**Study of the Surface Topografy of Biological and Synthetic Materials for Atomic Force Microscopy Surgery**

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**Abstract.** The paper presents a study of the surface topography of a number of synthetic materials used in surgical practice. Atomic force microscopy (AFM) was used for the analysis, providing high spatial resolution and the ability to assess the parameters of surface roughness, structure and homogeneity. Comparative analysis showed significant differences in the microrelief of biological tissues and synthetic implants, which allows us to judge the potential influence of topography on adhesive properties, biocompatibility and healing processes. The results obtained can be used to select optimal materials for surgical interventions and develop new biocompatible coatings.

**Keywords:** synthetic materials, biocompatible coatings, copolymers, surface topography.

**INTRODUCTION**

With the development of tissue engineering, one of the promising areas for bone grafting is the creation of cell grafts from biodegradable materials in combination with donor cells capable of restoring a damaged organ or tissue. However, for successful induction of osteogenesis at the implantation site, it is necessary to create a high initial concentration of cells, so there is a need to find an adequate carrier for fixing the transplanted cells in the recipient's body. Among the materials being developed and studied for these tasks, polyhydroxyalkanoates (PHA), linear polyesters of microbiological origin, are the most promising. PHA are of great interest for orthopedics due to their high biocompatibility, slow biodegradation and mechanical strength [1-3]. However, it remains unclear how the chemical composition, structure, properties of polymers (crystallinity, mechanical strength, temperature characteristics, biodegradation rates), surface properties of various polymer-based forms can affect the growth and adhesion of osteoblasts [4-6].

The relevance of the presented work is determined, on the one hand, by the importance and novelty of the objects of research, intensively studied by many scientific teams due to the interest in their fundamental characteristics and prospects for practical application in modern technologies. On the other hand, by the novelty of the developed experimental methods of nano diagnostics of the surface of materials with different degrees of structure ordering and relief structure using the atomic force microscopy method.

The aim of the work is to study the surface, physical and mechanical properties and surface morphology of forms based on PGA polymers using the AFM method [7-9].

**METHODS**

The work used samples of highly purified PHAs of various chemical compositions obtained at the Federal State Scientific Institution “Scientific Center for Biomedical Technologies of the Federal Medical and Biological Agency”: Homopolymer poly-3-hydroxybutyrate (P3HB) with polypolymer poly-3-hydroxyvalerate (P3HV) poly-3-hydroxyhexanoate (P3HH) poly-4-hydroxybutyrate (P4HB).

Based on highly purified PHA samples, products of various shapes were obtained: film matrices, 3D - porous and pressed matrices.

The microstructure of the film surface was studied using an automated AFM SOLVER NEXT.

Film matrices were obtained by pouring a 2% polymer solution in chloroform (0.4 g of polymer sample/20 ml of solvent) onto the degreased surface of Petri dishes. The films were dried in a dust-free laminar flow hood (Labconco, USA) at room temperature for several days.

Porous matrices were obtained using the leaching method. Sugar pieces (0,5×0,5× 0,5 sm) were impregnated with a 3% polymer solution in chloroform, then dried at room temperature. After evaporation of the solvent, the samples were washed in distilled water until the sucrose was completely removed.

To obtain pressed matrices, samples of highly purified PHA were ground in a laboratory mill. The cold direct pressing method was used on a laboratory automated Carver press. Auto Pellet 3887 (Carver, USA) under conditions of force equal to 8000 F, tablet forms were obtained.

The surface properties of the film samples: contact angle, surface energy, dispersed component, polar component, were calculated using a DSA -25 E contact angle measuring device (Krüss, Germany), using DSA -4 software for Windows. To study the surface properties, drops of liquid with different polarity (water, diiodomethane) were applied one by one to a strip cut from the film samples (1×4 sm). The moment of interaction between the liquid and the surface was recorded on a video camera and then processed in the software. At least 5 measurements were taken for each surface; the contact angle, surface energy, dispersed component, polar component, wetting were calculated, the average value and standard deviation were determined.

Physical and mechanical studies of strength and deformation were carried out using a universal electromechanical testing machine Instron 5565 (UK). Young's modulus (MPa), ultimate strength (MPa), failure load (N) and failure deformation (%) were measured.

The surface microstructure of the films was studied using a fully automated AFM for scientific research SOLVER NEXT. In this work, a probe sensor of the NT-MDT company of the DCP 01 series was used.

