QPI and Atomic Force Microscopy in a comparative evaluation of biopolymer composites for a biomimetic matrix

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INTRODUCTION

Biopolymers are practically inexhaustible polymeric raw materials with different structures and properties: hydrophilicity, ability to biodegradation under the influence of various environmental factors and microorganism strains, unique chemical modifying ability, possibility to obtain universal products with desired morphological parameters1,2. Modern technologies allow us to develop new composite materials based on modified biopolymers with unique desired properties: biodestruction and bioreaction ability, bioinertness, resistance to mechanical loads, etc. Such materials are used as coatings, films, membranes, various types of additives in the production of medical, packaging materials and food products, in oil production processes, biotechnology and pharmaceutics

Over the past few decades, optical non-contact assessment methods have been actively developed, complementing tactile measurements of the surface topography of biopolymers. These methods are improved by incorporating and adapting well-known techniques and methods based on the geometric nature of light propagation: deflectometry, confocal microscopy, structured light, projection moire, and ultra-precise focusing. Interference laser microscopy is one of these high-tech, high-speed and non-invasive methods that have high sensitivity regardless of magnification, allow recording and visualizing features of the structure and properties of the surface, as well as processing the data obtained using modern mathematical algorithms. The purpose of the work is to identify the possibilities of interference microscopy for studying the structural, functional and biological properties of biopolymer matrices based on fibroin and chitosan.

METHODS

The analysis of the thickness, relief features, and structure of biopolymer matrices was carried out using methods of superresolving interference and atomic force microscopy. We used a MIM-340 laser modulation interference microscope (Shvabe, Russia) with following technical characteristics: resolution in the lateral plane - 13 nm; vertical resolution - 0.1 nm; the range of measurement of linear vertical dimensions - 0.01–0.10 μm, in the lateral plane (for a 20x lens) - 0.1–8.0 μm; field of view of the measuring channel in the lateral plane of not less than 20 microns (**Figure 1**).

As a comparative method to study the surface topography of film coatings on silicon-aluminum wafers and slides we used atomic force microscopy based on the NtegraPrima (NT-MDT, Russia) micro-consol system in the semi-contact mode. The obtained data were subjected to processing and comparative analysis in the program "Nova" based on INTEGRA and Solver platforms (**Figure 2**).

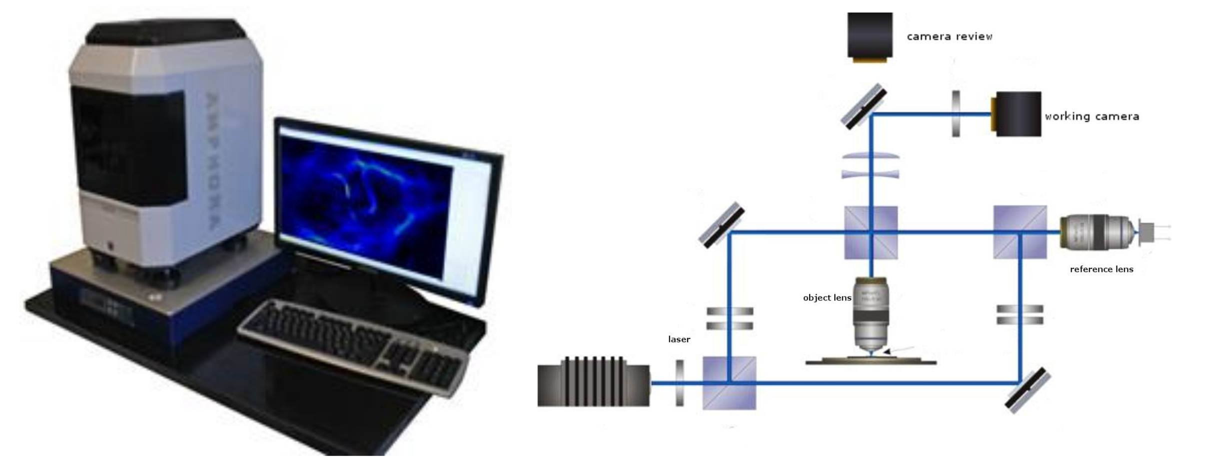

Figure 1. Computer Phase-Inteference Microscope

Figure 2. Atomic force Microscopy based on the NtegraPrima

RESULTS

At the first stage, the nature and roughness of the substrates (sublayer) were investigated, which were supposed to provide the necessary adhesive interaction with the polymer solution, but not cause physical and chemical changes in its structure. Experiments have shown the advantages of silicon-aluminum disks characterized by a uniform smooth surface with low roughness.

Figure 3 shows the results of roughness estimation of the polished substrate using interference and atomic force microscopy. As can be seen from the data obtained, the surface of the substrate is characterized by minimal roughness and, according to this indicator, can be used to produce thin films by centrifugation.

