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Synthesis, physicochemical characterization and antibacterial activity of carbon coatings

Polina Lapina, Evgeny Gorodnichiy, Dmitry Raikov, Irina Bazhukova *, Yulia Kuznetsova, Andrei Korelin, Viktor Kuznetsov

Institute of Physics and Technology, Ural Federal University, Ekaterinburg 620002, Russia
* Corresponding author: i.n.sedunova@urfu.ru

Abstract

In recent years, significant interest has emerged in synthesizing new carbon materials with novel physicochemical properties for applications in regenerative medicine, particularly as antibacterial coatings for implants. A pronounced biological effect is observed for diamond-like carbon (featuring sp^3- and sp^2-hybridized carbon atoms) and linear-chain carbon (with sp^1-hybridization), which can be engineered due to carbon's polymorphic nature. This work presents the results of synthesizing carbon coatings at different assisting voltages and investigating their physicochemical properties and antibacterial activity. The coatings were characterized using Raman spectroscopy and X-ray fluorescence analysis. Antibacterial activity was assessed using lyophilized luminescent bacteria from the "Ecolume" system, where the bioluminescence intensity correlates with bacterial viability. Luminescence quenching indicates inhibition of bacterial enzymatic activity upon interaction with the coating surface. It was shown that an increase in the assisting voltage reduces the content of short chains of linear chain carbon (LCC) in the coatings. The coatings with high diamond phase content were found to be chemically inert when interacting with a biological environment. Modification of the coatings with silver ions enhances the antibacterial activity of the material. The maximum bactericidal effect is achieved when bacterial cells interact with a coating containing predominantly long chains of linear chain carbon.

Key findings

- An increase in assisting voltage results in a reduced proportion of short-chain linear carbon (LCC) in the coatings.
- The coatings with high diamond phase content demonstrate chemical inertness in biological environments.
- Doping the DLC coatings with silver ions enhances their antibacterial activity.
- The antibacterial effect is achieved when bacterial cells interact with coatings containing predominantly long-chain linear carbon (LCC).

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Sustainable Development Goals

1. Introduction

Carbon is a unique chemical element capable of forming four covalent bonds, which underlies the wide diversity of its allotropic forms. The existence of these modifications is associated with the different geometry of electron orbitals of the carbon atom, determining three main types of hybridization: sp^3 (diamond), sp^2 (graphite, graphene, carbon nanotubes, fullerenes), and sp (carbine). The presence of numerous allotropic forms of carbon underlies the wide range of mechanical, electronic, and physicochemical properties of carbon nanomaterials [1].

Carbon coatings, synthesized as thin films, possess properties of bulk allotropic modifications, and their

functional characteristics are directly determined by the ratio of sp^3-, sp^2-, and sp-hybridized phases. Coatings with a predominance of the sp^3-phase, known as diamond-like carbon (DLC) coatings, are characterized by exceptional hardness, wear resistance, low coefficient of friction, and high chemical inertness, making them ideal for applications requiring increased abrasion resistance [2, 3]. Materials dominated by the sp^2-phase (graphene-like structures) exhibit high electrical conductivity and flexibility, opening prospects for flexible electronics and sensors. The most challenging for synthesis and study are coatings with significant proportions of sp-hybridization, known as coatings based on linear chain carbon (LCC). They contain polyynic (alternating single and triple bonds) and cumulenic (double bond system) carbon chains, providing them with a unique combination of longitudinal strength and transverse elasticity, as well as higher chemical reactivity [4, 5]. An additional factor influencing LCC properties is the length of the carbon chain: longer chains contribute to structural stabilization and modify their electronic properties. The ability to control the phase composition during synthesis opens pathways for the targeted creation of coatings with tailored properties for specific applications, especially relevant in biomedicine [5-8].

Due to their biocompatibility and complex set of operational properties, carbon nanomaterials have found widespread application in medical and biological practice. In recent years, diamond-like coatings [6, 7] and coatings based on linear chain carbon, notable for their strong adhesion to polymer and metal substrates [8], have attracted the greatest interest in modifying surfaces of medical implants. This determines the feasibility of their use as wear-resistant films for artificial joints subjected to significant motion loads, as well as for functionalization of the surface of various implantable devices, such as stents and orthopedic screws.

