitosan at pulp and periapical tissues; the results clearly demonstrates that chitine/chitosan could be used as an effective medicament especially for periapical wounds. In dentistery, chitin/chitosan is also applied as a dressing for oral mucous wound and a tampon following a radical treatment of maxillary sinusitis; furthermore, it is being

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investigated as an absorbing membrane for periodontal surgery (N°104).
Application to reinforcing plate, new method of chitosan spinning: this work consists in developing a new concept of reinforcing plates (especially for hernia reduction) principally made of chitosan fibers preventing from post-operative adhesion and promote the tissue regeneration, in addition to their stiffening function. Chitosan is not only known to be bioabsorbable, biocompatible and bioactive, but also anti-adhesive and tissue regenerative promoter. Finally, in vivo experiments contribute to comfort the use of this material for herniaplasty (N°103).

References list

N°2: The Effect of Chitin of Chitosan on Fibroblast-populated Collagen Lattice Contraction
Graeme I. Howling, Peter W. Dettmar, Paul A. Goddard, Frank C. Hampson, Michael Dornish and Edward J. Wood Biotechnol. Appl. Biochem. (2002) 36, 247-253

N°3: Topical Use of Chitosan in Opthalmology: Tolerance Assessment and Evaluation of Precorneal Retention
O. Felt, P. Furrer, J. M. Mayer, B. Plazonnet; P. Buri, R. Gurny International Journal of Pharmaceutics 180 (1999) 185-193 Received 23 September 1998, received in revised form 7 December 1998, accepted 11 December 1998

N°4: A Review of Chitin and Chitosan Applications
Majeti N.V. Ravi Kumar Recative & Functionnal Polymers 46, 2000, 1-27

N°5: Implantable Applications of Chitin and Chitosan
Eugene Khor and Lee Yong Lim Biomaterials 24, 2003, 2339-2349

N°7: Chitosan-Alginate Films Prepared with Chitosans of Different Molecular Weights
Xiao-Liang Yan, Eugene Khor, Lee-Yong Lim February 2001

N°8: Chitosan as an antimicrobial agent
Jonathan Rhoades and Bob Ratsall

N°9: Accelerating effects of chitosan for healing at early phase of experimental open wound in dogs
Hiroshi Ueno, Haruo Yamada, Ichiro Tanaka, Naoki Kaba, Mitsunobu Matsuura, Masahiro Okumura, Tsuyoshi Kadosawa, Totu Fujinaga Biomaterials 20, 1999, 1407-1414

N°10: Antibacterial Properties of Chitosan in Waterborne Pathogen
Yen-Meng Chen, Ying-Chien Chung, Li-Woan Wang, Kung-Tung Chen, Shyh-Yuan Li Journal of Environnemental Science and Health, Part A, Vol.A37, n°7, pp.1379-1390, 2002

N°11: Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: a review
J.-K. Francis Suh, Howard W.T. Matthew Biomaterials 21, 2000, 2589-2598

N°13: Effects of Chitin and Chitosan on Collagen Synthesis in Wound Healing
Kazuo Kojima, Yoshiharu Okamoto, Kazuya Kojima, Katsuyuki Miyatake, Hiroshi Fujise, Yoshihiro Shigemasa, Saburo Minami J. Vet. Med. Sci. 66, (12): 1595-1598, 2004

N°14: Effect of Molecular Weight and Deacetylation Degree of Chitin/Chitosan on Wound Healing
Tatsuya Minagawa, Yasuhiko Okamura, Yoshihiro Shigemasa, Saburo Minami, Yoshi-

References list

haru Okamoto Carbohydrate Polymers 67, 2007, 640-644

N°17: A preliminary investigation of chitosan film as dressing for punch biopsy wounds in rats.
Tanveer Ahmad Khan, Kok Khiang Peh J Pharm Pharmaceut Sci 6(1) 2003

N°21: Chitin: New Facets of Research
PR. Austin, C.J. Brinz, J.E. Castle, J.P. Zikakis Science 212: 749, 1981

N°23: Theapeutic Potential of chitosan and its derivatives in regenerative medicine
Chunmeng Shi, Ying Zhu, Xinze Ran, meng Wang

N°24: Use of chitosan bandage to prevent fatal infections development from highly contaminated wounds in mice
Marina Burkatovskaya, George P, Emilia Swietlik

