

Silver micro- and nanoparticles filled silicone for limb prosthetics

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ABSTRACT

The threat of bacterial growth on the skin under the prosthetic liners or sleeves is an important problem, which can cause various serious diseases up to the repeated amputation. One of the promising ways to solve this problem is to use antibacterial materials as a liner/sleeve material. Among others composite based on the silicone polymer with silver particles additive is may be a simple and effective solution, since the silicone is the main material for the prosthetic liners and sleeves and silver demonstrates pronounced antibacterial effect. However, the questions related to the optimal concentration of silver in silicone that results in maximum antibacterial efficiency without harming human skin are still open. In the present work, synthesis of metallic silver powder from a mixture of micro- and nanoparticles was performed and composite samples based on silicone polymer with different silver concentrations were fabricated. The antibacterial properties of fabricated samples were studied using the microdilution method against gram-positive spore-forming bacteria *Bacillus subtilis*. The cytotoxic effect of the tested samples was evaluated on healthy human fibroblast cell (NAF1nor). Moreover, the effect of adding silver micro- and nanoparticles to silicone on its extensibility and hardness was studied. The results showed that the addition of silver has a noticeable effect on the antibacterial properties of silicone polymer reaching more than 50%. Furthermore, all tested silicone-silver composites were shown to be non-toxic. The presence of silver does not significantly affect the relative elongation of the samples. However, hardness increases with higher silver concentrations. In the final phase, prototypes of the silver-filled silicone prosthetic sleeve were fabricated for utilisation by the patient at the prosthetic-orthopaedic clinic. The testing of the prototype was successfully completed by the patient, thereby demonstrating practical functionality and suitability for clinical use.

Keywords:

Antibacterial materials; Limb prosthetics; Medical materials; Nanomaterials; Silicone; Silver

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How to cite this article:

Eksharova, S.; Poletaeva, Y.; Kurenkova, A.; Mishchenko, D.; Aydakov, E.; Serdyukov, V. Silver micro- and nanoparticles filled silicone for limb prosthetics. *Biomater Transl.* 2025

doi: [10.12336/bmt.24.00073](https://doi.org/10.12336/bmt.24.00073)



1. Introduction

Since an increase on road accidents, musculoskeletal diseases, and injuries sustained during military service, the development and design of prosthetic limbs is a pressing issue. The global population of amputees is nearly 58 million, and falls (36.2%), road injuries (15.7%), other transportation-related injuries (11.2%), and mechanical forces (10.4%) are the leading traumatic causes.¹

The implementation of exoprostheses for upper or lower limbs typically necessitates the utilisation of specialised liners (or sleeves in cases of transtibial amputation) to facilitate the usage of a prosthesis.²⁻⁴ Liners are elastic protective covers that envelop the residual limb surface uniformly, ensuring a robust skin bond (**Figure 1**). Wearing a liner prevents

skin friction within the socket, protects against mechanical damage, enhances bonding strength between the residual limb and prosthesis, and promotes an even load distribution across the limb surface due to the elastic properties of the liner material. Liners are therefore essential for optimising the fit and function of the prosthesis while preventing damage.

Liners vary according to attachment type (e.g., vacuum, locking) and the materials used (silicone, polyurethane, copolymer).⁵ The choice of material determines the liner's key properties, including resilience, hypoallergenicity, cost, and ease of production.^{6,7} Silicone is the preferred material for liners due to its durability, hypoallergenicity, and long wearability. It also provides effective control and safety when

