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Investigation into Biological, Composite Surgical Meshes

Abstract

A method is proposed to prepare composite surgical meshes modified by chitosan. The polypropylene mesh OPTOMESH™ Macropore is used as the half product onto which a coating of microcrystalline chitosan with a lowered pH is deposited. The thus modified surgical mesh is sterilized by means of ethylene oxide and assessed in respect to physical–mechanical and biological properties. Cytotoxicity, irritating and allergenic testing was carried out in specialized medical institutions. All examinations made of the microcrystalline chitosan-coated meshes confirmed their biocompatibility as acceptable for potential uses in surgery. Hydrolytic and enzymatic degradation of the meshes was estimated, too. It was found that after 180 days the chitosan coating had lost 50% of its original weight when immersed in a bath at 37 °C containing 10 µg/cm³ of lysozyme.

Key words: surgical mesh, chitosan, composite, biodegradation, biocompatibility.

Introduction

Hernia is a common ailment afflicting at least 2% of the human population. Today, most hernia cases are surgically repaired by the so-called tension-free method, which employs knitted meshes made of non-resorbable synthetic fibres [1, 2]. More benign to the patient would be the use of partially resorbable meshes including composite implants. Such surgical materials bear the advantage of avoiding the stiffening of the implant caused by streaking with fibrous tissue and, as a consequence, surgical complications in the nearby organs [3].

Some companies have appeared on the market with partially resorbable hernia meshes: just to name a few, VYPRO® I, VYPRO® II and PROCEED® by Ethicon Co. or PARIETEX® by Sofradim Co. [4]. The VYPRO® meshes are made of resorbable polyglactin 910 fibres (copolymer of glycolic and lactic acids) and non-resorbable polypropylene yarn (VYPRO® I – multifilament; VYPRO® II – monofilament). PARIETEX® is prepared from polypropylene yarn onto which is deposited a coating of bovine cross-linked collagen. The manufacture of such surgical materials has yet not been implemented in Poland.

The aim of the research was to prepare a method for the production in Poland of composite, partially resorbable surgical meshes suitable for the repair of hernia, employing for the purpose the biocompatible and resorbable polysaccharide – chitosan. Two methods were adopted in the research to solve the problem: using a multifilament chitosan yarn (in cooperation with the Department of Knitting Technology and Structure of Knitted Products of the Technical University of Lodz, Po-

land), and alternatively the finishing technique [5]. The first of the two methods did not produce satisfactory results due to the knitted modified meshes showing too high a surface weight, exceeding 100 g/m², and the thickness not being acceptable for the implants. The use of a multifilament chitosan yarn in the preparation of surgical meshes did not result in satisfactory products either. There is presently no chance of preparing, even on a lab scale, a chitosan monofilament with physical-mechanical properties suitable for joint knitting with polypropylene fibres.

It was, therefore, resolved to adopt from the two versions implants prepared by a combined method [6] in which a microporous, resorbable chitosan coating is deposited on the surface of the non-resorbable mesh OPTOMESH™ Macropore (TRICOMED Co.). Various useful forms of chitosan prepared at the Institute of Biopolymers and Chemical Fibres (IB-ChF) [7-9] were applied for the purpose.

The physical–mechanical properties and the chemical and biological purity of the modified surgical meshes were estimated after sterilization with ethylene oxide (EO). Further steps of the research examined the biocompatibility in respect of the cytotoxicity of the chitosan coating and the susceptibility to enzymatic degradation as well as irritating and allergenic action.

The biocompatibility tests are a precondition to enter into the *in vivo* estimation.

Materials and methods

Preparation of the chitosan-modified surgical meshes

In the selection of the chitosan form and method to deposit the resorbable coat-

ing, a durable adherence of the coating layer to the basic mesh without limiting the elasticity and flexibility of the formed composite is crucial. The ultimate assessment of the modified meshes can only be made by future *in vivo* investigation including surgical handiness, sorption time and implantation effect. The *in vivo* estimation is expected to be carried out within a separate research project.

The research within this project included the following issues:

1. The preparation of flat knitwear as a support for depositing the resorbable layer.
2. The selection and preparation of chitosan forms with good film-forming properties and adhesion to the polypropylene surface of the supporting mesh.
3. The formulation of the composition of the coating preparations.
4. The preparation of a method to deposit the chitosan coating on the polypropylene mesh.
5. The adoption of a method and conditions for the drying of the chitosan-modified mesh.
6. The selection of an optimal variant.