N°25: Production of Chitosan and chitin
Inventeurs: Chen Mei-Huei, Chuang Su-Hui, Yuang Gwo-Fang, Chan Hing-Yuen, Chen Yen-Lin, Hwang Ing-Er, Wu Chih-Lu
Déposant: Food Industry RES and DEV I (TW) 2002-11-26

N°26: A spécial report on the chitosan-based hemostatic dressing: experience in current combat operations
Ian Wedmore, MD, John G, McManus,MD, Anthony E.Pusateri,PhD, and John B.Holcomb,MD.
The journal of Trauma, VOL 60 , Number 3

N°33: Control of wound infections using bilayer chitosan wound dressing with sustainable antibiotic delivery
Fwn Long Mi, Yu-Bey Wu, Shin Shing Shyu, Jen Yu Schoung 2001

N°38: Potential use of collagen-chitosan-hyaluronan tri-copolymer scaffold for cartilage tissue engineering
Jihong Yan, Xuemin Li, Lingrong Liu, Tissue Engineering Vol 8 Number 6, 2002

N°39: The effect of carboxymethyl-chitosan on proliferation and collagen secretion of normal and keloid skin fibroblasts
Xi Guang Chen, Zhen Wang, Wan-Shun Liu 2006

N°41: Topical formulations and wound healing applications of chitosan
Hiroschi Ueno, Takashi Mori, Toru Fujinaga 2001

N°45: Feasibility of chitosan-based hyaluronic acid hybrid biomaterial for a novel scaf-

References list

fold in cartilage tissue engineering
Shintaro Yamane, N. Iwasaki, T. Majima, T. Fumakoshi, T. Masuko, K. Harada, A. Minami, K. Monde, S. Nishimura Department of Orthopaedic surgery, Japan 2004

N°46: Chitosan-Copper complex as antibacterial agent: synthetis, characterization and coodinating bond-activity correlation study
S.Mekahlia, B.Bouzid, Faculté des sciences, Algérie 2009

N°48: Integration of extracellular matrix with chitosan adhesive film for sutureless tissue fixation
Antonio Lauto PhD 2009

N°50: New technologies for burn wound closure and healing
Review of the literature Bishara S. Atiyeh a,*, Shady N. Hayek a, S. William Gunn b 2005

N°51: Production of Fungal Chitosan by Enzymatic Method and Applications in Plant Tissue Culture and Tissue Engineering: 11 Years of Our Progress, Present Situation and Future Prospects
Nitar Nwe, Tetsuya Furuike and Hiroshi Tamura, Faculty of Chemistry, Osaka, Japan

N°52: Chitin content of cultivated mushrooms Agaricus bisporus, Pleurotus ostreatus and Lentinula edodes
Janos Vetter*, Faculty of Veterinary Science, Budapest, Hungary 2006

N°54: Carboxymethyl chitosan and its applications
V.K Mouryaa*, Nazma N. Inamdara and Ashutosh Tiwarib,c 2010

N°56: Chitosan Medical Dressings
Antoni Niekraszewicz

N°58: ChitoSkin: A new Chitosan-based wound dressing for healing especially chronic wounds
Prof. Dr.Dr.Dr.DR. W. Barnikol, A. Teslenko, M. Burow
Brazilian Archives of Biology and Technology 2006

N°59: Synthesis and Physicochemical Characterization of Chemically Modified Chitosan by Succinic Anhydride
Karine Gargioni Pereira Correa de Mello*, Leandra de Câssia Bernusso, Nogueira de Moraes Pitombo and Bronislaw Polakiewicz

N°61: Dressing Sponges Made of Chitosan and Chitosan-Alginate Fibrids
Magdalena Kucharska, Antoni Niekraszewicz, Maria Wiśniewska-Wrona, Kinga Brzoza-

References list

Malczewska Institute of Biopolymers and chemical Fibres, Poland, 2008

N°62: The use of physical hydrogels of chitosan for skin regeneration following third-degree burns
Nadège Boucard, Christophe viton, Diane Agay, Eliane Mari, Thierry Roger,Yves Chancerelle, Alain Domard 2007

N°65: Effect of a Chitosan-Based Hemostatic Dressing on Blood Loss and Survival in a Model of Severe Venous Hemorrhage and Hepatic Injury in Swine
Anthony E. Pusateri, PhD, Simon J. McCarthy, PhD, Kenton W. Gregory, MD, Richard A. Harris, DVM, MS, Luis Cardenas, Albert T. McManus, PhD, and Cleon W. Goodwin, Jr., MD The Journal of Trauma, 2003

