The Role of Morphological Structure of Fibroin/Alginate Blended Biomaterials on Their In Vitro Behavior

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Abstract: Blends of silk fibroin (SF), Bombyx mori and alginate (SA), in form of films and sponges, were investigated. The films were prepared by drying at room temperature, the sponges – by lyophilization. In vitro behavior test was performed in Simulated Body Fluid media (SBF) for 3, 14 and 25 days. It was found strong effect of biomaterial’s morphological structure on their behavior in 1.5 SBF. The films of SF/SA exhibit bioactivity. The sponges of SF/SA exhibit degradation. Two observed processes - bioactivity and degradation, depending on the ratio of SF and SA of the investigated blends. The changes were confirmed by FTIR, EDX, changes of sample’s mass and SEM. The effect of SA at the conversion of silk I to silk II into the blends was obvious, because the samples were not treated with alcohols. Alginate induced the conformation transition of fibroin’s macromolecules and recrystallization of β-sheets. It was found the interactions between the fibroin’s amido groups and carboxyl groups of the alginate that was generated by hydrogen bonds. As well FTIR was determined formation of ionic interaction (COO−)2 Ca2+ between alginate’s COO− groups and Ca2+ from 1.5 SBF. Forming of hydroxyapatite (HA) upon these places was confirmed by EDX and was a premise for the statement of films bioactivity. The morphology of films and sponges was examined by SEM.

1. Introduction

1.1. Silk fibroin/Alginate blended films
Natural biopolymer materials have good biocompatibility, biodegradability and bioactivity through that implement a wide range of applications in medicine and pharmacy. These properties make them irreplaceable in comparison to the variety of biomaterials based on synthetic polymers. Their application in the field of medicine requires a complex of properties, including physical, physicochemical and chemical, as well as opportunities for their modification, expressing the specifics of application fields. Recent years of investigations have shown an increased interest in silk fibroin (SF), which in the form of films, gels, membranes, sponges and fibers are used in various fields of medicine [1-8]. Alginic acid and its variety salts are used to treat wounds and burns because of their haemostatic properties. Their first application was in the form of a gel, but more effective turns sodium alginate (SA) in the form of a porous material [9,10].

Obtaining and application of blends in various fields of medicine can be described as rapid and serious growing research area involved in achieving optimal healing conditions. There are known investigations for the preparation of blends based on SF and a second component comprising gelatin, alginate, chitosan, etc., that achieve a desired change in the properties of the SF [11-14]. There is no generally shared view in the literature about interaction between SF and SA into their composites. Some authors, as a result of their investigation have concluded that there is no interaction between SF and SA [13]. Others [14] interpreted achieved results with interaction between SF’s amide groups and the carboxyl group of alginate [15], or the connection between the carbonyl groups of the SF and the hydroxyl groups of alginate [16].

1.2. Silk fibroin/Alginate blended sponges
There are many articles about SF porous materials which have been widely investigated in controlled drug delivery system, anticoagulant blood materials, biosensors, artificial skin, etc. [13, 17-25].

Degradation and bioactivity are two opposite processes, because the first one leads to a loss in the mass of the material, while the second - to its growth. The rate and extent of that they went off depend on two groups of factors. The first one included structure and morphological features of the polymers. The second is kind of physiological media - treatment conditions as well as type of reagent, concentration, time and temperature, that influence on these process. That complex of conditions, their
participation and their mutual influence, can make one of the two processes dominant.

In vitro degradation, unlike the in vitro biodegradation takes place in a similar physiological media, but in the absence of enzymes. Listed factors remain valid for these case and also carry very important information in achieving optimal properties for materials referred for medical applications [2, 26-33]. Many studies have found that the degradation of the porous materials depend on the conditions of their preparation and relates to the content of the crystalline form of β-sheets [24]. Depending on the method of preparation there are various kinds of porous materials, as of course, they have a different morphology [17].

Obtaining porous structures by freeze drying is a widely used method [22,33]. Morphological structure of sponges is characterized by a large number of pores, different by size and the distribution per unit surface area. This structure provides a large internal and external surface of the material. This is the essential difference with films that have a well-compacted structure and the absence of pores. Moreover, the sponges have the amorphous structure and the resulting much less intermolecular interactions, which is another factor making them readily available to the media. This whole complex of properties facilitates the penetration of the physiological media, which can alleviate and support the ongoing processes degradability or bioactivity. In the literature there are studies in this direction, as they are very important for reconstructive medical materials in terms of full recovery of living tissue in vivo conditions, and the use of medicine forms.

