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Dressing Sponges Made of Chitosan and Chitosan-Alginate Fibrils

Abstract

This paper presents a manufacturing process for biological chitosan and chitosan-alginate dressing sponges as well as their biological and physical-mechanical properties. The aim of the research was the preparation of such sponges based on chitosan and chitosan-alginate fibrils prepared according to a method elaborated at Institute of Biopolymers and Chemical Fibres (IBWCh). The sponges ought to display physical (absorption ability) and biological features (cytotoxic and haemostatic properties) which would qualify the materials obtained for the healing of wounds throughout all healing phases.

Key words: chitosan, calcium alginate, microfibrils, freeze-drying, dressing sponges.

Introduction

For several years demand has been increasing for biological dressings capable of protecting the wound throughout the various healing phases [1]. Demands placed on dressings designed for healing during the granulation and epithelisation phase are, to a large extent, met by biomaterials that contain healing-stimulating polymers. Polysaccharides, chitosan and alginates in particular are, thanks to specific biological properties like haemostatic, granulation and epithelisation, ideal materials for the construction of dressings suitable for wounds during the various healing phases [2].

In the preparation of a bioactive dressing material, it is essential to select a proper bioactive polymer and a useful form for it.

Chitosan and chitosan-alginate micro- and nanofibrils are suitable for the construction of dressings in sponge or non-woven form.

Commercial dressings based on chitin, chitosan and alginates are offered in large assortment. So far, there have been no announcements in technical literature concerning fibrils used for the preparation of dressing sponges.

Kaltostat[®] (ConvaTec), Melgisorb[®] (Molnlycke), SeaSorb[®] (Coloplast) and Sorbsan[®] (UDL Laboratories, INC) are examples of alginate dressings which are offered as non-woven plates for surface wounds and ropes for deep wounds [1]. Calcium ions delivered from the alginates to the wound activate the platelets and accelerate homeostasis due the dressings having good exudate absorbency and haemostatic properties. Such materials are designed primarily for wound healing in the first phase.

Also well known are dressings based on chitin, chitosan and their derivatives. Japan and the USA are the biggest producers of such materials. JEX KK Co. produces dressing composites from synthetic resins and chitosan or from collagen and acetylochitosan [3, 4]. Eisai Co. offers chitin dressings in sponge form (chitopack S[®]), non-woven made of chitin-modified PET (chitopack P[®]) and cotton-chitosan non-woven (chitopack C[®]). The Japanese concern Nikita Co. sells a dressing non-woven made of chitosan fibres [5]. The University of Medical Center (USA) proposes the use of a chitosan dressing capable of accelerating skin regeneration after burns (II and III grade) [6]. The dressing contains an addition of (EFG) protein in calcium alginate micro-capsules, which acts as a growth factor. The American firm 3M offers a chitosan preparation in gel (Tegasorb[®]) or hydrocolloid form (Tegaderm[®]) designed for the healing of wide internal wounds [5]. Also available is a palette of chitosan-based haemostatic dressings that are like a sponge made of chitosan salt called HemCon (HemCon Medical Technologies, Inc.), a chitin sponge RDH (Marine Polymer Technologies, Danvers, MA), as well as a chitosan-modified cellulose non-woven under the trade name Syvek Patch (Marine Polymer Technologies) [7, 8].

The Sree Chitra Tirunal Institute for Medical Sciences & Technology, (India) has reported about an experimental chitosan-alginate dressing [9].

For several years research and development works have been conducted in biomaterials, and in particular polysaccharides at the Institute of Biopolymers and Chemical Fibres (Instytut Biopolimerów i włókien Chemicznych - IBWCh), Łódź, Poland for uses in medicine, pharmacy, and veterinary medicine [10–19].

This paper presents a manufacturing process for biological chitosan and chitosan-alginate dressing sponges as well as their biological and physical-mechanical properties. The aim of the research was the preparation of such sponges based on chitosan and chitosan-alginate fibrils prepared according to a method elaborated at IBWCh. The sponges ought to reveal physical (absorption ability) and biological features (cytotoxic and haemostatic properties) which would qualify the materials obtained for the healing of wounds throughout all healing phases.

