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# Novel Dressing Materials Accelerating Wound Healing Made from Dibutrylchitin

## Abstract

*Dibutrylchitin (DBC), a soluble chitin derivative, is a polymer with confirmed biological properties. DBC was obtained in the reaction of shrimp chitin with butyric anhydride, carried out under heterogeneous condition, in which perchloric acid was used as a catalyst of reaction. Production of DBC batches was carried out on a half-technical scale line. DBC parties were examined by infrared spectrometry, size exclusion chromatography and viscometry. DBC with molar mass of  $132 \times 10^3$  daltons was used for the manufacturing of DBC fibres and DBC non-woven materials. DBC non-woven fabrics after  $\gamma$ -sterilisation were applied to a group of nine patients with different indications. DBC dressing materials were used excluding the use of other medical products. Satisfactory results of wound healing were achieved in most cases, especially in cases of burn wounds and postoperative/posttraumatic wounds and various other conditions causing skin/epidermis loss.*

**Key words:** shrimp chitin, dibutrylchitin, non-woven dressing, bioactivity, clinical research, wound healing.

## Introduction

Chitin, the most abundant natural polysaccharide containing nitrogen, undergoes degradation by enzymes, induces human cells to promote the restoration of wounds, enhances the healing process of wounds and has high permeability for substances of medium molar mass in serum [1 - 11]. Chitin might be an ideal raw material for bioactive dressing materials, but due to its low solubility it cannot easily be converted into useful forms like films, fibre or non-woven matrices.

An original method of synthesis of di-O-butyrylchitin (DBC), a soluble derivative of chitin, was worked out at the Technical University of Łódź, Poland [12, 13]. The proposed method applied to chitin of different origin (crab, shrimp & krill shells, and insect chitin) gave the products of definite chemical structure a degree of esterification very close to two. DBC is easily soluble in common organic solvents and has both film and fibre-forming properties [14 - 20]. Such properties of DBC created the possibility of manufacturing a wide assortment of DBC materials suitable for medical applications in the form of films, fibres, non-woven, knitted materials and woven fabrics. The first investigations of the biological properties of DBC materials showed good biocompatibility of the polymer [22 - 28] and its ability to accelerate wound healing [22, 27].

The first clinical investigations into the medical properties of DBC were carried out at the Polish Mother's Health Institute in Łódź, Poland. The DBC samples under investigation were used in the form of non-woven materials made at Technical University of Łódź using krill chitin as a source of DBC. The results of their clinical investigations were presented at a 6<sup>th</sup> International Conference of the European Chitin Society [29] and published [30].

The latest results received using non-woven dressing materials made from DBC of shrimp shell chitin origin are presented in this paper.

## Manufacturing of wound dressings

### Preparation of DBC and DBC characteristic

DBC as a raw material used for manufacturing non-woven dressing materials was prepared from shrimp chitin. Shrimp shell chitin powder with particle size of 200 mesh and viscosity average molar mass  $\overline{M}_v = 454.6 \times 10^3$  daltons [31] was delivered by FRANCE CHITIN, Marseille, France. The chitin contained calcium carbonate in the amount of below 0.3% and was characterised by the degree of N-acetylation of circum 95%.

Production of DBC from shrimp chitin on a semi-technical scale was carried out at the Institute of Dyes and Organic Products, Zgierz, Poland, in accordance with a Polish patent [32]. The synthesis

of DBC was carried out under heterogeneous conditions using chitin, 98% butyric anhydride, and 72% perchloric acid in approximate proportion equal to 10:50:6.8 (g/g). The acylation reaction was carried out for 3 hours at circum 25 °C under slow mixing of the reagents. The final product of the reaction – DBC fully soluble in acetone, ethanol and several other organic solvents – was obtained with a yield of circum 80%.

The intrinsic viscosity value of DBC determined in DMAc solutions with DBC concentration within the range of 0.1 to 0.4 g/dl at 25 °C was 1.70 dl/g, the weight average molar mass, determined by the SEC method coupled with light scattering and viscometry, was  $\overline{M}_w = 132 \times 10^3$  daltons [33].

