Loosening the skin function due to a lesion or a disease determines a severe unbalance and a physiological incapacity that can be lethal. Skin substitutes manufactured in tissues have their limits related to clinical utilization. In this field of specialization, there is no therapeutic tissue substitute able to take over skin function through a sole procedure, and with an adequate thickness for a corresponding coating of thick wound [1].

In order to obtain material support, in medical applications one uses natural polymers, such as keratin which, by their biological and physico-chemical properties, transfer these properties to the designed materials [2-5]. Regardless its origin, keratin presents scientifically substantiated morphological details that needed over time special cognition efforts. The evolution of the understanding of structural details and the role played in fiber biology has determined opposite electrostatic interactions, with the formation of a polymer loop that incorporate water.

The evaluation of formed hydrogel was carried out by determining the swelling degree. We have used optical and SEM photomicrographs to determine morphological organization, and EDAX micropictures for chemical modifications. A cellulose film realized by growing Gluconoacetobacter xylinus bacteria was used as control sample.

Keywords: beta-keratose, gamma-keratose, hydrogel, film

Considerations on human keratin utilization as biomaterial

Human hair is a keratin fiber developed from skin follicle, having the role of protection agent, sensor and sexual attraction, being a characteristic common to both man and mammals. The human hair structure is chemically and morphologically similar to the sheep wool. The difference consists in the fact that for wool, the ortho-para structure is differentiated, with cell disposition under the form of two bands twisted along the fiber [14,15]. Keratin...
products are biocompatible mediums that facilitate the cell growth [5].

**Experimental part**

One has collected undyed hair, cut at lengths of 2-5 cm, from men regardless the age, delivered by a hair dresser; then the collected hair was cleaned for 24 h, in a Soxhlet by removing the lipids, using a mixture of CHCl₃ and CH₃OH in ratio of 2:1 (v/v). The hair is dried by night at 40°C. The cleaned hair and an amount of distilled water are introduced in a sealed stainless steel capsule, at a solid/liquid ratio of 2:35 (w/w). The capsule is introduced in an air-drier at 150°C for 2 h, as illustrated in figure 1.

**Hydrogel 1**

In order to obtain the hydrogel from beta keratose film according to procedure 1, after cooling the solution, the supernatant is extracted through centrifugation at 10,000 rpm for 10 min. The obtained solution is cooled and additionally oxidized with 4 mL H₂O₂ for 10 min, repeating the procedure 6 times. Then the solution remains for 72 h at room temperature. The solution gets slowly thicker, forming a cross-linked gel.

**Hydrogel characteristics**

Swelling degree

The swelling degree is evaluated in percentages, based on gravimetric tests:

\[
SD(\%) = \frac{(M_f - M_{in}) \times 100}{M_{in}} \quad (1)
\]

where: \(M_f\) - weight of material after swelling, \(M_{in}\) - weight of the initial (dry) material.

In the case of performed determinations, the initial mass of beta-keratose subjected to treatment was 2 grams. Table 1 presents the swelling degree values obtained through gravimetric measurements in the two cases of hydrogel.

**Elemental composition determinations**

Chemical composition of the elements was determined in percentages through EDAX technique. The obtained data are summarized in table 2.

**Morphological aspects**

For morphological visualizations, one has prepared the following biomaterials:

Hydrogel 1 is illustrated in figure 2, witness sample consisting of bacterial cellulose film is presented in figure 3, biomaterial consisting of bacterial cellulose and beta-keratose is illustrated in figure 4, while figure 5 presents the biomaterial consisting of bacterial cellulose and gamma-keratose film, and figure 6 represents a micrograph of hydrogel 2 after drying.

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![Diagram of hydrolytic hair treatment device](image)

Inside the capsule at 150°C, a water vapor pressure of 4.9 at. (about 48•10⁴ Pa) is developed, according to Pavlov [16].

After treatment, two phases were separated through centrifugation for 10 min at 10,000 rpm, namely: a solid phase insoluble in water, considered as beta-keratose, and a liquid water-soluble phase, gamma-keratose.

