# 7. BIOMATERIALS CONTAINING CHITOSAN AND FIBROIN

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## 1. Introduction

Since several years there is an increasing demand for medical dressings. An ideal dressing material has not been found yet [1], despite enormous progress in material engineering. This is the reason why investigations in that direction are still under way. Also in the Institute of Chemical Fibres (ICF) research has been begun in cooperation with the National Institute of Agrobiological Sciences (NIAS) to prepare novel biomaterials designed for dressings [2 - 4]. Amongst the most promising materials for that application are biomaterials containing chitosan and fibroin.

Fibroin is one of the natural polymers which has been extensively studied in recent year. It is a fibrillar protein produced by the silk-worm larvae *Bombyx mori* in form of the well known natural silk. Apart from the great historical role silk played in the clothing business, it also had been used as surgical sutures [5, 6] Macromolecules of fibroin are built up mainly of Gly, Ala, and Ser amino-acids in the 3 : 2 : 1 proportion. Such molecular construction enables the high arrangement of fibroin's macromolecules and their packing in form of secondary β-structures. The molecular and super-molecular structure of fibroin provides for the outstanding features of silk such as excellent mechanical properties, resistance against most of the common solvents, good permeability of oxygen and water vapor, and high biocompatibility with a living organism manifested by good wearing comfort [1, 5 - 9]. Fibroin was recently in a way rediscovered, since investigations in the last years have revealed that the polymer is featured by many valuable biological properties and lends itself very well to applications in medical dressings. It is known from literature that fibroin positively influences the wound healing process, accelerates the filling of skin defects with granulation tissue. It also controls the growth of fresh epidermis cells thus minimizing scar formation [10 - 13].

Unfortunately, films and other useful forms made of pure fibroin are too brittle and rigid to produce single-component dressing materials. The drawback can be overcome by blending of the fibroin with other natural or synthetic polymers like cellulose [14], chitosan [15 - 17] or polyvinyl alcohol [18 - 19].

Chitosan is a well known nature-derived biopolymer manifesting unique biological properties that are, like in fibroin, particularly useful in wound healing, notably biostimulation of wound healing, and acceleration of tissue reconstruction and vascularization without scar formation [20 - 21].

It is expected that by combining the two biopolymers, new dressings can be made with unique properties surpassing those made of the single components.

The purpose of the work presented here was to recognize the possibility of preparing blended chitosan-fibroin biomaterials in form of fibres, sponges and film that could be suitable for a future construction of dressing materials.

## 2. Materials and Methods

### 2.1. Aqueous fibroin

Natural fibroin in form of cocoons supplied by NIAS was used in the research. The preparation of an aqueous fibroin solution was accomplished in a two-step procedure:

I step - removing of sericin. Natural fibroin was warmed in a solution containing 0.2 % of soap and 0.5 % of sodium carbonate.

II step - preparing of the aqueous fibroin. An aqueous solution of fibroin was obtained by dissolving of the fibroin in saturated aqueous LiBr which was then dialyzed against distilled water for 3 days; cut off M.W. 12,400. The solution was eventually concentrated to adjust the required fibroin content.

### 2.2. Chitosan

Chitosan used in the research was supplied by the Vietnamese LA NI Co Ltd. Chitosan was processed either to a solution of its salt for the preparation of chitosan/fibroin fibres, or to microcrystalline chitosan (MCCh) in the part of the work concerning sponges and film. MCCh was prepared according to a method elaborated at ICF [22, 23]. The basic properties of the initial chitosan and its processed forms are presented in Table 1.

***Table 1.*** *Basic properties of initial and processed chitosans.*

| Chitosan form | Molecular weight $M_v$, kDa | Deacetylation degree DD, % | Polymer content, % | Heavy metals content, % | Water Retention Value WRV, % |
|---|---|---|---|---|---|
| Initial chitosan | 70 | 88 | - | 0.0047 | 82 |
| Chitosan acetate | 428 | 88 | 2.0 | - | - |
| MCCh | 460 | 88 | 2.5 | - | 1150 |

### 2.3. Methods

Average molecular weight (Mv) was determined by viscometry [24], degree of deacetylation (DD) by potentiometric titration [25] and water retention value (WRV) by gravimetrical method [26]. Fibroin content in chitosan/fibroin fibres was calculation by nitrogen determination using Kjeldahl method. Polymer content in polymer blend before liophilization was determined by gravimetrical method.
Mechanical properties of the obtained fibres, sponges and film were tested according to PN- EN-ISO 5079: 1999, PN-ISO 1973:1997, PN-EN ISO 527-3:1998 and PN-ISO 4593:1999 standards.