Infrared investigations were made using an Perkin-Elmer 2000 FTIR instrument. IR spectra were recorded for chitin film prepared from a solution of chitin in DMAc/5% LiCl and for DBC film cast from an acetone solution. Recorded spectra are shown in Figure 1. In the IR spectrum of the DBC sample there was no band of absorption at about 3450 cm<sup>-1</sup> due to hydroxyl groups which were present in the IR spectrum of chitin, and a new band of strong absorption at 1741 cm<sup>-1</sup>, characteristic for the esters of fatty acids, is/was noticed. Furthermore, bands of stronger absorption appeared at around 2950 cm<sup>-1</sup>, corresponding to aliphatic groups –CH<sub>2</sub>– and –CH<sub>3</sub>, the content of which is much higher in DBC than in chitin.

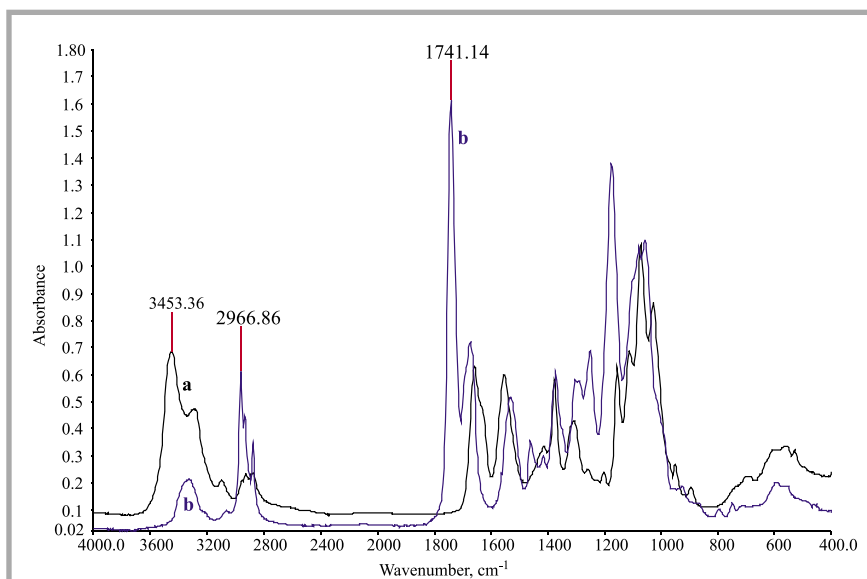


Figure 1. IR spectra of: a) shrimp chitin and b) DBC obtained from shrimp chitin.

### Preparation of dibutrylchitin fibres

Wet spinning of DBC fibres was made at the Institute of Chemical Fibres, Łódź, Poland, on an apparatus commonly used for the preparation of rayon fibres [18, 20, 21]. Dope containing 12 % of DBC in dimethylsulphoxide was added to the reservoir of the spinning system and extruded through a spinneret (300 holes, 80  $\mu\text{m}$  diameter of the hole) to a coagulation bath. The filaments were coagulated in water, drawn in hot water, collected on rollers with a rate of 40 m/min and dried.

### Preparation of non-woven dressing materials

New technologies of creating flat textile products from DBC solutions were described in [34 - 36]. In the present research classical needle punching technology was used to prepare DBC non-woven samples obtained from staple fibres.

The DBC fibres from shrimp chitin with a linear density of 3.5 dtex, and tenacity of 10.5 cN/tex were cut into pieces with a length of 80 mm. The web was formed at Technical University of Łódź on a laboratory carding machine (produced by Befama, Poland), without initial processes. Feeding was equal to 30 g/field. The web obtained was transported to a needle punching loom (produced by Asselin) to complete a needle punching process with following technological parameters: number of needling equalled 60  $\text{cm}^{-2}$ , the needling depth was 8 mm, and the web's velocity in the machine was 0.338 m/min. The surface mass of non-woven

fabrics was equal to 34.8  $\text{g}/\text{m}^2$ . The thickness of the samples was equal to 0.44 mm. To obtain the medical dressings, non-woven fabrics were cut into pieces of 5  $\times$  5 cm and sterilised with an absorbed dose of 25 kGy of  $\gamma$ -rays (Figure 2.). Earlier investigations showed that such a dose of  $\gamma$ -rays radiation does not cause significant degradation of the polymer [13].

