Chitin is an Effective Material for Sutures

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ABSTRACT: Chitin is an absorbable suture material with suitable mechanical properties. Tissue reaction is not specific and the good healing which ensued provided evidence for a satisfactory biocompatibility. Toxicity tests, including acute toxicity, pyrogenicity, mutagenicity were negative in all respects. The chitin suture was absorbed in about four months in rat muscles. The persistence of the tensile strength of the chitin was better than Dexon (TM) or catgut in bile, urine and pancreatic juice but weakening occurred early in the presence of gastric juice. Application in 132 patients proved satisfactory. Adverse effects were nil.

KEY WORDS: chitin, clinically absorbable suture, tensile strength, wound healing

INTRODUCTION

The selection of an appropriate suture material for each surgical procedure has led to development of a variety of sutures. With regard to absorbable sutures, there are commercially available suture materials, such as catgut, chromic catgut, polyglycolic acid and polyglactin. All of these materials are used for various types of surgical repair but are not the ideal, as degradation properties in various biological conditions leave much to be desired.

Chitin (β-(1-4)-N-Acetyl-D-Glucosamine, Fig. 1) a main component of the outer shells of Crustaceans is a biodegradable material with good biocompatibility and beneficial biological effects such as acceleration of wound healing.[1-3] Furthermore, as chitin is abundant in nature, both industrial and medical applications are feasible.[4] Although the advantages of a chitin suture have long been recognized, and the chitin powder was used as a clinical wound healing accelerator, and with good results,[5] practical sutures have not been prepared because of the difficulty in obtaining sufficient tensile strength.

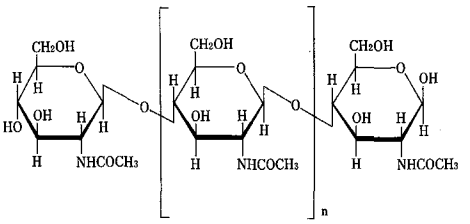

Fig. 1. Chemical structure of chitin, $(C_8H_{13}NO_5)n$.

We developed a chitin absorbable suture with practical strength and flexibility and evaluated it, in *in vitro* and *in vivo* applications.

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Materials and Methods

Manufacturing of the chitin suture

Crude Japanese pink crab (Chionecetes Opilio) shells were crushed and treated with acid and alkali to remove the calcium carbonate and protein. The purified and slightly modified chitin powder (without altering the chemical structure) was dissolved in amide solvent to form a transparent and high viscosity preparation. After filtration, fine multifilaments of about 5 μm in diameter were spun using isopropyl alcohol as a coagulant and the preparation rinsed. The dried multifilaments after complete coagulation were transparent and the flexible fibers had a tensile strength of about 50 kg/mm^2. After the filaments had been treated with a surfactant, 16-20 bundles were braided and rinsed in water.

Strength and elongation of the suture

Tensile strength of the suture was measured using a tensometer. Here, the material was either dry or had been moistured in saline for twenty minutes. The material was either straight or in a surgical knot. Elongation was measured by dividing the length of the suture at the break-point, by the original length.

Toxicity evaluation

Toxicity of the chitin suture was studied in terms of 1) Ames' mutagenicity test using salmonella strains for base pair substitutions and frame shift mutations, with and without metabolic activation by S9 mix, by the plating and preincubation method, 2) pyrogen test by intravenous administration of 10 ml/kg•Bw chitin extract, and 3) skin reaction test by inoculation of the 0.2 ml chitin extract into rabbits, 4) acute toxicity test by intraperitoneal administration of 1.0 ml chitin extract into mice, and 5) hemolysis test by adding the chitin extract to the defibrinated rabbit blood. In test 1), finely grained chitin powder was sonicated and suspended in dimethylsulfoxide. Concentrations of 10-5000 μg/plate were tested. In test 2) to 5), the chitin extract was prepared by adding 150 ml of saline to 15 g of the chitin and autoclaving the preparation at 121°C for one hour, followed by filtration through 0.85 μm filter.

Histological evaluation

The sutures were implanted in the calf muscle of rats and histological evaluation was performed 1, 4, 8 and 16 weeks later.

Scanning electron microscopy

The surfaces of the original and implanted sutures were observed using a scanning electron microscope.

