The vertically aligned carbon nanotubes arrays as biointerface for the E. Coli strain M-17

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Abstract. The biological interaction materials study is necessary when creating biocompatible implantable devices, including biosensors. Important criteria for their creation are the bactericidal properties of such materials. In this paper, we study the bacteria with vertically aligned carbon nanotubes interaction. In this work we examined the bioaffinity of multi-walled carbon nanotubes samples with E. Coli strain M17 bacteria. We synthesized carbon nanotubes with various structural features on the surface of silicon wafers. Then we studied of the wettability of the obtained samples and tested bioactivity of E. Coli bacteria using spectrometry and photometry methods. It was found that E. Coli bacteria of strain M-17 demonstrated the best vital signs when interacting with the surfaces of hydrophobic samples of vertically oriented carbon nanotubes.

1. Introduction
For more than 30 years carbon nanotubes have been the subject of growing interest among researchers around the world [1]. There are two main types of carbon nanotubes: single-walled and multi-walled. Moreover, multi-walled are the same carbon modification as single-walled, but at the same time, consisting of many coaxial single-walled nanotubes. However, in contrast to their single-walled analogs, multi-walled carbon are not affected by quantum effects, therefore they demonstrate the monotony of physical, electronic and optical properties, despite the variety of structural configurations growing with the number of layers.

Carbon nanotubes are used in many applied fields of mechanics (creation of ultra-strong nanoscale filaments, composite materials [2]), microelectronics (field effect transistors [3], nanowires), optics (displays, LEDs [4]), energy (fuel cells [5]), chemistry (water purification [6-7] catalysis), as well as in biotechnology (highly sensitive biosensors [8-11]) and medicine (artificial muscles [12-13], targeted drug delivery [14], bioprostheses [15]). For a safer use of nanotubes in biotechnology and medicine, it
is necessary to study their effect on living organisms and tissues [16]. Carbon nanomaterials are widely used to create biointerfaces in such areas as neurology [17], implantology [18-20]. In works on the creation of nanosized carbon biomaterials, their toxicity [21], immunological response [22], biochemical effect [23], bactericidal effect [24] are studied, and the latter property plays an important role in the design of materials based on carbon (carbyne, graphene, carbon nanotubes, nanodiamonds and their derivatives) to create biocompatible materials that do not introduce infectious contamination into a living system [25]. Such nanostructures are used for drug delivery [26].

The biological interaction of carbon nanotubes with various biological objects can have a negative impact: toxic, allergic, carcinogenic effects, can be a source of infection, as well as a positive effect: antispetic and regenerative [27]. The type of impact is a consequence of the structural features and sizes of nanotubes. From the above it follows that at the moment the problem of the biological interaction of nanotubes with living systems still needs the close attention of researchers, since it is impossible to clearly predict how this or that modification of carbon nanotubes will behave.

According to [16], when studying the bacterial biocompatibility of *E. Coli* with various materials, it was shown that the structural features of the surface, as well as its wettability, influence biocompatibility. Thus, the authors of [16] showed that hydrophobic surfaces are more favorable for *E. Coli* growth than hydrophilic ones.

Our goal was to study the relationship between the structural properties of the surface of the samples under study with their wettability and bacterial biocompatibility as a ferment response using the example of interaction with *E. Coli* M-17.

2. Methods and materials

In the course of the study, we set the following tasks: 1) Synthesis of carbon nanotubes with various structural features on the surface of silicon wafers; 2) Study of the wettability of the obtained samples; 3) Study of the effect of samples containing multi-walled carbon nanotubes on the growth of *E. Coli* M-17 bacteria using spectrometry and photometry methods.

2.1. Synthesis and characterization of carbon nanotubes samples

Vertically aligned multi-walled carbon nanotubes were synthesized by the chemical vapor deposition (CVD) method (Moscow State University, Russia) in a muffle furnace in an N\textsubscript{2} atmosphere, while acetylene was passed in a mixture with a ferrocene catalyst as in [28]. Multi-walled nanotubes were deposited on a silicon substrate. The synthesis parameters of the three samples used in the work are shown in Table 1.

| Temperature of synthesis T, °C | FP-336 | FP-340 | FP-341 |
|-------------------------------|--------|--------|--------|
| The part of catalyst in mixture, % | 830    | 850    | 870    |
| Time of heating, t1, min      | 49     | 11     | 35     |
| Time of synthesis, t2, min    | 20     | 24     | 39     |

The microstructure of the multi-walled carbon nanotubes samples was investigated using a scanning electron microscope (SEM) Tescan LYRA 3 (Tescan Orsay Holding, Czech Republic) equipped with a high brightness Schottky cathode. The SEM allows one to obtain the size of a focused electron probe ~1.2 nm thick at a working distance of no more than 3 mm at the energy of the primary electron beam E0 of 30 keV. This allows the size of the multi-walled carbon nanotubes to be estimated with an accuracy of 10% for the corresponding size less than 50 nm.