A series of experiments including methods of manufacture, finishing and sterilization was carried out to select suitable surgical meshes of polypropylene monofilaments. Four kinds of mesh prototypes with a varied knitting weave named Dallow M were prepared at TRICOMED Co. The physical-mechanical properties and the chemical and biological purity of the meshes before and after sterilization with ethylene oxide (EO) were examined. From the tested lot, a mesh was

Table 1. Mechanical properties of polypropylene meshes with one-side chitosan coating.

Quality parameter tested	Kind of chitosan coating			
	Without coating	ChL	MCCh	ChF
Surface weight, g/m ² PN-83/P-04891	80.3±0.8	99.0±2.0	100.7±2.2	98.3±2.0
Thickness, mm	0.72±0.02	0.74±0.02	0.75±0.02	0.75±0.02
Piercing force, cone method, kN PN-EN ISO 12236:1998	3.24±0.04	3.50±0.06	3.51±0.06	3.45±0.05
Piercing displacement, mm	19.5±0.4	23.0±0.5	22.8±0.5	22.2±0.5
Length of bending, cm	3.85±0.12	5.20±0.18	5.45±0.19	5.90±0.22
Total bending rigidity, mN·m PN-73/P-04631	0.050±0.01	0.160±0.05	0.201±0.05	0.250±0.07

selected with the highest piercing force, amounting to 38.2 N/cm (the demand is 16.0 N/cm), surface weight amounting to 60 g/cm², the lowest bending rigidity and high porosity. The chitosan used was delivered by Vanson HaloSource Co., USA; it was of adequate quality inclusive of chemical and biological purity. The chitosan was transformed into useful forms according to the IBChF methodology. After optimizing trials, the following coating preparations were selected:

- An aqueous solution of chitosan lactate with 1.5% concentration and increased pH (ChL)
- An aqueous suspension of chitosan fibrils (ChF) with 2.5% concentration
- An aqueous suspension of microcrystalline chitosan (MCCh) with a concentration of 1.9% and pH=6.88.

The chitosan preparations were applied either on one side of the mesh by the knife technique with a slot width of 1 mm (coating) or on both sides by padding.

To the chitosan-coated meshes, two different drying methods were applied:

- Conventional drying, blowing drier, temperature 30 °C, changeable drying time
- Freeze drying in the lyophilizing cabinet from Christ Co. type Alfa 1-4, temperature from -20 °C to +20 °C, time 20-24 hours

The drying procedure and amount of plasticizer in the preparation are crucial to obtaining a good quality of the coating, including elasticity, handiness and durability. **Table 1** shows the mechanical properties of meshes coated on one side with various chitosan preparations. The coating amounted to 20±2.5 g/m², and conventional drying was applied. The meshes with the best organoleptic properties were selected for the comparison shown in **Table 1**.

The best elasticity and adherence of the coating to the mesh surface could be found in meshes that were coated on one side with the addition of a plasticizer in the proportion of 9.2:1.0 on the chitosan, conventionally dried for 15 minutes at 30 °C and after-dried at an ambient temperature. All the meshes modified by various chitosan preparations revealed a slightly higher strength and a lower flexibility (higher bending rigidity) than the unmodified ones.

It was found that the various chitosan preparations used are equally suitable for the one-side coating technique. Hence, the selection ought to be made based on processability and chemical purity. When technological aspects are considered, chitosan lactate appears to be the best solution; however, the product with increased pH contains both lactic acid and sodium lactate.

MCCh is better in respect of composition since it does not contain such undesired compounds and, moreover, it does not dissolve in water and physiological solution. MCCh produces an even, elastic and firmly adhering coating on the mesh surface.

Sterilization and packing

Irradiation with fast electrons and X-rays with the recommended dose of 25 kGy causes degradation of the polypropylene yarn, resulting in a distinct deterioration of the mechanical properties of the meshes, hence radiation sterilization was abandoned.

In the preparatory phase, the samples intended for sterilization were transferred to the laboratory clean zone and microbiologically cleaned by irradiation with a UV lamp for 30 minutes. Next, the surgical meshes were packed in a double film/paper wrapping made by OPM Co., Poland. Such packaging, as it was documented in earlier works, enables the

sterilization with EO and assures sterility for at least 12 months.

Ready for sterilization, the meshes were examined in respect of microbiological contamination. It was found in quantitative tests carried out by the membrane filtration method that the chitosan-modified meshes satisfy the condition of FP (Polish Pharmacopeia) VI (2002). The application of the chitosan coating did not increase the danger of infection during the processing. The level of contamination is close to that of the unmodified surgical mesh.

