Deposition of thin films of nanoscale carbon onto solid samples as a method of suppressing surface colonization of bacteria

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Abstract. The paper presents preliminary results demonstrating the bactericidal properties of surface coatings created on metal and dielectric samples by evaporation of a colloidal solution of nanostructured carbon. A colloidal solution of nanocarbon was obtained using a high-voltage pulsed multi-spark discharge in ethanol with argon injection into the interelectrode space. The main properties of a colloidal solution and the parameters of its constituent nanoparticles are given. Bactericidal activity was assessed by seeding Escherichia coli (gram-negative) and Staphylococcus aureus (gram-positive) bacterial cultures on prepared slides. Results on suppression of bacterial colonization on samples coated with carbon nanoparticles are presented. Keywords: spark discharge, nanocarbon, colloidal solution, bactericide

1. Introduction
Recent research at GPI RAS in the field of gas-discharge physics has resulted in the development of an original method for producing a stable alcohol (ethyl alcohol) colloid containing carbon nanoparticles [1, 2], as well as a method for using the colloid to form surface nanocarbon films [3, 4]. Two application areas have stimulated interest in this type of research. This is space technology and the related problem of suppressing secondary emission discharges (multipactor) on board space objects that receive and emit microwaves (on communication satellites) [4, 5], as well as medicine and microbiology, meaning the search for coatings that reject bacterial and spore flows on parts used for implantation in biological objects and substances used in treatment. The main properties of anti-multipactor nanocarbon coatings identified in [1, 3] are as follows:
- Low level of secondary electronic emission ($\sigma_{\text{max}} < 1.5$) and high level of the first critical potential ($\varepsilon_1 > 75$ eV);
- Stability of secondary emission characteristics when staying in an air environment or in a vacuum for more than 24 months;
- High adhesion of the film coating (its independence from the vibrations characteristic of working with nanostructured carbon film samples in space);
- Preservation of secondary emission characteristics of the film from temperature jumps typical for operation in space;
- Possibility of covering satellite equipment elements with film without changing their functional characteristics.

The research conducted at GPI RAS [3] allows us to consider the problem of protecting a space object from secondary emission discharges that lead to the destruction of elements of microwave equipment on communication satellites to be solved in principle. However, until recently, the question of the protective role of nanostructured films in medicine and microbiology remained unresolved. This problem (the problem of bactericidal activity) devoted to the experimental work forming the basis of this article. One of the main reasons that stimulated research on the development and use of new technologies for the production of nanomaterials is to find new ways to counter the emergence of biofilms on medical instruments and devices [6]. Biofilms are one of the main causes of infectious diseases in humans [7]. Their elimination is particularly important in water distribution systems and in health care. Biofilms can occur on various surfaces and provide favourable conditions for the survival of microorganisms, which makes them resistant to traditional methods of mechanical protection and toxic substances usually used for disinfection. In this work, a high-voltage pulse-periodic multi-spark discharge in ethanol (95%) with argon injection into the interelectrode space was used to produce a colloidal solution of nanocarbon [1, 2]. The discharge is a set of microplasma formations with a specific energy storage of 1 kJ/cm³ [8]. The paper presents preliminary results demonstrating the bactericidal properties of nanocarbon particles (Fig. 1), obtained by discharge in ethanol [9].

2. Methods and materials

A. Experimental setup

Experiments on obtaining a colloidal solution of nanocarbon were carried out on the installation, the photo of which is shown in Fig. 1, where 1—the dielectric chamber, 2—the electrodes, 3—the electrical insulation material, 4—the pipe for injecting gas (argon) into the hole 5, 6—the terminals for supplying high-voltage. Power supply parameters: energy of the storage capacitor W ≤ 1.6 J; voltage U ≤ 20 kV; current I ≤ 300 A; pulse frequency f ≤ 100 Hz. The chamber is filled with 95% ethyl alcohol, the consumption of which was ~ 2 l/min.

