In this study, we prepared hybrid materials with C and Ag layers on the surface of polydimethylsiloxane polymer (PDMS). The prepared samples were subjected to thermal treatment and modification with high energy KrF excimer laser in single shot mode. The change in the surface morphology of the samples was investigated by Scanning Electron Microscopy (SEM), and the chemical composition of the prepared nanocomposites was studied by Energy Dispersive Spectroscopy (EDS). Finally, the samples were tested for antibacterial activity using two bacterial strains of Gram-positive *S. epidermidis* and Gram-negative *E.coli*. Antibacterial properties were observed on the prepared samples in both bacteria colonies.

**Introduction**

Some chemical elements can make up several different molecular structures from the same type of atoms - a unique property known as “allotropy”. These materials’ various chemical and physical properties are determined by the structural atoms’ geometry and the type of chemical bonds in the molecules. In this context, carbon is one of the most exciting elements with the ability to create a wide range of structures, often with fundamentally different properties. Only two natural carbon allotropes were known: diamond and graphite. In the meantime, various new allotropic forms (fullerenes, carbon nanotubes, graphene, etc.) have been described, including carbon nanomaterials. In general, nanomaterials are materials containing particles with a size of at least one dimension between 1 and 100 nm.

Meanwhile, carbon nanomaterials have many technical applications, including micro and nanoelectronics, gas storage, conductive plastics production, composites, displays, anti-pollution coatings, textiles, high-strength batteries, gas biosensors, and others [1]. Carbon nanomaterials were initially used in biosensing and as potential drug carriers. Their applications have expanded to regenerative medicine, tissue engineering, bioimaging, and therapeutics [2].

PDMS is widely used in micro-devices and systems due to its versatile and favourable properties, including non-toxicity, biocompatibility, flexibility, low cost, and easy production. To increase the selectivity of polymers, researchers have considered the use of fillers to form unique composites. These fillers can give composites the ability to tune properties such as conductivity and strength [3]. Polymeric composites with carbon nanoparticles naturally differ in their properties according to the type of polymer (silicone) used, but also according to the type of carbon nanoparticles, their representation, and spatial arrangement. However, carbon in the admixture improves the material’s mechanical, thermal, and primarily electrical conductive properties. Currently, perhaps the most common use of carbon nanocomposites is related to the adjustment of electrical conductivity. Silicones (especially PDMS) are biocompatible, and, together with non-toxic carbon to different cells (carbon dots), form an ideal combination for various bodily applications [4,5]. Another interesting group of materials is hybrid materials created by coupling/layering different materials, such as carbon (carbon layers, graphene layers) and noble metal. The resulting materials are usually not used in this original form. They are very often subject to UV or laser treatment. By the action of an excimer laser, for example, metal clusters and nanostructures can be formed. The structures prepared in this way can be used, for example, in optics, transistors, or sensor applications. The resulting properties of the structure and its "appearance" are strongly dependent on the substrate used and the parameters of the individual layers [6]. When silver is used as a noble metal, these composites also find excellent application in medicine [7]. The bactericidal properties of Ag are associated with the slow oxidation of nanoparticles and the release of Ag⁺ ions, thereby damaging the cell membrane. Low concentrations of nanosilver are more efficient against most bacteria and viruses. Microorganisms are unlikely to become resistant to silver during the mutation process because their ions attack large amounts of protein in cells. This property plays an essential role in drugs due to the ever-increasing antibiotic resistance.
resistance. The antibacterial effect of Ag nanoparticles depends on their size, shape, and inhibition of DNA replication. Notably, smaller nanoparticles exhibit better antibacterial properties because they have a larger specific surface area, which explains the increased biological activity of particles with a size < 10 nm [8].

This paper presents a simple procedure for producing large-area surfaces by excimer laser annealing of composites prepared from layers C and Ag on PDMS polymers. A unique high-energy excimer laser with power up to 1000 mJ allowed us to expose an area of 32 x 13 mm² with a single laser discharge. A single shot with an energy of 150 mJ.cm⁻² allowed us to modify the surface of the prepared samples and change their chemical composition, which has had a significant effect on antibacterial activity. The change in the height of the carbon layer significantly affected the morphology and chemical composition of the samples before and after laser deposition. Samples of the C/Ag system subjected to thermal stress showed excellent antibacterial results compared to the control and pristine PDMS. The combination of such simple and effective techniques has not yet been used, which opens up many possibilities in various areas such as electronics or medicine.