Confocal micro-Raman spectroscopy was performed on an NTEGRA Spectra setup in the backscattering mode at room temperature with an exciting laser wavelength of 632.8 nm (1.96 eV). The power of the exciting laser was kept below 1 mW/m\textsuperscript{2} to avoid overheating of the sample. A 100x/0.9NA objective lens focuses the laser light into a 2 μm spot. The scattered light spectra were recorded with a CCD camera for 5 min each.
2.2. Preparation of bacterial suspension

For the preparation of suspensions, the preparation “Kolibacterin dry” (Microgen, Russia) was used (1 vial of the preparation contains 3 doses), in one dose of which the main substance is contained: at least $10 \times 10^9$ CFU of live bacteria of *E. coli* strain M-17, and excipient: gelatin, sucrose. The contents of the vial were diluted with a sterile 0.9% sodium chloride solution at the rate of 5 mL per dose. The drug was dissolved within 5 minutes, until a homogeneous, opaque suspension of yellowish or beige color was formed.

In order to test the effect of the samples on the growth of bacteria, we prepared a sterile meat-peptone broth (MPB according to Lennox, Diaem, Russia), which was then poured into identical biological test-tubes. Subsequent studies were conducted using dilutions of the bacterial suspension in concentrations of $10^{-7}$ and $10^{-10}$ of the initial dose. To achieve the required concentrations, a serial dilution method was used, which provided for the use of: 10 test-tubes filled with 9 mL of sterile 0.9% sodium chloride solution, diluted “Kolibacterin”. Then, to obtain the first dilution, 1 mL of the bacterial suspension of the prepared preparation was transferred to the first tube, thereby, a $10 \times 10^{-1}$ dilution was obtained. After that, 1 mL of suspension was taken from the first tube and poured into the second tube – a dilution of $10 \times 10^{-2}$ was obtained, and so on up to 10 tubes. As a result, dilutions from $10 \times 10^{-1}$ to $10 \times 10^{-10}$ were obtained.

2.3. Cultivation of the bacterial culture

From the test tubes with the necessary dilutions using a Top Pe tte Pipette 100-1000 μL (Dragon Lab, China), using disposable sterile tips (tip for dispenser type-2, 100-1000 μL, Liptoplast-Med, Russia), 0.1 mL of the bacterial suspension was collected and transferred to test tubes with 10 mL of sterile MPB (MPB according to Lennox, Diaem, Russia). Then, 0.015 g of samples was introduced into test tubes with bacteria; for each sample, two test tubes with dilutions of $10^{-7}$ and $10^{-10}$, respectively. In addition to test tubes with test samples, there were two tubes: with culture control and with environmental control. After entering the samples, all tubes were placed in a thermostat (TS-1/80 SPU, Russia) for 24 h at 37 ºC. After 24 h, optical density and spectrometry of the obtained daily bacterial cultures were measured.

2.4. Study of the wettability of samples with distilled water

The wettability angle was measured by photographing (camera resolution 12 MPx) a solution drop on a setup shown on the figure1. Samples were pre-prepared and mounted on a table in a strictly horizontal position. A drop on the sample was placed using a TopPette Pipette 100-1000 μL (Dragon Lab, China), using disposable sterile tips (tip for type-2 dispenser, 100-1000 μL, Liptoplast-Med, Russia), supply distilled water was carried out over each sample until a whole drop came off the tip. After 3 min of holding the drop on the sample, photographing was carried out. Measurements of the wettability boundary angles of a still drop were carried out by the tangential method using the ToupCAM software.

![Figure 1. Scheme of the wettability experiment: 1 – stage, 2 - sample, 3 – drop, 4 – camera, 5 – tripod.](image)
2.5. Measurement of the bacterial culture optical absorption spectra
The spectra were measured in the daily bacterial culture of *E. Coli* grown in meat-peptone bullion (preparation procedure described above).

Measurements were taken on a Shimadzu UV-1800 spectrophotometer. The results were processed using spectrum processing UV probe version 2.42.software.

The 10–7 and 10–10 dilutions of the bacterial culture were used for measurements. Before the measurements, the absorption indices were zeroed; for this purpose, a sterile MPB was used; the spectral range was from 300 to 1100 nm with a step of 0.1 nm. For each tube, five spectrum scans were performed. Successively, each sample was measured at all dilutions. For this, 5 mL of culture broth was collected in a cuvette. Before each measurement, the cuvette was washed with distilled water.

2.6. Density measurement of bacterial culture cells
To measure the density of bacterial cells cultures, we used a Densitometer II – Mikrolatest (Erba Lachemas.r.o., Czech Republic and Slovakia), operating wavelength 535 nm. Measurements of optical density were carried out in a daily culture of *E. Coli* M-17, the growing method described above. Before measurement, the instrument was calibrated using reusable sterile tubes with meat-peptone bullion identical to those in which the cultures were grown. After that, the culture tubes were placed in a tube block, and a 3-fold measurement of the optical density of each sample was carried out.

The results were recorded, and then their statistical processing was carried out.

