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HEMOSTATIC, RESORBABLE DRESSING OF NATURAL POLYMERS- HEMOGUARD

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Abstract:

Investigations are presented for the preparation of a model hemostatic dressing that would exhibit an adequate hemostatic capacity in injuries and surgical wounds, an antibacterial activity to prevent primary and secondary infections, and offer safety in use. The Hemoguard dressing has been designed as a powder prepared from the complex chitosan/alginate Na/Ca in the form of micro- and nano-fibrids. Useful antibacterial and hemostatic properties of Hemoguard, which would qualify the material as first aid dressing and a temporary protection of injury wounds in field conditions, were assessed. Biocompatibility of the dressing was confirmed by biological in vitro examinations.

Keywords:

hemostatic, chitosan-alginate micro- and nano-fibrids, antibacterial activity

1. Introduction

An increasing demand has been observed lately in the medical market for new generation hemostatic dressings for first aid and temporary protection of injuries in field conditions. The demand is fueled by ever more frequent communication and terrorism accidents and building catastrophes.

The dressing of a new generation is expected to fulfill demands of an adequate hemostatic capacity in injuries and surgical wounds, antibacterial activity to prevent primary and secondary infections, and to offer safety in use.

Since many years, in the Institute of Biopolymers and Chemical Fibers (IBWCh), investigations are in progress in biomaterials with focus on polysaccharides for uses in medicine, pharmacy, and veterinary [1–10].

Presented here is a method of production and the assessment of applicable antibacterial and biological properties of a dressing in powder form called Hemoguard, which is expected to exhibit the ability to instantaneously control bleeding and offer safety of use.

Aim of the research was the preparation of a model of hemostatic dressing based on micro- and nano-fibrids of the chitosan/alginate Na/Ca complex. Its properties confirmed by *in vitro* and *in vivo* testing offer a chance of preparing a material suitable to the healing of injury and surgical wounds in field conditions.

2. Materials

Chitosan (Virgin chitosan ChitoClear hqg 95 from Primex ehf Iceland) characterized by: average molecular mass (M_v) = 373 kDa, deacetylation degree (DD) = 81%, ash content = 0.31%; Sodium alginate (Protanal LF 10/60 FT, from FMC Co); Calcium chloride anhydrous analytically pure (POCh, Co., Poland); Sodium hydroxide (Sigma-Aldrich Co); Lactic acid 88% analytically pure, by Avantor Performance Materials, Poland.

Commercial dressings: Celox™ and QuikClot ACS+™

The complex chitosan/alginate Na/Ca in the form of micro- and nano-fibrids named Hemoguard was prepared according to own IBWCh method [11–12] in which the flow reactor Dispax Reactor Labor- Pilot 2000/4 is used. The complex was featured by: polymer content (solid)-3.5–4.0% in the proportion of 80% chitosan, 20% alginate Na/Ca, water retention value = 1860%, particle dimensions wet: length-10–100 μm, dia.-1–3 μm, dimensions dry: length-1.5–24 μm, dia.-0.2–0.9 μm

3. Methods

3.1. Preparation of the dressing in powder form employing spray- and freeze-drying techniques

The dressing in powder form containing chitosan/alginate Na/Ca micro- and nano-fibrids was prepared by two methods: freeze-drying with the use of the lyophilizer Alpha 2-4 LSC of Christ GmbH, Germany, and spray-drying with the dryer Spray Dryer B-290 from Büchi Co. The freeze-drying proceeded for

20 to 24 hours at temperature from (−20) to 10°C and vacuum from 0.1 to 0.7 mbar. The prepared dry micro- and nano-fibrids were then disintegrated by means of the laboratory mill M-20, from IKA Werke. Conditions of the spray-drying: temperature of the drying head-210°C, temperature of circulating air-95°C, feeding rate 2.5–3 ml/min.

3.2.Testing of useful properties of the dressing in powder form

Dressings in powder form were tested in the Laboratory of Metrology of the Institute of Security Technologies "Moratex" according to following standards [13–14].