The aim of this work is to obtain blended biomaterials, in form of films and sponges, and to investigate theirs in vitro behavior.

2. EXPERIMENTAL

2.1. Materials

2.1.1. Obtaining Silk fibroin/Alginate blended films

The solution of SF was prepared from degummed silk fabrics. The preparation of the silk fibroin (SF) solution is performed by dissolving into the system CaCl₂: C₂H₅OH: H₂O (in a molar ratio of 1:2:8) [34] at a temperature of 75°C and duration 60 min. It was subjected to dialysis with distilled water for 3 days. Cellulose membranes are used /Slide-A-Lyzer/. The insoluble materials were removed by centrifuging 2 times at 3500 rpm. for 20 min. The final concentration of the aqueous solution of SF was 1.5%. It was determined by weight by drying at room temperature to constant weight.

SA used in the experiment was also in the form of an aqueous solution of 1%, purchased from the company Sigma-Aldrich. Blends were prepared by mixing solutions of the two components (SF and SA) with a different wt. % ratio – 75:25; 50:50; 25:75 (table 1). The blends were stirred for 30 min. The solutions were poured into polystyrene dishes and dried at room temperature.

Ratio SF/SA (25:75) obviously layered inhomogeneous structure with fibroin fibrils. Because of that reason this film has not been studied. Most authors studying composites based on SF/SA, observed homogeneous structure for all investigated ratios. On the other hand Moraes M., Silva M., Weska R., Beppu M., were found that in the composites where the amount of SF is more than 25%, exist the formation of SF fibrils [35].

<table>
<thead>
<tr>
<th>Sample numbers</th>
<th>Blends composition , %</th>
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<tr>
<td></td>
<td>Fibroin</td>
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<tr>
<td>1</td>
<td>100</td>
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<tr>
<td>2</td>
<td>75</td>
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<td>3</td>
<td>50</td>
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Drying at room temperature provides the preparation of films. The film-forming is a process which requires conditions of evaporation of the solvent, in this case water. It usually goes through three stages. During the last stage the polymer macromolecules become possible conformational changes, which result in organization of a film with good physical and mechanical properties [36].

To determine with greater clarity transforming structural changes in the blends, no post-treatment of the films with methanol, as it is done in almost all studies.

2.1.2. Obtaining Silk fibroin/Alginate blended sponges

Freeze-drying realized porous sponge-like structure, and therefore it is customary to call a sponge. Conditions for preparing the materials before freeze-drying are important, because they can lead to a different properties of the materials.

The solutions of SF and SA were prepared like the solution for films. Two components SF and SA in a different ratio - 100, 75:25; 50:50; 25:75, were mixed. The mixtures were stirred for 30 min. After that the solutions were poured into polystyrene
dishes and were frozen at -20°C overnight for 24 hours.

The examination includes prepared composites based on silk fibroin and sodium alginate, in the form of sponges with ratio of the components shown in Table 2.

<table>
<thead>
<tr>
<th>Sample numbers</th>
<th>Blends composition, %</th>
<th>Fibroin</th>
<th>Sodium Alginate</th>
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</thead>
<tbody>
<tr>
<td>1*</td>
<td>100</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2*</td>
<td>75</td>
<td>25</td>
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<td>4*</td>
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2.2. Methods

2.2.1. FTIR analysis

Fourier Transform Infrared Spectroscopy (FTIR) - a method for investigating the polymer’s structure occurred conformational transformation and other changes resulting from the interaction. The characteristic absorption bands of SF are known as amide I, amide II, amide III, amide IV and V amide. The initial SF fibers have values wave numbers, which are indicative for amide I at 1625 cm⁻¹ and 1701 cm⁻¹, amide II at 1518 cm⁻¹, amide III at 1229 cm⁻¹, amide IV at 1064 cm⁻¹, amide V at 725 cm⁻¹ and β-sheets regions have been existed. The structure of SF/SA blended films were analyzed by FTIR. In this case, the second natural polymer is SA. It is characterized by an intense absorption peaks of ionized carboxyl groups at 1610 cm⁻¹ and 1400 cm⁻¹; non-ionized carboxyl groups, posited at 1420 cm⁻¹; hydroxyl groups at 1110 cm⁻¹ and 1031 cm⁻¹ [37].