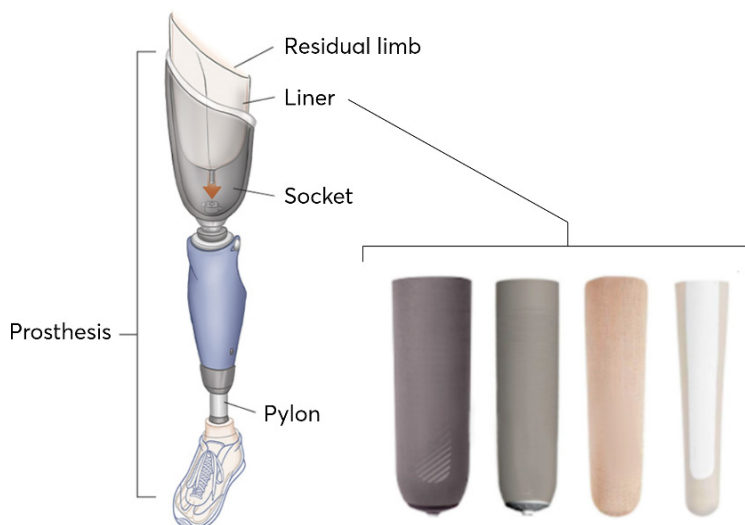


Figure 1. Transtibial prosthesis and liners for residual limbs. Created with CorelDRAW 2022.

wearing the prosthesis. The product range of leading manufacturers of prostheses and their accessories, such as Ottobock, Ossur, Willow Wood, and Alps, highlights silicone as the most preferred material for these products. Silicone is advantageous because it does not provoke skin diseases, is sufficiently durable, and develops microcracks only after extended wear. Additionally, silicone is biologically neutral and non-toxic.⁸⁻¹⁰ Furthermore, silicone liners used on the residual limb provide reliable control and safety, enhancing the prosthesis-wearing experience.

However, prolonged use of liners (exceeding 1 day) poses a risk of bacterial growth on the patient's skin. Studies report that up to 90% of amputees experience skin issues caused by liners,¹¹⁻¹⁴ including abscesses, botryomycosis, and cellulitis.¹⁵ Such conditions can necessitate discontinuation of prosthetic use or, in severe cases, further amputation. As such, solutions to inhibit or prevent bacterial growth on liner surfaces are critical.

Research has shown that certain micro- and nanoscale materials effectively combat bacterial growth.^{16,17} Among others, metal nanoparticles (NPs) exhibit remarkable antibacterial properties due to their small size and high chemical activity, driven by a large specific surface area. This enables them to penetrate bacterial membranes and interact with intracellular components. For example, iron oxide (Fe_3O_4) NPs inhibit bacterial growth and disrupt membrane-bound processes by generating reactive oxygen species (ROS). These ROS induce oxidative stress, damaging proteins and cell membranes, ultimately leading to bacterial death.^{18,19}

Studies have explored other metal-based compounds with antimicrobial properties. Copper ions (Cu^{2+}), for instance, exhibit significant activity in complexes such as copper-gallic acid-vancomycin (CuGA-VAN), effective against multidrug-resistant strains like methicillin-resistant *Staphylococcus aureus* (MRSA).²⁰ These compounds disrupt bacterial cell walls and

induce ROS generation. Similarly, copper cobaltite (CuCo_2O_4) NPs exhibit dual enzyme-like activities, generating hydroxyl radicals and superoxide anions, leading to bacterial membrane damage.²¹ Other compounds, such as ruthenium-procyanidin NPs (Ru-PC NPs) and zinc oxide/copper sulfide (ZnO-CuS) microspheres, exhibit pH-dependent ROS generation, providing versatility in antimicrobial applications.^{22,23}

Among metal NPs, silver NPs (AgNPs) are particularly effective due to their ability to release silver ions (Ag^+) in acidic environments.²⁴⁻²⁹ Ag^+ disrupt bacterial membranes, bind to proteins, impair enzymatic functions, and generate ROS.²⁴ This oxidative stress damages critical cellular components such as DNA, RNA, and proteins, compromising bacterial viability. Additionally, Ag^+ target enzymes like proton ATPase, disrupting energy production, and interfere with phosphate metabolism and nucleic acid functions, leading to bacterial death.³⁰ The "zombie effect" of Ag^+ further enhances their antimicrobial action, as silver-killed bacteria release internalised ions, spreading their toxic effects to neighbouring cells.²⁸

Comparatively, the smaller size of NPs, including AgNPs, enhances their antimicrobial efficiency over microparticles. The increased surface-to-volume ratio allows for greater ion release and interaction with bacterial cells, making them highly effective in applications requiring robust antibacterial activity, such as prosthetic materials and medical devices.