![Figure 1. Photo using an electron microscope of the nanocarbon coating obtained by evaporation of the colloidal solution.](image)

When applied to a multi-electrode ring (Fig. 2 D) high-voltage pulse in the interelectrode gaps spark channels are formed, characterized by the following parameters: the temperature of heavy particles T_g 4000 – 5000 K, the electron temperature T_e 1.0 – 1.5 eV, the concentration of charged particles n_e ≈ (2 – 3) 10^{17} cm^{-3} [8].

Fig.3 presents typical waveforms of current, voltage and power output when applying a high-voltage pulse to multi-spark ring plasma dischargers of the installation.

B. Colloidal solution of nanocarbon
The colloid parameters were studied by various methods using a transmission electron microscope JEM-2100 in combination with an energy-dispersive X-ray spectrometer JED-2300 based on Raman scattering (Raman) and dynamic scattering (DRS). The research has revealed the following features of the colloidal solution:

- When the specific energy storage is exceeded, a stable colloidal solution is formed in the colloid – forming liquid ~ \(10^{-15}\) J/cm\(^3\). The specific energy bill was defined as the electrical energy invested in the discharge and attributed to the unit volume of ethanol. The stability of the colloid is confirmed by the observation time (more than two years) with the measurement of the Zeta potential (about 32.3 mV) (Fig. 4). Heating the colloidal solution to a temperature close to the boiling point and subsequent cooling does not change its properties. The main mass of nanoparticles has a negative charge. The size and structure of nanoparticles depend on the specific energy pool (the distribution of particles by size at an energy pool close to the threshold is shown in Fig. 5).

Figure 2. A-the reactor in operation; B-the installation intended for the development of a nanocarbon colloid; C – one of the sections of the reactor, where 1 – a dielectric chamber, 2 – electrodes, 3 – an electrical insulating material, 4 – a pipe for injecting gas into the hole 5, 6 – terminals for supplying high-voltage.

Figure 3. Oscillograms of current, voltage and calculated power (U in V; I in A; P x10 in kW)

Figure 4. Size distribution of nanoparticles in the colloid.
Analysis of the elemental composition showed that (70 – 80)% of the colloid-forming particles is carbon, (1 – 2)% is accounted for by the atomized metals of the electrodes, and the remaining content is formed by oxygen atoms.

The characteristic X-ray spectra of elements and electronogram of vaporized colloidal droplet residues on copper PEM gratings are shown in Fig. 4. Spectra (Fig. 5, C) show the presence of metal nanoparticles formed when spraying the discharge electrodes made of stainless steel. Iron is the main component (70%) with chromium (18%), nickel (10%), silicon (0.8%), copper (0.3%). Metal particles in relation to carbon make up an average volume of ~ 8%.

On the Fig. 5d there are peaks D and G (1350 and 1595 cm⁻¹, respectively). This indicates that the bulk is disordered carbon in the form of graphite nanoparticles. From the ratio between the intensities of the components D and G of the spectra, the size of graphite clusters can be estimated at 1-1.5 nm [2].

C. Methods for assessing bactericidal activity

According to the method described above, two colloidal solutions (1 and 2) of nanocarbon in ethanol were produced. Stable colloids were manufactured under the same conditions but at different times. Then the solutions were applied to the surface of the slides. The method of application is extremely simple and consists of placing colloid drops on the glass surface with their spreading over it and forming a thin layer containing nanoparticles of colloid-forming liquid. The described process does not require either placing the sample in the pumped chamber, or additional exposure to the liquid film. Fairly fast simple evaporation of the liquid component (ethanol) leads to the appearance of a nanocarbon film on the glass surface, which is in a satisfactorily high adhesion to the substrate (high degree of adhesion).

The slides were then immersed in a culture of bacteria grown in an LB environment at 37°C for 24 hours. The slides were immersed in a standard bacterial suspension with an optical density (OD) of 0.1 for 24-28 hours. The control sample containing the film during water evaporation was also tested for bacterial colonization. The tests used a bacterial suspension consisting of from Escherichia coli (gram-negative) and Staphylococcus aureus (gram-positive) organisms. After incubating the slides for the required period, the film side of each slide was washed and colored using crystal violet and examined under a microscope (x1000) using oil immersion to attach bacteria (biofilm formation). The slides were
fixed by heating before staining. The optical distribution of bacteria on the surfaces was determined by examining multiple sites on different parts of the samples using light microscopy.