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FTIR spectra were recorded using equipment Bruker Tensor 27 spectrometer in the range of 4000 – 400 cm⁻¹, with the samples macerated in KBr matrixes.

2.2.2. In vitro test is widely used for determining the bioactivity, as it is easily applicable and reproducible works with a solution of 1.5 SBF, which has pH=7.4. The films and sponges were soaked in the solution, remaining for different time - duration of 3, 14 and 25 days. The 1.5 SBF aqueous solution consists: NaCl=11.9925g, NaHCO₃=0.5295g, KCl=0.3360g, KH₂PO₄.3H₂O=0.3420g, MgCl₂.6H₂O=0.4575g, CaCl₂.2H₂O=0.5520g and Na₂SO₄=0.1065g in 1L distilled water with buffering of pH=7.4; TRIS =9.0075g and 1M HCl [40].

In vitro behavior was defined by the change in mass of the samples. The calculation was done with the following equation:

\[
\text{Change in mass} = \left( \frac{W_0 - W_t}{W_0} \right) \times 100\% ,
\]

where:

- \(W_0\) - the mass of the initial sample,
- \(W_t\) - the mass of the sample after soaking in 1.5 SBF

The testing samples of known mass, were dried at 55°C for 1 hour, and then tempered in a desiccator and weigh. The weighing is accurate to the fourth digit. The procedure was repeated until a constant weight \(\langle W_0 \rangle\). After it is followed by soaking of the sample in solution of 1.5 SBF, in static conditions. The studies include varying soaking time in the solution at a temperature of 37°C. All samples were washed out with distilled water. Drying again at 37°C until a constant weight was achieved. All samples were weighed to the fourth digit.

2.2.3. Energy-dispersive X-ray spectroscopy (EDX) - an analytical method used for chemical characterization or elemental analysis of a sample. The energy of the X-rays are characteristic of the difference in energy between the two shells and of the atomic structure of the emitting element. With EDX was measured the elemental composition of the specimen. The visible light is in the range of 380 – 770 nm. To be able the measuring method to perform, the visible light length should be at least twice shorter than the length of the scanned object – \(\lambda<1\) [41].

Scanning electron microscopy with an energy dispersive X-ray spectroscopy detector (EDX/EDS) was performed using equipment Oxford Instruments - INCA Energy system to analyze the surface morphology and the element composition of the coatings after immersion in SBF.

2.2.4. SEM analysis - By scanning electron microscopy (SEM) can be observed microstructure and surface morphology of the studied materials. The morphologies (SEM) of silk particles were investigated using microscope Philips SEM 515 – model WEDAX 3A and Zeiss model EVO MA-15.

3. RESULTS AND DISCUSSION

3.1. Prepared blended films

3.1.1. Conformation transition and interaction between silk fibroin and sodium alginate
It is well known that the fiber-polymers are heterogeneous two-phase systems containing crystalline and amorphous regions. The geometry of the polypeptide macromolecular chain of SF is $\beta$-conformation, and it allows a high level of organization, including crystal areas /$\beta$-sheets/.

Dissolution of SF is associated with disruption of the compact network of Hydrogen bonds and destruction of orderliness in its structure - transforming the structural elements in the random coil, as well as $\alpha$-helix conformation for some parts of the macromolecular chains.

Study by FTIR analysis can determine the conformation transformation of Macromolecular chains from $\alpha$-helix to $\beta$-conformation and also the constituted hydrogen bonds between amido groups of SF and carboxyl groups of SA.

The process of film formation is connected to a reorganization of the SF structure from random coil and $\alpha$-helix as part of the macromolecular chains, which undergo a transformation to $\beta$-conformation and the resulting higher level of organization /$\beta$-sheets/. The protein of SF has the capacity to self-assemble and transition from silk I to silk II conformation [6].

