The structure of multi-walled carbon nanotubes as a factor affecting the life of E. Coli

A P Popov¹, A I Dimitrieva¹, A V Kovalenko¹, D S Yumanov¹, A V Stepanov¹, A A Shemukhin², E A Vorobyeva² and Emad M Elshehly³

¹Laboratory of Hematological Research, Chuvash State Agricultural Academy, 29, K. Marx street, Cheboksary, 428003, Russian Federation
²Skobeltsyn Institute of Nuclear Physics, Lomonosov Moscow State University, I(2), Leninskie gory, GSP-1, Moscow 119991, Russian Federation
³Faculty of Science, Physics Department, Damanhour University, El Gomhoureya St, Damanhour, El Beheira, Egypt

¹E-mail: popovaleksandr.petrovich@yandex.ru

Abstract. In this work, we investigated the relationship between the structural properties of materials based on multi-walled carbon nanotubes and the vital activity of bacteria E. Coli strain M-17. In the course of research using scanning electron microscopy and Raman spectroscopy, the structure of nanotubes was analyzed. Nanotube samples were tested for wettability. The effect of carbon nanotube samples on the growth of the bacterial culture of E. Coli strain M-17 using spectrophotometry was investigated. As a result, it was shown that samples containing more disordered defective nanotubes on the surface are more hydrophilic and also show worse biocompatibility properties for E. Coli bacteria.

1. Introduction
Carbon nanotubes (CNTs) are a form of carbon studied in the pioneering work of Iijima [1]. CNTs can be represented as a graphene sheet rolled into a cylinder. There are two main types of CNTs: single-walled and multi-walled. Multi-walled CNTs (MWCNTs) consist of many single-walled single-walled nanotubes.

CNTs have many applications in such fields as mechanics (the creation of heavy-duty nanoscale filaments, composite materials [2]), microelectronics (field-effect transistors [3], nanowires), optics (displays, LEDs [4]), energy (fuel cells [5]), chemistry (water purification [6], catalysis), as well as biotechnology (highly sensitive biosensors [7-10]) and medicine (artificial muscles [11], targeted drug delivery [12], bioprostheses [13]). When using CNTs in biotechnology and medicine, it is important to take into account the safety requirements for the materials obtained, and, as a result, to study their effect on living organisms and tissues [14].

The interaction of CNTs with various living systems can have a positive effect on them: for example, antispetic and regenerative on the tissues of various organs and systems [15-16], and negative: toxic [15], allergic, carcinogenic effects, can be a source of infection. The type of impact is a consequence of structural features, the presence of functional groups on the surface of carbon nanotubes, as well as their size. It follows from the foregoing 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 CNTs will behave.
Studies of bacterial biocompatibility of E. Coli with various materials in [14] showed that the structural features of the surface, as well as its wettability, directly affect biocompatibility. In the same work [14] it was shown 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 and the life of E.Coli M-17.

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) Investigation of the defectiveness and 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 optical density methods.

2. Materials and Methods

2.1. Growing carbon nanotubes
The synthesis of samples of vertically oriented multi-walled carbon nanotubes was carried out using the CVD method [17] using a muffle furnace in a nitrogen atmosphere, while passing acetylene in a mixture with a ferrocene catalyst. Nanotube deposition occurred on a silicon substrate. The synthesis parameters of the three samples used in the work are shown in Table 1.

| Sample No | 1     | 2     | 3     |
|-----------|-------|-------|-------|
| Label     | FP-336| FP-340| FP-341|
| CVD temperature T, °C | 830   | 850   | 870   |
| Δ mixtures (consumption of catalyst mixture in solution) | 49    | 11    | 35    |
| Heating time, t1, min | 20    | 24    | 39    |
| Synthesis time, t2, min | 33    | 61    | 30    |

Three samples of multi-walled carbon nanotubes were used in the study: FP - 336 (No. 1), FP - 340 (No. 2), FP - 341 (No. 3), which have differences in structural and surface properties. So, the FP-340 sample, in comparison with the FP-336 and FP-341 samples, contains a significant amount of loose carbon mass on its surface. The smallest amount of such a mass is contained on the surface of the AF sample - 336 (Fig. 1).