The absence of *Staphylococcus*, *Pseudomonas aeruginosa* and microorganisms of the *Enterobacteriaceae* family and other bacteria Gram (-) was certified in qualitative testing by direct inoculation on the surface with an area of 100 cm².

The sterilization was carried out at Technochemia Co., Grójec, Poland. After sterilization, the meshes were examined quantitatively for the presence of EO traces according to standard PN-PE 10993-7-2005 using the model strain *Staphylococcus aureus* (ATCC6528). The test confirmed the absence of EO capable of inhibiting the growth of the model strain.

Chemical and biological indicators were used to control the effectiveness of the sterilization. According to the validation report, the sterilization cycle proceeded correctly in respect of the physical parameters. The change of the hue in the active fields of the chemical indicator on the left and right sides of the strip confirmed the correct run of the EO sterilization. The biological indicator did not show any growth of the reference strain *Bacillus subtilis var niger* ATCC 9372.

Sterilization was also performed with overheated steam in an autoclave at 21 °C with time varying from 10 to 45 minutes. The treatment did not act upon the appearance of the meshes nor was the adhesion of the coating to the mesh affected, in contrast to the packaging material, which suffered deformation.

After sterilization, the chitosan-modified meshes were examined in respect of the presence of bacterial endotoxins according to the semi-qualitative test LAL. The results show that, at the applied dilution of 1:10, the amount of bacterial endotoxins in the sterilized material does not exceed 0.25 EU/ml

and in the whole product is below 2.5 EU/100 cm². The EO-sterilized surgical meshes satisfy the permissible limit of USP = 20 EU/product as described in the USP (United States Pharmacopoeia) Monograph 161.

Since the EO route provided for effective sterilization was confirmed by adequate tests, it was adopted for further investigations.

Investigation of the susceptibility of the selected surgical meshes to degradation

The polypropylene surgical meshes with a coating of modified chitosan (content of 20.5%) were examined in respect of susceptibility to hydrolytic and enzymatic degradation. The hydrolytic testing was carried out according to standard PN-EN ISO 10993-13: 2002 "Identification and quantitative estimation of degradation products of polymeric medical devices".

Susceptibility to hydrolytic degradation

Conditions:

- Time – 30, 90, 180 days
- Temperature – 37 °C
- Medium – H₂O, pH=6.95
- Bath ratio – 250:1 w/w

The test was carried out on two samples. After the respective time, the samples were taken out of the bath, washed with distilled water and dried. The degradation was assessed by the change of pH, loss of mass of the samples and concentration of reductive sugars (degradation products) in the bath.

For all the samples, photographic documentation was made by means of electronic and optical microscopy. **Table 2** presents the percentage mass loss of the samples in reference to the total weight of the meshes and to the weight of the chitosan coating.

The mass loss indicates a partial hydrolytic degradation of the chitosan coating of the meshes. After 180 days, the loss is the highest, making the degradation effect visible under an optical microscope. Oligomeric substances such as reductive sugars did not appear in the hydrolytic degradation.

Susceptibility to enzymatic degradation

The degradation was conducted in a citrate-phosphate buffer at 7.2 pH with varied lysozyme concentrations. Lysozyme

Table 2. Time-dependent mass loss of chitosan-modified surgical meshes.

Degradation time, days	pH	Mass loss, %		Concentration of reductive sugars, µmol/cm ³
		Mesh + chitosan	Chitosan coating	
0	6.95	0	0	0
30	6.90	0.1	1.2	0
90	6.90	0.3	2.8	0
180	6.85	0.9	9.2	0

Table 3. Impact of the degradation time and enzyme concentration on the mass change of the chitosan-modified surgical meshes at pH = 7.2.

Degradation time, days	Concentration of lysozyme, µg/cm ³	Mass loss, %		Concentration of reductive sugars, µmol/cm ³
		Mesh + chitosan	Chitosan coating	
0		0	0	0
30	0	0.10±0.03	0.8	0
	10	3.05±0.11	24.0	0.02±0.015
	100	3.85±0.05	30.8	2.84±0.065
	200	4.10±0.36	32.8	3.17±0.08
90	0	0.20±0.03	1.6	0
	10	4.60±0.05	36.4	0.69±0.06
	100	5.80±0.03	46.4	4.01±0.07
	200	6.60	52.8	5.34±0.06
180	0	1.20±0.06	9.60	0
	10	6.50±0.06	52.0	1.47±0.05
	100	7.30±0.15	58.4	4.40±0.10
	200	8.00±0.11	64.0	6.21±0.045

is an enzyme present in human body fluids. Conditions:

- Time – 30, 90, 180 days
- Temperature – 37 °C
- Enzyme – lysozyme (concentration 0, 10, 100, 200 µg/cm³)
- Bath ratio – 250:1 w/w

The degradation was assessed by the loss of mass of the samples and the concentration of reductive sugars (degradation products) in the bath. The pH value remained constant throughout the entire degradation time.