Obtained material with ratio of SF/SA (50:50) has a spectral curve (c), which is similar in terms of intensity to spectrum (b). It has wide absorption strips without formed and separated peaks. There is a shift towards shorter wave lengths, for the area of amide I which reached 1612 cm$^{-1}$, and for amide II is 1501 cm$^{-1}$. Absorption maximum of amide III have its value at 1231 cm$^{-1}$. The observed value of 1612 cm$^{-1}$ may belong to its ionized carboxyl groups. It can be assumed that it is due to the increased amount of SA in the blend. Carboxyl groups have absorption at 1412 cm$^{-1}$. The explanation is similar to the previous - macromolecular interactions between SA and SF based on hydrogen bonds. The observed change in the blended materials structure shown that in the presence of SA there is a reorganization process of the SF, that includes conformational change of the SF macromolecule from $\alpha$-helix to $\beta$-conformation as well as a process of recrystallization ($\beta$-sheets). Both the pure SF film and the SF/SA blended films have the random coil and $\beta$-sheets in their structure, which co-existed in the films.
Same results were observed by other authors [43,44], examining blends based on SF with other natural polysaccharides such as chitosan, cellulose. Structural changes in the SF are induced by the polysaccharides.

3.1.2. In vitro behavior – structure change and ionic bond

\[ \text{Fig. 2. FTIR of films before and after (*) soaking in 1.5 SBF in 25 days duration: 100\% SF, a and a'};\]
\[ \text{SF/SA (75:25), b and b'};\]
\[ \text{SF/SA (50:50), c and c'}.\]

Result about structural changes into the films after their stay in 1.5 SBF was shown in Fig.2. SF participation itself (curve a) is characterized by intense absorption bands that include large areas. The interpretation including structural changing into the resulting film contains both α-helix and the β-conformation of the polypeptide macromolecular chains. After 25 days stay in the physiological media reorganization processes was observed in the areas of amide I, amide II and amide III. The large areas are divided into well-defined separate bands (curve b) with well pointed peaks. In the area of amide II two new peaks occurs at 1678 cm\(^{-1}\) - 1701 cm\(^{-1}\), proving the existence of a fraction with a high level of organization - β-sheets. Two well-defined peaks were forms for amide II at 1540 cm\(^{-1}\), at 1508 cm\(^{-1}\). Shift to the right for amide III – from 1241 cm\(^{-1}\) to 1233 cm\(^{-1}\), can be observed. The 1.5 SBF solution catalyzed processes of reorganization and recrystallization in the structure of SF.

The incorporation of the SA, as a second component and the preparation of the blend SF/SA (75:25), results in the reorganization and recrystallization of the SF component. Stay in 1.5 SBF, make this process more complicated. There are intense, characteristic peaks of β-conformation and crystal structures in the area of amide I – at 1681 cm\(^{-1}\), 1701 cm\(^{-1}\) and 1623 cm\(^{-1}\), In the area of amide II – 1538 cm\(^{-1}\) and 1501 cm\(^{-1}\). The ionized carboxyl groups of the SA (absorption at 1610 cm\(^{-1}\) and 1400 cm\(^{-1}\)) may be involved in the formation of an ionic bond with Ca\(^{2+}\), delivered from 1.5 SBF - mechanism known and proven by other authors [45]. The first value (1610 cm\(^{-1}\)) cannot be observed due to the overlap with the area of amide I, which probably includes its participation as well in the peak at 1623 cm\(^{-1}\). The second one has a value 1409 cm\(^{-1}\) and after 25-day stay it is 1405 cm\(^{-1}\).

Blended film of SF/SA (50:50) has wide bands of amide I and amide II, including lengths of both the α-helix and β-conformation. The result shown fragmentation expressing with separation of the broad stripes separated with narrow stripes with clearly demonstrated maximums. This means that the 1.5 SBF solution support the transformation of the SF from α-helix and random coil (1650 cm\(^{-1}\) - amide I, 1540 cm\(^{-1}\) - amide II and 1233 cm\(^{-1}\) - amide III) to β-conformation (1624 cm\(^{-1}\) – amide I, 1523 cm\(^{-1}\) - amide II) and obtaining of crystalline structures in the field of amide I - 1681 cm\(^{-1}\) and at 1701 cm\(^{-1}\) and amide II – 1508 cm\(^{-1}\). Most probable explanation is interaction between two biopolymers by hydrogen bonds. The behavior of the ionized carboxyl groups of the SA is similar to the one observed in the blend (75:25). Prior to immersion in the solution film has absorption at 1412 cm\(^{-1}\). After 25 days stay absorption stripe is shifted to the right and has the value at 1406 cm\(^{-1}\).