These findings emphasize the potential of metal NPs, particularly silver, as effective antibacterial agents. Their ability to act through multiple mechanisms and sustain antibacterial effects makes them valuable tools in combating infections and enhancing the safety and efficacy of medical devices, including prosthetic. There is a sufficiently large number of studies devoted to the evaluation of antibacterial properties of various polymers with the addition of silver particles,³¹⁻³⁵ but such studies for silicones are limited in number.

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The relevance of the study is highlighted by the significant gap in existing research on incorporating AgNPs into silicone polymers for antibacterial applications. While silver's antimicrobial properties are well-known, studies specifically examining its integration into silicone - a material widely used in prosthetic liners and sleeves for its durability, biocompatibility, and non-toxic nature - are scarce. This creates an urgent need for targeted research to optimise the antibacterial efficacy of silicone composites through AgNP integration. Addressing this gap, the current study not only investigates the antimicrobial activity, cytotoxicity and mechanical properties of the developed composites but also successfully fabricates a test sample of a prosthetic sleeve, ready for patient use, offering innovative solutions for medical and prosthetic applications.

2. Methods

2.1. Preparation and characterisation of silver particles

Silver particles were obtained by reduction of Ag^+ in aqueous

solution using sodium borohydride (NaBH_4). For this purpose, a 0.1 M solution of AgNO_3 was prepared, then an excess of freshly prepared 0.1 M solution of NaBH_4 was added while stirring. The resulting suspension was stirred for another 30 minutes, then centrifuged several times and dried at 60°C in air (**Figure 2**).

To study the chemical characteristics of obtained particles, X-ray photoelectron spectroscopy was performed using X-ray photoelectron spectrometer (SPECS Surface Nano Analysis GmbH, Berlin, Germany). The spectrometer is equipped with an XR-50 X-ray source with a double Al/Mg anode and a PHOIBOS-150 hemispherical electron energy analyser. The core-level spectra were obtained using the Al $K\alpha$ radiation ($h\nu = 1486.6$ eV). The calibration of the binding energy scale was performed by the internal standard method based on the C1s carbon peak corresponding to the carbon impurities ($E = 285.0$ eV).

The crystal structure of the synthesised Ag particles was studied by X-ray diffraction analysis. X-ray diffraction patterns were



Figure 2. Synthesis of the silver particles. Created with Corel DRAW Graphics Suite 2020.

obtained using a D8 ADVANCE diffractometer equipped with a LYNXEYE linear detector (Bruker AXS GmbH, Karlsruhe, Germany) at room temperature in the 2θ range of 33° – 83° with a step of 0.02° using Ni-filtered Cu $K\alpha$ radiation ($\lambda = 1.5418$ Å). The size of coherent scattering region of Ag particles was obtained by performing the Rietveld refinement using GSAS-II software package³⁶ (Argonne National Laboratory, Lemont, IL, USA).

2.2. Preparation and characterisation of silicone samples

The silicone (EpoxyMaster LLC, Moscow, Russia) with the Shore A hardness 0, was used in the study as a material of the samples. The silicone was prepared by mixing in equal parts (by weight) components A and B in small moulds. Silver particles at a given concentration were added to the mixture and mixed thoroughly for 15 minutes (**Figure 3**). The mass control of the silicone component as well as the added silver was carried out using BM313M (Vesta LLC, St. Petersburg, Russia) precision scales.

To eliminate air bubbles from the resulting mixture, it was degassed by placing it into a vacuum chamber (pressure 10 kPa, degassing time - 25 minutes). After preparation, the mixture was poured into either 20 mm × 20 mm × 2 mm polyethylene terephthalate rectangular moulds (to evaluate the antibacterial properties of the samples) or into the cells of a culture plate (to evaluate the cytotoxicity of the samples). In both cases, the silicone

polymer was cured in an enclosed volume, inaccessible to dust, at room temperature (25°C) and 40% relative humidity for 24 hours.

The following concentrations of silver in silicone were studied: 0, 5, 13.7, 44.9, 135 and 225 ppmw. **Figure 4** shows a photo of the fabricated samples. As can be seen, the silicone sample in the absence of silver particles (0 ppmw) is almost transparent, with increasing silver concentration the samples take a pronounced gray colour with visible inclusions of silver clusters.