Numerous studies confirm the promising potential of carbon coatings as protective layers to enhance the performance of biomedical devices. It has been demonstrated that DLC not only increases the stiffness and wear resistance of products but also exhibits antimicrobial properties, preventing the adhesion and proliferation of microorganisms, in particular, due to the hydrophobic effect and minimization of the contact area with the bacterial cell [2, 9, 10]. In contrast, the antibacterial effect of LCC-based coatings is largely attributed to their chemical activity and generation of reactive oxygen species (ROS), causing oxidative stress in bacterial cells [11]. Current research actively studies the antimicrobial activity of carbon coatings, and several works showed that modification of implant surfaces with such coatings can significantly reduce the risk of postoperative complications related to bacterial infections [12, 13].

However, despite significant progress, the antibacterial activity of many carbon coatings is often insufficient for complete suppression of bacterial biofilm formation. Therefore, strategies aimed at enhancing their effectiveness are being extensively developed, the most promising of which is the doping of carbon matrices with ions of biocidal metals. It is well known that silver (Ag^+) and copper (Cu^{2+}) ions possess pronounced bactericidal activity against a wide range of pathogens [14–17]. In recent years, several studies have appeared demonstrating synergistic effects from combining a carbon matrix with metals. For example, it was shown that doping DLC with silver enhances the bactericidal effect through controlled ion emission while preserving the high tribological properties of the coating [16]. Similar studies with copper revealed its capability to induce oxidative stress in bacterial cells upon incorporation into sp^2-carbon matrices [17]. Furthermore, the biological mechanisms underlying the impact of both pure and modified carbon coatings on bacterial cells remain complex and not fully established.

Thus, the aim of this work is to synthesize carbon coatings and evaluate their antimicrobial activity.

2. Materials and Methods

2.1. Synthesis and characterization

Carbon coatings with different types of carbon atom sp-hybridization were synthesized on a UVNIPA-1-001 setup using ion-plasma sputtering with argon ion assistance. Samples deposited on titanium substrates at assisting voltages (U_{assist}) ranging from 0 to 1500 V, as well as samples doped with silver and copper ions, were investigated.

The principle of the coating synthesis method involves sputtering a composite carbon cathode in vacuum and subsequent condensation of its particles onto the substrate surface. Processing of samples with accelerated argon ions was carried out in a vacuum chamber (10^{-5} mm Hg) using an ion source. To clean the substrate surface, a metallic plasma source with flow separation was applied, which ensures the implantation of accelerated titanium ions into the surface layer without the ingress of droplets from the sputtered material. Doping of coatings with silver and copper ions was performed by embedding metal pins into the carbon cathode of the pulse-source of carbon plasma.

The structure of the coatings was studied by Raman spectroscopy on the Horiba LabRam HR800 Evolution spectrometer. A laser with a wavelength of 514 nm was used as the excitation source. The ruling density of the diffraction grating used was 600 lines/mm. The deconvolution of the Raman spectra was performed using the Origin 2025 software. A baseline correction was first applied using the asymmetric least squares method. The spectral profiles were then fitted with a multi-peak model based on Gaussian functions. The initial parameters for the peak positions and full widths at half maximum (FWHM) were constrained based on theoretical predictions and previously reported experimental data [18]. These parameters were subsequently iteratively adjusted to achieve the best fit between the cumulative curve and the experimental spectrum. The

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quality of the fit was assessed by the coefficient of determination (R^2), which exceeded 0.998 for all the spectra presented in this study.

The chemical composition of the coatings was determined using the ARL ADVANT'X X-ray fluorescence spectrometer with the Uniquant non-standard analysis program.

2.2. Antibacterial activity of coatings

The antibacterial properties of carbon coatings were studied using the bioluminescent sensor "Ecolum" based on a recombinant strain of *Escherichia coli* K12 TG1 (pF1) with an integrated cassette of *luxCDABE* genes from *Vibrio fischeri* MGU-6 (collection of Lomonosov Moscow State University). The lyophilized culture was rehydrated by adding distilled water cooled to 8 °C and then incubated for 30 min at a temperature of 2–4 °C. Immediately before measurements, bacterial suspensions were standardized according to optical density at 540 nm to a value of 0.5 units. Bioluminescence analysis was carried out in accordance with the methodology described in work [19]. Control samples contained only the solvents used.