The surface structure of fibres, sponges and film as well as the cross-section of the fibres were inspected by the use of the scanning electron microscope SEM Quanta 200 (FEI Co).

## 3. Experimental Part

The chitosan/fibroin fibres were formed according to the wet spinning method. A mixture of a 10 % aqueous solution of fibroin and a 5 % aqueous solution of chitosan acetate was used as spinning solution. Aqueous NaOH solution (10 %) containing some amount of ethyl alcohol and fibroin served as coagulation bath. The fibres were spun at speed of 16.8 m/min and draw ratio of 1.36. Un-modified chitosan fibres were also spun at above-description conditions, as a control.

Sponges containing chitosan and fibroin were prepared by lyophilization. Blends with different content of both polymers with addition of a plasticizer were subjected to lyophilization. The drying time was 20 hours at – 35 ˚C of the freezing temperature.
Film preparations were made by conventional drying. Polymer blends with the same composition as for sponges were dried at 20 ˚C .

## 4. Results and discussion

The obtained biopolymers shaped into fibres, sponges and film were estimated in respect of mechanical properties and surface structure. The investigations allowed to optimize the composition of the preparations.

The production conditions of chitosan/fibroin fibres were determined. The presence of fibroin in the fibres causes only an insignificant decrease of fiber tenacity (see Table 2) which still permits the consecutive processing into dressing materials. The content of 6 % fibroin in the fibres, in comparison with single component chitosan fibres, did not cause any significant change in the structure of the surface and cross-section. This is illustrated in Figures 1 – 4.

***Table 2.*** *Some properties of chitosan/fibroin fibres.*

| Fibre | Content of fibroin in fibre, % | Titre, dtex | Tenacity conditioned, cN/dtex | Elongation at break conditioned, % |
|---|---|---|---|---|
| Chitosan | 0 | 3.04 | 17.2 | 9.2 |
| Chitosan/fibroin I | 4 | 2.99 | 15.9 | 8.5 |
| Chitosan/fibroin II | 6 | 2.60 | 15.2 | 8.1 |

***Tabela 3.*** *Some mechanical properties of chitosan/fibroin sponges; * sponge composition expressed as weight parts calculated on dry polymer, ** polymer content in polymer blend before liophilization.*

| Sponge symbol | Sponge composition* MCCh : Fibroin : Plasticizer | Polymer content**, % | Specific strength, MPa | Elongation at maximum drawing tension % | WRV, % |
|---|---|---|---|---|---|
| a | 0.5 : 1 : 0.4 | 4.5 | 0.012 | 9.45 | 580 |
| b | 1 : 0.5 : 0.4 | 3.2 | 0.017 | 9.78 | 510 |
| c | 1 : 1 : 0.4 | 3.7 | 0.018 | 9.45 | 540 |
| d | 1 : 0 : 0.5 | 3.7 | 0.038 | 7.22 | 460 |
| e | 0 : 1 : 0.3 | 3.7 | 0.025 | 1.64 | - |

For the chitosan/fibroin sponges, it was found that the surface structure depends mainly upon the weight proportion of the two components (Figures 5 - 8). When fibroin is prevailing (MCCh/fibroin – 0.5 : 1), then the obtained sponges reveal structure of the surface and cross-section shown at Figure 3 and 5. Such preparations were featured by high WRV = 580% and good elasticity reaching nearly 10 % (Table 3). Sponges made of polymer blends with more chitosan than fibroin (weight proportion MCCh/fibroin – 1: 0.5) also show a developed structure, however, a different one rather resembling the structure of sponges prepared of pure chitosan (Figure 6, 8). These products, too, had a very good elasticity and a high WRV on the level of 500 % (Table 3). In the investigations it appeared that impossible to prepare sponges of pure fibroin, The relevant preparation, when taken out of the lyophilization chamber, lost its fluffiness and turned into a film with good mechanical resistance and poor elasticity (Table 3).