An NT-MDT microscope was also used as an AFM.

**results and discussion**

An important indicator that characterizes biological compatibility and affects cell adhesion and viability is the hydrophilic /hydrophobic balance of the surface of materials. The results of determining the surface properties of polymer films (wettability and energy characteristics of the surface), which were obtained on the basis of measured contact angles, are presented in Table 1.

**Table 1**. Surface properties of PGA film matrices.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Compound** | **Contact angle (water), deg** | **Surface energy, mN/m** | **Dispersed component, mN/m** | **Polar component, mN/m** |
| P(3GB) | 73,4 ± 1,37 | 39,5 ± 0,93 | 48,4 ± 0,74 | 4,4 ± 0,36 |
| P(3GB/P4GB) 90/10 mol, % | 56,1 ± 1,10 | 57,4 ± 2,44 | 42,4 ± 0,39 | 6,8 ± 0,25 |
| P(3GB/3GV) 89.9/10.1 mol, % | 64,4 ± 1,6 | 43,2 ± 1,41 | 47,5 ± 0,80 | 4,5 ± 0,74 |

The value of the contact angle of water wetting of the surface of the film made of the P(3HB/4HB) copolymer was lower than that of the film made of P(3HB) 73,4 ± 1,37º, and amounted to 56,1 ± 1,10º; for P(3HB/3GV) – 64,4 ± 1,6º. Surface energy is also an important characteristic that can influence the behavior of cells and the interaction of the polymer material with the tissues of the macroorganism. During the study of film materials made of PHA of different chemical compositions, it was shown that the lowest values of surface tension and polar component are characteristic of the homopolymer (39,5 and 4,8 mN/m, respectively), which have the lowest hydrophilicity according to this indicator (Table 2). For the copolymer P(3GB/4GB) these values are higher: 57,4 and 6,8 mN/m, respectively.

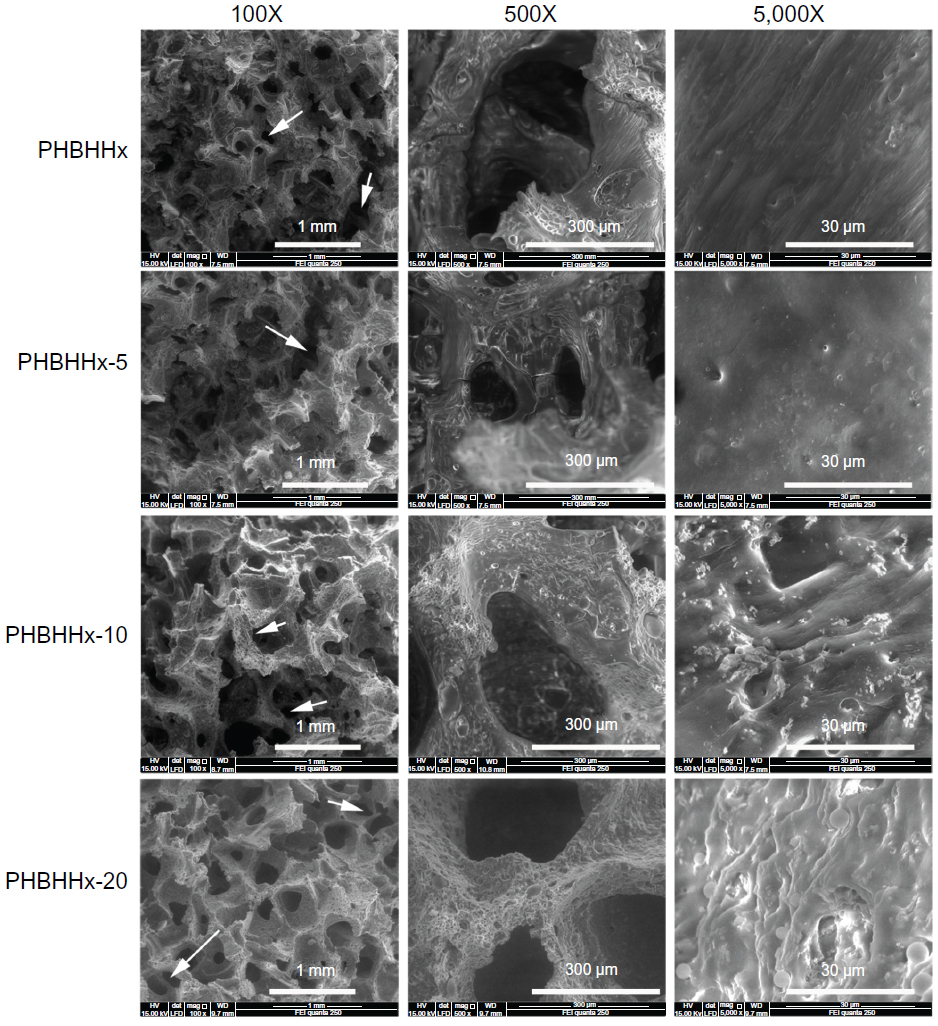
Physicomechanical characteristics: Young's modulus (modulus of elasticity, if a workpiece made of a known material is acted upon by a certain force, the material begins to resist this action: to compress, stretch or bend. The ability to such resistance can be estimated and expressed mathematically), ultimate strength, load at failure and deformation at failure of film matrices are presented in Table 2.