The "Ecolum" biosensor was selected for this study from a range of available bacterial bioluminescent systems due to its high sensitivity to carbon-based nanomaterials and metal nanoparticles, as established in comparative studies [20]. While certain sensors, particularly those based on Gram-positive bacteria like *Bacillus subtilis*, can exhibit higher sensitivity for some specific toxicants, the "Ecolum" system represents an optimal combination of high sensitivity, commercial availability, standardized protocols and validated application in nanotoxicology, ensuring the reproducibility and reliability of our antibacterial assessment.

Luminescence intensity was recorded using a photomultiplier tube FEU-85 in the spectral range of 300–600 nm. The signal from the photomultiplier consisted of a sequence of amplified pulses with an amplitude of 5 V and duration of 1 μs. The pulse repetition rate was between $(0.1–1.0)\cdot10^5$ pulses/s and was proportional to the light flux intensity. All experiments were performed in three independent replicates, ensuring statistical significance and reproducibility of results regarding bioluminescence quenching.

3. Results and Discussions

3.1. Phase composition and structure of coatings

Raman spectroscopy was used to determine the carbon modification of the studied coatings (Figure 1). The spectra demonstrate a complex structure typical of carbon materials with different hybridization states of atoms. Decomposition of the spectra reveals peaks indicating the presence of the sp^3-phase (D- and G-peaks in the region of 1350 and 1580 cm^{-1}, respectively) as well as characteristic features indicating the presence of a one-dimensional allotropic modification of carbon – two-dimensionally ordered linear chain carbon.

Analysis of the low-frequency modes in the 1000–2500 cm^{-1} region revealed a large number of bends in the carbon chains, indicative of their shortening [18]. The intensity of these modes varies significantly depending on the assisting voltage. As the number of bends decreases (reduction in the proportion of sp^2-type carbon), there is an increase in the intensity of the band near 2000 cm^{-1}, which is indicative of an increasing proportion of sp-hybridized carbon.

Analysis of the Raman spectra of silver ion doped coatings (Figure 1b) revealed noticeable changes in the spectral structure compared to undoped samples. A decrease in the intensity of the D-band and shift of the G-band indicate changes in the structural state of the carbon matrix upon incorporation of silver ions. Similar changes are also observed for copper ion doped samples.

Additional analysis of the Raman spectra was carried out according to the methodology proposed in [21], where a nonlinear dependence was found between the full width at half maximum (FWHM) of the G-peak and the content of sp^3-hybridized carbon. The nature of the obtained dependence is explained by the appearance of distortions in the sp^2-clusters due to the formation of defects of various natures with an increase in the proportion of the sp^3-phase.