3. Results and discussion
Carbon nanotubes studied in the work have different structural and surface properties. In particular, FP-336 and FP-341 (figure 2a and 2c), unlike FP-340 (figure 2b), practically do not contain a loose carbon film on the surface, and the FP-336 sample has the cleanest surface. The characteristic size of micro-roughness on the surfaces of FP-336 and FP-341 samples is no more than a few micrometers (~3 µm), while for FP-340 there are larger irregularities of 30µm.

![Figure 2](image-url)

*Figure 2*. SEM images of samples of FP-336 (a), FP-340 (b), and FP-341 (c).

It was previously shown [29] that samples FP-336, FP-340 and FP-341 were characterized by the following features of the Raman spectra shown in table 2. They show that the D and G peaks characteristic of carbon graphite materials are located at frequencies of 1362 and 1587 cm<sup>-1</sup>, respectively. For samples FP-340 and FP-341, the intensity of the D peak is higher than the intensity of the G peak, while for sample FP-336 I<sub>D</sub>/I<sub>G</sub> < 1. This indicates a greater crystallinity of the nanotubes contained in FP-336 and the presence of defects in FP-340 and FP-341. If we turn to the micrograph of FP-340, we can note the presence of a “cap” of misoriented carbon nanotubes on its surface, and as a result, more defects, as evidenced by Raman spectra.
Table 2. Raman data for samples containing multi-walled carbon nanotube arrays [29].

| Relative peak intensity | FP-336 | FP-340 | FP-341 |
|-------------------------|-------|-------|-------|
| I_D (1362 cm⁻¹)         | 0.87  | 1     | 1     |
| I_G (1587 cm⁻¹)         | 1     | 0.75  | 0.91  |

Experiments on measuring the optical density of the daily culture of bacteria *E. Coli* M-17 showed that the highest optical density was observed in test tubes with samples FP-336 and FP-342 (table 3), that testified the highest concentration of bacteria.

Table 3. The bacterial culture cells density measuring results of the in the presence of samples of multi-walled carbon nanotubes, 10¹⁴ cells/m³.

| Dilution | Sample | Control |
|----------|--------|---------|
| 10⁻⁷     | 2.7    | 1.2     | 2.7    | 1.2   |
| 10⁻¹⁰    | 0.9    | 0.9     | 0.9    | 0.9   |

In the measurement range from 300 to 1100 nm, the following features were noted. For all three samples, the presence of two peaks at a wavelength of 460 and 415 nm is recorded. These peaks correspond to the presence of pyruvate dehydrogenase (460 nm) and triamine deaminase (415 nm) enzymes in the studied suspensions. Both enzymes are involved in the metabolic processes of bacteria. Consequently, in cell cultures, bacteria do not stop in various forms of vital activity, and samples FP-336 and FP-341 favorably affect the intracellular processes of exchange of bacterial cells, as a result of which we can assume increasing of bacteria cells concentration in broths with these samples.

Figure 3. Optical absorption spectrum (Abs) of a bacterial suspension grown in the presence of three samples.

It is known that structure and surfaces affect hydrophobic properties [30]. The result produced on experimental setup (figure 1) is shown on, samples FP-336 (figure 4a) and FP-341 (figure 4c) have a larger contact angle of wettability (137±5 and 107±5°, respectively) compared to sample FP-340 (66±5°, figure 4b), and as a result, they have more hydrophobic surfaces that are worse wetted by distilled water.

As a result of the measurements, we can conclude that there is no negative effect of samples FP-336 and FP-341 on bacterial growth. Taking into account the results of [16], as well as the results of our experiments with the wettability of samples FP-336, FP-340 and FP-341of distilled water, we can conclude that samples of vertically oriented carbon nanotubes with hydrophobic surface properties have a more favorable biological interaction on vital activity of bacteria *E. Coli* strain M-17 according to the
spectra of suspension (figure 3). As a result, we can talk about positive bacterial biocompatibility of samples FP-336 and FP-341 E. Coli M-17.

![Figure 4. Drops on samples FP-336 (a), FP-340 (b), and FP-341 (c).](image)

4. Conclusion
As a result of the research, we came to the following conclusions. Depending on the structural features of the surface of nanotubes, they have different wettability. Samples with the highest hydrophilicity show the least bacterial biocompatibility. Metabolic processes in bacterial culture are manifested more actively in the presence of hydrophobic samples of nanotubes. Most likely, the characteristic dimensions of nanoscale surface irregularities of the samples of vertically aligned carbon nanotubes with a hydrophobic surface correlate better with the structural features of the cell wall of the bacterium E. coli M-17 [24], this is also indicated by the concentration of substances released in the life cycle of the bacterium E. coli M-17, in particular pyruvate dehydrogenase (460 nm) and triamine deaminase (415 nm). Note that the structure and composition of the surface can be effectively controlled by ion irradiation [31]. Thus, varying the surface relief and wettability can be used to create bactericidal or bacteriophilic biointerfaces with low infection property for bioimplants and implanted biosensors.

Moreover, as a result of local heating, the formation of defects occurs more or less uniformly [32]. Therefore, further experiments on the biological interaction of E. Coli strain M-17 with vertically oriented arrays of carbon nanotubes will be carried out with a different structure modified by ion irradiation.

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