HAEMOSTATIC, RESORBABLE DRESSING OF NATURAL POLYMERS - HEMOGUARD

Magdalena Kucharska[1*], Maria Wiśniewska-Wrona[1], Kinga Brzoza-Malczewska[1], Marcin H. Struszczyk[2], Magdalena Cichecka[2], Bożena Wilbik-Hałgas[2], Zbigniew Rybak[3], Maria Szymonowicz[3], Danuta Paluch[3], Krystyna Guzińska[1], Dorota Kaźmierczak[1]

[1]Institute of Biopolymers and Chemical Fibres,
ul. M. Skłodowskiej-Curie 19/27, 90-570 Łódź, Poland
e-mail: sekretarz_naukowy@ibwch.lodz.pl

[2]Institute of Security Technologies „MORATEX",
ul. M. Skłodowskiej-Curie 3, 90-965 Łódź, Poland

[3]Department of Experimental Surgery and Biomaterials Research,
Wroclaw Medical University,
ul. Poniatowskiego 2, 50-326 Wrocław, Poland

Abstract

Investigations are presented in the preparation of a first aid haemostatic dressing that would exhibit an adequate haemostatic capacity in injuries and surgical wounds, an antibacterial activity to prevent primary and secondary infections, and offer safety in use.

Key words: *haemostatic, natural polymers, biological and useful properties*

Received: 11.05.2015
Accepted: 10.06.2015

1. Introduction

An increasing demand has been lately observed in the medical market for new generation haemostatic dressings for first aid and temporary protection of injuries in field conditions. The clinical need is fueled by ever more frequent transport accidents, building catastrophes or terrorism risk. A new generation dressing is expected to fulfil demands of adequate haemostatic capacity in injuries and surgical wounds, antibacterial activity to prevent primary and secondary infections, and safety in use.

There is a number of dressing materials available on the medical market, with content of natural polymers designed for the healing of wounds throughout the entire healing process. Most popular are dressings prepared on the basis of chitosan and alginate [1,2,3,4]. Commercial dressings: Kaltostat® (ConvaTec), Melgisorb® (Mölnlycke), SeaSorb® (Coloplast) and Sorbsan® (Maersk) [5] count among the alginate-based materials. They are offered as nonwovens in the shape of either a plate for surface injuries, or a cord for deep wounds. High absorption and haemostatic capacity of the materials are due to calcium ions which, released from the alginate to the wound, activate platelets and accelerate haemostasis. The dressings are primarily intended for the first phase of healing [6,7].

Another group of dressing materials is based on chitin derivatives, like chitosan. It includes commercial dressings such as: HemCon (HemCon Medical Technologies, Inc.), RDH (Marine Polymer Technologies), Syvek Patch (Marine Polymer Technologies), Clo-Sur P.A.D. (Sion Cardin-Vascular, Inc.), ChitoSeal (Abbot), Traumastat (Ore-Medix), Excel Arrest (Hemostasis LLC Co.) [8, 9,10]. HemCon exhibits haemostatic action by direct adhesion to the wound, thus accelerating the incorporation of erythrocytes to the growing clot. Clo-Sur PAD, Syvek Patch and ChitoSeal are available in a form of haemostatic patches with chitosan as active substance.

There is also a first aid dressing TROMBOGUARD®, deployed into production by the national company TRICOMED S.A. in Lodz [11,12]. Moreover, Sam Medical company offers chitosan haemostatic dressing called Celox™ [13-14]. It is available in granulated form or in applicator. In literature, there is also a dressing reported in a form of foam applied by means of a pressurized container. It is being developed by the company Remedium Inc. and is presently in preclinical and clinical testing [15].

For many years, in the Institute of Biopolymers and Chemical Fibers (IBWCh) investigations in biomaterials have been in progress, with focus on polysaccharides for application in medicine and pharmacy [16-26]. This article reports the production method and assessment of performance, haemostatic and antimicrobial activity of model dressings in powder form, made from chitosan/alginateNa-Ca complex and/or chitosan/alginateNa-Ca/CMC complex in the form of micro- and nanofibrids. Evaluation of selected properties of model dressings were carried out in comparison to clinically used dressings of similar form (Celox™, QuikClot ACS+™).

