

New Bioplastic Material Based on Hyaluronic Acid Hydrocolloid

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Abstract

Relief of the typical pathological processes and the early activation of regenerative mechanisms are the conditions for the successful wound healing. Reparative potential of epithelial tissues is realized at a balanced migration and proliferation of their cellular elements, synthesis of fibrous proteins and extracellular matrix components, epithelisation. An in-depth assessment was made of the structure of the developed bioplastic material based on hyaluronic acid hydrocolloid (G-Derm) and of its properties, positive in terms of optimizing the reparative mechanisms. Scanning probe microscopy and ultrastructure evaluation of bioplastic material were performed. For investigating the visualized globular structures, homogeneous samples of the material length 101.5 ± 11.2 nm, width 110.3 ± 10.7 nm, height 23.4 ± 3.4 nm were taken. The space between globular formations was 127.2 ± 21.3 nm. It was found that the roughness coefficient of the surface relief (Rq) was 8.7 ± 0.5 nm. The method of fixing the contact angle of water was used to evaluate hydrophilic/hydrophobic properties of biomaterial. Value of the angle was 83° ; adhesion coefficient was 99.88 mN/m², which characterizes the surface of biomaterial as moderately hydrophilic. Mesenchymal stromal stem cells were cultured on a substrate of bioplastic material based on hyaluronic acid hydrocolloid. In the course of ultrastructural study cell migration into the material was recorded, presence between the interwoven fibrillar fibres of cell layers of an oblong shape 3.7 ± 0.5 μ m wide, which reflects their cooperation with the surface of the developed polymer suitable for the use as a growth substrate in case of biotechnological replacement of tissue defects.

Keywords: Hyaluronic acid; Bioplastic material; G-Derm; Cell carrier; Biomaterial ultrastructure; Mesenchymal stromal stem cells

Introduction

Relief of typical pathological processes (microcirculatory disorders, interstitial oedema, uncontrolled inflammatory reaction, respiratory burst, acidosis, hypercytokinemia) and early activation of regenerative mechanisms are the conditions for successful healing of defects in epithelial tissues of various etiologies. Reparative potential of epithelial tissues is realized at a balanced migration and proliferation of their cellular elements, synthesis of fibrous proteins and extracellular matrix components, epithelisation [1]. For the purpose of plastic reconstruction of epithelial tissue defects as well as for ensuring self-healing of wounds and burns, various bioplastic materials based on synthetic and natural (preferably) polymers are extensively used [2,3]. Determination of the optimal physicochemical, mechanical and biological properties of natural bioplastic materials used for replacement of the epithelial tissue defects is extremely important and relevant in particular in terms of the ability of these polymers to ensure the optimal conditions for skin cells adhesion, migration and proliferation [4,5]. Study of the structural features of biopolymer material is important for biomaterials intended for plastic reconstruction of the epithelial tissue defects [2]. Publications contain information about the evaluation of relief and hydrophilicity/hydrophobicity of a number of natural polymers such as collagen, cellulose, chitosan as well as the materials obtained from resorbable polyhydroxyalkanoates (PHAs) [6]. It is shown that the relief and surface roughness of polymers in the nanoscale range can determine the adhesion, spreading and motion activity of cells and also influence the synthesis of specific extracellular matrix proteins. The presence of the surface potentials, specific features of the surface structure as well as its composition are the necessary characteristic which can affect the viability and proliferation of cells at interaction with bioplastic materials [7-9].

Purpose of Investigation

Experimental study of ultrastructure and biological properties

of histoequivalent-bioplastic material based on hyaluronic acid hydrocolloid.