Generation of N-Acetylglucosamine (NAG) in lysozyme solution

To study the chemical degradation process of chitin to NAG, the chitin material was placed in lysozyme solution, (regraded as a major enzyme for degradation *in vivo*), and generation of NAG was measured by the Morgan-Elson reaction.

Tensile strength in tissue and in body fluids

The sutures were either implanted in the dorsum of rabbits or immersed in aseptically collected body fluids and human urine contaminated with *E. Coli*, then incubated at 37°C and removed periodically to measure the tensile strength. The fluids included calf serum, gastric juice (pH 1.2) and bile from dogs and pancreatic juice from humans.

Results

Strength and elongation of the suture

The size of the sutures were USP 4-0 and the tensile strength of the chitin suture in a dry straight condition was 2.25 ± 0.05 kg, that is much the same as Dexon (TM) and much stronger than catgut. In straight pull, the strength of the chitin and catgut suture was less in a wet than in a dry condition, but in a knot pull, the strength of the chitin suture was not less in a wet condition. A characteristic property of the chitin suture was the smaller elongation, that is 12.3 per cent in a straight pull in a dry condition. In a wet condition, the elongation of the chitin was much the same as other suture materials (Table 1).

Table 1. Mechanical Properties of Suture Materials

	USP Size		Chitin 4-0	Dexon 4-0	Catgut 4-0
Straight Pull	Dry	Strength (Kg)	2.25±0.05	2.35±0.06	1.39±0.33
		Elongation (%)	12.3±0.8	25.3±1.3	23.9±2.6
	Wet	Strength	1.96±0.06	2.33±0.09	1.01±0.19
		Elongation	21.2±1.8	24.8±1.8	25.3±3.2
Knot Pull	Dry	Strength	1.21±0.05	1.47±0.08	0.89±0.16
		Elongation	12.6±0.7	23.9±0.9	22.1±3.4
	Wet	Strength	1.25±0.09	1.48±0.06	0.54±0.06
		Elongation	18.9±1.2	24.1±1.5	17.0±1.9

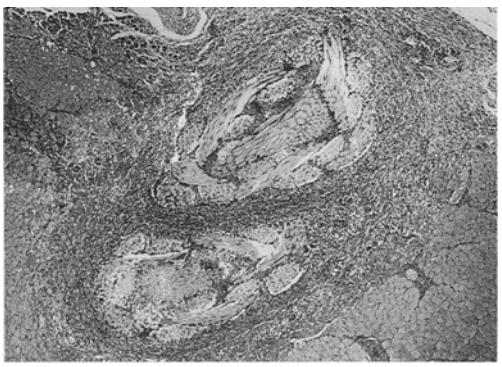
(n=10, mean ± SD)

Toxicity evaluation

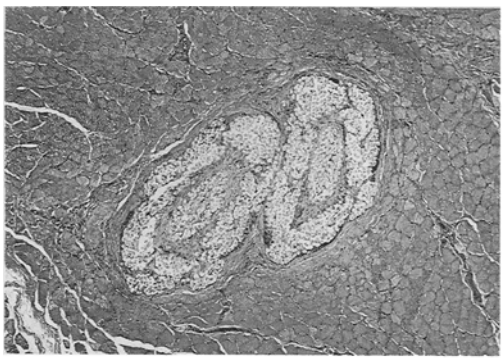
1) Various mutagenicity tests revealed no activity. 2) Pyrogen tests showed no significant elevation of body temperature during three hours of observation. 3) On skin reaction test, 72 hours of observation revealed no erythema, edema, bleeding or necrosis at the injected sites. 4) On acute toxicity test, no abnormal behavior or death occurred during 5 days of observation. 5) Hemolysis was not observed with this sample.