2. Materials and Methods

2.1. Materials

1. Chitosan: virgin chitosan ChitoClear hqg 95, Primex ehf., Iceland, average molecular mass (M_v) = 373 kDa, deacetylation degree (DD) = 81%, ash content = 0,31%.
2. Sodium alginate Protanal LF 10/60 FT, FMC Co.
3. Sodium carboxymethylcellulose, Sigma-Aldrich Co.
4. Calcium chloride anhydrous, analytically pure, POCh Co., Poland.
5. Sodium hydroxide, Sigma-Aldrich Co.
6. Lactic acid 88% analytically pure, Avantor Performance Materials, Poland.
7. Commercial dressings: Celox™ and QuikClot ACS+™.

2.2. Methods

2.2.1. Testing of application properties of the dressings in powder form

Dressings in powder form were tested in the Laboratory of Metrology at the Institute of Security Technologies "Moratex" according to following standards:
- PN-EN 13726-1: 2005 - "Methods for the direct testing of wounds. Part 1: Aspects concerning absorption"
- PN-EN 13726-2:2005 - "Methods for the direct testing of wounds. Part 2: Transmission of moisture through the dressing with a semi-permeable film" for model dressing"

2.2.2. Examination of the structure of dressings in powder form

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.

2.2.3. Sterilization of the dressings in powder form

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

2.2.4. Examination of antibacterial activity of dressings in powder form

The antibacterial activity of the dressings was tested in the Accredited Laboratory of Microbiology of IBWCh according to procedure PB 1 "Examination of antibacterial action of textile products. Quantitative test" prepared on the basis of JISL 1902:2002. The strains *Escherichia coli* (ATCC 11229) and *Staphylococcus aureus* (ATCC 6538) from the American Collection of Pure Cultures were used in the testing.

2.2.5. Examination of haemostatic properties of dressings in powder form

The dressings were examined in the Department of Experimental Surgery and Testing of Biomaterials of Medical University in Wroclaw, according to standards: EN ISO 10993-12:2009; PN-EN ISO 10993-4:2006; ASTM, F 756-08:2008; ASTM, F 756-00:2000.
The examination comprised of assessment of haemostatic capacity of the dressing:

DOI: 10.15259/PCACD.20.12

a) after a temporary contact of an extract from the dressings with full blood – estimation of haemoglobin concentration, computing of haemolytic index, definition of haemolytic class,
b) *in vitro* examination of the plasma clotting system after a temporary contact of the dressings with full blood and clot formation and fibrinolysis of the clot.

3. Results and discussion

3.1. Preparation of dressing materials in the form of powder

Binary fibrids: FDR/Chit/AlgNa-Ca, containing: chitosan 80%wt., AlgNa-Ca 20%wt., and ternary fibrids: FDR/L/Chit/AlgNa-Ca/CMC of composition: chitosan 75%wt., AlgNa-Ca 15%wt., CMC 5%wt. were used to produce dressing material in powder form. The fibrids were obtained by wet molding using a flow reactor in accordance with the method developed in IBWCh [19, 20]. The resulting polymer forms were subjected to freeze drying and spray drying. The process of drying by the freeze drying technique was performed using laboratory freeze-dryer Alpha 2-4 LSC Christ GmbH. Lyophilization time was 20-22h in the vacuum range of 0.1-0.42 mbar. After lyophilization, the dried polymer was subjected to the grinding process. Spray drying of the slurry of fibrids was conducted using a spray-dryer Büchi Mini Spray Dryer B-290. The drying process was conducted using a nozzle ϕ 0.7. Spray drying parameters were: dosage 5-7 ml/min, inlet temperature 210 ^{o}C, outlet temperature 94-96 ^{o}C, aspirator flow 37 m^3/h, spray flow 357 L/h.

Collected material in powder form was assessed in respect of the structure and particle size. These studies were carried out using a scanning electron microscope Quanta 200, FEI Co. (USA), and the measurement of particle size was performed on the basis of Analysis Docu program adapted to operate in Quanta. Results are presented in Fig. 1.

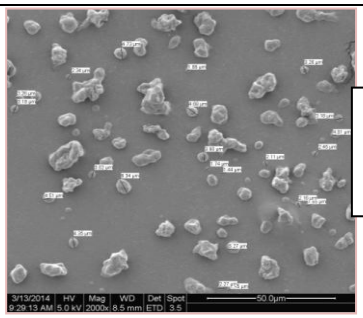
Studies have shown that the developed method of production enable to obtain polymeric materials in powder form of proper internal surface area and particle size at the micro- and nanometric level.