All tested experimental samples (100, 75:25, 50:50) were showed the same trend toward the influence of the 1.5 SBF solution on the properties of the blends – the transformation of SF macromolecule from α-helix to β-conformation, and the process of recrystallization of crystalline regions /β-sheets/.

Under these conditions it is possible forming an ionic bond with the carboxyl groups of the SA and the Ca\(^{2+}\), contained in a solution of 1.5 SBF , of the type (COO\(^{-}\))\(_2\) Ca\(^{2+}\). There are studies demonstrating the formation of such an ionic bond, and for some of them it is implemented and crosslinking of SA [45].

The most common method to prepare alginate hydrogels from an aqueous solution is to combine the alginate with divalent cations, ionic crosslinking agents [46,47]. The divalent cations cooperatively interact with blocks of G monomers to form ionic bridges. Usually, Ca\(^{2+}\) is one of the most commonly used divalent cations used to ionically cross-link alginate and calcium chloride (CaCl\(_2\)) is one of the best choices [48,49].
3.1.3. Change in mass

Performed study includes four test samples of different blends in the form of films, which are immersed in 1.5 SBF solution for different time - 3, 14 and 25 days. The obtained results are shown in Fig. 3. The experiment monitors and analyzes the influence of following factors: blended component ratio; soaking time in 1.5 SBF solution; morphological structure.

![Fig. 3. Mass increase of the films depending on blended ratio* and soaking time in 1.5 SBF solution. *number of samples according to Table 1.](image)

Figure 3 shows that the behavior of test samples, depending on their composition, have different increase in weight after soaking in 1.5 SBF solution. The observed general trend is an increase in mass with increasing soaking time. A higher mass increase of the samples was achieved in the blends including SA, compared with the same of SF itself. Also more amount of SA in the blends leads to a higher increase in the mass of the samples. The reason for these significant differences is that the pure silk SF has very compact structure, that hinders the penetration of the SBF components (Sample 1). The combination of SF with SA achieved the blends with "a loose structure" which is more hydrophilic, due to the hydrophilic nature of the SA (large amount of -COOH groups and natural porosity). Under these conditions it is possible forming an ionic bond with the carboxyl groups of the SA and the Ca$^{2+}$, contained in a solution of 1.5 SBF, of the type (COO)$^{-2}$Ca$^{2+}$. There are studies demonstrating the formation of such an ionic bond, and for some of them it is implemented and crosslinking of SA [45, 50-53]. It is known that crosslinking the polymer leads to a stabilization of their structure. The reported increase in the weight of the film by increasing the residence time in the physiological media may be associated with the preparation of a stable structure. The comparison between growth mass of the films from pure SF with that of the blends indeed confirms the positive role of the SA for obtaining materials with greater accessibility for physiological media.

The observed increase in the weight of the films is proportional to the increase in the amount of SA in the blends and the soaking time in 1.5 SBF solution.

3.1.4. Form of hydroxyapatite – bioactivity

Identified shifts from an absorption bands of carboxyl groups after aging the samples in 1.5 SBF, warranting the claim of formed ionic bond between COO$^{-}$ and Ca$^{2+}$. The mechanism of its formation is known and proven by other authors [45, 50-53]. It should be noted that Ca$^{2+}$ is supplied to the solution from 1.5 SBF. The connection position of (COO)$^{-2}$Ca$^{2+}$ are playing role of “seed sites” to the formation of a carbonate-containing calcium phosphate, as hydroxyapatite.

Kong et al.[54, 55] investigated mineralization of HA over SF surface in SBF at pH=8 at room temperature. They find out strong chemical interaction between SF and HA. In other article [56] regenerated SF films, containing calcium, were coated with HA after soaking in 1.5 SBF solution for 6 hours. Other authors [57] proved that SF can induce HA deposition at 37° C in 1.5 SBF.

Surface EDX analysis was performed to determine forming a hydroxyapatite after 25 days soaking in SBF of the sample with ratio SF/SA (75:25). The date can be seen on Fig. 4 (spectrum a, image b): a - EDX spectrum; b – SEM image.