The key physico-mechanical properties of the fabricated samples, which can affect the functionality of the final prosthetic product, were also studied. The hardness of the silicone polymer samples with silver addition was measured according to international standard ASTM D 2240-15 using portable durometer HT-1208 (HTi Xintai, Dongguan, Guangdong, China). In order to evaluate the effect of silver addition to silicone on its extensibility, one end of the sample was rigidly fixed with the help of a vise, and a weight of $800 \times g$ was suspended on the other end. The relative elongation of the sample was measured using a ruler.

2.3. Evaluation of antibacterial properties

The antibacterial properties of the mixture of nano- and microparticles of silver (AgMNPs) in the silicone polymer were evaluated using the microdilution method against gram-positive

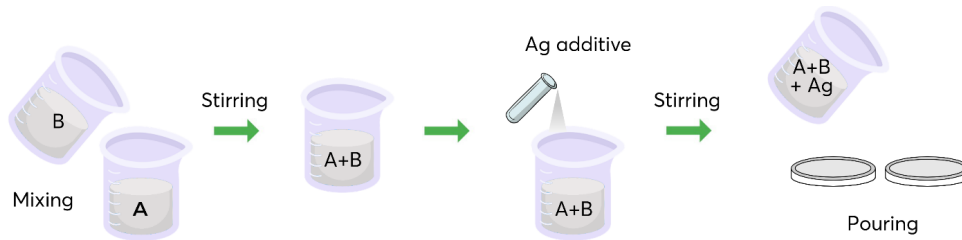


Figure 3. Fabrication of the silicone-silver composite. Abbreviation: Ag: Silver.

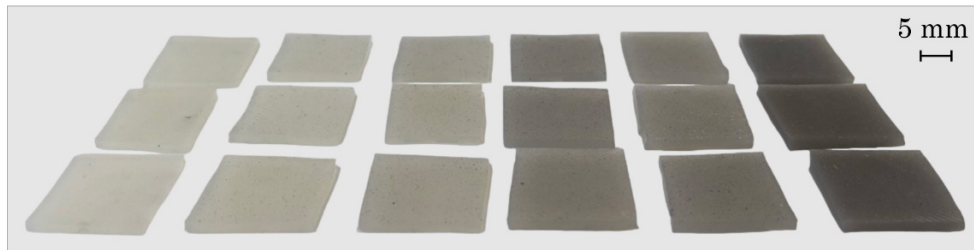


Figure 4. Triplicates for each concentration of the mixture of silver micro- and nanoparticles in silicone polymer. From left to right: 0, 5, 13.7, 44.9, 135 and 225 ppmw.

spore-forming bacteria *Bacillus subtilis* 1A2. The original strain was obtained from the Bacillus Genetic Stock Center (BGSC) at Ohio State University (USA). This is a classical model object and the most studied organism of the Gram-positive lineage, fast growing and easy to cultivate.³⁷ The studied silicone polymer samples were taken as colonised surfaces.

All samples were sterilised with ethanol (70%) and exposed to ultraviolet irradiation for 30 minutes. The bacterial strain was incubated in Lysogeny broth medium for 24 hours at 37°C in a shaker-incubator (ES-20, Biosan SIA, Riga, Latvia) at 1 × g in a sterile aerobic 15 mL plastic tube under aerobic environment. After, the bacterial density was brought to a concentration of 1 × 10⁴ colony-forming units/mL by serial dilution with control by spectrophotometry. A spectrophotometer SmartSpec Plus (Bio-Rad Inc., Hercules, CA, USA) at optical density equal to 600 nm was used for measurement. The studied samples were placed in three disposable 6-well culture plates one sample per well. Then, 100 µL of bacterial suspension was applied to each sample and covered with sterile 25 mm × 25 mm high density polypropylene square film. Each sample was incubated in a thermostat at 37°C for 24 hours. The samples were rinsed with 5 mL of Lysogeny broth medium and washed. Then the polypropylene films were removed and samples were again incubated for 24 hours at 37°C in a thermostat to activate the release of silver ions from the silicone polymer (**Figure 5**).

The final bacterial concentration was also measured by spectrophotometry at optical density at 600 nm.