Materials and Methods

The object of study was histoequivalent-bioplastic material based on hyaluronic acid hydrocolloid (G-Derm) produced photochemically (RF patents No. 2425694, 2367476, 2458709, 2481127). Samples of biomaterial were studied by scanning atomic force microscopy (AFM) in contact mode (multimicroscope "SMM-2000", Open Joint Stock Company OAO "Proton-miet", and Russia). In the process of scanning cantilevers "MSCT-AUNM" manufactured by "Veeco Instruments Inc." (United States of America) were used with beam stiffness 0.05 N/m, of a pyramid shape and curvature radius of the probe 10 nm. Quantitative morphometric analysis was performed using regular software for the microscope. Mechanical properties of bioplastic material were studied using AFM in power spectroscopy mode, at which the cantilever deflection is no longer dependent on the distance between the needle tip and the sample surface. For assessing the hydrophilic properties of the material as a parameter integrally characterizing its interaction with the polar solvent (water) the work of adhesion (Wa) was used determined from the evaluation results of the equilibrium limiting wetting angles [2,3]. Five mcl of distilled water (at temperature of 20-21°C.) were

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Received January 31, 2014; **Accepted** March 17, 2014; **Published** March 24, 2014

Citation: Zinoviev EV, Rakhmatullin RR, Almazov IA (2014) New Bioplastic Material Based on Hyaluronic Acid Hydrocolloid. J Clin Exp Dermatol Res 5: 215. doi:10.4172/2155-9554.1000215

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applied on the surface of biomaterial; 30 seconds later, photographing was accomplished, and determination of the values of limiting wetting angles (θ) was carried out at the liquid/solid phase interfaces [2,3]. The work of adhesion was calculated from the formula:

$$W\alpha = \sigma \cdot (1 + \cos\theta) \quad (1)$$

where σ is the surface tension coefficient of water at 20°C taken as 72.86×0.001 mN/m; θ , value of static limiting wetting angle. Taking into account the porosity and developed relief characterized by high roughness values, the calculation of the adhesion forces on the basis of the limiting wetting angle was carried out taking into account the roughness coefficient (the ratio of true surface area to geometric area) [10].

The results of the investigation were processed by the analysis of variance.

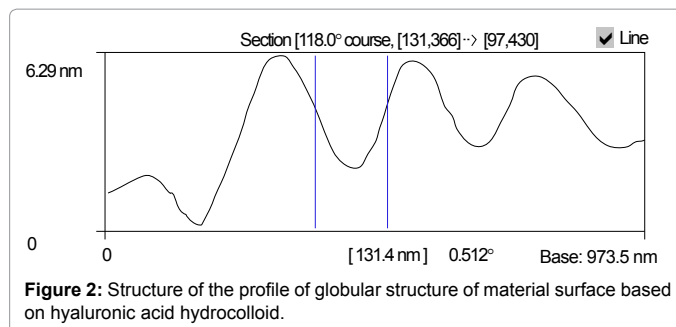
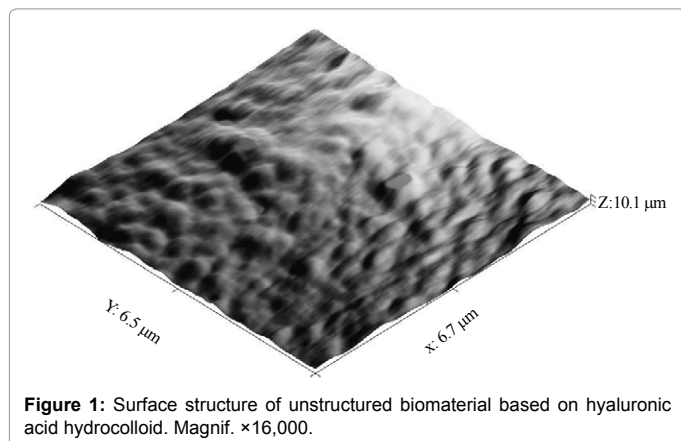
Results and Discussion

It has been ascertained that histoequivalent-bioplastic material based on hyaluronic acid hydrocolloid visualized by scanning AFM has a morphologically homogeneous surface, the surface ultrastructure being represented by globular formations of the same type (Figure 1).

One of the most important characteristics of the images obtained by scanning AFM is 3D geometry of the objects which allows a detailed analysis of their morphology along the drawn profiles and performing morphometry (Figure 2).