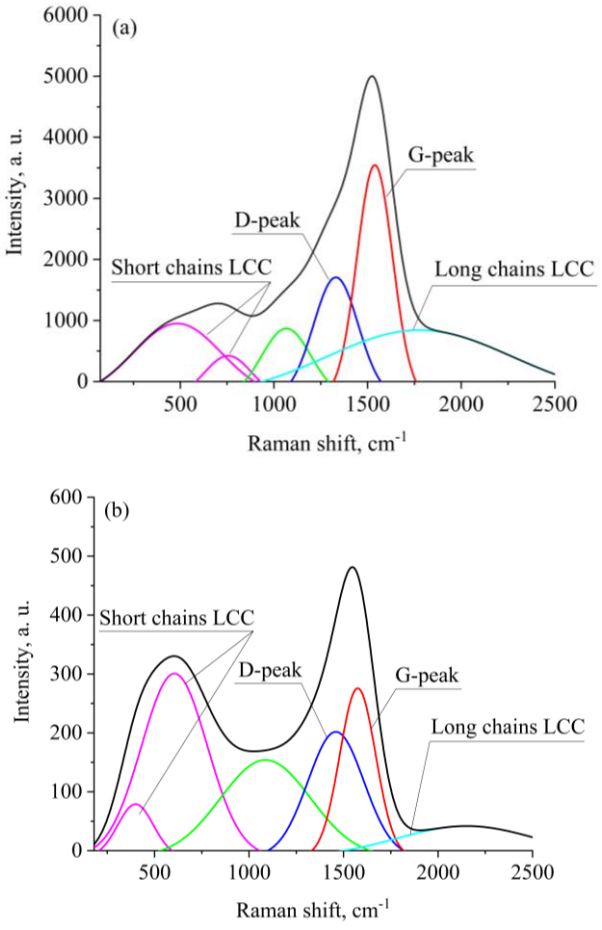

Figure 1 Raman spectra of: the carbon coating (a); the carbon coating doped with silver ions (b).

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The calculated values of the sp^3-component content in the samples are presented in Table 1. These data correlate with the results of studies on similar samples [1, 22].

We observed the reduction in sp^3-content upon Ag and Cu doping. We propose that the presence of metal vapors (Ag, Cu) in the plasma discharge affects the condensation kinetics of carbon ions onto the substrate. The metals may act as catalytic centers, promoting the formation of sp^2-clusters over sp^3-bonds, explaining the observed decrease in the diamond-like phase. Thus, in work [23] it was shown that if metal ions enter the growing carbon film, they act as a catalyst for graphitization, reducing the proportion of the sp^3 phase.

Analysis of the distribution of LCC chains of different lengths revealed a dependence on the assisting voltage (Figure 2). In the interval U_{assist} = 0–340 V, short LCC chains dominate, while at voltages U_{assist} = 740–1500 V, a redistribution in favor of long chains occurs, which presents practical interest [24]. The highest content of long LCC chains is observed at U_{assist} = 1250 V, which correlates with the minimum content of sp^3-phase in this sample. At the same time, the sample with the maximum relative fraction of diamond-like carbon was synthesized at U_{assist} = 0 V.

X-ray fluorescence analysis was performed to evaluate the chemical composition of the modified coating. The results confirmed the presence of silver and copper ions in the sample. The effective surface dopant concentration amounted to approximately 0.008–0.013 mg/cm².

Table 1 Results of sp^3-content assessment.

Coating	U_{assist}, V	sp^3-content, a. u.
C		0.866
C+Ag	0	0.748
C+Cu		0.698
C	340	0.831
C	540	0.824
C	740	0.727
C	940	0.772
C	1250	0.694
C	1500	0.784

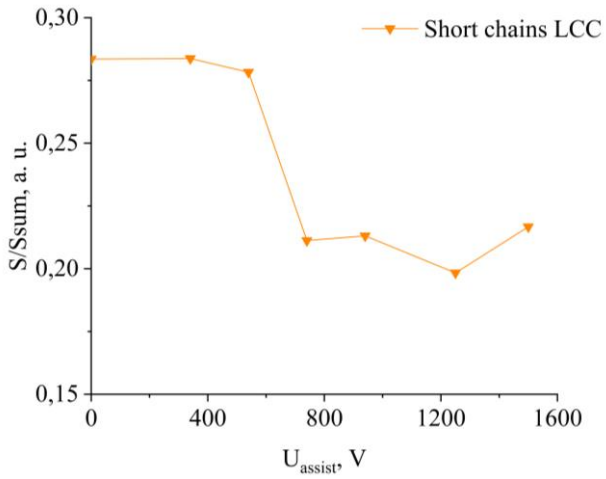

Figure 2 Evaluation of fractional content of chains with different lengths.

3.2. Antibacterial activity

Antibacterial activity of carbon coatings was investigated using the «Ecolum» bioluminescent sensor based on a recombinant *Escherichia coli* K12 TG1 strain, following a standardized methodology thoroughly described in [19]. The bioluminescence intensity in this system correlates with bacterial metabolic activity and viability. The changes in bioluminescence intensity were registered. The luminescence intensity of the biosensor in distilled water decreases exponentially due to inhibition of bacterial enzymatic activity (Figure 3).

The results of the bioluminescence study upon interaction with carbon materials are presented in Figure 4. The significant differences in the mechanisms of action of coatings with different structures were revealed. For the sample with a high content of diamond-like carbon (U_{assist} = 0 V), the mechanism of bioluminescence quenching turned out to be almost identical to that in the control sample (Figure 4a). This agrees with literature data about predominantly mechanical antibacterial effect of DLC, associated with damage to cell membranes upon contact with the coating surface [25].