**Table 2.** Physical and mechanical characteristics of film matrices made of PGA.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Compound mol, %** | **Young's modulus, MPa** | **Tensile strength, MPa** | **Load at failure, N** | **Deformation at failure, %** |
| P(3GB) | 1502,11 ± 12,45 | 11,63 ± 2,04 | 14,0 ± 1,9 | 1,36 ± 0,37 |
| P (3GB/P4GB) 90/10 mol, % | 1184,45 ± 10,7 | 25,34 ± 2,6 | 11,0 ± 2,14 | 10,6 ± 1,2 |
| P (3GB/3GV) 89,9/10,1 mol, % | 1370 ± 11,7 | 33,12 ± 3,7 | 14,0 ± 4,03 | 12,4 ± 3,0 |

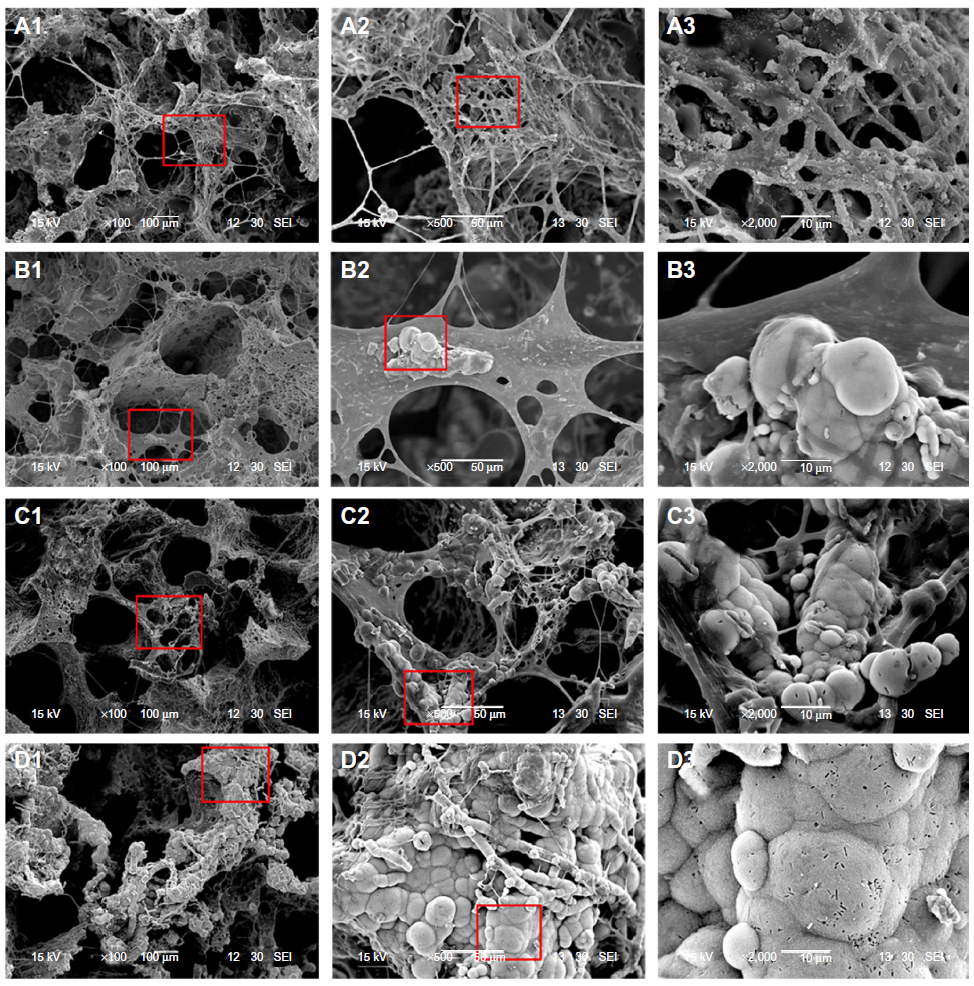
According to the obtained results, the Young's modulus was almost 1,5 times higher for films made of P(3GB) homopolymer compared to copolymers made of P(3GB/4GB). At the same time, the tensile strength of copolymer products was almost twice as high, and the deformation index at failure was an order of magnitude higher, i.e., films made of P(3GB/4GB) and P(3GB/3GV) withstand the highest mechanical stress, which is obviously associated with the structure of the copolymers and their properties, primarily reduced crystallinity.