The changes proceeding in the course of the degradation were observed under microscope, too. The results compiled in **Table 3** were reckoned as the arithmetic mean of three separate runs of the degradation.

Samples of the composite meshes were, prior to the degradation, washed in demi water to remove the plasticizer and dried. After that treatment, the content of chitosan amounted to about 12.5% of the total weight of the chitosan/PP composite. The chitosan coating deposited on the mesh of polypropylene fibres underwent degradation caused by the lysozyme. After 180 days, the mass loss exceeded 50% even at the lowest enzyme concentration (10 µg/cm³). The increase in the enzyme concentration imperceptibly accelerated the degradation of the coating, but sig-

nificantly increased the amount of delivered reductive sugars. That part of the work was carried out in cooperation with the Institute of Technical Biochemistry of the Technical University of Lodz.

The SEM photos presented in **Figure 1** (see page 120) illustrate the progressing degradation of the chitosan coating deposited on the surgical mesh (at a lysozyme concentration of 100 µg/cm³).

Estimation of the biocompatibility and biological activity

The following biological characteristics of the chitosan-coated surgical meshes were assessed: antibacterial activity, cytotoxicity, and irritation and allergenic features.

Antibacterial activity

The MCC-modified surgical meshes were assessed in respect of antibacterial activity against gram (-) *Escherichia coli* and gram (+) *Staphylococcus aureus* bacteria. The tests were carried out *in vitro* in an accredited microbiological laboratory of IBChF by the quantitative method according to the international standard JIS L 1902:2002. One-side coated meshes were tested with the content of about 20 g/m² MCC. The modified meshes with such an amount of MCC did not reveal antibacterial activity against the test bacteria strains.

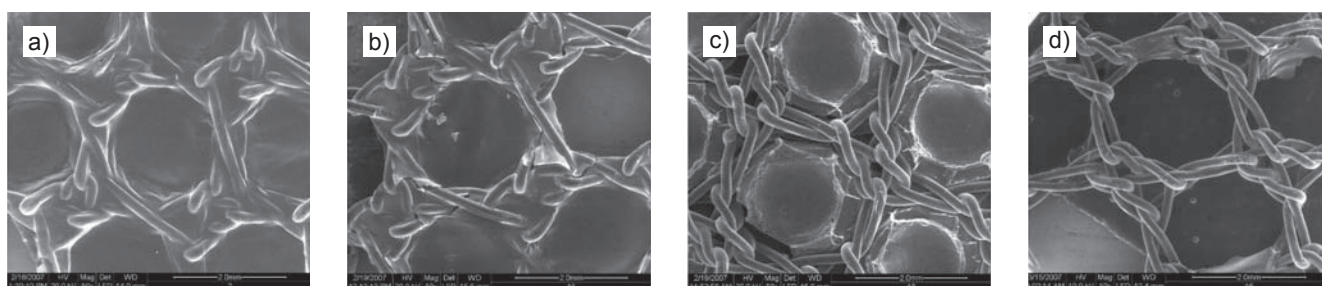


Figure 1. SEM micro-photos of chitosan-modified surgical meshes during enzymatic degradation: a) before degradation, b) after 30 days, visible impairment of the coating on the surface of the knitting weaves, c) after 90 days, distinctly exposed knitting weaves, d) after 180 days, the surgical mesh is devoid of the chitosan layer.

It is hoped that the effect could be achieved with other forms of chitosan, like chitosan lactate or other bioactive substances in the coating. Underpinning such an assumption are the results of earlier investigations at IBChF.

Cytotoxicity

The cytotoxicity of the chitosan-coated surgical meshes was tested in the Department of Experimental Surgery and Biomaterial Testing, and in the Department of Cell Culture of the Faculty of Histology and Embryology of the Medical University of Wrocław according to integrated standard PN-EN ISO 10993-5:2001 "Biological assessment of medical devices". The examination of extracts was made *in vitro* on a reference cell line of mouse fibroblasts 3T3/Balb by the direct method. The tested samples had earlier been EO-sterilized.