N°67: Chitosan and Alginate Wound Dressings : a short Review
Willi Paul and Chandra P. Charma Trends Biomater. Artif.Organs, Vol 18 (1), pp 18-23 (2004)

N°70: The preparation and in vivo evaluation of the wound-healing properties of chitosan microspheres B.Conti, P.Giunchedi, Genta and U,Conte Revue STP PHARMA Science , 2000

N°71: Chitosan as tear sbstitute: a wetting agent endowed with antimicrobial efficacy
Felt O, Carrel A, Baehni P, Buri P, Gurny R. 2004

N°72: Potential applications of chitosan in vétérinary medicine
Sevda Senel, Susan J. McClure 2004

N°74: Using and properties biofibers based on chitin and chitosan on medical applications
N. Onar, M. Sariisik

MPN°76: Production of chitosan by Fungi
K. Nadarajah, J. Kader, Mohd. Mazmira and D.C. Paul Pakistan Journalof Biological Science 4, 2001

N°78: Photocrosslinkable Chitosan Hydrogel as a Wound Dressing and a biologic adhesive
Ishihara, Masayuki

MPN°79: Fungal Chitosan production and its characterization
P. Pochanavanish & W. Suntomsuk

MPN°80: Chitin and Chitosan in fungi

References list

Prof. Dr. Martin G. Peter

N°83: Chitosan and method of preparing Chitosan
Fan Weiyu, Hwang Ki-oh, Bohlmann John A, Steinke James D, Trinkle James R, Henning Joseph P

N°85: Reporting degree of deacetylation values of Chitosan: the influence of analytical methods
Tanveer Ahmad Khan, Kok Khiang Peh, Hung Seng Ch'ng

N°89: Biocompatilility of potentiel wound management products: Hydrogen peroxide generation by fungal chitin/chitosan and their effects on the proliferation of murine L929 fibroblasts in culture
Lip Yong Chung, Richard J. Schmidt, Paul F. Hamlyn, Brian F. Sagar, Andrea M. Andrews, Terence D. Turner

N°90: Antioxydant properties of fungal chitosan from shiitake stipes
Ming-Tsung Yen, Yu-Hsiu Tseng, Ruei-Chian Li, Jeng-Leun Mau

N°91: Chitosan: a versatile biopolymer for orthopaedic tissue ingeneering
Alberto Di Martino, Michael Sittinger, Makarand V. Risbud

N°92: Chitin,Chitosan, and co-products chemistry, production, applications, and health effects
Fereidoon Shahidi and Reem Abuzaytoun

N°93: Wound Filling Compositions US Patent
Mosbey, Deral T. SanguiBioTech/D-Witten (Ruhr)

N° 95: Chitosan kills bacteria through cell membrane damage
Hui Liu, Yumin DU, Xiaohui Wang, Liping Sun, Department of Environmental Science, Wuhan, China, 2004

N°96: The influence of molecular weight of chitosan on the physical and biologic properties of collagen/chitosan scaffolds
Chalonglarp Tangsadthakun, Sorada Kanokpanont, Neeracha Sanchavanakit, Rath Pichyangkura, Tanom Banaprasert, Yasuhiko Tabata and Siriporn Damrongsakkul

N°100: Characterization of chitosan isolated from Aspergillus Niger Industrial waste Mycelium
Lubomir Adamek, Milos Beran, Petr Molik, Department of Quality Features and Microbial products, Prague, Czech Republic

References list

N°101: Effect of Chitin and its derivatives on skin regeneration
Yasuhiko Okamura, Seiko Katakoa, Yoshiharu Okamoto, Saburo Minami, Department of clinical veterinary Medicine, Tottori University, Japan, Advance in Chitin Science- Vol VIII

N°102: Application of Chitosan films in Sulcoplasty operation
Mustafa Atac, Sevna Senel, Aysegul Eren, Semra Kustimur, Nadir Gungor, Gazi University, School of dentistery, Turkey, Advance in Chitin Science, vol VIII

N°103: New Method of Chitosan spinning : Application to reinforcing Plate
Laure Notin, Christophe Viton, Laurent David, Cyrille Rochas, Thierry Roger and Alain Domard, Sociét Europlak, La Garde, France, Advance in Chitin Science vol.VIII