**Beta keratose film**

In order to prepare the beta-keratose film, the material is subjected to a oxidation treatment with hydrogen peroxide, 3h, using at boiling 4 g solid phase and H₂O₂ at a ratio of 1:4 (w/v). The obtained solid phase is filtered and washed with 100 ml distilled water, by 6 times. It is dried for 24 h at 40°C. Then, a reduction process follows, using Na₂S₂O₄ added in 6 equal portions of 0.3 g each, in beta-keratose emulsion, at one hour intervals each, at 72°C. This is followed by solution cooling and its deposition on bacterial cellulose mass, and by lyophilization. The cellulose is in the form of a nanofiber veil, obtained by seeding Gluconobacter xylium.

**Gamma keratose film**

The liquid water-soluble gamma-keratose phase obtained from the hair subjected to a hydrolytic process at 150°C is deposited over the bacterial cellulose film.

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| No. | Sample          | C (%) | N (%) | O (%) | S (%) |
|-----|-----------------|-------|-------|-------|-------|
| 1   | Control         | 47.87 | -     | 51.12 | 1.01  |
| 2   | Beta-keratose   | 45.35 | 2.38  | 47.65 | 4.41  |
| 3   | Gamma-keratose  | 50.75 | 2.98  | 41.75 | 4.52  |
| 4   | Hydrogel        | 64.93 | 2.18  | 25.70 | 7.19  |

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![Table 2: Experimental values of EDAX elemental composition](image)
Fig. 2 - Optic microscopy image x100 of hydrogel 1 from beta-keratose in wet condition

Fig. 3. SEMx1000 micrograph of witness sample made of bacterial cellulose

Fig. 4. SEMx1000 micrograph of biomaterial consisting of cellulose film and beta-keratose

Fig. 5. SEMx1000 micrograph for the film of bacterial cellulose and gamma-keratose
Results and discussions

Taking into account that the skin represents a human organ with a big surface [17], its regeneration implies a series of peculiarities. According to the opinion of the authors of this study and to other bibliographic references [2, 5], keratins preparation with the formation of a biomaterial constitutes a basic sustaining element for artificial tegument realization.

The treatment of hair in the metallic capsule at 150°C and pressure of 4.9 atm. in the presence of water determines a hydrolytic process of protein material splitting in two parts: a solid part, considered to be beta-keratose, and a water-soluble phase, namely gamma-keratose [5,18,19].

In the case of the studied hydrogel, one has obtained different swelling degrees, as the result of procedures differences. Under the action of temperature and pressure as common treatment, both procedures participate in hair hydrolytic degradation in the two types of keratose, beta and gamma. This treatment is followed by an oxidative treatment with hydrogen peroxide, which facilitates the splitting of cystine disulfide bonds in sulphonic groups, as illustrated in figure 7.

According to the above presented images, one can obtain a hydrogel that follows the organization of the hair in quasi-fibrillary state (images from figs 2 and 6) or of more homogeneous formations (figs.3-5). Depending on keratin treatment and processing procedure, one can obtain more compact (beta-keratin and cellulose) or more porous formations.

According to EDAX data presented in Table 2, where the witness sample is a cellulose structure of bacterial origin, the presence of sulphur in the structure can be exclusively due to Acetobacter xylinum bacteria. For the other representatives illustrated in table 2, sulphur comes from hair cystine structure, while the nitrogen certifies the protein structure as the result of the presence of constitutive amino acids.

Different oxidation degrees indicate differences at the level of the implied chemical modifications and of keratin sources, from beta-keratin and gamma-keratin respectively.

Conclusions

The keratin structure of the human hair is susceptible of chemical transformations that permit to obtain a film or hydrogel precursors for the generation of a biomaterial as tegument substitute.

The obtained hydrogel determines the swelling degrees necessary to optimize the preparation factors that compete to hydrogel performance as biomaterial adequate as artificial tegument.

The obtained hydrogels, as well as the cellulose film are precursors for biomaterials meant for tegument regeneration.

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