2.4. Evaluation of cytotoxicity

The cytotoxic effect was evaluated by methylthiazolyldiphenyl tetrazolium bromide (MTT) assay³⁸ using healthy human fibroblast cell (NAF1nor cells; Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia, Cat# HSAF00064). Cells

were seeded in Dulbecco's modified Eagle medium (Sapphire, Moscow, Russia) with 10% fetal bovine serum for 24 hours in a CO₂ incubator (Innova CO-170, New Brunswick Scientific Co. Inc., Edison, NJ, USA). After 24 hours of incubation, an MTT assay kit (Sputnik-K, Moscow, Russia) was used to calculate the cytotoxicity. 10% MTT was added to each well, incubated for 4 hours, then all the liquid fraction was removed, leaving the precipitate at the bottom. Using isopropyl alcohol at a rate of 650 mL per well, the precipitate was dissolved in a shaker at 3 × g and 37°C for 10 minutes. The optical density of the resulting solution was then measured at 570 nm using a spectrophotometer (Tecan Austria GmbH, Grödig, Austria).

2.5. Prototype test

At the final stage, a sleeve was manufactured for use by the patient. Typically, liners and sleeves are manufactured using metal moulds for silicone casting. In this work, such moulds were made of plastic using FDM 3D printing, which also significantly reduces the cost of the final product. The Designer XL S2 3D printer (Picaso LLC, Moscow, Russia) was used in this work. Glycol-modified polyethylene terephthalate (PETG) was used as a mould material, which has low shrinkage and hygroscopicity, which is important for repeated use of moulds. The mould is two parts connected by bolts. The surface of the moulds was treated with sandpaper to achieve a smooth inner and outer surface of the moulded sleeve. In order to ensure a comfortable wear with a large girth difference between the distal (D) and proximal (P) ends, the sleeve was cone-shaped. The size of the final product was: length – 400 mm, width P/D – 35/65 mm, wall thickness – 3 mm. Silver was added at a concentration of 135 ppm to be sure that the cytotoxicity level of the resulting composite was 0. On the outer side, the silicone was covered with a fabric that prevents the sleeve

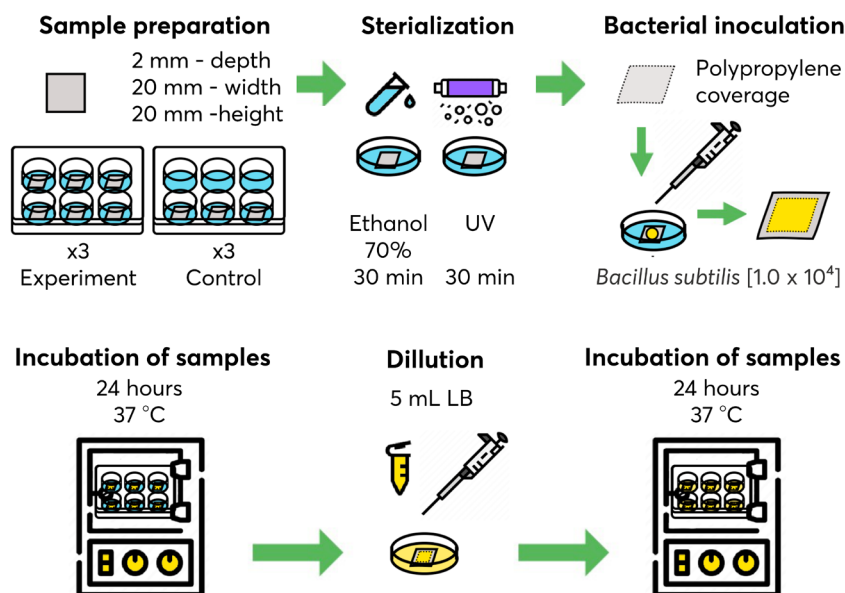


Figure 5. Evaluation the antibacterial properties of silver filled silicone. Created using Adobe Photoshop version 21.1.1. Abbreviations: LB: Lysogeny broth; UV: Ultraviolet.

from excessive stretching and serves as a protective layer that prevents the silicone from rapid wear or tearing. This prosthetic prototype was tested on a volunteer (male, 40 years old). The experiment has received informed consent from this volunteer and approval from the relevant ethics committees.