3.3. Examination of the dressing structure

The scanning electron microscope Quanta 200, FEI Co. (USA) served to assess the structure of the dressings. The software Analysis Docu from Soft Imaging System Co enabled the measurement of the powder particle dimensions.

3.4. Sterilization of the dressing

The dressings were sterilized in Institute of Applied Radiation Chemistry. Irradiation dose was 25 kGy.

3.5. Biological tests *in vitro*

3.5.1. Examination of antibacterial activity

The antibacterial activity of the dressings was tested in the Accredited Laboratory of Microbiology of IBWCh [15]. The strains *Escherichia coli (ATCC 11229)* and *Staphylococcus aureus (ATCC 6538)* from the American Collection of Pure Cultures were used in the testing.

3.5.2. Examination of hemostatic properties

The materials were biological examined in the Department of Experimental Surgery and Biomaterials Research, Wroclaw Medical University. The biological examinations comprised the assessment of hemolytic action, plasmatic activation of coagulation system [16–19]. The tests were performed on a nameless human blood group 0 Rh+ taken for preservative fluid CPD (citrate-phosphate-dextrose). Consent of Bioethical Commission of Wroclaw Medical University was obtained for the tests (No.KB-319/2013).

4. Results and discussion

4.1. Preparation of dressing materials in the form of powder from micro- and nano-fibrids containing Ca ions

It was studied if the micro- and nano- forms of polymers prepared at IBWCh were suitable to the manufacture of powder-form dressing materials. Structure and particle size of the prepared powder material were SEM- analyzed and compared with commercial reference products. Results are presented in Figure 1.

The results indicate that the prepared method offers the chance of obtaining polymeric materials in powder form with a well developed internal surface and with particle size in the micro- and nano-range. After spray-drying, a dressing material was obtained in powder form containing beads sized 0.9–6.0 μm, while particle size of 10–60 μm resulted from freeze-drying. Particle size in commercial dressings offered in form of powder or granules was: in Celox ™ – 0.1–0.9 mm (powder) and in QuikClot ACS+™ – 1.8–2.0 mm (granules).

4.2. Assessment of useful properties

In the Laboratory of Metrology of the Institute of Security Technologies, the dressings were examined with respect to the absorption that occurs at free imbibition, and moisture vapour transmission rate (MVTR). Absorption is a parameter to assess proper functioning of the dressing when applied on medium or profuse exudate wounds. MVTR gives an estimation of the permeability of materials that permits water molecules to pass from the skin to atmosphere at given humidity and temperature.

Examined were dressings prepared by spray- and freeze-drying. They contained micro- and nano-fibrids from the complex sodium–calcium chitosan/alginate. Commercial hemostatic dressings

Celox ™ and QuikClot ACS+™ served as reference. The materials were sterilized with fast electrons at dose of 25 kGy. Results of the investigation are compiled in Table 1.

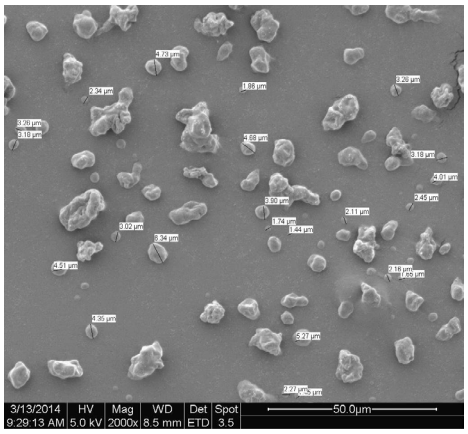
The results lead to the conclusion that both the freeze-dried dressing FDR/L/Chit/Alg Na/Ca and the spray-dried one FDR/R/Chit/Alg Na/Ca exhibit adequate useful properties, that is, absorption and MVTR. The two parameters are close to each other in the two materials. Absorption of the two prepared dressings is only about half of that of the commercial Celox™, while MVTR is about two times higher than in the commercial product. The results are an indication of what can be expected in useful properties and safety of use.