In accordance with Fig. 4, image a, there are observed spectrum of Ca and P, which compose hydroxyapatite and are situated on “suitable sites” [50] with low intensity. The result is logical, because of the organic matrix of the films, containing C, N, O and their percentage share was respectively – 44.21, 33.04, 21.71. The Ca/P molar ratio is similar to obtained from X. Ma et al. [58] and is very close to stoichiometric ratio Ca/P =1.67.
SEM image shows small particles over the film’s surface with very different morphology. It can be suggested the formation of hydroxyapatite, which is proved from other authors [50, 58] and is in agreement with ours results.

3.1.5. Morphological structure

Resulting films can be described and distinguished visually depending on their composition. Pure SF gives a smooth, transparent, homogeneous film with good strength. The presence of the SA in the blends changes the type of the film when the ratio of the blend is SF/SA (50:50), and the film becomes cloudy and in some point inhomogeneous. The SF/SA (25:75) blended film has not been studied, as it gives an in-homogeneous structure with SF fibrils.

SEM analysis Scanning electron microscopy of the tested samples was performed at a magnification of 5000, in order to observe more detailed topography of the surface.

Obtained films can be described and distinguished visually depending on their blend. Pure SF gives a smooth, transparent, homogeneous film with good strength. SEM micrographie of pure SF (Fig. 5, image a) exhibit beads and irregular morphology. Analogical results have been observed [1] and the explanation of that topography is a conversion of the amorphous structure to the β−pleated structure (silk II). The presence of the SA in the blends changes the type of the film and it was depended on the ratio of the blends. Adding SA to SF in the ratio SF/SA (75:25) made the films surface smoother, compared with that of the pure SF film (Fig. 5, image “a” and image “b”). The film with ratio SF/SA (50:50) are smooth but becomes cloudy, opaque and in some point nonuniform (Fig. 5, image c). Blend with ratio SF/SA (25:75) will yield in-homogeneous structure with SF fibrils. Ratio SF/SA
(25:75) obviously layered in-homogeneous structure. Most authors studying composites based on SF/SA, observed homogeneous structure for all investigated ratios. On the other hand Moraes M., Silva M., Weska R., Beppu M., were found that in the composites where the amount of SF is more than 25%, exist the formation of SF fibrils [35].

There were no monitored pores for all investigated test samples.

3.2. Prepared blended sponges - conformation transition and interaction between silk fibroin and sodium alginate

Absorption spectrum of the pure SF material (Fig. 6, curve a) does not have well-defined bands of clearly shaped peaks. The area of amide I covers 1651-1697 cm⁻¹, of amide II is highly diffuse as well as of amide III. Blended materials (Fig. 6, curve b) - SF/SA (75:25) and (Fig. 6, curve c) - SF/SA (50:50) have similar spectral curves. Compared to pure SF their spectra have intense and wide absorption bands. For amide I the area includes amide I 1651-1697 cm⁻¹, for amide II the area is 1540-1515 cm⁻¹ and for amide III the area is 1239 cm⁻¹ and these values are similar for both composites. This result indicates that the structure of the resulting sponges contain both as α-helix and β-conformation of the polypeptide chain macromolecules. There is no sufficient difference between obtained sponges and it depends on freeze-dried technology.

Carboxyl groups of SA has a strong absorption in 1420 cm⁻¹, either of the two blends, SF/SA (75:25) and SF/SA (50:50), is at 1412 cm⁻¹. Shift right and obtaining well-formed peaks can be explained by interactions with leaking hydrogen bonds. To compare the effect of morphological structure between the films and the sponges it can be concluded that the films have more well-organized structure, while sponges have the structure of a lower level of organization.

3.2.1. In vitro behavior – degradation

In vitro degradation behavior of the blended sponges were systematically investigated up to 25 days in 1.5 SBF solution at 37°. The study includes four test sample (table 2), which are immersed in 1.5 SBF solution for different time - 3, 14 and 25 days. The obtained results are shown in Figure 7. The experiment monitors and analyzes the influence of following factors: blends component ratio; soaking time in 1.5 SBF solution; morphological structure.

Fig.7. Mass loss of sponges depending on blends component ratio and soaking time in 1.5 SBF solution.