## Clinical investigations of DBC non-woven dressing materials

### Biological properties of DBC material: *in vitro* and *in vivo* assays

Biological properties of DBC in the form of fibres or DBC films were previously widely investigated and the results of these investigations were published [22 - 27]. The investigations were carried out in accordance with the requirements of the standard EN ISO 10993 ("Biological evaluation of medical devices"). They included investigation into the cytotoxic effects of DBC aqueous fibre extracts and wound dressings coated with DBC, investigation into the haemolytic effects of DBC aqueous fibre extracts, the fulfilment of intracutaneous reactivity tests of aqueous extracts, evaluation of local tissue reactions after the implantation of DBC fibres into the gluteal muscles of Wistar rats and the peritoneal cavity of mice BALB/C. Moreover, the investigation of the tissue regeneration process after the covering of damaged rabbit skin with DBC type wound dressings was completed. In order to define the inflammatory and immunomodulation effects of DBC containing

wound dressings, an assessment of their influence on the induction of cytokines and on the synthesis of nitrogen oxides in human leukocytes was conducted. Evaluation of the influence of DBC on the activation of the blood coagulation system was completed in the latter research.

Based on the results of biological investigation the following final conclusions were drawn:

- Tests of aqueous DBC extracts from fibres and non-wovens performed *in vitro* proved that cytotoxicity and haemolytic effects were not stated [23, 26, 28].
- After animal tests conducted *in vivo* on rabbits, no reaction could be noted as the result of intradermal DBC fibre application [23, 26].
- No intensification of the tissue reaction after implantation of DBC fibres into the gluteal muscles of the Wistar rats [26] and into the peritoneal cavity of mice BALB/C [23] could be stated. The results of microscopic observation were the same as those obtained in the investigation of the reactions in the surrounding of Maxon surgical thread [23].
- Tests of IL1 $\beta$  and IL6 interleukin levels in the peritoneal fluids of the experimental mice BALB/C did not prove the existence of statistically essential differences when comparing two animal groups: the group represented by animals with DBC fibres inserted into the peritoneal cavity with those of peritoneal implanted Maxon surgical thread [23].
- It was confirmed that DBC films do not demonstrate cytotoxicity and primary irritation effects, do not cause an increase in the activities of TNF- $\alpha$ , IFNs or nitrogen oxide level and have an active, positive influence on the wound healing process [22, 27].
- DBC materials showed the activity of blood pro-coagulation and have no



Figure 2. The petal of DBC.



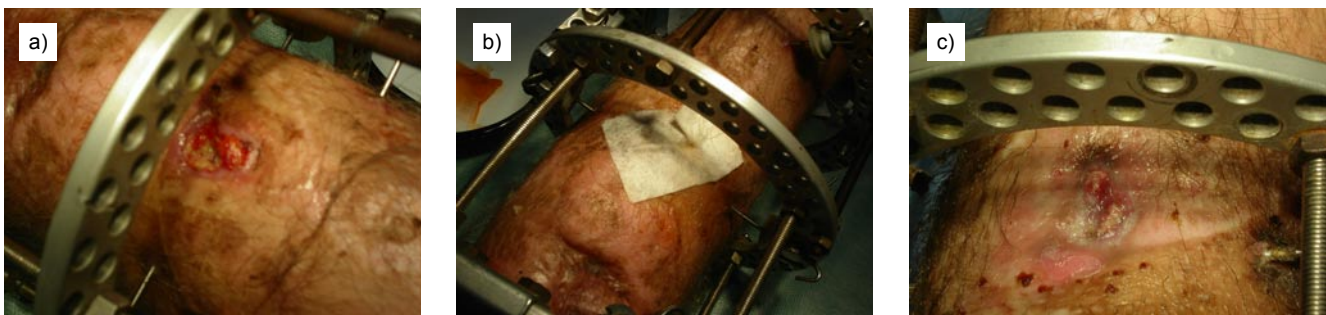
**Figure 3.** a) Burn of chest and abdomen; b) DBC application; c) the wounds are healed.