Approaches to atomic force microscopy methods have been studied. The results obtained from atomic force microscopy are presented in the matrices pictures.

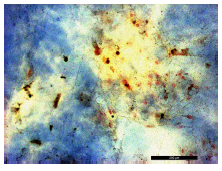


**Figure 1.** Scanning electron microscopy results of pure P3HB (poly-3-hydroxybutyrate) and porous P3HB (poly-3-hydroxybutyrate) with siliceous mesostructures.

Scanning of pure P3HB (poly-3-hydroxybutyrate) and porous P3HB (poly-3-hydroxybutyrate) with siliceous mesostructures (Figure 2) revealed that the content of siliceous mesostructured cellular foam does not have a significant effect on the pore size and the interconnections between pores (white arrows).



**Figure 2.** Results of atomic force microscopy of the matrix after immersion in the simulated liquid.



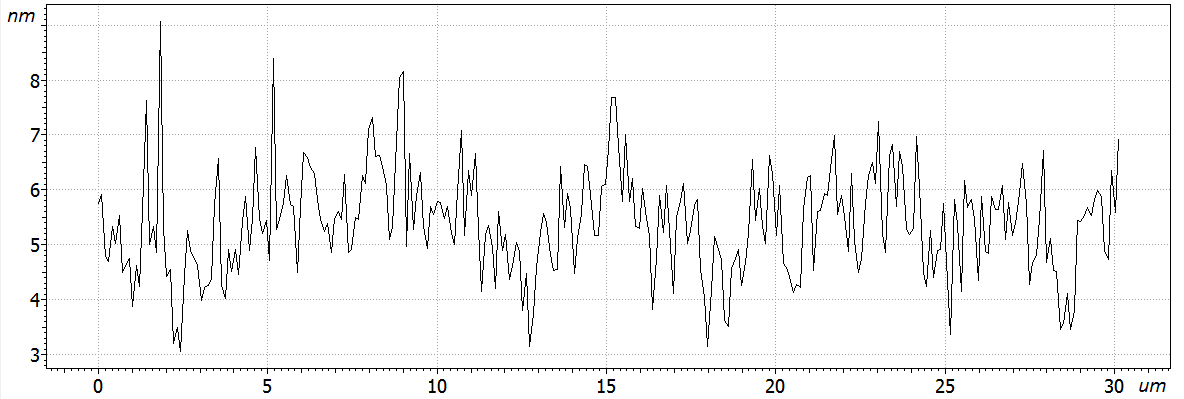
**Figure 3.** Pressed matrix

No obvious changes were seen on the surface of P3GB (A1–A3). Few particles were formed on the surface of PHBHHx-5 (B1–B3). Many spherical particles were formed on the surface of PHBHHx-10 and PHBHHx-20 (C1–C3 and D1–D3, respectively). The red area represents the magnification area.

Solver -NEXT atomic force microscope using a DCP 01 diamond-coated probe.

The first to be examined were pressed matrices measuring 3x3 mm (Figure 3). Clear traces of pressed particles were observed on the surface. Also, various "light dots" of different sizes were present in the images - these were all kinds of surface contamination (dust particles).

Figure 4 shows the surface profile of the pressed matrix. When examining it, one can notice wavy bumps with alternating sizes from 3 to 7 µm.