2.6. Statistical analysis

The experimental data obtained were compared with the data of control samples using a multivariate analysis of variance, where each mechanical property acts as the dependent variable and the presence and concentration of AgMNPs acts as independent factors. Statistical analysis of obtained data was performed using the program XLMiner Analysis ToolPak Statgraphics Centurion XV (Frontline Systems Inc., Incline Village, NV, USA). Values with *P*-value less or equal to 0.05 were considered statistically significant (at 95% confidence level). For each measured averaged value standard deviation of data also was measured, while the corresponding graphs presented further demonstrate averages values with confidence intervals.

3. Results

3.1. Characterisation of silver particles

Chemical state and surface composition of the obtained Ag samples were studied by X-ray photoelectron spectroscopy (Figure 6A). The Auger parameter of the sample was 726.1 eV, so it can be concluded that silver in the obtained sample is in the metallic state.

The X-ray diffraction results of the phase composition (Figure 6B) demonstrated that the synthesised silver is in a single phase of metallic Ag with space group Fm-3m (lattice parameter 4.0859 ± 0.0001 Å). The coherent scattering region, characterising an average crystalline size, is 200 ± 20 nm.

3.2. Antibacterial properties and cytotoxicity of the fabricated samples

The result of antibacterial properties against *Bacillus subtilis* 1A2 was shown in Figure 7. There was a significant increase of antimicrobial activity from 13.7 to 225 ppmw. In contrast, no statistically significant difference was found among the mixture of micro- and nanoparticles of 0 and 13.7 ppmw.

As seen in Figure 8, the addition of silver has a noticeable effect on the antibacterial properties of silicone polymer reaching more than 50%.

The MTT results showed that the relative growth intensity of NAF1nor cells did not decrease below 75%. It can be seen that there is an increasing trend of cytotoxicity with increasing silver concentration (Figure 8). The maximum cytotoxicity value is up to 25%, which as will be shown below allows to refer the fabricated composites to non-toxic materials.

3.3. Physicomechanical properties of silver micro- and nanoparticles filled with silicone

The results of the relative elongation study of the fabricated samples showed that silver addition to the silicone does not affect this parameter significantly, all samples showed similar value of relative elongation within the measurement error. This is in agreement with Sonnahalli's study³⁹ and is due to the extremely small concentration of silver particles, which does not noticeably affect the extensibility of silicone.

Figure 9 showed that increasing the silver concentration leads to an increase in the hardness of the silicone. Despite the fact that for the maximum silver concentration the hardness of the sample increases more than twice in comparison with the base silicone without silver, the

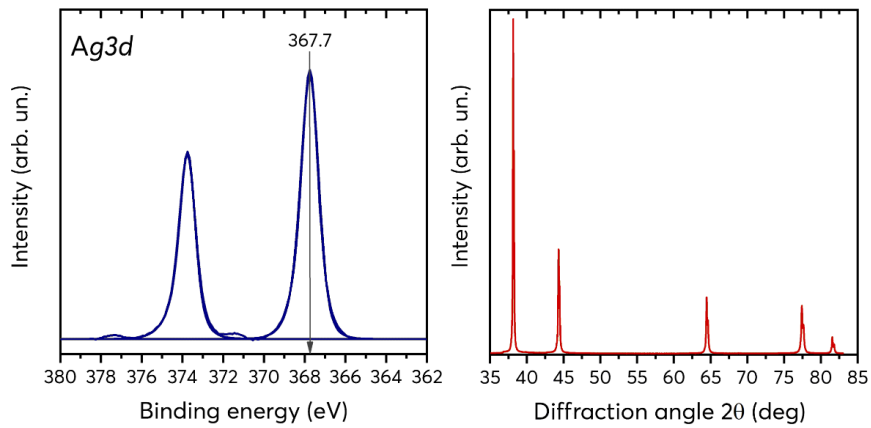


Figure 6. Ag3d spectrum (A) and X-ray diffraction pattern (B) of the silver particles