N°104: Relationship between Lysozyme activity and clinical symptoms following the application of chitin/ chitosan in endodontic treatment
T. Ikeda, K. Yanagiguchi, I.L. Viloria and Y. Hayashi
Department of endodontics and operative dentistry, Nagasaki, Japan

N°105: Chitosan Physical Hydrogels: a new therapeutic way to burn injuries treatment
N. Visio, D. Agay, E. Marie, Th. Roger, C. Rochas, C. Viton, L. David, A. Domard, Umr cnrs 5627, Advances in chitin science VIII

N°106 : Safety of Chitosan bandages in shellfish Allergic Patients
LTC Kirk H. Waibel, MC USA; CPT Brian Haney, MC USA; Lt Col Merrideth Moore, USAF MC; Bonnie Wishman, MS; Robert Gomez, BS.

N°107:Evaluation of the safety of chitosan (chitoclear) by the allergological point of view
Ilkka Harvima, Professor of Dermatology, Department of Dermtology and Allergology, University of Kuopio, Kuopio, Finland.

N°108: GRASS Assessment Chitoclear shrimp derived chitosan for Primex, ehf. Siglufjordur, Iceland
Richard C. Kraska, Ph.D; Robert S. Mequate, Ph D; Madhusudan G. Soni, Ph D, FACN, FATS, 2012

N°109: Relationship between antibacterial activity of chitosan and surface characteristics of cell wall
Ying-chien CHUNG1,Ya-ping SU 2, 7, Chiing-chang CHEN 3, Guang JIA4, Huey-lan WANG5, J C Gaston WU2, Jaung-geng LIN6, 2004

N°111: The systematic effects of chitosan on fibroblasts derived from hypertrophic scars and keloids
Lv C, Dai H, Xing X, Zhang J., 2012

References list

N°112: Biological properties of the chitosan-gelatin sponge wound dressing
Chun-Mei Deng a, Lan-Zhen He a, Ming Zhao b, Dan Yang a,*, Yi Liu a, 2007

N°113: Safety of chitosan bandages in shellfish allergic patients
LTC Kirk H. Waibel, MC USA, CPT Brian Hany, MC USA, Lt Col Meddireth Moore, USA MC, Bonnie Wishman, MS, Robert Gomez, BS.

N°114: Analgesic effects of chitin and chitosan
Y. Okamoto, K. Kawakami, K. Miyatake, M. Morimoto, Y. Shigemasa, S. Minami 2001

N°115: Chitosan nerve tube for primary repair of traumatic sensory nerve lesions
Florian Neubrech, Sina Heider and Thomas Kremer, 2015

N°116 Chitosan film enhanced chitosan nerve guides for long distance regenration of peripheral nerves
Meyer, Cora; Stenberg, Lena; Gonzalez-Perez, Francisco; Wrobel, Sandra; Ronchi, Giulia; Udina, Esther; Suganuma, Seigo; Geuna, Stefano; Navarro, Xavier; Dahlin, Lars; Grothe, Claudia; Haastert-Talini, Kirsten, 2016

N°117: Abstract : Analgesis and wound healing effect of chitosan and carboxymethyl chitosan on scalded rats
Shuya HuangBaoqin HanKai ShaoMiao YuWanshun Liu

N°118: Use of chitosan scaff olds for repairing rat sciatic nerve defects
M. Simoes, S. Amado, A . Gartner, P. Armada-da-Silva, S. Raimondo, M. Viera, A. Luis, Y. Shirosaki, A. Veloso, J. D. Santos, A. Varejao, S. Geuna and A. Mauricio, 2010

N°119: Development of a new chitosan hydrogel for wound dressing
Maximiano P. Ribeiro, MD1; Ana Espiga, MD2,3; Daniela Silva, MD1; Patrı´cia Baptista, MD1; Joaquim Henriques, MD1; Catarina Ferreira, MD1; Jorge C. Silva, PhD4; Joa˜o P. Borges, PhD2; Eduardo Pires, PhD2,3; Paula Chaves, PhD1; Illı´dio J. Correia, PhD1 , 2009

N°120: An open multicenter comparative randomized clinical study on chitosan
Mo X1, Cen J1, Gibson E2, Wang R3, Percival SL4.

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