In contrast to DLC, the coating with an increased content of LCC (U_{assist} = 1250 V) demonstrates an antibacterial effect (Figure 4b). During the experiment, a «spike» in bacterial luminescence was recorded at the beginning of each measurement interval (Figure 4b), which is consistent with literature data and is explained by the short-term activation of the luciferase enzyme upon contact of the bacterial suspension with atmospheric oxygen [26]. The absence of a characteristic luminescence «spike» for the LCC sample is likely due to the chemical activity of LCC, leading to the generation of reactive oxygen species (ROS) and the development of oxidative stress in bacterial cells. Unlike LCC, coatings with a high diamond phase proportion did not exhibit a similar effect, confirming their predominantly mechanical (rather than chemical) mechanism of antibacterial action [11].

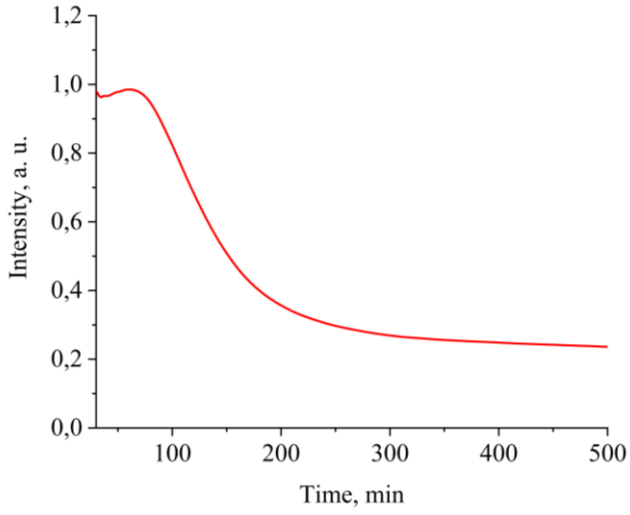

Figure 3 Kinetics of bioluminescence quenching of the "Ecolum" biosensor.

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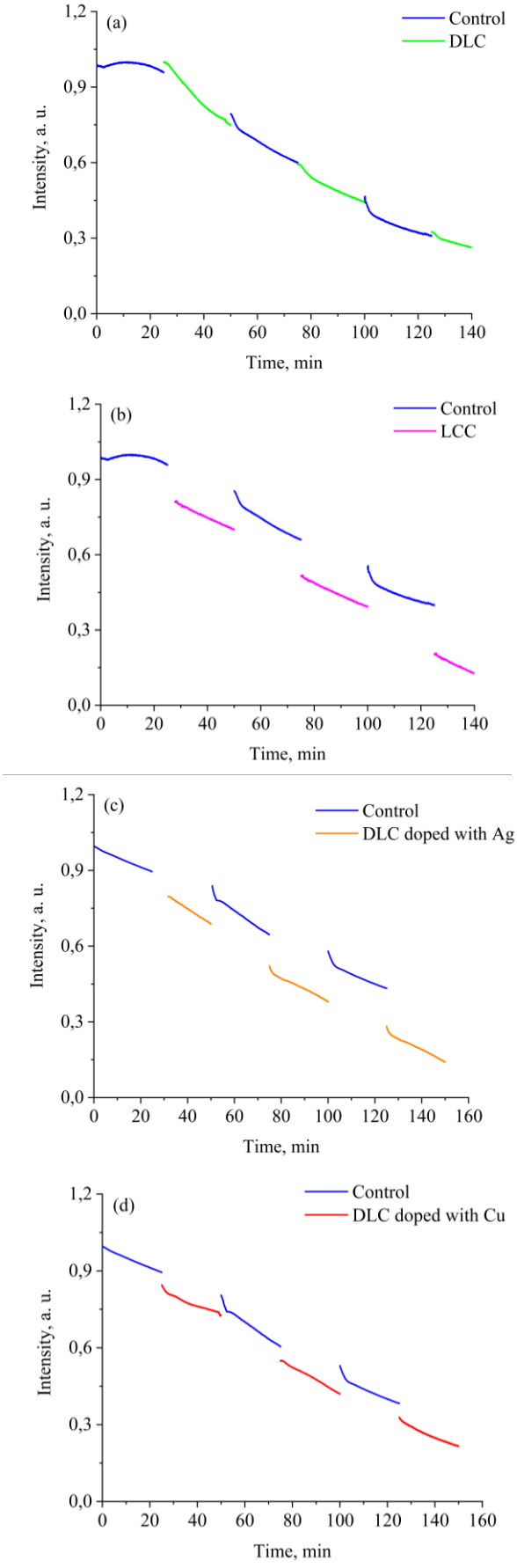