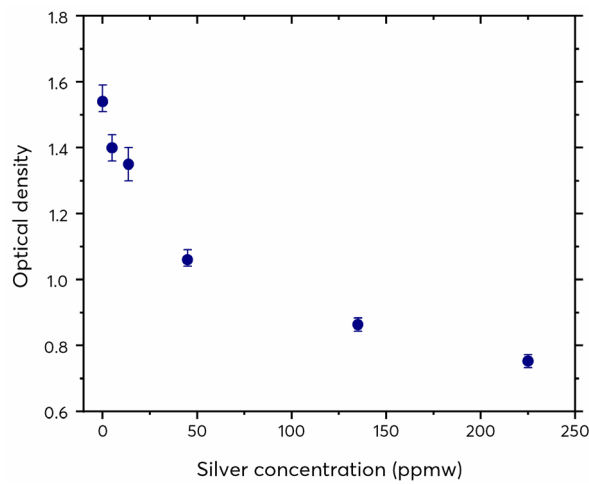


Figure 7. Optical density of *Bacillus subtilis* 1A2 on silver filled silicone. The data are shown as mean \pm SD, and were analysed by multivariate analysis of variance.

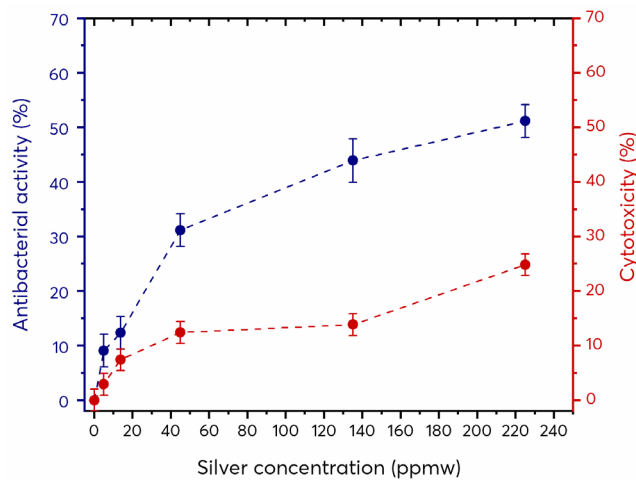


Figure 8. Antibacterial activity and cytotoxicity of silver filled silicone. The data are shown as mean \pm SD, and were analysed by multivariate analysis of variance.

obtained values are rather low and do not allow to classify the fabricated samples as Shore 1 silicone. Thus, the addition of silver in the concentrations studied in the present work

has an insignificant effect on the mechanical properties of the silicone polymer, which should not affect its use by the patient.

3.4. Prototype test of prostheses

The volunteer successfully completed the prototype testing without any abnormalities (Figure 10).

4. Discussion

The study indicated the potential for utilizing the outlined method of silver particle synthesis (reduction of Ag^+ in aqueous

solution using NaBH_4) in the development of polymer composites that possess antibacterial properties. The method's simplicity and cost-effectiveness are particularly noteworthy, as these qualities are essential in meeting industry demands. Additionally, the method's scalability and the absence of requirements for costly equipment are advantageous. It is recognised that the resulting powder is a mixture of nano- and microparticles, as evidenced by

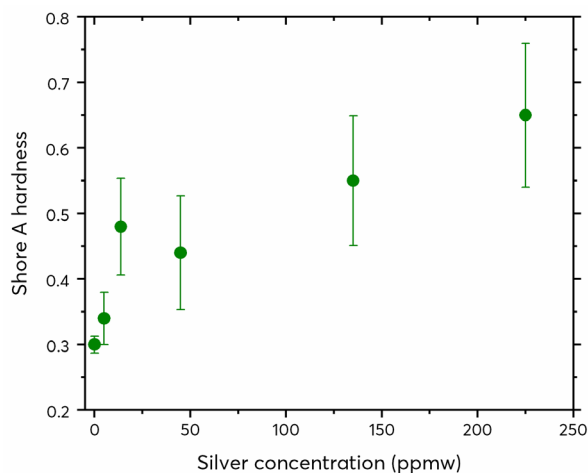


Figure 9. Hardness of silver filled silicone. The data are shown as mean \pm SD, and were analysed by multivariate analysis of variance.



Figure 10. Fabricated prototypes of the silver filled silicone sleeves

SEM image analysis. As will be discussed below, this distinguishes the properties of the fabricated silver-filled silicone polymer from those studied in other papers where primarily NPs were synthesised. However, in our opinion, this is not a reason that can in any way prevent the solution proposed in this study from being used in real practice, since even microparticles of about $100 \mu\text{m}$ in size will be either weakly or not at all felt by the human skin.