In the course of morphometry it was determined that the dimensional parameters of the visualized globular structures of bioplastic material based on hyaluronic acid hydrocolloid were as follows: width 110.3 ± 10.7 nm, height 23.4 ± 3.4 nm. The space between globular formations reached 127.2 ± 21.3 nm. The analysis of the development degree of relief using the parameter of mean square roughness (R_q , deviation of profile points from its midline) showed that the surface of bioplastic material based on hyaluronic acid hydrocolloid was a uniform texture with R_q values about 8.7 ± 0.5 nm (Figure 3).

A possibility of adhesion and migration of somatic cells of epithelial tissues on the surface of bioplastic material based on hyaluronic acid hydrocolloid is determined, on the one hand, by its hydrophilic/hydrophobic properties; and, on the other hand, by microrelief. It is known that the fastening of fibroblasts and keratinocytes is more likely to occur on the surface of material having high surface energy, on a hydrophilic surface. At the same time, basic cellular processes (growth, differentiation, migration) are strongly affected by geometric and dimensional characteristics of the substrate relief [11,12].



In the course of assessing hydrophilic/hydrophobic properties of biomaterial it was established that the value of the contact angle of water was 83°, and W_a force was 99.88 MN/m², which characterizes the surface of histoequivalent-bioplastic material as moderately wettable [12,13].

Additional visualization of biopolymer surface in registration mode of W_a forces allowed localizing nanoregions with elevated adhesion (Figure 4).

When considering the obtained data on physical properties of the developed bioplastic material based on hyaluronic acid hydrocolloid and linking them with a possibility of its use as a substrate when culturing somatic cells it was revealed that the morphological structure of biomaterial was mainly represented by globular formations forming a branched 3D structure with pore complexes from tens to hundreds of nanometres in diameter. Such 3D configuration determines a possibility of free diffusion of tissue fluid through the entire volume of the material. An important feature of this material is the stability of its morphological and mechanical parameters in response to wetting, which appears to be a favourable condition for using the material in the wound healing process.

Evaluating thermodynamic action of water on the surface of histoequivalent bioplastic material based on hyaluronic acid hydrocolloid from the limiting contact angle it was found that the recorded values of adhesion forces characterize it as moderately hydrophilic material. Such a result can be explained by the presence of air in cavities and pores of biomaterial, which prevents initial penetration of water. Thus, hydrophilic/hydrophobic properties of biopolymer appear to be dependent not only on physicochemical properties, but also on its structural features. This was confirmed by direct visualization of biomaterial surface in the adhesion-contact AFM mode. The obtained results point to occurrence on the surface of biomaterial of areas with adhesive properties which are rather large with respect to the visualized area.

When evaluating the cultivation of mesenchymal stromal stem cells on the substrate of histoequivalent-bioplastic material based on hyaluronic acid hydrocolloid by AFM method it was found that the cells of an oblong shape and 3.7 mm wide are detected on its surface (Figure 5a). In-depth study revealed the presence on the cell surface of intertwined fibres of, presumably, secondary and tertiary structure of collagen (Figure 5b).

Conclusions

1. Histoequivalent-bioplastic material based on hyaluronic acid polymer (G-Derm) maintains the stability of morphological and mechanical parameters in response to wetting, which appears to be favourable for creating the optimal conditions of humid environment