4.3. Assessment of antibacterial properties.

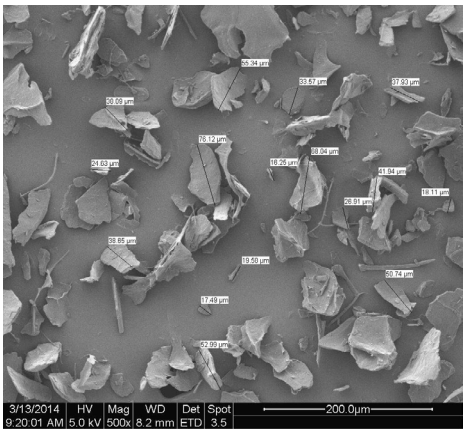
Results of the antibacterial activity testing of the two prepared dressings in comparison to commercial products are shown in Table 2.

Results presented in Table 2 witness that both prepared new dressings and the commercial ones used as reference exhibit a higher bactericidal activity against bacteria Gram (−) *Escherichia coli* than bacteria Gram (+) *Staphylococcus aureus.* The spray-dried dressing FDR/R/Chit/Alg Na/Ca is much more active than the freeze-dried FDR/L/Chit/Alg Na/Ca and the commercial products Celox™ and QuikClot ACS+™.

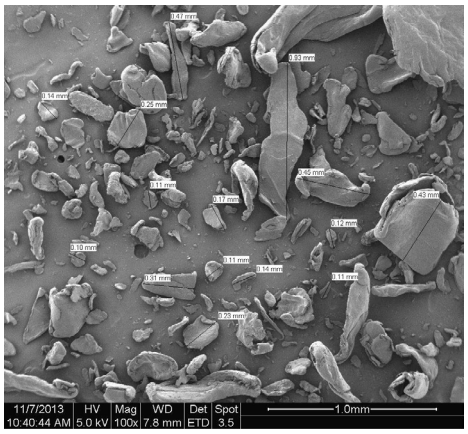
It may be assumed that it relates to the way the dressing material is dried in result of which a dressing is prepared as a powder with bead-shaped particles and to the mechanism according to which the dressing reacts upon the cell wall of the bacteria.



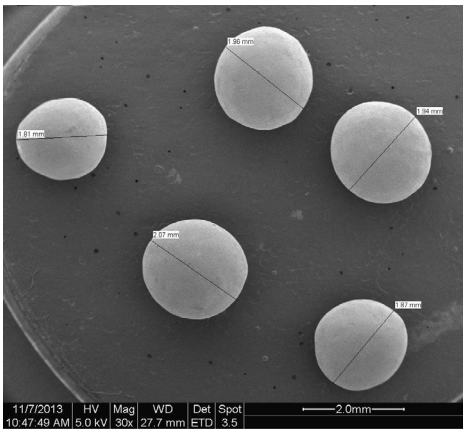
SEM of powder dressing FDR/R/Chit/Alg Na/Ca after spray-drying (mag. 2000 x)



SEM of powder dressing FDR/L/Chit/Alg Na/Ca after freeze-drying (mag.500 x)



SEM of commercial powder dressing Celox™, (mag.100 x)



SEM of a dressing in granules QuikClot ACS+™, (mag.30 x)

Figure 1. Assessment of the surface structure and particle size of selected dressing and commercial product

Table 1. Assessment of useful properties of dressing materials in powder form.

Materials	Absorption at free imbibition [per 1 g of sample]	MVTR [g. $m^{-2}.24^{-1}$]
FDR/L/Chit/Alg Na/Ca	8.70	4717
FDR/R/Chit/Alg Na/Ca	8.10	4581
Celox™	17.70	2556
QuikClot ACS+™	0.20	1558

L-freeze-dried
R-spray-dried

Table 2. Antibacterial activity of the prepared dressings in powder form and of commercial products (quantitative method).