It may be concluded that the addition of fibroin to MCCh allows for the preparing of chitosan/fibroin biomaterials in sponge form. Compared with sponges made of pure chitosan, the fibroin-containing ones are characterized by lower strength but, on the other hand, by better elasticity and higher WRV (Table 3). A possibility is given to alter the polymer composition, and obtain, in consequence sponges in a palette of properties.

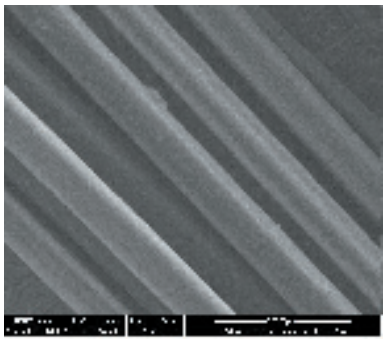

***Figure 1.*** *SEM image of chitosan fibres Longitudinal view.*

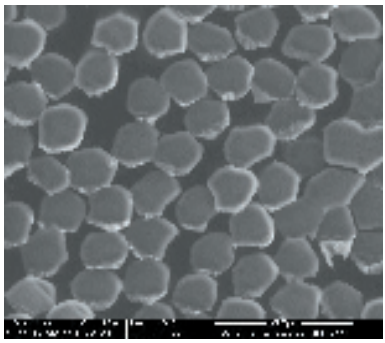

***Figure 2.*** *SEM image of chitosan fibres Cross-section.*

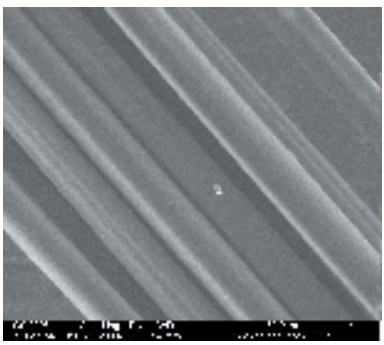

***Figure 3.*** *SEM image of chitosan/fibroin fibres. Longitudinal view.*

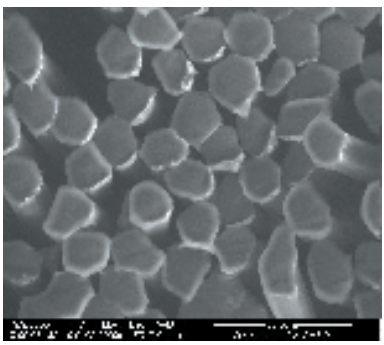

***Figure 4.*** *SEM image of chitosan/fibroin fibres. Cross-section.*

***Table 4.*** *Some mechanical properties of chitosan/fibroin films; * polymer content in polymer blend before drying.*

| Film symbol | Film composition MCCh : Fibroin : Plasticizer | Polymer content*, % | Specific Strength, MPa | Elongation at maximum drawing tension, % |
|---|---|---|---|---|
| a | 0.5 : 1 : 0.4 | 4.5 | 6.35 | 17.4 |
| b | 1 : 0.5 : 0.4 | 3.2 | 3.35 | 17.3 |
| c | 1 : 1 : 0.4 | 3.7 | 4.03 | 15.7 |
| d | 1 : 0 : 0.5 | 3.7 | 2.37 | 19.3 |
| e | 0 : 1 : 0.3 | 3.7 | 2.55 | 6.7 |

Table 4 presents mechanical properties of chitosan/fibroin films with varied composition. The obtained results allow to conclude that the addition of fibroin provides the possibility to double or even treble the specific strength of the film in comparison to those made of pure chitosan or fibroin. The modified film is also characterized by an adequate elasticity. Maximum strength parameters could be achieved with the MCCh/fibroin proportion amounting to 0.5 : 1. All obtained films are characterized by proper

strength, elasticity and a homogeneous surface which is shown in Figure 9. From the research ensues the possibility of preparing bio-preparations in form of film from polymer blends containing chitosan and fibroin.