As a result of spray drying a model dressing material in powder form was obtained (FDR/R/Chit/AlgNa-Ca and FDR/R/Chit/AlgNa-Ca/CMC) having a particle size of 0.9-6.0 μm and shape similar to commercial dressing QuickClot ACS+^{TM}.

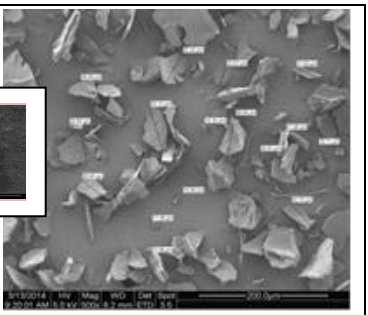
Freeze drying allowed to obtain dressings (FDR/L/Chit/AlgNa-Ca and FDR/L/Chit/AlgNa-Ca/CMC) having a shape similar to commercial dressing Celox^{TM} and the particle size at the level of 10-60 μm. The particle sizes of commercial dressings were: 0.1-0.9 mm for the Celox ^{TM} powder, and 1.8-2.0 mm for QuikClot ACS+^{TM} granules.

3.2. Assessment of useful properties of dressings in powder form

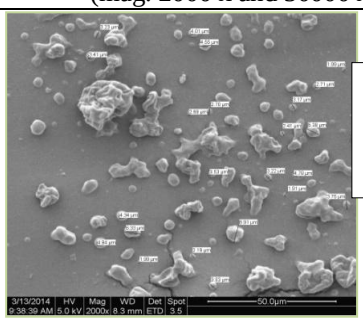
The model dressings in the powder form: FDR/R/Chit/AlgNa-Ca and FDR/R/Chit/AlgNa-Ca/CMC obtained by spray drying, and FDR/L/Chit/AlgNa-Ca and FDR/L/Chit/AlgNa-Ca/CMC obtained by freeze drying, were evaluated



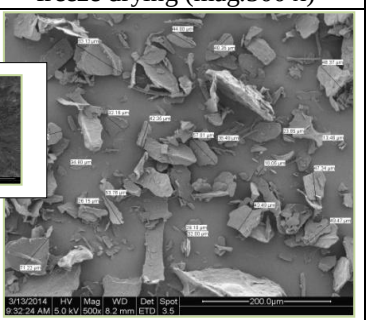
SEM of powder dressing FDR/R/Chit/AlgNa-Ca after spray drying (mag. 2000 x and 50000 x)



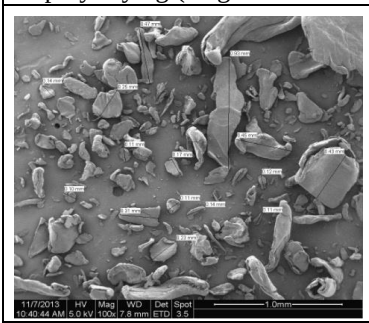
SEM of powder dressing FDR/L/Chit/AlgNa-Ca after freeze drying (mag.500 x)



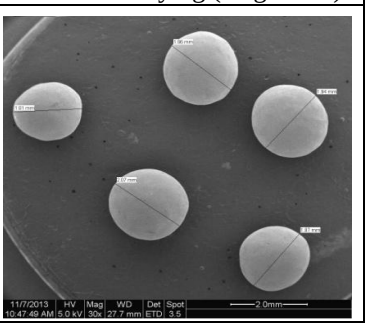
SEM of powder dressing FDR/R/Chit/AlgNa-Ca/CMC after spray drying (mag. 2000 x and 50000 x)



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



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



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

Figure 1. Assessment of the surface structure and particle size of selected dressings and commercial products

DOI: 10.15259/PCACD.20.12

in respect to operating parameters including the free soaking absorbency and moisture vapor transmission rate (MVTR) for the dressing in contact with steam.

The absorption capacity is a parameter that allows to assess the proper operation of dressings used on abundant or moderately oozing wounds, and MVTR is the parameter determining the permeability of the material. Assessment of the performance of model dressings was performed in accordance with standards: EN 13726-1:2005 "Test methods for primary wound dressings. Part 1: Aspects of absorbency.: In this study, the control were commercial haemostatic dressings Celox ™ and QuikClot ACS+™. The materials were sterilized with fast electrons at dose of 25 kGy. Results of the investigation are compiled in Tab. 1.