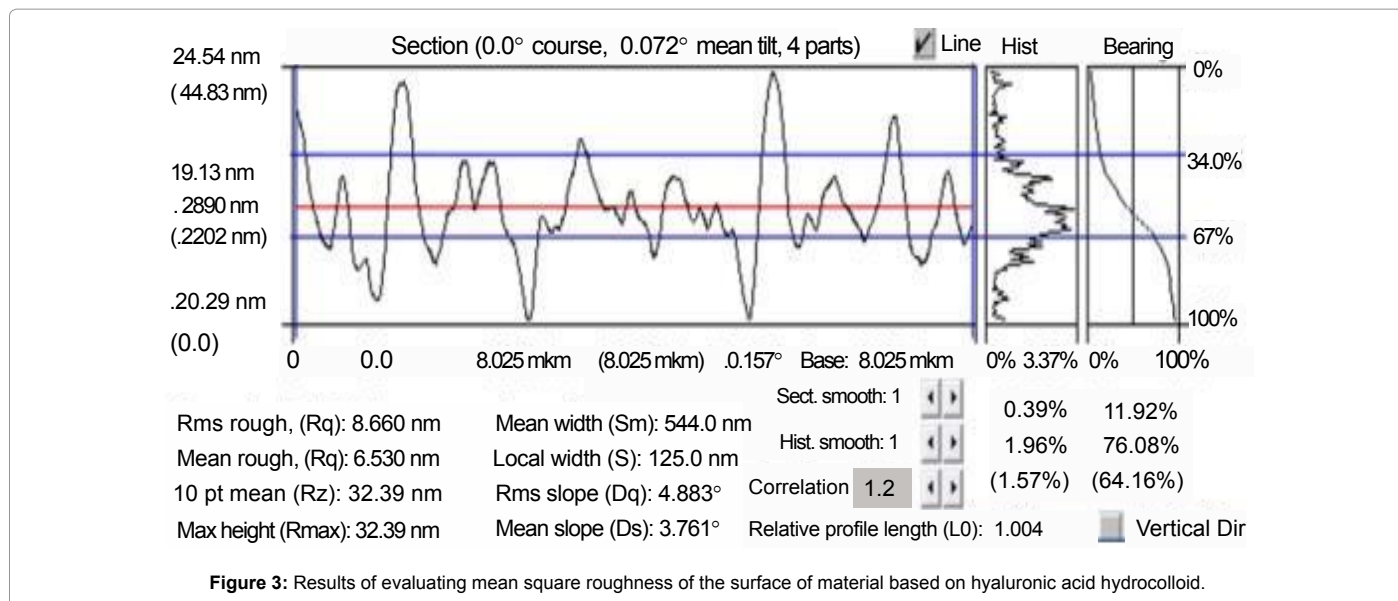


Figure 3: Results of evaluating mean square roughness of the surface of material based on hyaluronic acid hydrocolloid.

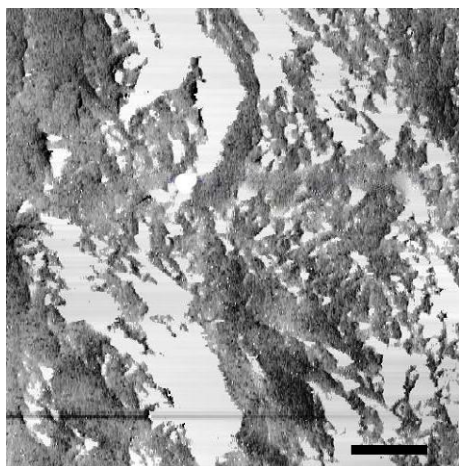


Figure 4: Distribution structure of Wa forces on the surface of material based on hyaluronic acid hydrocolloid. Scale-500 nm.

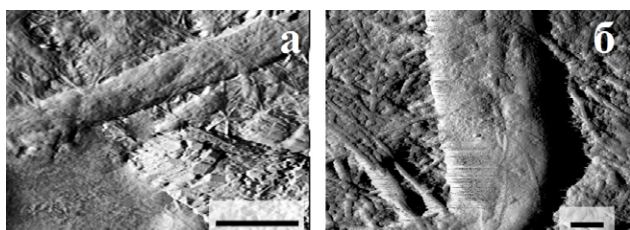


Figure 5: Mesenchymal stem cell on the surface of biomaterial: a, 5 mcm scale; b, 2 mcm scale. Arrangement of mesenchymal stem cells on the surface of biomaterial points to their migration processes, synthesis of fibrous proteins, active interaction with the substrate surface as well as penetration of hyaluronic acid hydrocolloid into deep layers.

during adhesion and migration of mesenchymal stem cells.

2. Material based on hyaluronic acid hydrocolloid is moderately hydrophilic and the hydrophilic/ hydrophobic properties of biopolymer appear to be dependent on physicochemical and structural properties.

3. After culturing of mesenchymal stromal cells, their presence is recorded in the structure of biomaterial as well as migration and protein-synthetic activity both on the surface, and in the structure of the polymer.

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