Materials	Bacteria	Bacteriostatic activity	Bactericidal activity
FDR/L/Chit/Alg Na/Ca	*Staphylococcus aureus* ATCC 6538 Bacteria Gram (+)	0.60	−1.10
FDR/R/Chit/Alg Na/Ca		5.70	3.30
Celox™		3.80	1.50
QuikClot ACS+™		2.90	0.60
FDR/L/Chit/Alg Na/Ca	*Escherichia coli* ATCC 11229 Bacteria Gram (−)	5.70	2.60
FDR/R/Chit/Alg Na/Ca		6.80	3.70
Celox™		6.70	3.60
QuikClot ACS+™		2.80	−0.30

L-freeze-dried
R-spray-dried

From literature reports, it may be assumed that a relation exists between the chitosan antimicrobial activity and the characteristic of the bacteria cell wall. It was found that chitosan exerts a higher bactericidal activity against bacteria Gram (−) than Gram (+) because of the composition of phospholipids and carboxylic acids that are contained in the cell walls of bacteria [20–21].

4.4. Assessment of hemostatic properties

Two dressing materials prepared within the project: FDR/L/Chit/Alg Na/Ca (freeze-dried) and FDR/R/Chit/AlgNa/Ca (spray-dried) were examined in comparison to commercial materials Celox™ and QuikClot ACS+™, which served as reference. The examination was carried out with human full blood 0 Rh+ collected. The assessment was grounded on the examination of hemolytic activity-after a temporary contact of an extract from the dressing with full blood, computing of hemolytic index, definition of hemolytic class [16]. Examination *in vitro* of the plasma clotting system after a temporary contact of the dressings with full blood and observation of clot forming and fibrinolysis [16–19].

4.4.1.Examination of hemolytic properties

The hemolytic activity was tested after a temporary contact of the dressing samples and of extracts from the dressings with full blood. The examination was made with the use of isotonic extracts from the tested dressings, which were put for 4 hours to a contact with diluted full blood at 37°C.

Indicate hemolytic index (H. in the range of 2–5 in the dressings FDR/R/Chit/Alg Na/Ca (H.I./4.18) and Celox™ (H.I./3.23), which results in hemolytic grade named “slightly hemolytic.” In the freeze-dried dressing FDR/L/Chit/Alg Na/Ca (H.I./0.03) and the commercial QuikClot ACS+™ (H.I./0.06), the hemolytic index fell into the appropriate 0–2 range indicating absence of hemolysis.

4.4.2. Examination in vitro of the plasmatic clotting system

The prepared dressings FDR/L/Chit/Alg Na/Ca and FDR/R/Chit/Alg Na/Ca, and commercial materials were *in vitro* examined with the purpose of determining their impact upon the plasmatic clotting system. The commercial granulated product QuikClot ACS+™ was, prior to testing, milled to powder.

The citrate plasma was joined together with the tested materials at a ratio of 0.0030 g/2 cm³ full blood, and after 15 and 30 minutes, the selected parameters of the plasmatic clotting systems were estimated.

The activation of the plasmatic clotting system, which depends on the contact factors (endogenous system), was estimated on base of the tests: APTT-partial thromboplastin time after activation. Activation of the clotting system depending on the tissue thromboplastin (exogenous system) was assessed by PT-prothrombin time. The measurement TT-thrombin time is characteristic to both systems. The fibrinogen-Fb concentration was marked. Results of the examinations are presented in Table 3.

The presented results indicate that with the freeze-dried dressing FDR/L/Chit/Alg Na/Ca, the PT was shortened, concentration of Fb decreased and the APTT and TT values were comparable with the reference.

With the dressings FDR/R/Chit/Alg Na/Ca, APTT and PT were shortened, Fb concentration was decreased. The observed changes witnessed in the plasma were the presence of the material components accelerating the activation of clotting factors both of the endogenous and exogenous systems. In the commercial dressing Celox™, an extension of the PT and TT was observed. After a contact with dressing QuikClot ACS+™ both in form of beads and powder, the values of APTT, PT, TT, CT in the plasma were reduced slightly exceeding the reference range.