*number of samples according to Tabl. 2.

Results show that all the test samples have a loss of mass, which may be explained by predominating of degradation process. Comparing the behavior of the films in the same solution under the same conditions pointed the main role of morphology. Sponges of the pure SF having the smallest loss in mass are below 10%. The presence of the SA, as a second component in the blends and increase the amount of it results in a clear increase in the losses of weight - to 40%. The factor time of stay in 1.5 SBF solution, also plays an important role in decomposition process of all tested samples. There are higher losses in the mass with an increase in the residence time. The result shows that in media of 1.5 SBF, blended materials with the examined composition when they are in the form of porous structure undergo a process of degradation. The sample blended of SF-SA (25:75) alone is an exception as it "stops" the degradation process.

The observed loss of sponges weight is proportional to the increase in the amount of sodium SA in the blended materials, and the time of stay in 1.5 SBF solution. SF/SA blended sponges were decomposed in the 1.5 SBF solution.
3.2.2. Morphological structure

The surface of samples, prepared by freeze-drying, can be described visually. They have very different morphology than that of the films. Pure SF has formations mimicking "crystal" and not enough pores to be described as a spongy structure. The incorporation of SA as a component of the blended material, resulting in a reduction of "fibrillar entities" and the presence of more pores - the structure more clearly look a like a sponge. Freeze-drying afforded the porous structure well suited as a carrier for medicaments.

Scanning electron microscopy of the tested samples was examined at a magnification of 1000, in order to observe more detailed topography of the surface.

![Fig. 8. SEM images of prepared blended sponges: 100% SF sponge, a; SF/SA (75:25), b and SF/SA (50:50), c.](image)

Sample of pure SF (Fig. 8, image a), look as a zipper, which is broken at random locations of cracks with small holes. Last ones were measured about 6-7 μm, as the average area of 5 cm² were obtained 0.03 numbers (or 0.02 at 5 μm²). There were also observed entities like crystals. The material has a spongy structure, but not porous enough.

The presence of the SA in the blends significantly improves the porous nature (Fig. 8, image b). There were observed also better openings or pores with larger size - 5-15 μm, whose number has grown by an average of 5 cm² were obtained 0.05 numbers (or 0.04 at 5 μm²).

Increasing the amount of SA (Fig. 8, image c), in the blends made the sample more well-defined spongy structure like - increased pore sizes - 7-21 μm, and their number per area. Average at 5 cm² gave 0.34 numbers (or 0.26 at 5 μm²). The results are logical, since a higher degree of "hydrophilic" of the blends causes the retention of more water and after its evaporation obtaining more "empty" space in the structure - pores.

To compare the effect of morphological structure between the films and the sponges it can be concluded that it is absolutely differently and is the main cause for their opposite in vitro behavior.

4. CONCLUSION

In vitro behavior of investigated films and sponges was showed two different processes - bioactivity and degradation. In spite of equal composition of materials and the same condition of in vitro test the results are opposed. The films of SF/SA exhibit bioactivity. The sponges of SF/SA exhibit degradation. The main reason is different morphological structure of films and sponges. On the other hand these results are the important possibility to obtain biomaterials of medical application for very different arias.

1.5 SBF causes structural changes in the biomaterials, forming ionic bonds (COO-)₂Ca²⁺ between -COO⁻ of the SA and Ca²⁺ from the solution into the films. The formation of hydroxyapatite over film’s surface means that they exhibit bioactivity. The other results were showed mix structure of the obtaining film contains both also α-helix, as well as β-conformation. SA in the blended materials is induce fibroin’s macromolecule conformational changes from α-helix to β-conformation, as well as a process of recrystallization(β-sheets). Interaction between fibroin’s amido groups and alginate’s carboxyl groups was happened and was realized by hydrogen bonds.

The sponge structure contain both as α-helix and β-conformation of the fibroin’s macromolecules. The presence of SA in the blends significantly improves the porosity of materials. SF/SA blended sponges were decomposed in media of 1.5 SBF.
5. CONFLICT OF INTERESTS

The authors have declared no conflict of interest.

6. COMPLIANCE WITH ETHICS REQUIREMENTS

This article does not contain any studies with human or animal subjects.

REFERENCES