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

Type of dressing	Absorption at free imbibition [per 1 g of sample]	Moisture vapor transmission MVTR [g. $m^{-2}.24^{-1}$]
FDR/L/Chit/Alg Na-Ca	8.7	4717
FDR/R/Chit/AlgNa-Ca	8.1	4581
FDR/L/Chit/AlgNa-Ca/CMC	7.9	4602
FDR/R/Chit/AlgNa-Ca/CMC	7.7	4401
Celox™	17.7	2556
QuikClot ACS+™	0.2	1558

L - freeze-dried; R - spray-dried

The research has shown that model dressings FDR/L/Chit/Alg and FDR/L/Chit/Alg/CMC obtained by freeze drying, and FDR/R/Chit/Alg/CMC and FDR/R/Chit/Alg obtained by spray drying, of preprogrammed composition (FDR/Chit/AlgNa-Ca: chitosan 80%, AlgNa-Ca 20%; FDR/Chit/AlgNa-Ca/CMC: chitosan 75%, AlgNa-Ca 20%, CMC 5%) have suitable properties, i.e. absorbency and MVTR. The ability to absorb liquids by the dressings is twice lower than of the control dressing Celox™, and the MVTR value is twice as high. However, in comparison to the patch QuikClot ACS+™ performance characteristics of designed dressings are significantly higher.

3.3. Assessment of antibacterial properties

The evaluation of antimicrobial activity of the model dressings in powder form was conducted in accordance with test procedure PB 1 "Examination of antibacterial textiles. Quantitative test" by JISL 1902:2002. The study used microbial strains from the American Type Culture Collection Pure Culture: *Escherichia coli* (ATCC 11229), *Staphylococcus aureus* (ATCC 6538). Evaluation of the activity of model dressings was compared to the commercial haemostatic dressings Celox™ and QuikClot ACS+™. Results of the

antibacterial activity testing of prepared dressings in comparison to commercial products are shown in Tab. 2.

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

Type of dressing	Bacteria	Bacteriostatic activity	Bactericidal activity
FDR/L/Chit/AlgNa-Ca	*Escherichia coli* ATCC 11229 Gram (-)	5.7	2.6
FDR/R/Chit/AlgNa-Ca		6.8	3.7
FDR/L/Chit/AlgNa-Ca/CMC		3.6	0.5
FDR/R/Chit/AlgNa-Ca/CMC		6.8	3.7
Celox™		6.7	3.6
QuikClot ACS+™		2.8	-0.3
FDR/L/Chit/AlgNa-Ca	*Staphylococcus aureus* ATCC 6538 Gram (+)	0.6	-1.1
FDR/R/Chit/AlgNa-Ca		5.7	3.3
FDR/L/Chit/AlgNa-Ca/CMC		3.4	1.7
FDR/R/Chit/AlgNa-Ca/CMC		5.7	3.3
Celox™		3.8	1.5
QuikClot ACS+™		2.9	0.6

L - freeze-dried; R - spray-dried

The study showed that most of the dressings exhibit bacteriostatic and bactericidal activity against Gram (-) and Gram (+) bacteria. The model dressings: FDR/R/Chit/Alg and FDR/R/Chit/Alg/CMC obtained by spray drying exhibited the best bacteriostatic and bactericidal activity against both *E. coli* and *S. aureus*, however they showed greater ability to inhibit growth of *E. coli* than of *S. aureus*. These dressings have also higher antimicrobial activity than dressings obtained by freeze drying and commercial dressings Celox™ and QuikClot ACS+™.

3.4. Assessment of haemostatic properties

The examination was carried out with human full blood 0 Rh+ collected on preservative fluid containing sodium citrate, citric acid, glucose and sodium diphosphate. The samples of dressings in proportion of 0.003g/3cm^3 and 0.003g/2cm^3 of full blood were incubated at 310K (37±1)ºC for 15 and 30 min. The assessment was grounded on the examination of haemolytic activity. The activation of the plasmatic clotting system which depends on the contact factors (endogenous system) was estimated on the basis of the tests: APTT - time of partial thromboplastin after the activation.. Activation of the clotting system depending on the tissue thromboplastin (exogenous system) was assessed by PT - prothrombin time. The measurement of thrombin time (TT) is characteristic to both systems. Concentration of fibrinogen (Fb) was measured too. The clotting

DOI: 10.15259/PCACD.20.12

process in plasma as well as the tested samples and their fibrinolysis were assessed. Results of the testing are shown in Tables 3-5.