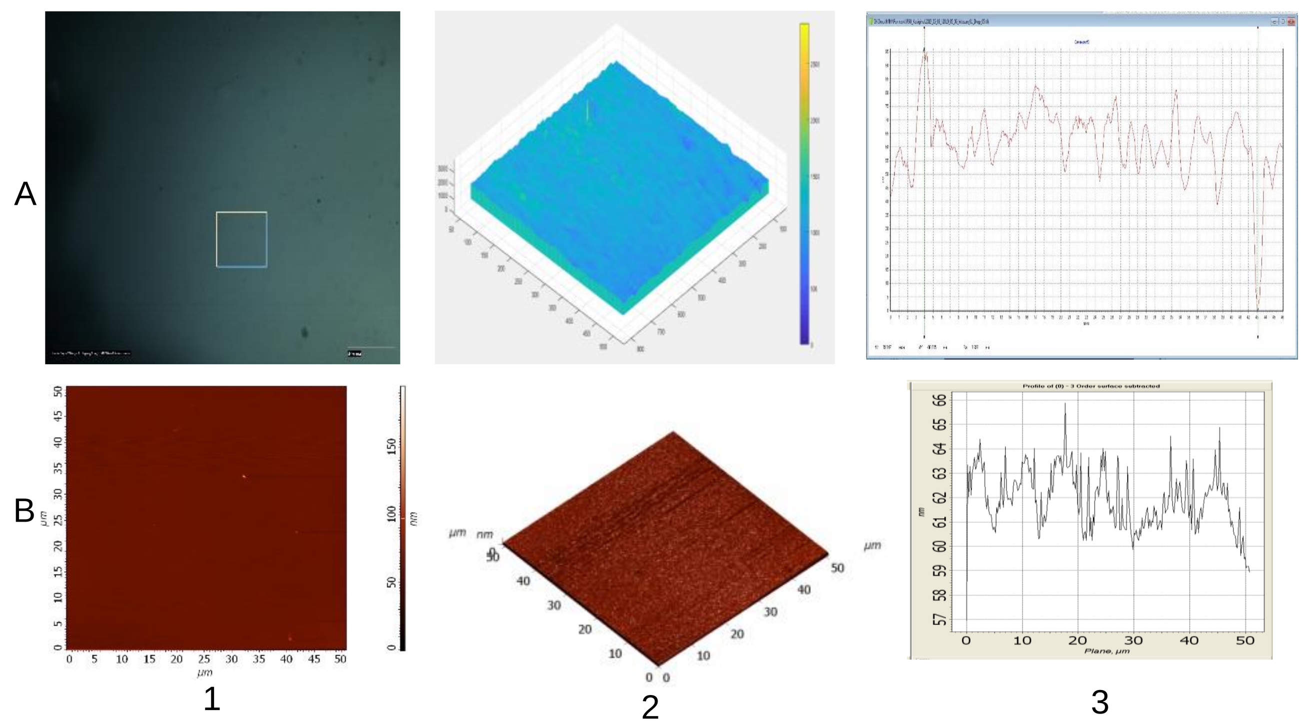

Figure 3. A – Modulation interference microscopy; B - Atomic Force Microscopy. 1 - visualizing the surface; 2 - evaluation of surface geometric parameters; 3 - averaged surface roughness index.

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In order to regulate the morphofunctional properties of the biopolymer matrix (composition and morphology of the surface), the molding composition was changed: the structural protein fibroin was added to the polysaccharide chitosan. This approach made it possible to form thin polymer coatings with a composition similar to the extracellular matrix – the natural environment for the formation of living tissues. The presence of amino groups in the resulting composites allowed simultaneous chemical crosslinking of polymers and the inclusion of fibroin in the three-dimensional structure of jenipine-crosslinked chitosan.

The surface morphology and structure of fibroin-containing films were studied by interference and atomic force microscopy. Optical, AFM and phase interference images of the surface of films obtained from mixed solutions of fibroin and chitosan are shown in Figure 3, and crosslinked with jenipine – in Figure 5.

The irregularities registered on the interferograms, which reach several microns when fibroin is added, are due to the inhomogeneity of the relief of the composite matrix coating formed as a result of phase separation during solvent removal. Crosslinking with jenipine smoothes the surface relief, but retains a strong surface roughness in the form of fiber-like formations that affect the thickness of the resulting sample and the roughness of its surface.