Table 3. Examination of plasma clotting activation after a temporary contact of the dressing with full blood.

Materials	Activation of clotting							
	APTT		PT		TT		Fb	
	15¢	30¢	15¢	30¢	15¢	30¢	15¢	30¢
FDR/L/Chit/Alg Na/Ca	N	↓ *	↑	N	↑ #	N	↓ #	↓
FDR/R/Chit/Alg Na/Ca	↓ *	sk.	↓ #	sk.	↑ #	sk.	↓ #	sk.
Celox™	N	N	↑ #	N	N	N	N	N
QuikClot ACS+™ (granules)	↓ *	↓ #	↓ #	↓ #	N	N	↓ #	↓ #
QuikClot ACS+™ (powder)	↓ *	↓ #	↓ #	↓ #	↑ *	↓ *	↓ #	↓ #
Plasma/control	N	N	N	N	N	N	N	N

APTT-time of partial thromboplastin after activation; PT-prothrombin time; TT-thrombin time; Fb-fibrinogen
N-conforms to standard; ¯-time shortened, decrease of value; -time prolonged, increase of value; sk-clot
**-changes in the range of reference values; #-value in slight excess of reference values*

4.4.3. Macroscopic evaluation of clotting process and clot fibrinolysis

The process of clot formation and fibrinolysis breakdown with the examined dressings is presented in Table 4.

Macroscopic observation of the clot formation in citrated plasma with the tested dressings and in plasma without the dressing was carried out after addition of 25 mmol/L of calcium chloride at a temperature of 37°C. After the formation of a complete clot, it was observed at temperature 37°C and next, the liquefaction -fibrinolysis process of the formed clots.

Clot formation was fastest in the plasma with samples of the commercial dressing QuikClot ACS+™; fibrinolysis in that case was quick, too. From the materials prepared within this project, the spray-dried FDR/R/Chit/Alg Na/Ca was the first to initiate clot forming. The process was prolonged clotting. The forming of the clot and its decomposition-fibrinolysis were longest when the commercial material Celox™ was applied. In the case of dressing FDR/R/Chit/Alg Na/Ca, clotting was comparable with the reference while fibrinolysis was prolonged in comparison to reference and dressing FDR/L/Chit/Alg Na/Ca.

4. Conclusions

1. The outcome of the investigation are modern hemostatic material in the form of powder prepared from chitosan and alginate micro- and nano-fibrids. The materials satisfy demands of applicable, antibacterial, and hemostatic properties, which make them potential candidates for the construction of modern, functional dressings designed for first aid and a temporary protection of injuries and post-surgical wounds in field conditions.

2. Two differently prepared dressings marked FDR/R/Chit/Alg Na/Ca and FDR/L/Chit/Alg Na/Ca exhibit adequate useful properties, that is, absorption and transmission of moisture (MVTR). Ability to imbibe fluids is half of that of the reference commercial Celox™, while the MVTR parameter is of double value.

3. The FDR/R/Chit/Alg Na/Ca dressing prepared by spray-drying reveals a much higher antibacterial activity than the freeze-dried FDR/L/Chit/Alg Na/Ca and the reference Celox™ and QuikClot ACS+™ dressings.

4. Both FDR/L/Chit/Alg Na/Ca and FDR/R/Chit/Alg Na/Ca dressings show adequate hemostatic properties. In a direct contact with plasma, they activate clot formation faster than the commercial Celox™ material.

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Table 4. The process of clot formation and fibrinolysis of the clot.

Materials	Sequence of clot formation				Sequence of fibrinolysis
	becomes turbid	thread fibrin clot	thread fibrin	clot	
FDR/L/Chit/Alg/CMC	6	7			4
FDR/R/Chit/Alg/CMC	4		8	11	5
Celox™	9		10	12	6
QuikClot ACS+™ (granules)		2			3
QuikClot ACS+™ (powder)		1			1
Plasma	3			5	2

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