Table 3. Examination of haemolytic action of extracts from the dressings

Symbol of dressing	pH	Hbs* mg/cm^3	H.I.**	Haemolytic class
FDR/L/Chit/AlgNa-Ca	6.98	0.005 ± 0.002	0.029	No haemolysis
FDR/R/Chit/AlgNa-Ca	6.48	0.75 ± 0.050	4.18	Minor haemolysis
FDR/L/Chit/ AlgNa-Ca/CMC	6.85	0.007 ± 0.001	0.042	No haemolysis
FDR/R/Chit/ AlgNa-Ca/CMC	6.55	0.53 ± 0.070	3.15	Minor haemolysis
Celox™	6.56	0.54 ± 0.080	3.23	Minor haemolysis
QuikClot ACS+™	7.09	0.014 ± 0.002	0.064	No haemolysis

* - haemoglobin concentration in supernatant (Hbs); ** - haemolytic index (H.I.)

Table 4. Examination of plasma clotting activation after a temporary contact of the dressing with full blood.

Type of dressing	Activation of clotting															
	APTT				PT				TT				Fb			
	0,0030g/ 3cm^3 of blood		0,0030g/ 2cm^3 of blood		0,0030g/ 3cm^3 of blood		0,0030g/ 2cm^3 of blood		0,0030g/ 3cm^3 of blood		0,0030g/ 2cm^3 of blood		0,0030g/ 3cm^3 of blood		0,0030g/ 2cm^3 of blood	
	15′	30′	15′	30′	15′	30′	15′	30′	15′	30′	15′	30′	15′	30′	15′	30′
FDR/L/Chit/ AlgNa-Ca	N	N	N	N	N	N	↓	↓	N	N	N	N	N	N	↓	N
FDR/R/Chit/ AlgNa-Ca	↓	↓ #	↓ #	sk	↓ #	↓ #	↓ #	sk	↑ #	↑ #	↑ #	sk	N	N	↓ #	sk
FDR/L/Chit/ AlgNa-Ca/ CMC	N	↓ *	N	↓ *	N	N	↑	N	N	N	↑ #	N	N	N	↓ #	↓
FDR/R/Chit/ AlgNa-Ca/CMC	↓	↓ *	↓ *	sk	↓ #	↓ #	↓ #	sk	↑ *	↑ #	↑ #	sk	N	N	↓ #	sk
Celox™	N	N	N	N	↑ #	↑ #	↑ #	N	↑ #	N	N	N	N	N	N	N
QuikClot ACS+™	N	↓ #	↓ *	↓ #	↓ *	↓ *	↓ #	↓ #	N	N	N	N	N	↓	↓ #	↓ #
Plasma	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

APTT – time of partial thromboplastin after activation; PT- prothrombin time; TT- thrombin time; Fb - fibrinogen concentration; 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

Table 5. The process of clot formation and fibrinolysis of the clot.

Sequence	Clot formation in the plasma with samples of the dressings and reference	Sequence of fibrinolysis	Fibrinolysis of the clot
1	QuikClot ACS+™ (powder)	1	FDR/R/Chit/ AlgNa-Ca
2	QuikClot ACS+™ (granules)	2	QuikClot ACS+ ™ (powder)
3	FDR/R/Chit/AlgNa-Ca (becomes turbid)	3	Control
4	FDR/L/Chit/AlgNa-Ca	4	QuikClot ACS+™
5	Control (K)	5	FDR/L/Chit/ AlgNa-Ca
6	FDR/L/Chit/AlgNa-Ca/CMC	6	FDR/L/Chit/ AlgNa-Ca/CMC
7	FDR/R/Chit/AlgNa-Ca	7	FDR/R/Chit/ AlgNa-Ca/CMC
8	FDR/R/Chit/AlgNa-Ca/CMC	8	Celox™
9	Celox ™ becomes turbid	-	-
10	FDR/R/Chit/AlgNa-Ca/CMC	-	-
11	Celox ™	-	-