Figure 4 Kinetics of bioluminescence quenching of the "Ecolum" biosensor upon exposure to carbon coatings: (a) diamond-like carbon (DLC); (b) linear chain carbon (LCC); (c) silver-doped DLC (DLC-Ag); (d) copper-doped DLC (DLC-Cu). The control measurement is shown for comparison.

The observed absence of the initial luminescence «spike» upon bacterial contact with the LCC coating is of significant practical interest. This phenomenon, reflecting the rapid suppression of cellular metabolic activity, could be utilized as a simple and rapid qualitative test for the preliminary screening of coatings with high chemical antibacterial activity.

Beyond the chain length, the chemical reactivity of LCC is strongly influenced by its terminal groups and bond type. Cumulene and polyynic chains have oscillation frequencies of the same order [18], so it is quite difficult to determine the exact nature of the chemical bonds in LCC chains based on Raman spectra. We assume that polyynic structures (alternating single and triple bonds), which are more stable in the free state compared to cumulenic ones, predominate in our coatings. The high reactivity of these chains, responsible for the antibacterial effect, is likely associated with the presence of reactive terminal groups (e.g., hydrogen or oxygen-containing functional groups) formed during synthesis or upon air exposure. These groups likely initiate oxidative reactions, including ROS generation upon contact with an aqueous environment, leading to oxidative stress in bacterial cells [27].

Doping DLC with silver and copper ions leads to an additional enhancement of antibacterial activity (Figures 4c,d). Analysis of the bioluminescence kinetic curves showed an increase in the quenching rate for the sample doped with silver ions. Modification of the carbon coating with copper ions did not lead to a noticeable reduction in the luminescence quenching rate. The weak antibacterial effect of the copper-doped coating compared to the silver-doped one may be explained by several factors. One probable factor is the surface oxidation of copper and the formation of poorly soluble compounds within the inert DLC matrix, reducing the bioavailability of Cu^{2+} ions. Further studies, for instance, using X-ray photoelectron spectroscopy (XPS) to analyze the chemical state of copper in the coating, are required to precisely determine the reasons [17].

At the same time, the decreased activation of luciferase in the initial moments of measurement for all modified samples indirectly indicates the suppression of bacterial metabolic activity [28, 29].

The results demonstrate a correlation between the structure of carbon coatings and their antibacterial activity. Coatings with a predominance of the sp^3-phase show minimal biological activity, while samples with a high content of sp-hybridized carbon (LCC) demonstrate a pronounced antibacterial effect. Doping with silver ions enhances this effect, likely due to the synergistic action of ROS generation mechanisms and ionic toxicity.

The greatest bactericidal effect was achieved for the coating synthesized at U_{assist} = 1250 V, which is characterized by maximal content of long LCC chains and minimal fraction of diamond-like carbon. This confirms the potential use of LCC-based coatings for creating biocidal surfaces of medical implants.

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The observed degree and rate of bioluminescence quenching for our most effective LCC- and Ag-DLC coatings are comparable to or exceed the effects described for other carbon nanomaterials tested using similar E. coli-based bioluminescent sensors [19, 28], confirming the high potential of the developed coatings.

Despite the high antibacterial activity, the practical application of LCC-based coatings in implantology requires addressing the challenge of their long-term stability in physiological conditions. The high chemical reactivity of carbyne chains, which underlies their bactericidal effect, may lead to their gradual degradation in protein- and enzyme-rich environments. Protein adsorption on the surface could passivate the coating, reducing its activity. Furthermore, enzymatic cleavage of the chains is possible. To ensure stable long-term performance, further research aimed at stabilizing LCC is needed, for example, via creating multilayer structures where LCC acts as an active layer, and a protective coating controls its release rate [27].