**Figure 4.** a) Burn of right forearm; b) DBC application; c) healing nearly complete.



**Figure 5.** a). Postoperative wound, after excision of huge naevus; b) application of DBC petal; c) wound almost healed.



**Figure 6.** a) Old open fracture with bone exposed; b) application of DBC petal; c) the wound around the bone is healing.

influence on the activity of the plasma protein coagulation system [37].

Biocompatibility of the polymer in form of non-woven dressing materials made from DBC of shrimps chitin origin was confirmed in the recent investigations published by Muzzarelli et. al. [28]. It was also showed that DBC obtained from shrips chitin is not cytotoxic for fibroblasts and keratinocytes. All pre-

sented results confirm the bioactivity of DBC material.

#### **Results of clinical investigations of DBC wound dressings**

Based on the above-mentioned results, the Ethic Committee of the Polish Mother's Health Institute has given their acceptance for the project, forecasting the application of DBC in surgical paediatric patients.

In previous experiments dressing materials made from DBC of krill chitin origin were tested and the results have been published [29, 30]. In this paper further results of medical tests of DBC are presented. However, in this case, the tested petals were manufactured from DBC obtained from shrimp chitin. Sterilised DBC dressings were applied to a group of nine children with the following indications:

- thermal burns -four cases,
- wounds of various aetiology - five cases.

DBC non-wovens applied to patients were resorbed in the wound area and did not require changing during the healing process. No other medicines were used additionally.

The first group of patients, composed of four persons, suffered from burns. The total area of thermal burns of the patients changed within the range of 5 - 20% of the whole patient's surface area. The depth of burns was classified in each case as 2a and 2b. All the burns healed up within 1 - 2 weeks after DBC petal application. The healing processes are documented in Figures 3 and 4.

The second group of patients suffered from wounds other than burns with various aetiology such as postoperative, posttraumatic and posttraumatic/postoperative. Three patients complained from postoperative wounds. All of them had small areas of granulation tissue healing "per secundam". One of them was after excision of huge naevus as illustrated in Figure 5.

In the posttraumatic group was one patient who sustained injury to the digits with partial necrosis of soft tissue. After removal of necrotic tissue, the DBC petal was applied and there was no need of further surgical interference. One patient from the posttraumatic/postoperative group sustained severe injuries to the lower limbs resulting in open fractures of the tibia with the loss of soft tissue. It was repeatedly operated on by orthopaedic surgeons. As a consequence residual chronic ulcers, with the tibial bone exposed, were seen. The application of DBC petals resulted in the healing of the wound. The healing processes are documented in Figures 6.

## ■ Discussion and conclusions

As far as thermal burn patients are concerned, the depth of the wound was evaluated as 2a, 2b. This means that there was no necrotic tissue and no indication for operative treatment. In all cases healing was quick and uneventful, and in no case infection developed. The non-septic wounds are, by definition, an indication for surgical treatment. As a matter of fact, all the patients presented were primarily treated this way. In all cases, after plastic operation, small unhealed patches with

granulation were stated. No signs of infection and no necrotic tissue were indicated. All these places healed up soon after the application of DBC. Another child suffered mechanical injury, and the wounds were complicated by the presence of necrotic tissue. The first step in all cases was the removal of necrosis followed by the use of DBC. It healed up soon enough to avoid surgery. With great surprise the progress was uneventful, which took place with one child repeatedly operated on for open, complicated leg fracture. The wounds were clean, without necrosis but with exposed bone in the centre. In spite of this, they healed up quickly.

In all cases DBC dressings were applied to the clean wound, and not removed till the end of the healing process during which DBC disintegrated in the area of the wound. No other medical products were applied for the healing of the wound.