Control - plasma

The study showed that all dressings FDR/L/Chit/AlgNa-Ca, FDR/L/Chit/AlgNa-CaCMC, FDR/R/Chit/Alg/ Na-Ca CMC and the FDR/R/Chit/Alg Na-Ca exhibit hemostatic properties. Dressing FDR/L/Chit/Alg Na-Ca less activates the coagulation system. The haemolytic index, plasma haemoglobin concentration values was normal. Dressing FDR/L/Chit/Alg/Na-Ca CMC activates clotting. The haemolytic index value and morphological image of blood cells was normal. Dressing FDR/R/Chit/AlgNa-Ca significantly activated coagulation and fibrinolysis. Activation of the coagulation process was characterized by shortening of the clotting time of plasma coagulation system, prolonged process of clot formation and shortened process of fibrinolysis. The dressing showed increased haemolytic index. Dressing FDR/R/Chit/AlgNa-CaCMC largely activates the clotting process. The activation of clotting system was characterized by abbreviated plasma coagulation times, prolonged process of clot formation and prolonged process of fibrinolysis. The dressing showed increased haemolytic index. Dressings FDR/L/Chit/AlgNa-Ca, FDR/L/Chit/AlgNa-CaCMC, FDR/R/Chit/AlgNa-Ca CMC and

DOI: 10.15259/PCACD.20.12

FDR/R/Chit/AlgNa-Ca in contact with full blood showed rapid absorption and change from the form of powder into the form of amorphous gel. Similarly it was reported in the reference dressing Celox™. Dressing FDR/R/Chit/AlgNa-Ca in the clot formation process behaves like Celox™, but the activation of coagulation process is faster.

4. Conclusions

1. In the study model dressings in powder form were developed:
   - FDR/L/Chit/AlgNa-Ca composed of chitosan 80%, AlgNa-Ca 20%, prepared by freeze drying
   - FDR/R/Chit/AlgNa-Ca composed of chitosan 80%, AlgNa-Ca 20%, prepared by spray drying
   - FDR/L/Chit/AlgNa-Ca/CMC composed of chitosan 75%, AlgNa-Ca 15%, CMC 5%, prepared by freeze drying
   - FDR/R/Chit/AlgNa-Ca/CMC composed of chitosan 75%, AlgNa-Ca 15%, CMC 5%, prepared by the spray drying
2. Generated model dressings have suitable properties, i.e. absorbency and moisture vapor transmission rate (MVTR). The ability to absorb liquids by these dressings is twice lower than of the control dressing Celox™, and the MVTR value is twice as high. Compared to the dressing QuikClot ACS+™ performance characteristics of developed dressings are significantly higher.
3. The model dressings obtained by spray drying show much higher antimicrobial activity than the dressings obtained by freeze drying and the commercial dressings Celox™ and QuikClot ACS+™.
4. The model dressings prepared by spray drying or by lyophilization have adequate haemostatic properties. In direct contact with plasma they activate the coagulation process more rapidly in comparison with the commercial dressing Celox™.
5. The highest degree of activation of blood coagulation process was demonstrated by model dressing FDR/R/Chit/AlgNa-Ca produced by spray drying.

5. Acknowledgment

The research was carried out within a project contained in The Programme of Applied Research No PBS1/B7/5/2012 sponsored by National Centre of Research and Development

6. References

1. Struszczyk M.H., Olejnik M.; (2010) Obecne i przyszłe zapotrzebowanie rynku na włókiennicze wyroby medyczne, Techniczne Wyroby Włókiennicze 19-25.
2. The EU market for wound care and wound closure products, CBI Market Survey, 2009, http://www.icci.com.pk/data/downloads/10/1169838317_1.pdf [2012-03-02]