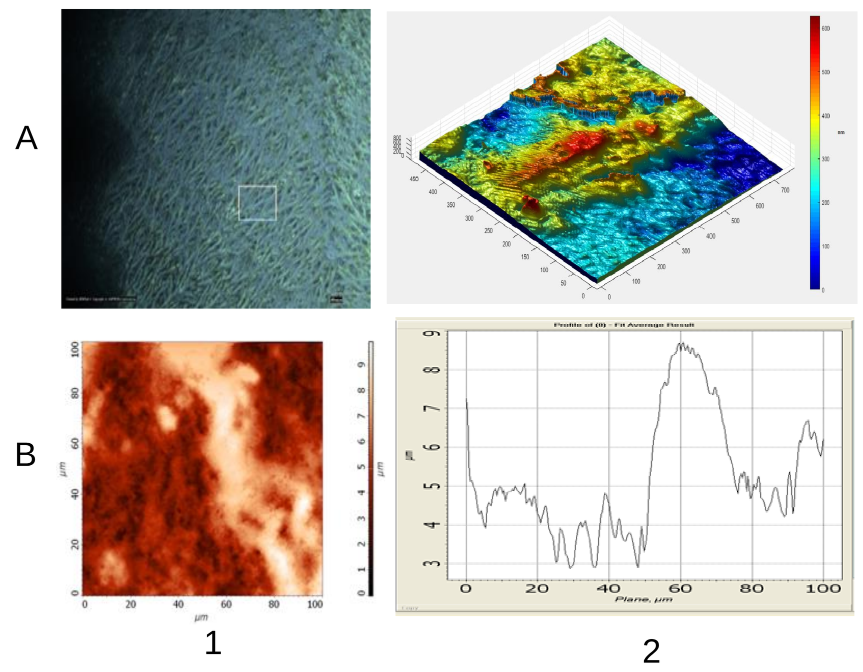

Figure 4. Investigation of the morphology of chitosan thin films containing fibroin. A – Modulation interference microscopy: optical (1) and 3D-phase (2) images of the surface. B - Atomic Force Microscopy: image (1) and surface micro-profile (2).

The high roughness of the chitosan-fibroin-containing composite films cross-linked with jenipine allows us to conclude that this surface can promote cell adhesion and spreading when used as matrices for tissue engineering. Predictions about the prospects of using films based on the studied composites as matrices for tissue engineering were tested in in vitro studies with various cytoobjects (leukocytes, platelets, mesenchymal stem cells).

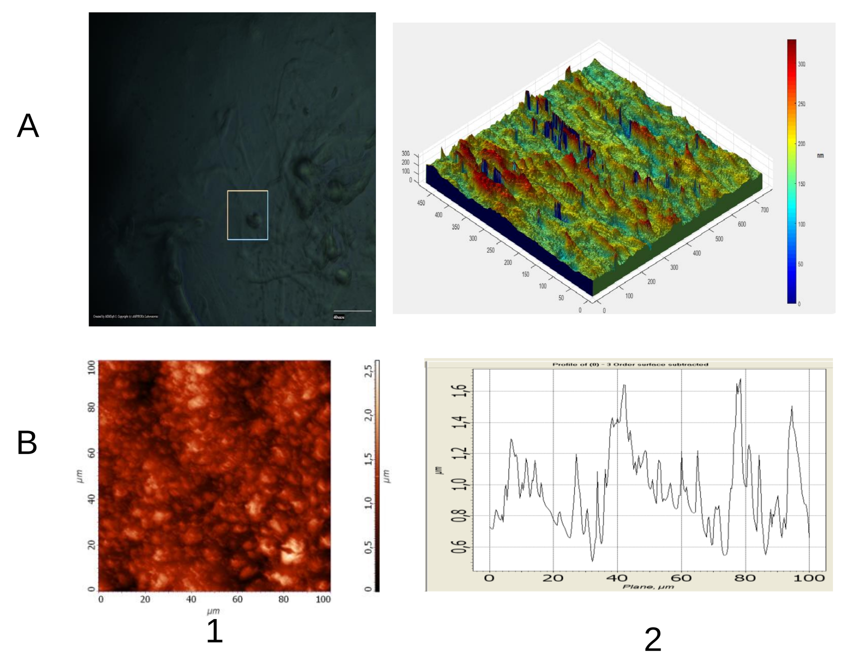

Figure 5. Investigation of the surface morphology of chitosan thin films cross-linked with jenipin and containing fibroin (1:1). A – Modulation interference microscopy: optical (1) and 3D-phase (2) images of the surface. B - Atomic Force Microscopy: image (1) and surface micro-profile (2).

CONCLUSIONS

The possibility of using modulation interference and atomic force microscopy to assess the structural and functional features of biopolymers intended for contact with the environment of a living organism is argued, their limitations are established, and a research methodology is proposed.

Using the technology of quantitative phase imaging, 3D visualization and non-invasive assessment of the structure of the obtained biopolymer composites were performed, densitometric criteria were tested, new data on the topography, functional properties of the surface and structure of the obtained biopolymer composites were obtained to identify the most promising samples.

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