3. RESULTS AND DISCUSSIONS

Fig. 6 shows the results after suspending slides in the presence or absence of a nanocarbon coating using a standard suspension of cultures of gram-negative bacteria *E. coli* and gram-positive *S. aureus*, respectively. It can be seen that both types of bacteria were able to colonize the surface of slides when they did not have a nanocarbon coating. Numerous sites were examined under a microscope after a two-day incubation period.

![Image of bacterial colonization](image)

**Figure 6.** Bacterial colonization of surfaces borosilicate glass with colloidal nano-carbon coating and without it.

Fig. 6 also shows images of the surface of slides covered with two samples of colloid 1 and 2. They show that bacterial colonization is significantly suppressed on these surfaces. The degree of colonization in *S. aureus* was higher than in *E. coli* bacteria, indicating a large inhibitory effect on the gram-negative organism.

As noted earlier, the stability of a colloidal solution of nanocarbon in ethanol depends on many factors, such as the Zeta potential (which is a measure of the Coulomb interaction of colloidal particles), the ratio of the size of nanoparticles and functional groups that may be present on their surface after a new synthesis. Stability is also a function of increasing the energy density of the solution to $\sim 10^{15}$ J/cm$^3$.

The stability of the colloid as a whole suggests that metal particles are related to carbon particles. Surface modification of the nanographite makes these particles soluble and obviously improves their reactivity and bactericidal properties. It has been shown that when selecting suitable carrier gases and plasma parameters, functional groups such as -COOH or -NH$_2$ can be inserted into the outermost graphite shells of nanoparticles [10]. The mechanism by which carbon nanoparticles act on bacteria is complex and depends on the composition and modification of the particle surface, the types of microorganisms, and the type of medium in which microbe–nanoparticle interactions occur [11].

Disordered graphite material obtained by spark discharge is considered diamond-like carbon (DLC) in structure. DLC structures obtained in several ways were considered for solving a number of biological
problems. DLC colloid particles are highly stable in water, some organic solvents such as alcohols and possibly, from Escherichia coli (gram-negative) and Staphylococcus aureus (gram-positive) organisms. After incubating the slides for the required period, the film side of each slide was washed and colored using crystal violet and examined under a microscope (x1000) using oil immersion to attach bacteria (biofilm formation). The slides were fixed by heating before staining. The optical distribution of bacteria on the surfaces was determined by examining multiple sites on different parts of the samples using light microscopy.

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The following reasons can cause antimicrobial activity in DLC:
- Strong hydrophobicity of DLC can cause changes in the bacterial cell membrane;
- DLC films reveal antibiophilic / antibacterial properties depending on the surface profile;
- Properties of DLC films depend on the manufacturing conditions;
- The sp3/sp2 ratio often plays an important role in the biological activity of DLC [17].

Conclusion
The technology for obtaining nanocarbon colloidal solution by electric spark method was originally developed for creating antimultipaktor coatings (to reduce secondary electron emission in microwave space communication devices, in high-power microwave generators and in electronic accelerators) [9], as well as for growing diamond films and solid microcrystalline diamond plates [18]. Colloidal solution has high stability. The films obtained on its basis are characterized by high adhesion and hydrophobicity. In this paper, the bactericidal properties of films obtained by evaporation of colloidal ethanol droplets containing nanostructured carbon on the surface of a metal or dielectric were studied for the first time. The results of the experiments conducted and described in the article allow us to expand the use of nanocarbon coatings as rejecting bacterial and spore deposition, which counteracts the appearance of biofilms on the surface of medical instruments and devices. The use of colloid as an affordable and cheap nanomaterial for creating antibacterial coatings that prevent the appearance of biofilms is very promising for modern medicine. However, the research program on how to obtain nanostuctured carbon gel and use it in the interests of modern technology and science cannot be considered complete. There is an obvious need for further research to assess the specific structure and functional groups of nanoparticle surfaces, as well as the mechanisms of their impact on the microbiological components of the air environment.

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