4. Limitations

While this study demonstrates the potential of carbon coatings for biomedical applications, several limitations should be acknowledged.

Regarding the synthesis process, the method of doping coatings by embedding metal pins into the carbon cathode, while effective, presents challenges in achieving perfectly uniform distribution of dopant atoms (Ag, Cu) throughout the coating matrix. This heterogeneity could lead to local variations in antibacterial efficacy and physicochemical properties. Furthermore, precise control over the stoichiometry and concentration of the incorporated metal ions remains complex, as the final dopant concentration is influenced by multiple dynamic plasma parameters.

In terms of antibacterial activity assessment, the use of a single, genetically modified bacterial strain (*Escherichia coli* K12 TG1) in a standardized laboratory setting, while providing valuable and reproducible data, may not fully represent the behavior of coatings against complex, multispecies bacterial communities encountered in clinical environments, such as biofilms. The bioluminescence assay, though highly sensitive, primarily reflects metabolic activity and may not directly correlate with other viability indicators or physical adhesion under dynamic flow conditions. The experimental setup, involving contact between bacterial suspension and coating sample in a controlled volume, simplifies the complex and prolonged interaction that occurs in the human body. Furthermore, the proposed antibacterial mechanism of LCC-based coatings involving reactive oxygen species (ROS) generation is based on indirect evidence. Direct measurement of ROS production in future studies would unambiguously confirm this mechanism.

Future studies should employ advanced elemental mapping techniques to quantitatively assess dopant distribution, expand biological testing to include clinically relevant bacterial strains and biofilm models, and evaluate the long-term performance and biocompatibility of these coatings in *in vivo* settings.

5. Conclusions

This work involved the synthesis of carbon coatings and a comprehensive investigation of their physicochemical properties and antibacterial activity.

Carbon coatings were characterized using Raman spectroscopy and X-ray fluorescence analysis. It was established that the Raman scattering technique allows efficient evaluation of the content of carbon atoms with different hybridizations and identification of structural features of the coatings. It was found that increasing the assist voltage during synthesis leads to an increase in the fraction of long LCC chains in the coating structure.

The study on antibacterial activity revealed the differences in the biological activity of coatings depending on their structure. It was established that DLC exhibits chemical inertness when interacting with biological environment, whereas doping coatings with silver ions causes a pronounced toxic effect. The highest antibacterial activity was demonstrated by the sample with a predominance of long LCC chains, confirming the effectiveness of the ROS generation mechanism as the main factor of bactericidal action.

Thus, the research results confirm the promise of using carbon coatings for creating biocidal surfaces of medical implants. The obtained results open up possibilities for the targeted design of carbon coatings with specified biological properties for application in medical practice.

Supplementary materials

No supplementary materials are available.

Data availability statement

Data will be made available on request.

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Author contributions

Conceptualization: V.P.K., D.V.R.
Data curation: A.V.K.
Formal Analysis: I.N.B., Y.A.K.
Funding acquisition: A.V.K.
Investigation: P.K.L., E.K.G.
Methodology: I.N.B., Y.A.K., D.V.R.
Project administration: D.V.R.
Resources: A.V.K., D.V.R.
Software: E.K.G.
Supervision: I.N.B., Y.A.K.
Validation: V.P.K.
Visualization: P.K.L.
Writing – original draft: P.K.L.
Writing – review & editing: I.N.B., Y.A.K.

Conflict of interest

The authors declare no conflict of interest.

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Additional information

Author IDs:

Polina K. Lapina, Scopus ID -

Evgeny K. Gorodnichy, Scopus ID 59722947800;

Dmitry V. Raikov, Scopus ID 8658061100;

Irina N. Bazhukova, Scopus ID 57204033553;

Yulia A. Kuznetsova, Scopus ID 57214958458;

Andrei V. Korelin, Scopus ID 6507930476;

Viktor P. Kuznetsov, Scopus ID 57191966571.

Website:

Ural Federal University, https://urfu.ru/en/.

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