2.2. Preparation of bacterial suspension
For research, a bacterial suspension was prepared from the preparation “Kolibacterin dry”. The drug is packaged in glass bottles, each bottle contains 3 doses, one does is at least $10 \times 10^9$ Colony Forming Unit (CFU) of live bacteria of E. coli strain M-17. To obtain a suspension, the contents of the bottle were mixed
with 15 ml (5 ml per 1 dose) of a sterile isotonic solution NaCl. Then the contents of the vial were mixed for 5 minutes until completely dissolved.

To study the effect of MWCNT samples on bacterial growth, we used a daily bacterial culture grown on meat and peptone broth (MPB according to Lennox, Diaem). Ready medium was poured into identical sterile laboratory tubes. Studies were conducted using dilutions of the bacterial suspension at a concentration of $10^{-7}$ and $10^{-10}$ from the initial dose. The necessary concentrations were obtained by the method of successive dilutions. For this purpose, 10 tubes with 9 ml of sterile isotonic NaCl solution were used. The first dilution ($10 \times 10^{-1}$) was obtained by transferring 1 ml of the bacterial suspension into the first tube with 9 ml of physiological saline. Then, 1 ml of the obtained suspension was transferred from the first tube to the second (dilution $10 \times 10^{-2}$). From the second tube, 1 ml of suspension was transferred with a third ($10 \times 10^{-3}$ dilution), and so on, until $10 \times 10^{-10}$ dilution was stirred.

2.3. **Bacterial culture**

To obtain the necessary dilutions of the bacterial culture during its preparation, we used a laboratory dispenser (TopPettePipette 100-1000 ul, DragonLab, with an error of $\pm 1.5 - \pm 10 \mu l$) together with disposable tips (tip for the dispenser type-2, 100-1000 μl, Liptoplast-Med): 0.1 ml of the bacterial suspension was transferred into a test tube with 10 ml of MPB. After that, 0.015 g of nanotube samples were added to each tube with bacteria and MPB, for each sample there were two tubes with dilutions of $10^{-7}$ and $10^{-10}$, respectively, there were also two tubes with a culture control and one with a medium control. Then all the tubes were kept for 24 hours in a thermostat (TS-1/80 SPU) at +37°C.

Further studies (measurement of optical density and spectrometry were carried out using daily bacterial cultures.

2.4. **Study of the wettability of samples with distilled water**

The wettability of carbon nanotube samples was studied by measuring the wettability angle (Fig. 2). To do this, drop of distilled water put on samples horizontally placed on a micro table. Distilled water was supplied over each sample until a whole drop was torn off the tip. Then, after 3 minutes of holding the drop on the sample, photographing was carried out (camera resolution 12 MPs). The contact angles of wettability of a still drop were measured by the tangential method using the ToupCAM software.

![Figure 2. The experimental design for measuring wettability: 1 - stage, 2 - sample, 3 - drop, 4 - camera, 5 - tripod](image)

2.5. **Measurement of optical absorption spectra of a bacterial culture**

Spectra were measured in a daily culture of Escherichia coli grown on MPB. The measurements were carried out using a Shimadzu UV-1800 spectrophotometer. The results were processed using UV probe ver. 2.42 (spectral processing software). Spectra were measured in bacterial
cultures grown from dilutions $10^{-7}$, $10^{-10}$, in which MWCNT samples No. 1 – No. 3 were added, as well as in environmental controls and culture controls.

Before measuring the spectra, the absorption values were zeroed; for this, sterile MPB was added to the cuvette. The measurement range of the spectra is from 300 to 1100 nm, the measurement step is 0.1 nm. After a sequential measurement of each sample was carried out in all dilutions, the contents of each tube were scanned 5 times. After each measurement, the cuvette was washed with distilled water.