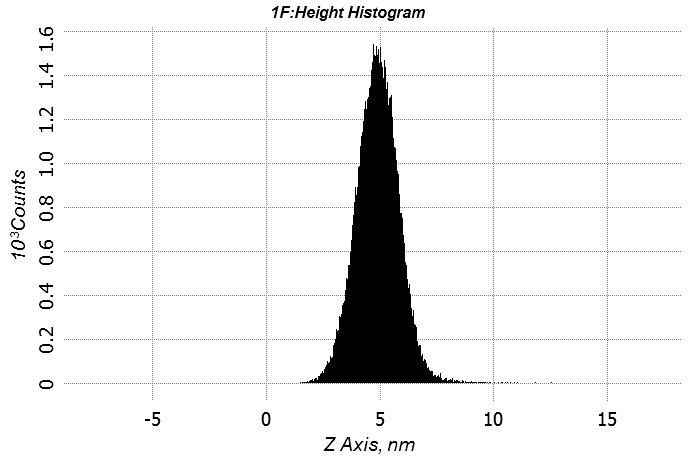


**Figure 4.** Surface profile 1.

These grooves are nothing more than pressed particles, which were also found in the drawings. Thus, it is possible to estimate the approximate sizes of the particles.

Next in the Image program Analysis P 9 subsequent mathematical evaluation of the morphology of the studied sample was carried out. This program has the ability to evaluate more than 20 different parameters of the structure. All calculated parameters are included in GOST 25142-82 and ISO 4287-1:1984. The main statistical characteristics of morphology are: c arithmetic mean roughness S a (nm), mean square roughness S q (nm) and the average height of the five highest and five lowest points of the surface S z (nm). It is worth noting that Roughness Analysis works in two modes: without selection and with selection of areas on the original 2D image.

Figure 5 shows a histogram of the distribution density of the values of the original 2D function (in our case, the magnitude of the roughness).

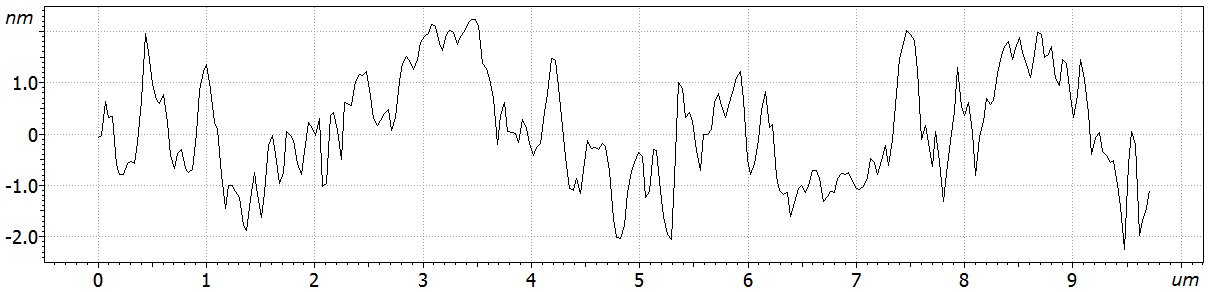


**Figure 5.** Distribution of surface roughness values for sample 1.

The field below the histogram displays the length of the partial interval, Histogram step, corresponding to the set number of partial intervals. In our case, Histogram step = 0,0376 nm. From the histogram data it can be seen that the average roughness value is approximately 5 nm.

In the same dialog box, on the Statistic panel Parameters displays in the form of a table the results of calculating the main statistical parameters for the original 2D function.

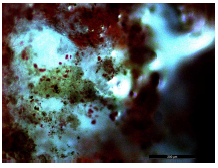
The next sample 2 (Figure 6) also represents pressed matrices of 3x3 mm. Traces of particles are also visible on this sample.



**Figure 6.** Surface profile of the pressed matrix.

According to the analyzed histogram of roughness height distribution, it was found that the average roughness lies within 5 nm, Histogram step = 0,0165 nm. According to the statistical parameters of the sample 2Sa = 0.92 nm and Sz = 9,906 nm.