Experimental

Materials and preparation

Polymer polydimethylsiloxane (PDMS, density 1.5 g.cm⁻³) in the form of 50 μm thick sheets (supplied by Goodfellow, Ltd.) was used as a substrate on which the C and Ag layers were deposited. The carbon layers were deposited using an SCD 050 Carbon Thread Evaporation Device (Bal-Tec) by the flash evaporation process using a carbon filament (Leica). The carbon fibre was degassed at a current of 1.5 A. The polymer substrate was deposited from the carbon fibre at a distance of 3, 5, or 7 cm (corresponds to the thickness of 18 resp. 8 or 4 nm). Silver layers were sputtered on Quorum 300 T sputter coater for 300 s at 40 mA, and the thickness of the deposited Ag was 20 nm. Thermal treatment of prepared samples was performed at the upper working temperature of PDMS (285 °C) for 1 h in a Binder oven with a thermostat. The annealed samples were cooled in the air at room temperature. Prepared samples were also modified with a high energy pulsed excimer KrF laser (Coherent Inc., Leap 100 K) with a wavelength of 248 nm and a pulse duration of 20–40 ns, repetition rate of 1 Hz with stabilized energy range 900 – 1,000 mJ and beam dimensions of 32 x 13 mm². An aperture of 30 x 10 mm² was used in the experiments.

Analytical methods

To monitor the material surface morphology, we used the scanning electron microscope LYRA3 GMU (Tescan, Czech Republic). The acceleration voltage was set up to 10 kV. The elemental composition was determined by energy dispersive X-ray spectroscopy (EDS, analyzer XMaxN, 20 mm² SDD detector, Oxford Instruments, United Kingdom). The accelerating voltage for SEM-EDS measurement was of 10 kV.

Antibacterial properties

Gram-negative and Gram-positive bacterial strains of E. coli (DBM 3138) and S. epidermidis (DBM 2124) were used to determine the antibacterial activity of the prepared C/Ag nanocomposites. The bacterial strains were transferred from agar plates; one colony was transferred to 20 respectively 5 ml of liquid Luria – Bertani (LB) medium. The inoculum thus prepared was then cultured overnight at 37 °C on an orbital shaker. The next day, the bacteria were diluted in sterile PBS to a concentration of approximately 1.10⁶ bacteria per 1 ml. 100 μl of bacterial suspension was pipetted onto the test samples, which were stacked in five replicates at the bottom of a sterile Petri dish and were statically incubated at room temperature. As a control, 100 μl drops were pipetted onto the bottom of a sterile petri dish. After 2 hours, the bacterial suspension was mixed, and 3 drops of 25 μl were pipetted from each PC onto a culture dish. These plates were cultured overnight at room temperature of 37 °C. Subsequently, the number of colony forming units (CFU) was determined, which was compared with the number of CFUs in control. The experiment was performed under sterile conditions.

For clarity, we present the labelling of the samples as used in this experiment: the letter “C” indicates the deposited carbon layer, the number 3, 5, or 7 means the deposition distance (in cm), and the letters “T” indicates the thermal stress and the letter “L” - laser modification.

Results and discussion

Surface morphology using SEM method

The SEM method investigated changes in the surface morphology of the prepared samples. Fig. 1 compares SEM images of samples at two scales: larger 30 x 30 μm (corner image) and smaller 10 x10 μm (main image). Samples with a combination of layers C (C3, C5, C7) and Ag, followed by thermal treatment (left column) or possible laser modification (right column), were studied. The sample with the highest C content (C3/Ag/T) before laser exposure formed a slightly wrinkling structure on its surface (corner image) supplemented by homogenously spread globular nanoclusters over a surface with the narrow size of distribution (main picture). This phenomenon could occur thanks to decreasing tension between the C layer and the PDMS substrate surface after thermal treatment. With the increasing thickness of the C layer (C5 and C7), the prepared samples had a higher wrinkling of surface structures (larger surface area). After laser exposure to the prepared samples, there was a significant change in the morphology of all types of samples. On sample C3, there was slight damage to the eventual rupture of the structure, while on the sample with a lower layer C (C5), the wrinkled structure became more pronounced. The C7 (its surface
layer) sample did not withstand the laser modification and was completely torn, which is better shown in the corner image with a larger scanning area. The reason may be a combination of C layer thickness and rapid temperature change.

![Image](https://aml.iaamonline.org)

**Fig. 1.** SEM images of PDMS substrate with deposited layer C (C3,C5,C7) and Ag with the consequent thermal treatment: 285 °C, 1 hour (left column), compared to C/Ag/T samples with single laser shot: 150 mJ.cm$^{-2}$ (right column). The inspected area of the main image was 10 × 10 µm$^2$ and corner image was 30 × 30 µm$^2$.