Histological evaluation

On macroscopic examination of the implanted sutures, no apparent infection or tumor formation was observed with either chitin or Dexon (TM), at any postoperative stage and the tissue reaction was not specific. Microscopically, at one week, the chitin suture showed a greater extent of inflammatory cell infiltration than did Dexon (TM). At 4 weeks, the inflammatory cells disappeared



a



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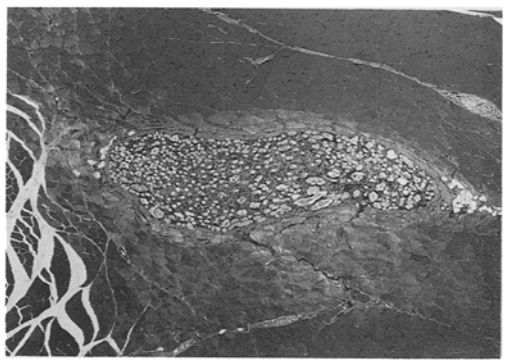
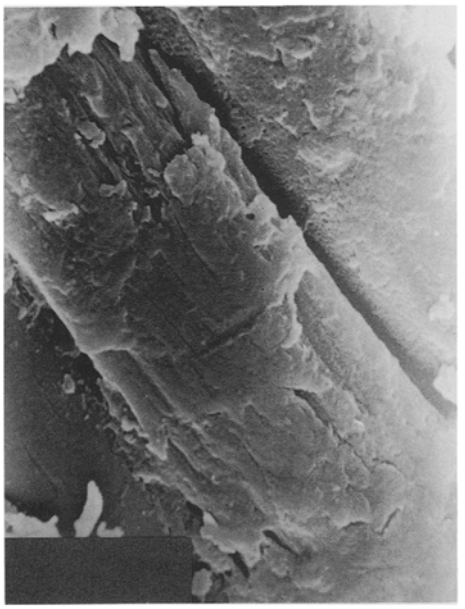


c

Fig. 2. Histology of the chitin suture implanted in the rat calf muscle (HE ×40). Acute inflammatory cell infiltration at 1 week (**a**), inflammation subsides at 4 weeks (**b**), fibers are thinned on 8 weeks (**c**) after implantation

a



b

Fig. 3. Scanning electron microscopy of the chitin suture. Smooth uniform surface before implantation (a, ×1000). A scale-like degradation after 30 days of implantation (b, ×100).

and fibroblastic and histiocytic cell proliferation was intense in the chitin samples. On the other hand, giant cells which are typical in the Dexon (TM) sample were fewer in case of chitin. At 8 weeks, each fiber of the chitin suture is thin and encapsulated with fibrous cells. Degradation of the Dexon (TM) suture is enhanced to a greater extent but giant cells were present. The absorption of the chitin at this stage was less than the Dexon (TM) but at 16 weeks, the sutures and the surrounding capsules were hardly discernible in most applications of Dexon (TM) and chitin (Fig. 2).

Scanning electron microscopy

Scanning electron microscopy (SEM) of the chitin suture before implantation revealed a rather smooth surface of fibers and uniformity in fiber diameter. SEM taken after 30 days of implantation in rabbits showed a surface irregularity and a scale-like degeneration of the fiber at a high magnification, thereby indicating the process of degradation and absorption, *in vivo* (Fig. 3).

Generation of N-Acetylglucosamine

Up to 8 days of incubation with lysozyme, NAG was not detected in the solution, but after this period, NAG increased with time and degradation by this enzyme became evident (Fig. 4).

Tensile strength in tissue and in body fluids

1) In the dorsum of rabbits, the tensile

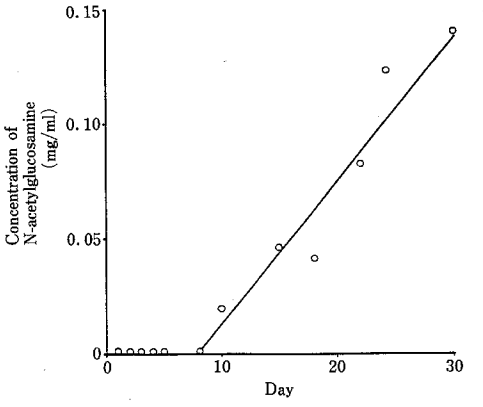


Fig. 4. Generation of N-acetylglucosamine (NAG) in lysozyme solution (37°C), measured by the Morgan-Elson reaction.