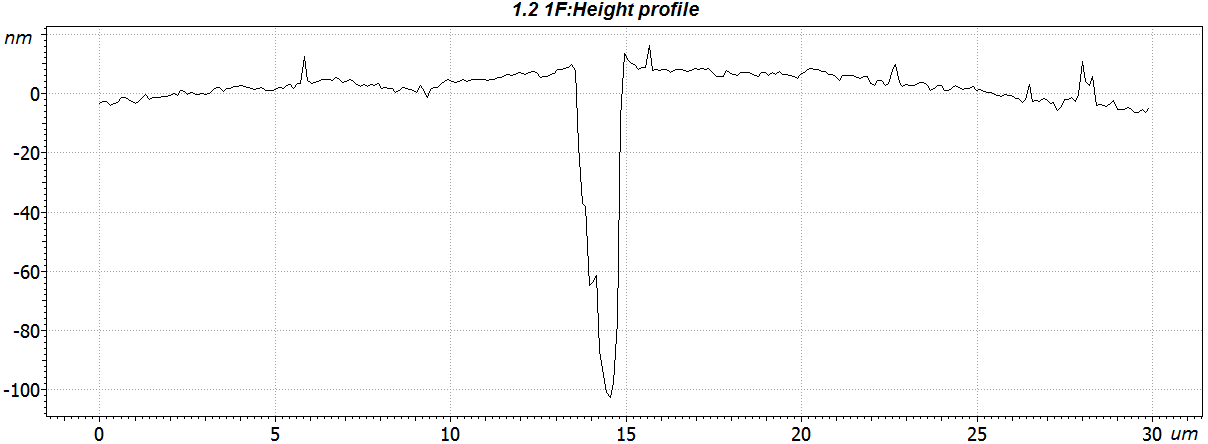
Sample 3 is a 3x3 mm plate with visible pores: blue area (Figure 7).



**Figure 7**. 3D porous matrix.

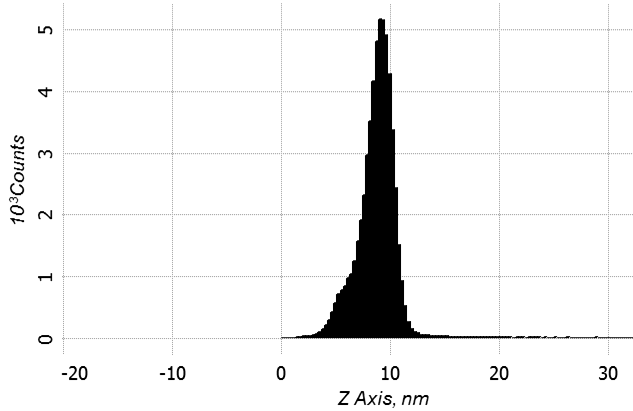
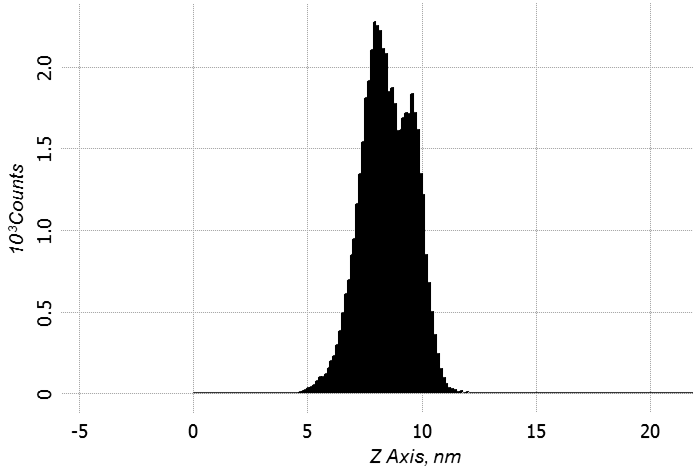
This sample clearly shows pores. This type of structure may indicate compliance with the technological process of creating this sample.

Figure 8 shows a 3D scan and surface profile of the 3D porous matrix, which clearly shows a pore in the structure in the form of a narrowing funnel with a size of 191 nm.



**Figure 8.** 3D scan and surface profile of the 3D porous matrix.

For this sample, a scan area without a pore was selected to determine the statistical parameters so that the histogram distributions would give us information about the average surface roughness without taking the pore into account (Figure 9).