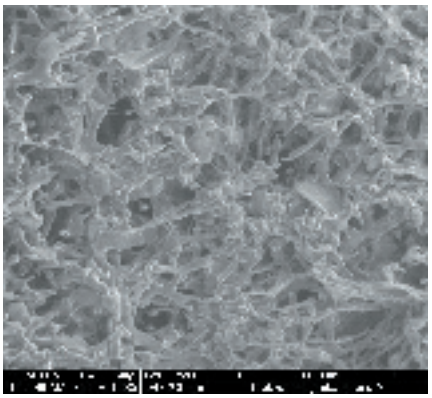

***Figure 5.*** *SEM image of chitisan/fibroin sponge type a . Surface.*

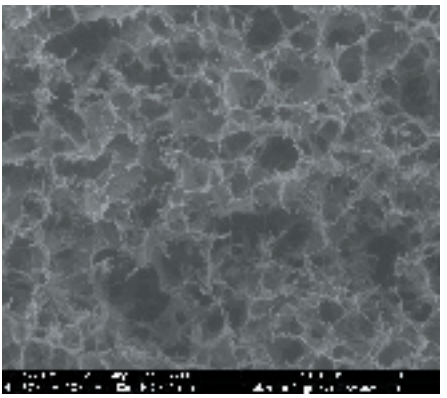

***Figure 6.*** *SEM image of chitosan/fibroin sponge type b. Surface.*

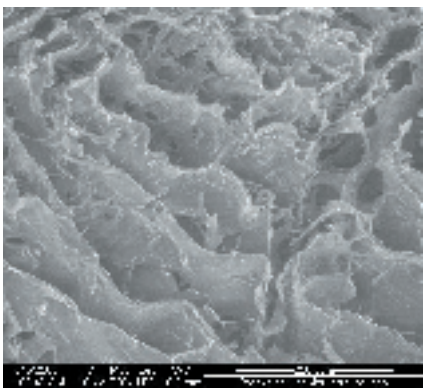

***Figure 7.*** *SEM image of chitosan/fibroin sponge type a. Cross-section.*

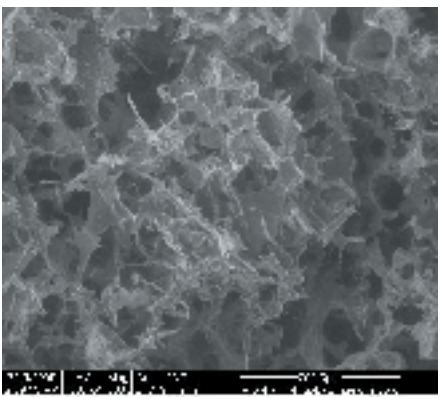

***Figure 8.*** *SEM image of chitosan/fibroin sponge type b. Cross-section.*

The research results presented here constitute just a fragment of a wider scope of investigations on biomaterials containing fibroin. Presently, biological properties of selected samples are tested *in vitro*.

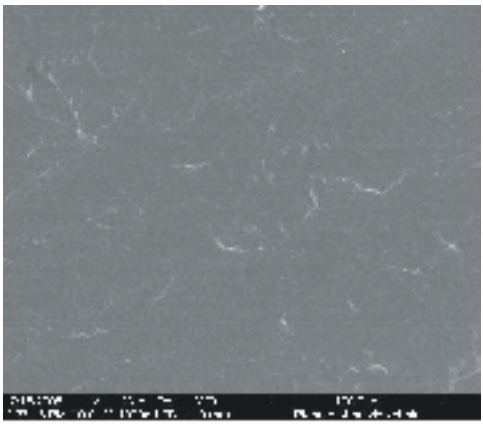

***Figure 9.*** *SEM image of chitosan/fibroin film surface, marked –b.*

## 6. Conclusions

Following conclusions may be drawn from the results:

1. The possibility is documented of preparing chitosan/fibroin - containing biopolymers in form of fibres, sponges and films.
2. Chitosan/fibroin fibres are featured by good mechanical properties which allowed to find them useful for a further processing into dressing materials.
3. Chitosan/fibroin sponges reveal different structures of surface and cross-section depending upon composition.
4. Chitosan/fibroin sponges are characterized by strength and elasticity which gives a well grounded chance to use them to the construction of dressings.
5. An addition of fibroin to chitosan provides the possibility to manufacture film with proper strength and adequate elasticity.

## 7. References


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