Experimental

Materials

1. Chitosan:
As a starting material, chitosan from the Primex Co, whose trade name is Chito Clear FG90 was used, which is characterised by an average molecular weight (M_v) = 344 kD, a deacetylation degree (SD) = 82%, an ash content = 1.7%, and a heavy metal content = 0.0%.
2. Sodium alginate (Protanal 10/60), by Biopolymer Engineering, Inc.
3. Calcium chloride, analytically pure by POCh, S.A.
4. Plasticiser (glycerol), by Riedel.
5. Microfibrils.

The chitosan and chitosan-alginate microfibrils used were prepared at IBWCh according to a newly prepared method with the use of the flow reactor DISPAX REACTOR LABO-PILOT 2000/4 [20, 21]. The fibrils were characterised by:

Chitosan microfibrils:

chitosan content = 3.13%,
average molecular weight (M_v) = 314 kD,
deacetylation degree (DD) = 82%,
water retention value (WRV) = 2700%,
dimensions wet: length = 20–100 μ m,
diameter = 1–3 μ m,

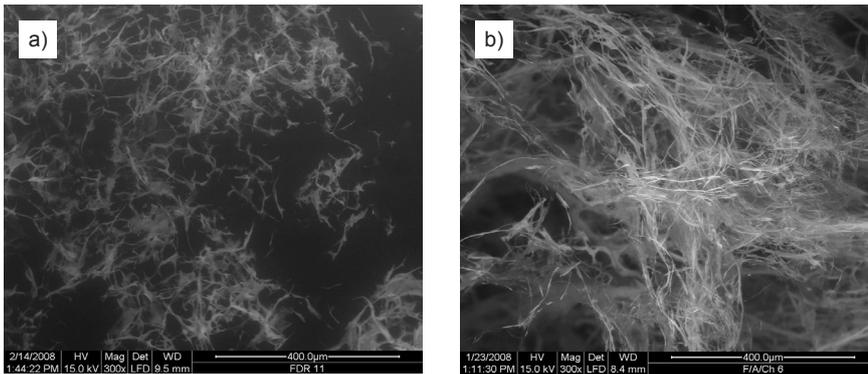


Figure 1. SEM images of the chitosan and chitosan-alginate microfibrils dry; a) chitosan microfibrils, b) chitosan-alginate microfibrils; magnification – 300×.

Table 1. Mechanical properties of the sponge dressings; * sponge from chitosan microfibrils, ** sponge from chitosan/alginate microfibrils with Ca addition.

Sponge type	Thickness, mm	Tenacity, MPa	Elongation at break, %
mFCh*	2.52	0.092	12.7
mFCh/25 kGy	2.50	0.102	11.3
MFCh/AlgCa**	2.51	0.057	10.0
MFCh/AlgCa /25 kGy	2.50	0.059	10.3

dimensions dry: length = 10 - 60 μm, diameter = 0.1-0.3 μm, (Figure 1).

Chitosan-alginate microfibrils:

content of solids = 1.8% (81% chitosan, 19% calcium alginate), WRV = 2850 %, dimensions wet: length = 30 - 200 μm, diameter = 1 - 4 μm, dimensions dry: length = 10 - 100 μm, diameter = 0.2-0.7 μm (Figure 1)

Methodology

Mechanical properties

The mechanical properties of dressing sponges were determined in the Accredited Laboratory of Metrology at IBWCh (certificate No. AB 388) in accordance with the following standards:

- thickness of sponge - PN-EN ISO 9073-2:2002,
- tenacity and elongation at break – PN- EN 29073-3:1994.

Evaluation of the sorption properties

The sorption properties of dressing sponges were determined according to the weighing method. 1.0 ml of DM water was poured into a container with a flat bottom, and the sponge sample was inserted in the water. The sample was 1×1 cm in size and weighed with 0.0001 g accuracy. After fixed time intervals (10, 30, and 180 minutes), the sample was taken out of the water and weighed. The sorption ability of the material was expressed as a sorption coefficient. The sorption co-

efficient was calculated by the following equation:

$$W = [(M_w - M_a) / M_a] \times 100\%$$

where: M_a – mass of dry sponge
 M_w – mass of sponge with water.

Evaluation of the internal and external structure of the dressing materials

The structure of the sponges was evaluated with the use of a SEM, type Quanta 200, made by FEI Co.