Summarising, it is possible to conclude:

- The results of application of dressing materials made from DBC of shrimps chitin origin are highly promising and does not differ from results previously obtained for dressing materials made from DBC of krill chitin origin. In both cases DBC promotes the healing of wounds.
- Selection of the patients for this treatment has to be meticulous: the tissues to be covered with DBC should be viable, possibly without infection.
- Further randomised trials with referential groups should be carried out to obtain evidence-based proof of the beneficial effects of DBC wound dressings.

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## References

1. Muzzarelli R. A. A., Pasiser E. R., editors. *Chitin/Chitosan*. MIT Sea Grant. Program 78-7, 1978. p.108.
2. Hirano S., Tokura S., editors. *Chitin and Chitosan*. Tottori: Japanese Chitin Society; 1982.
3. Zikakis J. P., editor. *Chitin, Chitosan and Related Enzymes*. New York: Academic Press, 1984.
4. Muzzarelli R. A. A., Jeuniaux C., Gooday W., editors. *Chitin in Nature and Technology*. New York: Plenum Press, 1986.
5. Oshimoma Y., Nishino K., Yonekura Y., Kishimoto S., Wakabayashi S.; *Eur. J. Plast. Surg.* Vol. 10 (1987) pp.66-69.
6. Skjak-Braek G, Sandford P., editors. *Chitin and Chitosan: Sources, Chemistry, Biochemistry, Physical Properties and Applications*. New York: Elsevier, 1989.
7. Brine C., Sandford P., Zikakis J. P. editors. *Advances in Chitin and Chitosan*. New York: Elsevier Applied Science, 1992.
8. Tokura S., Azuma I., editors. *Chitin Derivatives in Life Science*. Sapporo: Japanese Chitin Society, 1992.
9. Roberts G. A. F., *Chitin Chemistry*, London: Macmillan, 1992.
10. Muzzarelli R. A. A. *In vivo biochemical significance of chitin-based medical items*. In: Dimitriu S, editor. *Polymeric Biomaterials*. New York: Marcel Dekker, Inc., 1993. p.179-197.
11. Muzzarelli R. A. A.; *Chitin*. In Salamone J.C., editor. *Polymeric Materials Encyclopedia*. Boca Raton FL, USA: CRC Press; 1996.
12. Szosland L., Janowska G.; *Patent PL No 169077 B1*, 1996.
13. Szosland L.; *J. Bioactive and Compatible Polymer*, Vol. 11 (1996) pp. 61-71.
14. Muzzarelli R. A. A., Peter M. G., editors. *Chitin Handbook*. Ancona-Potsdam: European Chitin Society, 1997.
15. Szosland L, East G. C.; *J. Appl. Pol. Sci.* Vol. 58 (1995) pp. 2459-2466.
16. Szosland L. *A simple method for the production of chitin materials from the chitin ester derivatives*. In: Muzzarelli R. A. A., Peter M. G., editors. *Advances in Chitin Science 1996*; v.I: pp. 297-302.
17. Szosland L. *Di-O-butryrylchitin*. In: Muzzarelli R. A. A., Peter M. G., editors. *Chitin Handbook*. Germany, 1997. pp. 53-60.
18. Szosland L., Stęplewski W.; *Rheological characteristic of dibutryrylchitin semi-concentrated solutions and wet spinning of dibutryrylchitin fibre*. In: Domard A., Roberts G. A. F., Varum K. M., editors. *Advances in Chitin Science 1998*; v.II: pp. 531-536.
19. Szosland L. *Fibres & Textiles in Eastern Europe* Vol. 4 No. 14-15 (1996) pp. 76-79.
20. Struszczyk H., Ciechańska D., Wawro D., Stęplewski W., Krucińska I., Szosland L., Van de Velde K., Kiekens P.; *Some properties of dibutryrylchitin fibres*. *Proceedings of 6<sup>th</sup> International Conference of the European Chitin Society, EUCHIS'04*. Poznań-Poland: 2004.
21. Wawro D., Stęplewski W., Ciechańska D., Krucińska I., Wesółowska E.; *Fibres & Textiles in Eastern Europe*, vol. 15, No. 62 (2007), pp. 14-18.
22. Szosland L., Cisto R., Krucińska I., Paluch D., Staniszevska-Kuś J., Pielka S., Solski L., Żywicka B.; *Dressings made from dibutryrylchitin and chitin accelerating wound healing*. *Proceedings of International Conference MEDTEX'2002*. Łódź-Poland, 2002. pp. 35-41 (ISBN 83-89003-41-4).
23. Staniszevska-Kuś J., Paluch D., Szosland L., Kofodziej J., Staniszevska-Kuś J.,