**Surface chemistry analysis using EDS method**

The surface chemistry of the prepared samples was determined by the EDS method, and the graph of the atomic concentration is shown in **Fig. 2**. The EDS method can analyze the surface up to the depth of several hundreds of nm [9]. The main characteristic of PDMS polymer is the presence of Si and O. A large amount of both elements was detected in its structure. It is evident from **Fig. 2** that with an increasing primary layer of carbon (from C7 to C3, where C3 represents the sample with the highest C thickness), the atomic concentration of Ag decreases. This surprising fact is probably caused by the properties of the initial C layer, which affect the formation of subsequently deposited Ag and its ability to penetrate the substrate, more importantly, by the combination/ratio of elements themselves. With increasing primary carbon layer, even not precisely detected by increasing carbon atomic concentration (due to the presence of carbon in the PDMS chain), significantly affects the atomic ratio of elements (C/Ag) on the deposited surface. After laser modification, the number of O atoms increased in all samples. Sample C5 at about 3 at. % and in samples C3 and C7 at about 10 at. %, caused by the oxidation of the Ag layer. For this reason, the amount of Ag atoms declined and even disappeared in the resulting analysis after the laser shot was applied. The second reason is partial ablation of both oxidized and original silver layer by the applied laser fluence affecting predominantly the upper surface layers with silver. The diffusion of silver elements and the formation of polymer composite may also affect the surface concentration of Ag.

![Graph](https://aml.iaamonline.org)

**Fig. 2.** Percent atomic concentrations (Ag, Si, O and C) of prepared hybrid samples C/Ag with different thickness of C layer (C3,C5,C7) and thermal treatment (285 °C, 1 hour) determined by EDS method. In comparison with samples after laser modification (150 mJ.cm$^{-2}$).

**Antibacterial properties**

The next step was to determine the antibacterial properties of the prepared samples by using two species of Gram-negative (G$^-$) *E. coli* and Gram-positive (G$^+$) *S. epidermidis* strains. The graph in **Fig. 3** compared the antibacterial activity of C/Ag/T samples before and after laser modification with pristine PDMS. Almost all samples within the error had a higher/excellent antibacterial effect than the control. For the C/Ag/T/l system samples, bacterial death occurred for both types of bacteria. The cause is increased oxidation of Ag, thus releasing Ag$^+$ ions, which have an increased antibacterial effect (mentioned in the introduction). Non-treated samples showed weaker antibacterial activity than laser samples but still higher activity than control or pristine PDMS. As a result of lower efficiency, these samples’ changes in surface morphology can be shown in the SEM images in **Fig. 1**. According to the article [10], some nanostructures may prevent bacteria from adhering to their surface. This confirms the theory that samples C3 (globular nanostructure) and C7 (wrinkled nanostructure) had higher antibacterial activity than sample C5.
Fig. 3. The numbers of colony-forming units (CFU) of S. epidermidis and E. coli, which were applied on the surfaces of the prepared samples for 2 h. PDMS substrate with layer C (C3,C5, C7) and Ag with the consequent thermal treatment (285 °C, 1 hour) compared to C/Ag/T samples with single laser shot (150 mJ.cm⁻²) and samples pristine PDMS were tested. The samples were compared with the control.

Conclusion

This experiment suggested a simple and efficient method for creating hybrid materials from an evaporated base C layer and a sputtered Ag layer on a PDMS polymer substrate. The height of the carbon layer varied depending on the distance of the polymer from the source carbon fibre. The C/Ag composite was thermally stressed at 285 °C and subsequently modified with a single laser shot on an area of 30 × 10 mm². The aim was to determine and compare the effect of morphology and chemical composition on the antibacterial activity of the prepared samples. The laser modification changed both the morphology and chemical composition and significantly affected antibacterial activity. By combining carbon and silver layers in a specific ratio combined with subsequent excimer laser exposure, we have prepared the surface with outstanding antibacterial properties again in both E. coli and S. epidermidis.

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Conflicts of interest

There are no conflicts to declare.

Keywords

Carbon, silver, nanocomposites, antibacterial activity.

Supporting information

Supporting informations are available online at journal website.

References


Authors biography

Klaudia Hurtuková graduated from the University of Chemistry and Technology Prague in the field of study of Drug Production at the Faculty of Chemical Technology. Currently, she is a student in the 2nd year of PGS studies in the field of Drugs and Biomaterials, and now she mainly deals with surface modification and preparation of nanostructures using an excimer laser. She is the first author/co-author of 4 scientific publications.

Graphical abstract

| PDMS | Flash evaporation C nanolayer | Sputtering Ag nanoparticles | Laser single shot |

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