Sterilisation of dressing sponges

Sterilisation was performed at the Institute of Applied Radiation Chemistry, Technical University of Łódź, by irradiating the samples with a 25 kGy dose of γ radiation.

Cytotoxicity testing of the dressing sponges

This was done at the Department of Cellular Breeding of the Medical University of Wrocław in accordance with Standard. PN-EN ISO 10993-5 – ‘Biological Evaluation of Medical Devices. Cytotoxicity testing *in vitro*’ – March 2001. An indirect testing method was employed with the use of extracts.

Testing of the haemostatic properties of dressing sponges

This was accomplished at the Department of Experimental Surgery and Biomaterials Testing of the Medical University of Wrocław. The testing concerned the im-

part of the dressings on the clotting of blood *in vitro* with measurement of the APTT and PT times.

Results and discussion

Preparation of dressing sponges from chitosan and chitosan-alginate sponges with Ca ions.

The objective of the investigation was to estimate the suitability of chitosan microfibrils for the preparation of dressings in sponge form.

The sponges were prepared from a mixture composed of an aqueous suspension of chitosan or chitosan/alginate microfibrils (content of solid components at about 2.4%) and glycerol with a weight proportion of 1 : 0.5 (on dry polymer). Firstly, the preparation was carefully homogenised and next freeze-dried in a ALFA 1-4 lab freeze dryer, made by Christ Co, within a temperature range of (-20) to 10 °C and vacuum of 10 to 70 Pa for a period of 20 to 24 hours, depending upon the size of the charge.

This type of drying resulted in the preparation of sponges with a smooth surface without defects.

Physical- mechanical properties of the sponges

The physical - mechanical properties, sorption capacity and external structure of the prepared dressings were estimated before and after radiation sterilisation. Results are compiled in **Tables 1 – 3** and **Figures 1** and **2**.

From the results above, it may be concluded that γ ray sterilisation with a 25 kGy

Table 2. Estimation of the sorption capacity of sponges prepared from chitosan microfibrils (mFCh); * sterilized sponge.

Measurement time, min	Sorption coefficient, %	
	mFCh	mFCh/25 kGy*
10	186.1	631.5
30	265.2	807.8
180	311.3	822.0

Table 3. Estimation of the sorption capacity of sponges prepared from chitosan/alginate microfibrils containing Ca ions (mFCh/AlgCa); * sterilised sponge .

Measurement time, min	Sorption coefficient, %	
	mFCh/AlgCa	mFCh/AlgCa/ 25 kGy*
10	187.1	1612.1
30	260.1	1616.4
180	435.8	1750.2

dose does not inconveniently influence the mechanical properties. Sponges of chitosan/alginate microfibrils display about a 45% lower tenacity and slightly lower elasticity when compared with sponges made of chitosan microfibrils.

Results presented in **Tables 1** and **2** lead to the conclusion that the sorption capacity of both sponges increases with the detention time of the samples in the water bath. After 180 min. the chitosan/alginate sponge and the chitosan sponge absorbed 4.5 and 3 times more water than their initial weight, respectively. The good absorption properties can be explained by the porous internal structure, as well by the adopted form of the polymers.

It must be noted that the sorption capacity increases substantially after γ irradiation, which probably disturbs the integrity of the material. For the chitosan/alginate microfibril sponges, a 16-fold increase, compared to the initial weight, occurred after 10 and 30 min. caused by the imbibed water, while after 180 min the increase was 17-fold. For the chitosan microfibril sponges, the water absorption capacity after irradiation was lower by one half. After testing times of 10 and 30 min, a 6 - 8 - fold mass increase occurred compared to the initial mass; after 180 min. the increase was 8-fold. With reference to research results presented in [22] and the SEM inspection (see **Figures 2** and **3**), the reasons for the sponge sorption increase must be the 25 kGy γ irradiation, which induces changes in the internal and surface structure, resulting

Table 4. Toxicity grades for the direct contact test.

Grade	Toxicity	Description of changes in the culture
0	non	Single internal cytoplasmic granules, cellular lysis not found
1	insignificant	about. 20% of cells rounded,, shrunk, deglutinated from the base, without condensation of cytoplasm, single cells disrupted
2	moderate	about. 50% of cells rounded, without granules, wide lysis of cells and voids between cells
3	average	about. 70% of cells rounded, cells underwent lysis
4	strong	Cell culture almost destroyed

in more intensive porosity and, in consequence, a higher water absorption capacity. This is enhanced by the presence of alginate: a polymer with a higher susceptibility to γ irradiation than chitosan.