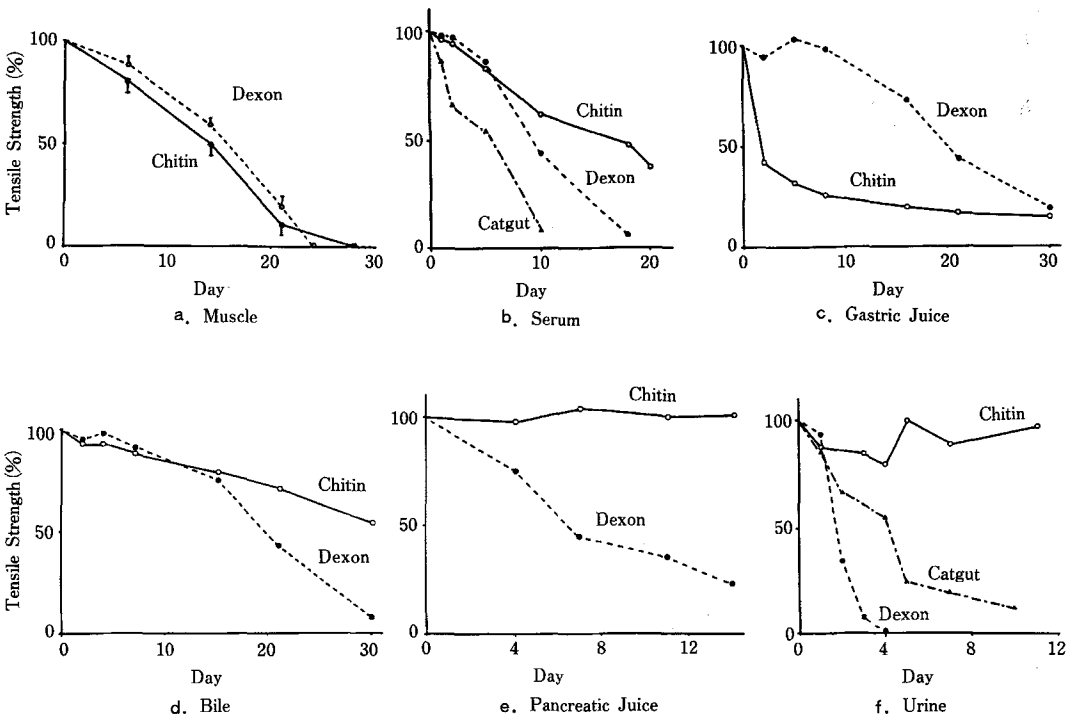


Fig. 5. Tensile strength of the chitin and Dexon (TM) and catgut suture, **a.** in rabbit dorsum muscle, **b.** in calf serum, **c.** in dog gastric juice, **d.** in dog bile, **e.** in human pancreatic juice, **f.** in human infected urine. (n=10, bars indicate standard deviations)

strength of the chitin decreased with time and was half the initial strength on day 14. Both the chitin and Dexon (TM) suture lost strength on days 24 to 28 (Fig. 5a). 2) In calf serum, the tensile strength of the chitin was 35 per cent on day 20 while that of the Dexon (TM) or catgut disappeared (Fig. 5b). 3) In canine gastric juice pH 1.2, the chitin material weakened more rapidly than did the Dexon (TM). This weakening occurred early in the incubation period but this slowed later on (Fig. 5c). 4) In canine bile, the strength of the Dexon (TM) decreased more rapidly than did chitin which retained 71 per cent of the initial strength on day 21. Weakening was not accelerated throughout the course (Fig. 5d). 5) In human pancreatic juice, the strength of the Dexon (TM) decreased to 32 per cent on day 11, whereas that of chitin retained practically all of the initial strength (Fig. 5e). 6) In human contaminated urine, the chitin suture

Table 2. Clinical Application of Chitin Sutures

Skin	48
Gastric	6
Gastroduodenostomy	3
Gastrojejunostomy	1
Intestinal	9
Ileoileostomy	3
Ileocecostomy	1
Ileotransversostomy	1
Cystic duct ligation	9
Liver bed closure	4
Thyroid	2
Anal	2
Fascia	15
Subcutaneous	28
	84
Total	132

retained 80 per cent of the strength on day 4 while the Dexon (TM) lost strength almost completely (Fig. 5f).

Clinical results

Clinical application of the chitin suture was made on 132 patients ranging from 1 to 90 years of age and who underwent various surgical procedures (Table 2). As a first step to evaluate the clinical effect of this material, the nonabsorbable sutures were used for skin closure (48). The duration of observation was from 1 to 15 days. Changes in the skin were not specific, namely, the slight inflammatory reaction for 5 to 7 days rapidly disappeared after removal of the suture. There was no tendency toward suppuration or unusual discharge, nor were there any allergic or hypersensitive reactions. Acceleration of wound healing was not apparent in comparison with other sutures. The SEM observation revealed a slight scale-like degradation on the suture surface on day 7.

