NEW BIOACTIVE TEXTILE DRESSING MATERIALS FROM DIBUTYRYLCHITIN STIMULATING WOUND HEALING

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ABSTRACT

Dibutyrylchitin (DBC) is an ester derivative of a natural polysaccharide – chitin. DBC is obtained by reaction of chitin with butyric anhydride in the presence of a catalyst. The production methods of DBC were elaborated and optimized. DBC is easily soluble in common organic solvents and has film- and fibre forming properties. Such characteristic allows obtaining classical fibres from the polymer solutions. DBC is also a raw material for manufacturing yarn and for a broad range of textile dressing materials. Fibres with good mechanical properties were obtained by an optimized spinning process from the DBC solutions.

The excellent biomedical properties of the DBC were confirmed by different experimental results which proved that DBC is a biocompatible and biodegradable polymer and stimulates regeneration of damaged tissues.

Tests of these DBC dressing materials under clinical conditions were done and proved the excellent results of DBC-based dressing materials for the ordered healing of tissues and wounds. The DBC dressing materials accelerate the healing of the wound and are biodegraded during the healing process. From the clinical tests, it was clearly observed that the DBC dressing materials were absorbed into the fresh tissue formed during the healing process of the wounds.

DBC and DBC-based dressing materials are good bioactive textile materials for wound healing and for understanding the biological properties of chitin derivatives. The obtained results proved the importance of the O-substitution of the hydroxyl groups present in chitin, not only for the solubility of the derivatives and the mechanical properties of the produced fibres, but still more important for the biological properties of these ester derivatives of chitin containing butyric acid. This development creates a link between textile products, based on material properties, and human health, based on the biological properties of the basic material.

The mechanical properties of DBC were further optimized by blending it with poly(ε-caprolactone). Good transparent and flexible products, such as films, with a high elongation to break were obtained by blending 10 to 20wt% of poly(ε-caprolactone) with DBC. This creates new possible bioactive applications for DBC or poly(ε-caprolactone).

Key Words: chitin, biomedical properties, mechanical properties, blends

1. INTRODUCTION

Chitin, the natural most abundant polysaccharide containing nitrogen, undergoes degradation by enzymes, induces human cells to promote the restoration of wounds, enhance and enhances the healing process of wounds and has high permeability for substances of medium molar masses in serum [1-3]. Chitin might be an ideal raw material for bioactive dressing materials, but due to its low solubility can be only hardly converted into useful forms like films, fibre or non-woven matrices.

An original method of synthesis of di-O-butyrylchitin (DBC), the soluble derivative of chitin, was worked out at the Technical University of Łódź, Poland [4]. The proposed method applied to chitin of different origin (crab, shrimp & krill shells, and insect chitin) gave the products of definite chemical structure with a degree of esterification very close to two. DBC is easily soluble in common organic solvents and has both film and fibre-forming properties

[5]. 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. Thus, O-butyrylation of chitin gives the possibility of practically unlimited manufacturing of DBC dressing materials comfortable and easy in use. The first investigations of biological properties of DBC materials, carried out in vitro and in vivo in accordance with the European standards EN ISO 10993 ("Biological evaluation of medical devices"), showed good biocompatibility of DBC [6, 7] and his ability to accelerate wound healing [6, 8]. The recent investigations published by Muzzarelli et. al. [9] confirms the biocompatibility of DBC. The presented results indicated that DBC is not cytotoxic for fibroblasts and keratinocytes.

The first clinical investigations into medical properties of DBC have been carried out at the Polish Mother's Health Institute in Łódź, Poland. DBC samples under investigation have been used in the form of non-woven materials. Results of their clinical investigations were presented on the 6th International Conference of the European Chitin Society and published [10].

2. MANUFACTURING OF WOUND DRESSINGS

2.1. Preparation of Dibutyrylchitin (DBC) and DBC Characteristics

DBC as a raw material used for manufacturing of non-woven dressing materials was prepared from shrimp chitin. Shrimp shell chitin powder with particle size of 200 mesh and viscosity average molar mass M_v = 454,6x10^3 g/mol was delivered by FRANCE CHITIN, Marseille, France.

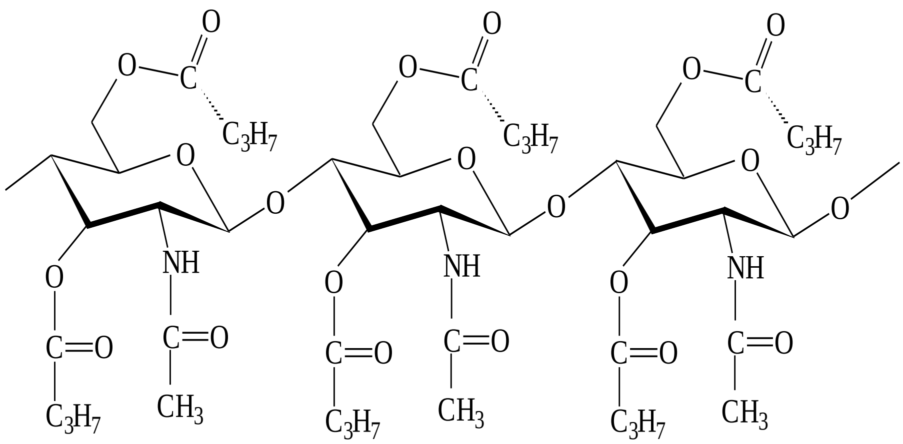

Figure 1. Dibutyrylchitin (DBC): chemical structure after complete o-butyrylation