- Szymonowicz M., Solski L.; *Eng. of Biomaterials* Vol. II, No. 7-8 (1999) pp. 52-60.
24. Paluch D., Pielka S., Szosland L., Staniszevska-Kuś J., Szymonowicz M., Solski L., Zywicka B.; *Eng. of Biomaterials* Vol. 12 (2000) pp. 17-22.
  25. Paluch D., Szosland L., Staniszevska-Kuś J., Solski L., Szymonowicz M., Gębarowska E.; *Polymers in Medicine* Vol. XXX No. V. (2000) pp. 3-32.
  26. Szosland L., Krucińska I., Cisko R., Paluch D., Staniszevska-Kuś J., Solski L., Szymonowicz M.; *Fibres & Textiles in Eastern Europe* Vol. 9, No. 34 (2001) pp. 54-57.
  27. Pielka S., Paluch D., Staniszevska-Kuś J., Zywicka B., Solski L., Szosland L., Czarny A., Zaczyńska E.; *Fibres & Textiles in Eastern Europe* Vol. 11, No. 41 pp. 79-84.
  28. Muzzarelli R. A. A., Guerrieri M., Goteri G., Muzzarelli C., Armeni T., Ghiselli R., Cornellisen M.; *Biomaterials* Vol. 26 (2005) pp. 5844-5854.
  29. Chilarski A., Szosland L., Krucińska I., Błasińska A., Cisko R.; *Non-wovens made from dibutyrylchitin as novel dressing materials accelerating wound healing. Proceedings of 6th International Conference of the European Chitin Society, EUCHIS'04. Poznań-Poland, 2004.*
  30. Chilarski A., Szosland L., Krucińska I., Błasińska A., Cisko R.; *The application of chitin derivatives as biological dressing in treatment of thermal and mechanical skin injuries. The Annual of Pediatric Traumatic Surgery, The Division of Pediatric Traumatic Surgery 2004; 8 (XXXII): 58-61.*
  31. Terbojevich M., Cosani A.; *Molecular weight determination of chitin and chitosan. In: Muzzarelli R. A. A., Peter M. G., editors. Chitin Handbook. Ancona-Potsdam: European Chitin Society, 1997. pp. 87-101.*
  32. Kopczacki P., Rutkowska-Olma E., Muszyński M., Szuster L., Szosland L., Janowska G., Krucińska I.; *Patent PL No. P 370755, 2004.*
  33. Szumilewicz J., Szosland L.; *Determination of the absolute molar mass of chitin and dibutyrylchitin by means of size exclusion chromatography coupled with light scattering and viscometry. In: Uragami T., Kurita K. and Fukamizo T., editors. Chitin and Chitosan. Tokyo: Kodansha Scientific Ltd, 2000. pp. 99-102.*
  34. Krucińska I., Błasińska A., Komisarczyk A., Chrzanowski M., Szosland L.; *Przeгляд Włókienniczy* Vol. 11 (2003) pp. 3-5.
  35. Błasińska A., Krucińska I., Chrzanowski M.; *Dibutyrylchitin nonwoven biomaterials manufactured using electrospinning method. Proceedings of World Textile Conference - 4th AUTEX Conference, Roubaix - France, 2004.*
  36. Komisarczyk A., Krucińska I., Szosland L., Strzembosch W.; *Influence of the rate of air flow onto the structure of nonwovens made from dibutyrylchitin solution. Proceedings of International Conference on "Magic World of Textiles", Dubrovnik-Croatia, 2004.*
  37. Szymonowicz M., Paluch D., Solski L., Pielka S., Błasińska A., Krucińska I., Szosland L.; *Engineering of Biomaterials* Vol. 7 (2004) pp. 123-125.

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