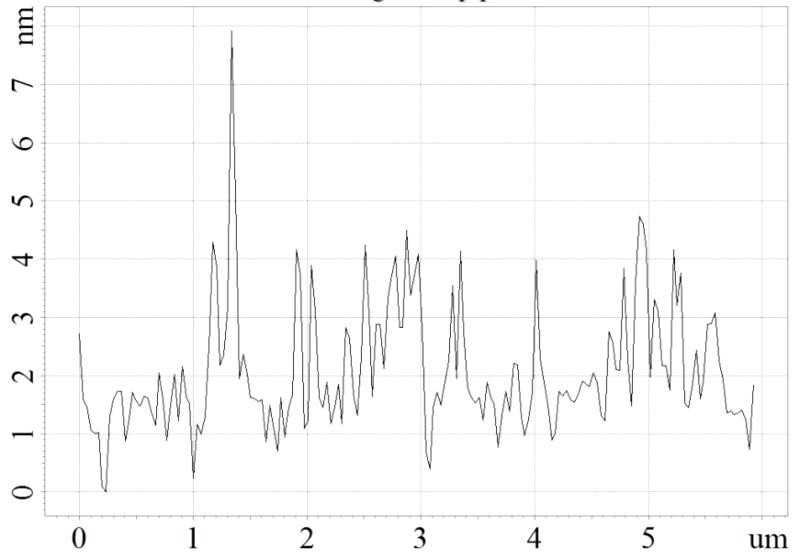


**Figure 9.** Distribution of surface roughness values in two areas near a pore.

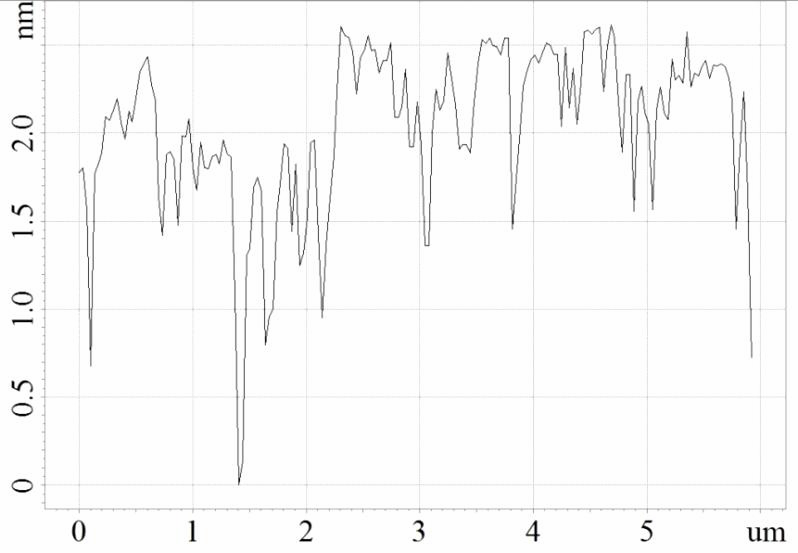
Based on the comparative data of the histograms, it can be concluded that the average roughness value in the non-porous region is different and is about 7 nm and 9 nm.

Sample 4 was a film matrix. The sample clearly shows the difference in the roughness of the areas: smoother areas and areas with more intensely expressed irregularities are distinguished.

By comparing the surface profiles in different areas (Figures 10,11) and also evaluating the obtained height distribution histograms in each sector (12 nm and 7 nm), it can also be confirmed that the morphology of the areas is different.



**Figure 10**. Surface profile of area 1.



**Figure 11.** Surface profile of area 2.

The obtained results prove that the use of AFM in the study of surface topography of synthetic materials is a promising method for obtaining in-depth information.

Thus, based on the conducted studies, it was established that an important indicator that characterizes biological compatibility and affects the adhesion and viability of cells is the hydrophilic /hydrophobic balance of the surface of materials.

When studying the physical and mechanical properties, samples with a high tensile strength were identified, which is an extremely important indicator of products in orthopedics.

The analyzed morphology of the studied objects shows a high degree of surface preparation, most samples belong to a high class of surface purity. A comparative analysis of the morphology of the studied samples was also performed.

**Conclusion**

In the process of carrying out this work, the features of synthetic materials for surgery were considered. Then, approaches to atomic force microscopy methods were studied.

Methods for studying the surface properties of the material, as well as its physical and mechanical properties, were studied.

When choosing a diagnostic method for the structures under study, it was important to consider the precision and reliability of the results obtained. In this work, the atomic force microscopy method was used as a method for studying surgical materials.

When analyzing the morphology of the studied structures, special attention was paid to the degree of roughness of the studied surface. A comparison of the morphology of various P3GB matrices was also carried out.

The conducted study allows us to qualitatively and quantitatively characterize the properties of a promising material for surgery.

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