Dressing Sponges Made of Chitosan and Chitosan-Alginate Fibrids

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 fibrids 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, microfibrids, 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 bio-materials 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 nanofibrids 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 fibrids 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 alginites to the wound activate the platelets and accelerate haemostasis 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 an Chemical Fibres (Institytut 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 fibrids 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 (Mn) = 34 kDa, a deacetylation degree (SD) = 82%, an ash content = 1.7%, and a heavy metal content = 0.0%.
2. Sodium alginate (Prostan 10/60), by Biopolymer Engineering, Inc.
3. Calcium chloride, analytically pure by POCCh, S.A.
4. Plasticiser (glycerol), by Riedel.
5. Microfibrids.

The chitosan and chitosan-alginate microfibrids 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 fibrids were characterised by:

- **Chitosan microfibrids:** chitosan content = 3.13%, average molecular weight (Mn) = 314 kDa, deacetylation degree (DD) = 82%, water retention value (WRV) = 2700%, dimensions wet: length = 20-100 µm, diameter = 1 - 3 µm,
Suspension 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 coefficient was calculated by the following equation:

\[ W = \frac{(M_a - M_w)}{M_a} \times 100\% \]

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 Quant 200, made by FEI Co.

**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 coefficient was calculated by the following equation:

\[ W = \frac{(M_a - M_w)}{M_a} \times 100\% \]

where: \( M_a \) – mass of dry sponge, \( M_w \) – mass of sponge with water.

**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 \( \gamma \) ray sterilisation with a 25 kGy dose is effective for sterilising these dressings.

**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 impact 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 microfibrids 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 microfibrids (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.

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

<table>
<thead>
<tr>
<th>Sponge type</th>
<th>Thickness, mm</th>
<th>Tenacity, MPa</th>
<th>Elongation at break, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>mFCh*</td>
<td>2.52</td>
<td>0.092</td>
<td>12.7</td>
</tr>
<tr>
<td>mFCh/25 kGy</td>
<td>2.50</td>
<td>0.102</td>
<td>11.3</td>
</tr>
<tr>
<td>MFCh/AlgCa**</td>
<td>2.51</td>
<td>0.057</td>
<td>10.0</td>
</tr>
<tr>
<td>MFCh/AlgCa/25 kGy</td>
<td>2.50</td>
<td>0.059</td>
<td>10.3</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Measurement time, min</th>
<th>Sorption coefficient, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>mFCh</td>
<td>mFCh/25 kGy*</td>
</tr>
<tr>
<td>10</td>
<td>186.1</td>
</tr>
<tr>
<td>30</td>
<td>265.2</td>
</tr>
<tr>
<td>180</td>
<td>311.3</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Measurement time, min</th>
<th>Sorption coefficient, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>mFCh/AlgCa</td>
<td>mFCh/AlgCa/25 kGy*</td>
</tr>
<tr>
<td>10</td>
<td>187.1</td>
</tr>
<tr>
<td>30</td>
<td>260.1</td>
</tr>
<tr>
<td>180</td>
<td>435.8</td>
</tr>
</tbody>
</table>
dose does not inconveniently influence the mechanical properties. Sponges of chitosan/alginate microfibrids display about a 45% lower tenacity and slightly lower elasticity when compared with sponges made of chitosan microfibrids.

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 microfibrid sponges, a 16-fold increase, compared to the initial weight, occurred after 10 and 30 min. caused by the imbied water, while after 180 min. the increase was 17-fold. For the chitosan microfibrid 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 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 radiation than chitosan.

**Biological testing of dressing sponges made of microfibrids**

**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 microfibrids (mFCh).
- A sponge of chitosan/alginate microfibrids (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 the 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.

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### Table 4. Toxicity grades for the direct contact test.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Toxicity</th>
<th>Description of changes in the culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>non</td>
<td>single internal cytoplasmic granules, cellular lysis not found</td>
</tr>
<tr>
<td>1</td>
<td>insignificant</td>
<td>about. 20% of cells rounded, shrunk, deglutinated from the base, without condensation of cytoplasm</td>
</tr>
<tr>
<td>2</td>
<td>moderate</td>
<td>about. 50% of cells rounded, without granules, wide lysis of cells and voids between cells</td>
</tr>
<tr>
<td>3</td>
<td>average</td>
<td>about. 70% of cells rounded, cells underwent lysis</td>
</tr>
<tr>
<td>4</td>
<td>strong</td>
<td>cell culture almost destroyed</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Culture</th>
<th>Testing time - 24 h</th>
<th>Testing time - 48 h</th>
<th>Testing time - 72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Morphological change</td>
<td>Dead cells, %</td>
<td>Degree of toxicity</td>
</tr>
<tr>
<td>0</td>
<td>Non</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>Non</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>Non</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
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 microfibrids used in the preparation of the dressing. It is well known that the polymeric 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 microfibrids 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 microfibrids a 17-fold imbibition capability, whereas those made of chitosan microfibrids 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 microfibrids, with an addition of calcium, excluded undesired effects upon mouse fibroblasts 3T3 Balb/C.
3. The sponge of chitosan/alginate microfibrids, 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 the endogenous and exogenous systems when compared with the sponge made of chitosan microfibrids.

### Acknowledgment

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### References

Additional Information about:

**EL-TEX 2008 Symposium**

**‘Electrostatic and Electromagnetic Fields New Materials and Technologies’**

26 - 27 November 2008, Łódź, Poland.

Opening of the Symposium - Jolanta Mamenas, M. Sc. Eng, Director of the Textile Research Institute, Łódź

Two plenary sessions, a poster session (P) and five sessions devoted to the following topics are provided:

- **Electrostatic phenomena (A)**
- **Methods protecting against electrization (B)**
- **Test methods (C)**
- **New materials (D)**
- **Textiles of special properties (E)**

**Plenary lectures:**

- **Eckhard Schollmeyer, Thomas Bahners, Uwe Schlosser, Modern Concepts of Electrical and Optical Integrated Sensors in Textiles**
- **Halina Aniołczyk, Electromagnetic Fields in Natural and Occupational Environment of a Modern Man - Assessment of Exposure and the Need of its Limitation**
- **Jerzy Kołodziejski, The Effects of Electric Overstress(EOS) and Electrostatic Discharges (ESD) in Semiconductive Instruments and Systems - Selected Issues**
- **Jan Vrba, Milan Stejskal, Marika Pourová, Jaroslav Fábera, Ondřej Žák, Microwave Drying of Textiles**

Additional information of the EL-TEX Symposium you will also find on pages 107 and 116.

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