(degree of substitution = 2)

The synthesis of DBC was carried out under heterogeneous conditions using chitin, butyric anhydride, and 72% perchloric acid in approximate proportion equal to 10: 50: 6.8 (g/g). The intrinsic viscosity value of DBC determined in DMAc solutions at 25^{o}C was 1.70 dL/g, the weight average molar mass, determined by SEC method coupled with light scattering and viscometry, was M_w =132x10^3 g/mol.

Another possible reaction to produce DBC is the so-called homogeneous reaction in methanesulphonic acid. Intrinsic viscosity value of the obtained DBC by the homogeneous reaction determined in DMAc solutions with DBC was 5.70 dL/g, the corresponding weight average molar mass was 456 10^3 g/mol.

A DBC with a higher molecular weight was obtained by the homogeneous reaction in methanesulphonic acid. The starting chitin has a mean amount of 2800 monomers in the polymer chain. The mean amount of monomers for the DBC obtained by the homogeneous reaction is 1340 and for the DBC obtained from the heterogeneous reaction is 450. There is less degradation of the polymer chains for the homogeneous reaction than for the heterogeneous reaction.

2.2 Mechanical Properties

The mechanical properties were measured on films cast from an acetone solution. The elasticity modulus, elongation and maximum stress were obtained from the tensile stress-strain data on the films using an Instron 3369 tensile tester. The product TUL-DBC was the DBC with an average molecular weight M_w of 153 10^3 g/mol and UGT-DBC the DBC with an average molecular weight M_w of 456 10^3 g/mol. The films were conditioned at room temperature and a relative humidity of 65%. The measured stress-strain curves are reproduced in Figure 2.

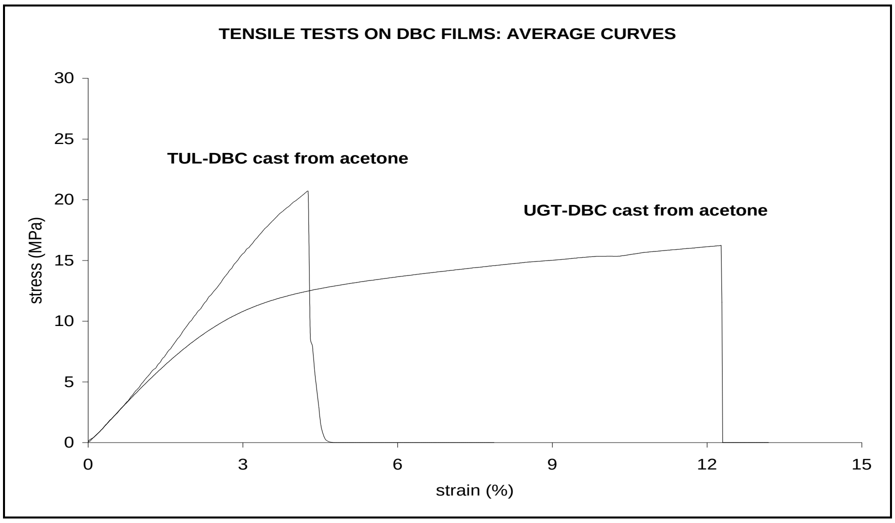

Figure 2. Stress-strain data for two different DBCs at room temperature.

The elasticity modulus, equal to 1025 MPa, was the same for the two samples, independent of the molecular weight, but the maximum elongation is increasing with increasing molecular weight. The maximum elongation is increasing from 4.3% for the lower molecular weight to 12.5% for the higher molecular weight. This resulted in better strength characteristics of the films, due to the more plastic behaviour of the DBC with the highest molecular weight.

2.3. Preparation Of Dibutyrylchitin Fibres

Wet spinning of DBC fibres was made at the Institute of Chemical Fibres, Łódź, Poland, on an apparatus commonly used for preparation of rayon fibres [5]. Dopes containing 15 % of DBC from krill chitin in dimethylformamide or 12 % of DBC from shrimp chitin in dimethylsulphoxide were added to the reservoir of the spinning system and extruded through a spinneret (300 holes, 80 μm 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.