3. US Markets for Wound Management Products, Medtech Insight, RP-181303
4. All Change in the Advanced Wound CareMarket 2009, A complete guide to this dynamic market sector, Espicom Business Intelligence Ltd, May 2009,
5. Budynek M., Nowacki C.; (1999) Wiedza o opatrunkach, Łódź.
6. Jap. Pat. No 8593868
7. Jap. Pat. No 8663798
8. Muzzarelli R.A.A.; (2009) Chitins and chitosans for the repair of wounded skin, nerve, cartilage and bone. Carbohydr. Polym. 76, 167–182. **DOI**: 10.1016/j.carbpol.2008.11.002
9. http://www.trends-in-medicine.com/Oct2003/Closure103p.pdf, Hemorrhage Control in the Battlefield: Role of New Hemostatic Agents, MILITARY MEDICINE, 170, 1:63, 2005.
10. Fischer T.H., Connolly R., Thatte H.S., Schwaitzberg S.S.; (2004) Comparison of Structural and Hemostatic Properties of the poly-N-acetylglucosamine Syvek Patch with Products Containing Chitosan, Microscopy Res. Technique, 63, 168-174. **DOI**: 10.1002/jemt.20017
11. Polish patent appl. P.390253 „Opatrunek hemostatyczny warstwowy i sposób wytwarzania opatrunku hemostatycznego warstwowego („Multilayer haemostatic dressing and method to produce the dressing")
12. Kucharska M., Niekraszewicz A., Ciechańska D., Witczak E., Struszczyk M.H., Gulba-Diaz A., Sujka W.; (2010) „TROMBOGUARD®- nowoczesny opatrunek pierwszej pomocy",(-modern first aid dressing) Military Pharmacy and Medicine, Vol. XV, No2, p. 22-28, 2010
13. Millner R.W.J., Lockhart A.S., Alexiou Ch.; (2009) A New hemostatic agent:initial Life-saving experience with Celox (chitosan) In cardiothoracic burgery, Ann. Thorac. Surg., 87, e13-e14. **DOI**: http://dx.doi.org/10.1016/j.athoracsur.2008.09.046
14. Gegel B., Burgert J., Cooley B., MacGregor J., Myers J., Calder S, Luellen R., Loughren M., Johnson D.; (2010) The effects of bleed-arrest, Celox, and trauma-dex on hemorrhage control in a porcine model, J. Surg. Res., 164, e125-e129. **DOI**: http://dx.doi.org/10.1016/j.jss.2010.07.060
15. http://kopalniawiedzy.pl/opatrunek-chitosan-rana-krew-wypadek-ofiara-zolnierz- 9127.html
16. Niekraszewicz A., Lebioda J., Kucharska M., Wesołowska E.; (2007) Research into Developing Antibacterial Dressing Materials, Fibres & Textiles in Eastern Europe 15, 101-105.
17. Kucharska M.**,** **N**iekraszewicz A., Lebioda J.**,** Brzoza-Malczewska K.**,** Wesołowska E; (2007) Bioactive Composite Materials. Progress on Chemistry and Application of Chitin and Its Derivatives XII, 131-138.
18. Niekraszewicz A., Kucharska M., Wawro D., Struszczyk M.H., Rogaczewska A.; (2007) Development of a Manufacturing Method for Surgical Meshes Modified by Chitosan. Fibres & Textiles in Eastern Europe 15, 105-109/

19. PL 214 380 B1 (2008)
20. PL 214 380 B1 (2008)
21. Niekraszewicz A., Kucharska M., Wiśniewska-Wrona M., Kardas I.; (2010) Biological and Physicochemical Study of the Implantation of a Modified Polyester Vascular Prosthesis, Fibres & Textiles in Eastern Europe 18, 100-105.
22. Niekraszewicz A., Kucharska M., Kardas I., Wiśniewska-Wrona M., Kustosz R., Jarosz A.; (2011) Chitosan Coatings to Seal Cardiovascular Prostheses, Fibres & Textiles in Eastern Europe 19, 106-111.
23. Kucharska M., Ciechańska D., Niekraszewicz A., Wiśniewska–Wrona M., Kardas I.; (2010) Potential use of chitosan–based materials in medicine. Progress on Chemistry and Application of Chitin and its Derivatives XV, 169-176
24. Wiśniewska-Wrona M., Kucharska M., Kardas I., Bodek A., Bodek K.H.; (2011) Polymer biocomposites used in bedsores treatment. Progress on Chemistry and Application of Chitin and its Derivatives XVI, 111-120.
25. Kucharska M., Niekraszewicz A., Kardas I., Marcol W., Właszczuk A., Larysz-Brysz M., Lewin – Kowalik J.; (2012) Developing a Model of Peripheral Nerve Graft Based on Natural Polymers. Fibres & Textiles in Eastern Europe 20, 115-120
26. Kucharska M., Struszczyk M.H., Cichecka M., Brzoza-Malczewska M, Wiśniewska-Wrona M.; Prototypes of Primary Wound Dressing of fibrous and Quasi-fibrous Structure in Terms of Safety of Their Usage. Fibres & Textiles in Eastern Europe 20, 142-148.
27. Gadelha de Carvalho M.M.S., Montenegro Stamford T.C., Pereira dos Santos E., Tenório P., Sampaio F.; (2011) Chitosan as an oral antimicrobial agent, Formatex 2011
28. Yadav AV, Bhise .B.; (2004) Chitosan: a potential biomaterial effective against typhoid. Cur. Sci. 87(9): 1176-1178.