2.6. Density measurement of bacterial cultures

The optical density of daily bacterial cultures was measured using a densitometer (Densitameter II - Mikrolatest), with a working wavelength of 535 nm. Densitometer was scaled in MacFarland standard optical density (McF).

Prior to the measurements, the device was calibrated using test tubes with MPB identical to those used in the experiment.

After calibration, 3-fold measurements of optical density were performed; for this, all tubes were placed in turn in the tube block and measurements were taken.

3. Results and discussion

It was previously shown that samples 1-3 are characterized by the following features of the Raman spectra shown in Table 2 [17]. They show that the D and G peaks characteristic of carbon graphite materials are located at frequencies of 1362 cm$^{-1}$, 1587 cm$^{-1}$. The G-peak shows us the nanotube crystallinity degree, while D-peak shows the nanotube defectiveness. For samples No. 2 and No. 3, the intensity of the D peak is higher than the intensity of the G peak, while for sample No. 1 $I_D / I_G < 1$. This indicates a greater crystallinity of the nanotubes contained in sample No. 1 and the presence of defects in samples No. 2 and 3. If we turn to the micrograph of sample No. 2, we can note the presence of disaligned carbon nanotubes on its surface, and also accordingly to Raman spectra we obtain that sample is more defective. The $I_G$-peak intensity indicates the number of layers in a nanotube; the higher the intensity, the fewer the layers and, accordingly, the smaller the diameter. Sample No. 1 and 3 are represented by minimal nanotube diameters and relatively high crystallinity; however sample No. 2 contains the nanotubes with larger diameters and greater defectiveness.

Table 2. Raman data for samples containing multi-walled carbon nanotube arrays [17].

| Relative peak intensity | Sample 1 | Sample 2 | Sample 3 |
|------------------------|----------|----------|----------|
| $I_D$ (1362 cm$^{-1}$)  | 0.87     | 1        | 1        |
| $I_G$ (1587 cm$^{-1}$) | 1        | 0.75     | 0.91     |
| $I_G'$ (2723 cm$^{-1}$)| 0.43     | 0.22     | 0.37     |

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 No. 1 and 3 (Table 3). Thus, in test tubes with samples No. 1 and 3, a large concentration of bacteria is noted.

Table 3. The results of measuring the density of the bacterial culture in the presence of samples of multi-walled carbon nanotubes, the density is presented in units of $10^8$ cells/cm$^3$

| Dilution | Sample No | Control |
|----------|-----------|---------|
|          | 1         | 2       | 3       |
| $10^{-7}$| 2.7       | 1.2     | 2.7     | 1.2     |
| $10^{-10}$| 0.9      | 0.9     | 0.9     | 0.9     |
It is known that structure and surfaces affect hydrophobic properties [18]. As shown in Figure 4, samples 1 and 3 have a larger contact angle of wettability (137° and 107°, respectively) compared to sample No. 2 (66°), and as a result, they have more hydrophobic surfaces that are worse wetted by distilled water.

![Figure 3. Drops on samples No. 1-3 (from left to right).](image)

As a result of the measurements, we can conclude that there is no negative effect of samples No. 1 and 3 on bacterial growth. Taking into account the results of [16], as well as the results of our experiments with the wettability of samples 1-3 of 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. As a result, we can talk about positive bacterial biocompatibility of samples FP-336 and FP-341 E. Coli M-17.

According to [15, 19], nanotubes have a bactericidal effect. Moreover, it is more pronounced for long nanotubes and nanotubes with functional groups or defects.

4. Conclusions
As a result of the research, we came to the following conclusions:
1. Depending on the structural features of the surface of nanotubes, they have different wettability, and as a result they have different effects on bacterial cells [14]. Samples with the highest hydrophilicity show the least bacterial biocompatibility.
2. Based on the article [20], we can conclude that the bacteriostatic action of the FP-340 sample is more pronounced in bacterial cells.
3. It can be assumed that the bacteriostatic effect of the FP-340 sample is due to structural features, namely, the presence of a layer of disordered nanotubes on the sample surface, imperfection, and also an average large diameter of the nanotubes, in comparison with other samples.

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