DIBUTYRYLCHITIN FIBRE						
Fibre's symbol	**dtex**	**Solvent**	**Tenacity (cN/tex)**	**Loop (cN/tex)**	**Elongation at break (%)**	**Max. Elongation (loop; %)**
8 E/1	3.3	Ethanol	6.5	2.9	4.8	2.1
22/3	2.1	DMF	15.7	3.1	6.2	2.3
35/1	2.8	N-MP	13.0	2.2	9.4	3.4
68	2.9	DMSO	11.2	3.5	12.6	2.6

Low susceptibility to deformation during the drawing stage at about 70°C is linked to the rigid structure of the DBC macromolecules. The results of this are rather poor strength properties in the fibres, especially the maximum elongation for bending. By using ethanol as the solvent for DBC, porous fibres were obtained.

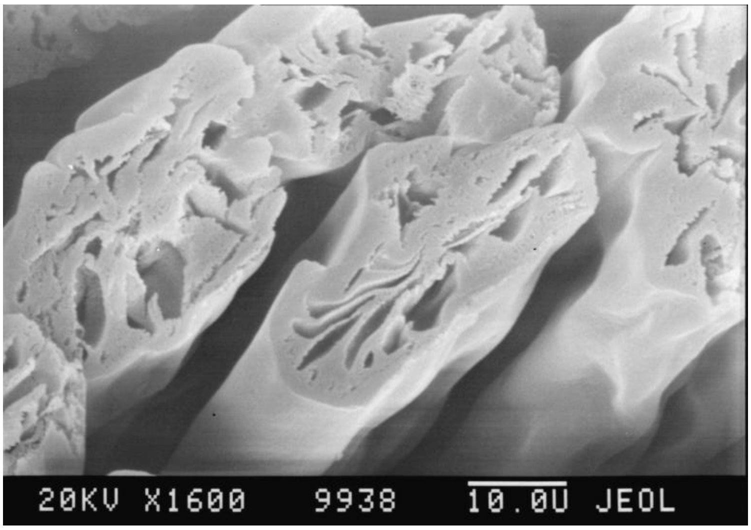

Figure 3. : SEM photos of DBC Fibres (solvent-ethanol); cross-section

The presence of very large pores in the cross-section view is visible in the SEM micrograph (figure 3). The DBC fibres from DMSO-solution were round without visible pores. These tests confirmed that the supramolecular structure of fibre-forming polymers depends upon the solvent and the character of the solvent-polymer interactions.

3. CLINICAL INVESTIGATIONS OF DBC NON-WOVEN DRESSING MATERIALS

3.1 Rationale for the Possible Provision of Butyrate

Short chain fatty acids, especially butyrate, play central metabolic roles in maintaining the mucosal barrier in the gut. A lack of butyrate, leading to endogenous starvation of enterocytes, may be the cause of ulcerative colitis and other inflammatory conditions. The main source of butyrate is dietary fibre, but they can also be derived from structured biopolymers like DBC. Butyrate has been shown to increase wound healing and to reduce inflammation in the small intestine (13). In the colon, butyrate is the dominant energy source for epithelial cells and affects cellular proliferation and differentiation by yet unknown mechanisms. Recent data suggest that the luminal provision of butyrate may be an appropriate means to improve wound healing in intestinal surgery and to ameliorate symptoms of inflammatory diseases. It was also suggested that butyrate may inhibit the development of colon cancer (14, 15). Butyrate has a relatively short metabolic half life. The half-life of butyrate in plasma is extremely short, as peak plasma butyrate concentrations occurred between 0.25 and 3 h after application and disappeared from plasma by 5 h after the application.

3.3 Results of Clinical Investigations

The surgical staff of the Department of Pediatric Surgery was provided with a number of DBC petals for medical application (Fig.6).

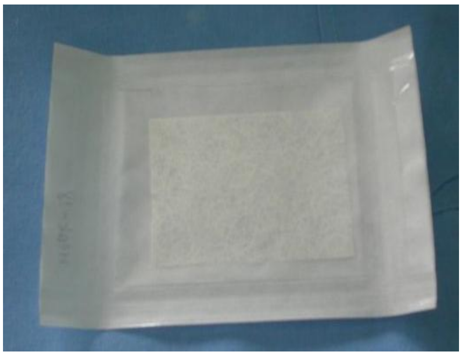

Figure 6: The flake of DBC

The first group of patients, composed of 10 persons, suffered from burns. The total area of thermal burns of patients changed within the range of 5-20%. The depth of burns was classified in each case as 2a. All the burns healed up within 1 – 2 weeks after DBC petal application. The healing processes are documented in Figure 7. The result of the healing process was very good.

Figure 7. Burn of right lower limb (left) and healing nearly completed (right)

4. CONCLUSIONS

As far as thermal burn patients are concerned, in all cases DBC dressings have been applied to the clean wound and not removed till the end of the healing process, while DBC has been disintegrated in the area of the wound. No other medical products have been applied for the wound healing. The presented observations are preliminary and further evaluation is necessary.

Summarising, it is possible to conclude from the preliminary results of DBC application that DBC seems to promote wounds' healing. Further randomised trials with referential groups should be completed to obtain evidence-based proofs of beneficial effects of DBC wound dressings.

Blending DBC with PCL gave very good flexible products, with a good value of elasticity modulus, a high elongation to break and a good transparency for films containing between 10 and 20wt% PCL.

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