Biological testing of dressing sponges made of microfibrils

Testing of the cytotoxic action of sponge dressings

The testing was carried out on a reference cell line - a 3T3/ Balb mouse fibroblast. Two types of sponge were tested:

- a sponge of chitosan microfibrils (mFCh),
- a sponge of chitosan/alginate microfibrils (mFCh/AlgCa).

The sponges were first γ sterilised with a 25 kGy dose.

The quantitative and morphological changes that occurred after contact with the tested material were estimated after 24, 48, and 72 hours on a reverse contrast-phase microscope. The degree of toxicity was evaluated on basis of the changes occurring in the cell morphology, their survival rate and ability to proliferate, according to the criteria shown in **Table 4**. Test results are presented in **Table 5**.

The investigation showed that after 24, 48 and 72 hours of testing in the culture of mFCh/25kGy, the cells adhered to the base and maintained regular morphology features. No agglutination, vacuolation, detaching from the base or cell lysis was observed. The proliferation of cells after 24, 48 and 72 hours was insignificantly higher in comparison to the stock culture. The percentage of dead cells was identical to that of the stock culture.

In the culture with extracts from mFCh/AlgCa/25kGy dressings, the cells adhered to the base and revealed regular morphology features. No agglutination, vacuolation, detaching from base or cell lysis was observed. Proliferation of cells after 24, 48 hours was significantly higher, and after 72 hours it was insignificantly higher in comparison to the stock culture. The cells formed colonies covering the whole plate. After 24 and 48 hours no dead cells were found in the culture with extracts from the mFCh/AlgCa/25kGy dressings tested. The percentage of dead cells after 72 hours was identical to that of the stock culture.

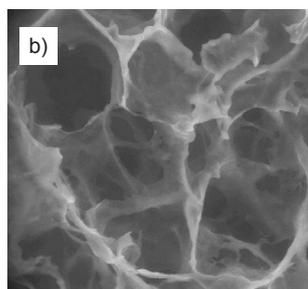
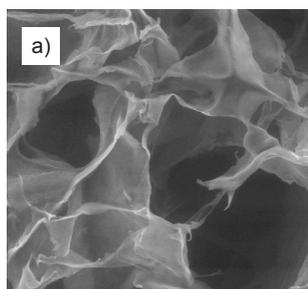


Figure 2. SEM photos of the surface of sponge made of chitosan-alginate microfibrils with Ca addition; sponge surface before (a) and after sterilisation (b); magnification 600 \times .

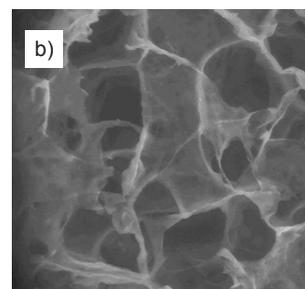
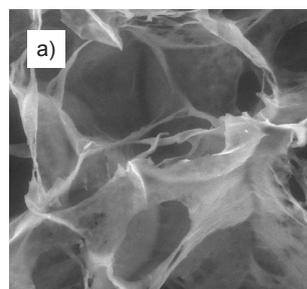


Figure 3. SEM photos of the surface of sponge made of chitosan microfibrils; sponge surface before (a) and after sterilisation (b); magnification 600 \times .

Table 5. Cytotoxic changes in 3T3Balb/C mouse fibroblast culture with control extracts and extracts from the dressing materials tested.

Culture	Testing time - 24 h			Testing time - 48 h			Testing time - 72 h		
	Morphological change	Dead cells, %	Degree of toxicity	Morphological change	Dead cells, %	Degree of toxicity	Morphological change	Dead cells, %	Degree of toxicity
0	Non	0	0				Non	2	0
0	Non	0	0				Non	2	0
0	Non	0	0				Non	2	0

Testing of the haemostatic properties of the dressing sponges

The *in vitro* investigation was aimed at estimating the impact of the mFCh/Alg/25kGy and mFCh/25kGy dressings on the plasmatic clotting system.

Citrate plasma was brought into contact with the materials tested, followed by the estimation of selected parameters of the plasmatic clotting system after 15, 30, 120 min and 4 hours.