The cells were bred according to recommended tissue culture in an incubator at 37 °C in an atmosphere with 5% content of CO₂ with constant humidification of the chamber. The culture was assessed after 24, 48 and 72 hours by means of a reverse phase-contrast microscope.

After 24 hours of breeding with the extracts from the modified surgical meshes, the cells were augmented, showing grain-like structures in the cytoplasm. The cell proliferation was distinctly lower than for the reference cultures. After 48 and 72 hours, the morphological changes were the same as after 24 hours and the cell proliferation much lower than for the reference. The content of dead cells after 48 and 72 hours was 5 and 20%, respectively.

Conclusive of such results is that the chitosan-modified surgical meshes show after 24 and 48 hours a low and after 72 hours a low to moderate cytotoxic activity against mouse 3T3 Balb/C

fibroblasts. The results do not limit the possible use of the chitosan-modified surgical meshes in surgery, considering the fact that unmodified surgical meshes did not manifest cytotoxic activity. The reason why the chitosan coating containing a plasticizer exerts a low cytotoxicity after sterilization calls for a separate exploration.

Irritation and allergenic characteristics

The irritation and allergenic testing was performed in the Pharmacology Department of the National Drug Institute in Warsaw according to standards harmonized with European Directive 93/42/UE. The examination was based on standard ISO 10 993-10:2002 and carried out *in vivo* on rabbits with sterilized surgical meshes coated on one side with chitosan (MCCh). Extracts of the meshes in normal saline were prepared for the tests. The response of the animal's skin to the tested material was assessed on points of the scale published in standard PN-EN ISO 10993-10:2003 after 24, 48 and 72 hours and compared with a reference (0.9% sodium chloride). The skin treated with the extract remained unchanged compared with regions on which the reference was applied, hence the result on the scale is 0. The chitosan-coated surgical meshes do not cause any irritation and satisfy the requirement of the PN-EN ISO 10993-10:2003 standard.

The allergenic action was examined on 20 healthy guinea pigs according to standard PN-EN ISO 10993-10:2003 (Buehler's method). Small samples of the tested meshes were fixed onto the animals' skin along with hygroscopic gauze swabs as a reference. No hypersensitivity reaction occurred; the skin with affixed test samples showed a regular appearance and was no different in comparison with the reference. The animals in both groups (tested and reference) were regularly gaining weight.

The results confirmed that the chitosan-coated surgical meshes are non-allergenic to guinea pigs and the risk of allergenic reaction in humans is very low.

Summary and conclusions

The surgical mesh OPTOMESH™ Macropore (TRICOMED Co.) is a proven medical device

implemented in surgical praxis as an implant in the repair of hernia. It is made of polypropylene knitted yarn. The manufacturer, TRICOMED Co., has an interest in the further improvement of the material quality by conferring enhanced biocompatibility and partial degradability upon the device. Combining the synthetic basic material of OPTOMESH™ Macropore with natural polymers could provide the preparation of a material that is over a longer period even better tolerated by the human body, thanks to a partial absorption of the implant's constituents. The use of such modified surgical meshes is expected to eliminate some disadvantages of the non-resorbable implants.

It was resolved to combine the basic material OPTOMESH™ Macropore as a half product onto which a chitosan preparation would be deposited as the biocompatible and resorbable component.

Useful forms of chitosan formulated earlier at IBChF were chosen to serve the purpose, notably modified chitosan lactate (ChL), chitosan fibrils (ChF) and microcrystalline chitosan (MCCh). Preparations of the substances were deposited on the basic OPTOMESH™ by one-side coating or double-side padding. The optimal results were attained with MCCh one-side coating, and the amount of deposited chitosan at about 20 g/m². It was documented that the chitosan coating underwent biodegradation under the action of lysozyme, and that the biodegradation

products were water-soluble. Methods to sterilize and pack the ready products were examined and recommended.

The chemical and biological purity of the prepared modified surgical meshes was tested in accredited laboratories and adequate departments of medical academies. Tests made on animals showed that the modified implants did not exert any irritating or allergenic action. The modified surgical meshes show a slight cytotoxic effect upon mouse fibroblasts caused by the chitosan in the coating itself or the used plasticizer. This is a disadvantage of the new materials, though it does not limit its application in the implants.

The prepared composite surgical meshes, modified by both MCCCh and ChL, are candidates for commercialization. Both chitosan preparations can be manufactured according to IBChF proprietary technology and the depositing of the coating is to be made by known, simple techniques.



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