Following the acceptable results of the aforementioned trials, the second step trials were performed on 84 with internal sutures. These were subcutaneous sutures (28), gastric procedures (9), intestinal procedures (14), hepatobiliary procedures (13) and others (19). Postoperative courses were followed by blood counts, blood chemistry and urinalysis. The clinical courses of these patients were uneventful and there was no abnormal laboratory data to indicate organ or hematological disorders related to the chitin suture. Some patients with gastrointestinal anastomoses were followed by fluoroscopic examinations and leakage or stenosis was nil. Fiberoptic observations with biopsy specimens at 2-3 months after operations revealed slight inflammation but there was no specific histological reaction around the anastomosis.

Discussion

Absorbable sutures must meet the following requirements, 1) strong and flexible enough to suture and knot tightly, 2) maintain strength long enough for the wound to heal and develop sufficient adaptation strength, 3) the suture material is to be absorbed without causing adverse tissue reactions, 4) the degradated material is nontoxic. Available absorbable sutures thus have limitations, e.g. polyglycolic acid (PGA, Dexon TM),[6] polyglactin 910 (Vicryl TM),[7] polydioxane[8] and catgut or chromic catgut. These sutures have such characteristics but are always inadequate for various conditions.[9]

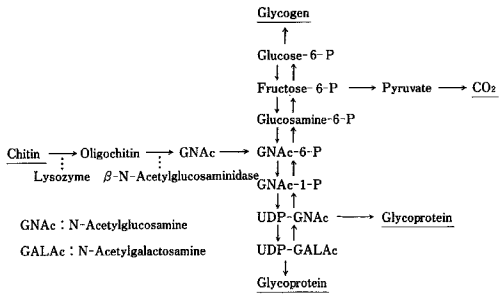


Fig. 6. Metabolic degradation of chitin *in vivo*.

Concerning the degradation of the chitin *in vivo*, it has been shown that lysozyme plays an important role, as shown in the generation of NAG *in vitro*, but a rapid decrease in tensile strength in certain biological states showed that other mechanisms might also be involved. The oligomeric chitin made by these processes is further hydrolysed to monomeric NAG by exo-glycosidases such as β-N-Acetylglucosaminidase or β-N-Acetylhexosaminidase. The generated NAG, a common aminoglucose in the body, enters the innate metabolic pathway to be incorporated to form glycoproteins or to be excreted as carbon dioxide gas in respiration[10] (Fig. 6). Thus, the chitin and its degradation products are natural or at least safe. Various toxicological tests in our study revealed a total absence of untoward effects.

Concerning mechanical properties of the chitin suture, it proved to be of sufficient strength and was stronger than chromic catgut and almost the same as the Dexon (TM) suture material. The characteristic of the chitin suture is its shortness in elongation. This suture is most flexible and soft, and easy

to tie securely, however, it is slightly fragile.

The main feature of the chitin suture is the good tensile strength which is maintained for a longer period in urine, serum, bile and pancreatic juice, than is Dexon (TM) or catgut suture. These events are related to its resistence to hydrolysis and digestive enzymes. When a poor risk patient is considered, the wound healing would take a longer period than usual and this property would be beneficial together with the possible wound healing acceleration. In contrast, the chitin suture is weak against strong acid such as gastric juice with a low pH. Therefore, care must be taken when chitin is exposed to a strong acid.

The pharmacological effects of the chitin such as wound healing acceleration,[2,5] suppression of the serum lipid levels,[1] to promote immunonoresponsiveness and other proven characteristics[3] of the chitin suture are promising factors for the development and clinical application of the chitin suture.

Clinical results of the chitin suture are acceptable, namely, there were no abnormal tissue reactions or infections and no allergic nor hypersensitive reactions. There was no hepatic, renal or hematological untoward event. The wound healing after application of the chitin suture was uneventful, but clinically, there was no apparent wound healing acceleration, in comparison to the conventional sutures.

Areas of application and testing in a larger number of patients are expected to provide further positive data indicating that chitin sutures can be safely applied in clinical surgery.

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