Activation of the clotting system was estimated by conducting a APTT (Activated Partial Thromboplastin Time) and a PT (Prothrombin Time) test. Results are presented in **Table 6**.

From the results presented in Table 6, it can be seen that in the plasma after incubation with the mFCh/Alg/25kGy dressing, the APTT and PT time was shortened in comparison to the pure blood plasma reference. The changes that occur bear witness to the presence of material components in the plasma, which accelerate the activation of clotting agents in both the endogenous and exogenous systems. Calcium alginate is the accelerating component, which is contained in the microfibrils used in the preparation of the dressing. It is well known that the polymer manifests haemostatic properties excellently.

No significant changes were observed concerning the APTT and PT values of the plasma after contact with the mFCh dressing.

Conclusions

The sponges of chitosan and chitosan/alginate microfibrils prepared comply with the basic physical-mechanical and biological criteria for application as dressing materials for wound healing throughout the various healing phases.

1. The sponges have sufficient mechanical strength and a very good sorption capacity. The sponges made of chitosan/alginate microfibrils a 17-fold imbibition capability, whereas those made of chitosan microfibrils can absorb an 8-fold amount of water compared to their initial weight.
2. The cytotoxicity testing of the sponges made of both chitosan and chitosan/alginate microfibrils, with an addition of calcium, excluded undesired effects upon mouse fibroblasts 3T3 Balb/C.

Table 6. Time of partial thromboplastin after activation (APTT) and prothrombin time (PT) of the plasma after contact with mFCh/AlgCa/25 kGy, mFCh/25 kGy dressings, and those for, the plasma without dressing as functions of time.

Material	Time, min	APTT		PT		
		s	Ratio	s	%	INR
Dressing mFCh/AlgCa/25 kGy	15	29.81***+++ ±0.34	0.916***+++ ±0.005	11.90*+ ±0.24	0.895*+ ±0.207	112.83*+ ±2.32
	30	30.63***+++ ±0.51	0.936***+++ ±0.015	11.68***+++ ±0.18	0.883***+++ ±0.011	113.83***+++ ±1.17
	60	30.75***+++ ±0.61	0.933***+++ ±0.012	11.93***+++ ±0.42	0.907***+++ ±0.037	111.67***+++ ±3.38
	120	32.26***+++ ±1.07	0.995***+++ ±0.025	12.42***+++ ±0.49	0.942***+++ ±0.035	107.00***+++ ±3.68
	240	32.30***+++ ±0.23	0.993***+++ ±0.006	12.20***+++ ±0.21	0.923***+++ ±0.013	108.00***+++ ±1.00
Dressing mFCh/25 kGy	15	33.05 ±0.47	0.990 ±0.012	12.63 ±0.55	0.952 ±0.029	106.50 ±3.61
	30	33.36 ±0.81	1.022 ±0.029	12.33 ±0.29	0.933 ±0.018	107.33 ±1.966
	60	33.90 ±0.28	1.045 ±0.005	12.58 ±0.25	0.959 ±0.017	104.83 ±1.79
	120	35.95 ±1.09	0.933 ±0.051	13.25 ±0.29	1.006 ±0.025	99.66 ±2.44
	240	37.56 ±0.35	0.930 ±0.011	13.36 ±0.25	1.013 ±0.015	98.66 ±1.58
Reference without dressing	15	33.03 ±0.51	0.998 ±0.017	12.46 ±0.38	0.945 ±0.029	105.83 ±3.31
	30	33.78 ±1.15	1.030 ±0.028	12.42 ±0.32	0.958 ±0.027	106.66 ±2.42
	60	34.28 ±0.37	1.053 ±0.005	12.71 ±0.29	0.968 ±0.015	104.66 ±2.34
	120	35.82 ±1.04	1.092 ±0.021	13.40 ±0.39	1.006 ±0.025	100.00 ±2.60
	240	37.00 ±0.25	1.120 ±0.010	12.96 ±0.13	0.983 ±0.005	103.00 ±1.00

3. The sponge of chitosan/alginate microfibrils, with an addition of calcium in the *in vitro* contact with citrate plasma, activates the plasma clotting system to a higher degree, resulting in the shortening of the clotting time of both of the endogenous and exogenous systems when compared with the sponge made of chitosan microfibrils.

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