## Human hair keratin-based film for biomedical applications



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Keratins extracted from human hair in aqueous solutions can self-assemble into the films which are successfully used in tissue engineering because they have the ability to support and improve cell growth, adhesion, migration and proliferation as well as for controlled drug delivery.

The keratin extract obtained from human hair was used for the production of the films. The hair samples were ground, washed in 1% sodium dodecyl sulfate solution, rinsed for three times with distilled water and dried at room temperature. For keratin extraction we used mixture which consists of 25 mM Tris-HCl, 5 M urea, 2.6 M thiourea and dithiothreitol. Protein extraction was performed at a temperature of 60°C at pH 8.5 for 72 hours. The obtained extraction mixture was filtered and dialyzed against distilled water for three days, protein concentration was determined by colorimetric method using Bradford's reagent. The protein composition was investigated by electrophoresis using the Lemmley buffer. The gels were stained with a 0.2% solution of Coomassie R-250, and then washed with a 7% solution of acetic acid. We prepared two types of films by casting method: keratin-glycerol film prepared from a 4% solution of keratin in distilled water and a 1% solution of glycerol and keratin-based film in the same concentration but without glycerol addition. Obtained solutions in a thin layer were poured into Petri dishes and incubated in a thermostat for 24 h at 37°C. After that, the films were fixed in water vapor for 24 h. Characteristics of the film surface and elemental composition were studied using a scanning electron microscope with an X-ray microanalyzer.

After keratin extraction using DTT we obtained a protein solution at a concentration of 3.75 mg/ml which consists of two polypeptide chains with a molecular weight in the range of 40–60 kDa and proteins with a molecular weight of 10–30 kDa. These proteins were used for film preparing. The keratin-glycerol based film after stabilization in water vapor acquired a gel-like consistency, which is explained by the property of glycerol to accumulate moisture. The keratin-only film was very fragile which is not good characteristic for biomedical purposes. Scanning electron microscopy shows that the surface of the keratin-glycerol film is homogeneous, without cavities and holes, when the surface of keratin-based film without glycerol is not so homogeneous, with a large number of recesses and protrusions. The results of X-ray microanalysis of both types of films indicate the high presence of Sulfur. This is due to the large number of disulfide bonds in the keratin molecule. In addition to Sulfur, such elements as Sodium, Silicon, Sulfur and Potassium were also found.

The obtained keratin-glycerol film due to its better mechanical properties can be used in reparative medicine and tissue engineering.

Key words: keratin, extraction, film, biomedicine, casting