The dependence of bacterial activity showed that the addition of silver has a noticeable effect on the antibacterial properties of silicone

polymer. The maximum value of antibacterial efficiency reaches 50%. As the analysis of the obtained graph shows, the dependence of antibacterial activity on silver concentration has a similar to logarithmic dependence, which makes it different from other study,²⁶ presented linear dependence. The absence of linearity in the growth of antimicrobial properties with increasing silver concentration, as observed in the present study, distinguishes it from another study.³⁵ This phenomenon may be attributed to the heterogeneity of the particles synthesised in the present study. The antimicrobial activity

of silver is contingent on the size of the particle, with larger particles exhibiting higher antibacterial efficiency due to a greater surface area relative to their volume. This relationship is a direct consequence of the nature of silver's action. Once in an environment with a certain acidity, silver begins to convert to its active ion form, which in turn is involved in a significant part of the antimicrobial activity scenarios. It is evident that the antibacterial efficiency of microparticles is lower than that of NPs.

Given the highly variable volume-to-area ratio of the particles in the synthesised silver powder, the increase in total concentration more strongly reflects the difference in activity of different-sized particles. At low silver concentrations, the difference in particle activity is hardly noticeable, but with increasing particle content in the polymer, characterised by geometric progression, the reduced antimicrobial properties of silver microparticles become more noticeable.

In addition, the study analysed the effect of silver addition on the cytotoxicity of the polymer. The results also showed all samples were non-toxic materials. Hence, all the samples studied can be used clinically.

The experiments showed that the investigated concentrations of silver in silicone provide a valuable improvement in antibacterial properties and allow its use in contact with the skin of patients.

While the findings of the present study confirm that the silicone-silver composite provides sustained antibacterial performance suitable for clinical applications, further research is also necessary. Particularly, the present study does not assess the long-term stability or sustained antibacterial properties of the AgMNPs in the silicone polymer. This omission is significant, as long-term efficacy is critical for practical applications in fields such as medical devices and industrial materials. Future work to address these limitations could focus on directly quantifying the release rate and amount of silver ions using advanced analytical techniques. In addition, *in vivo* testing is a critical next step to validate the antibacterial efficacy of the prosthetic materials studied in the research. For further studies, the authors propose using a subcutaneous pocket model in animals to evaluate antibacterial properties. Such methods have been widely employed to simulate prosthetic infections and evaluate antibacterial materials for hernia repair, offering a proven strategy to investigate *in vivo* antimicrobial properties. Authors believe that these data would further optimize the composite's formulation to balance antibacterial efficacy with cytotoxicity and ensure long-term safety for prosthetic use.

5. Conclusions

In conclusion, we have fabricated samples of composite based on silicone polymer with different silver concentrations and analysed the effect of such additives on the antibacterial, cytotoxicity and physicochemical properties. The results of the tests showed that the addition of silver has a noticeable effect on the antibacterial properties of silicone polymer reaching more than 50%. Furthermore, all tested silicone-silver composites were found to be non-toxic, indicating their potential for clinical use. The presence of silver does not significantly affect the relative elongation of the samples. However, hardness increases with higher silver concentrations.

In the final phase of the study, prototypes of the silver-filled silicone prosthetic sleeve were fabricated for utilisation by the patient at the prosthetic-orthopaedic clinic. The testing of the prototype was successfully completed by the patient, thereby demonstrating practical functionality and suitability for clinical use.

Acknowledgement

We thank Prof. Anatoly Beklemishev for providing the *Bacillus subtilis* 1A2 strain for this study. We also thank the staff of the Moscow Prosthetic-Orthopaedic Enterprise (Novosibirsk Branch) and personally the director Anton V. Kamenev for valuable advice and the opportunity to test the fabricated prosthetic sleeve on a patient.

Financial support

The study was supported by the Mathematical Center in Akademgorodok (No. 075-15-2022-282) with the Ministry of Science and Higher Education of the Russian Federation. The X-ray photoelectron spectroscopy and X-ray diffraction studies were performed using the facilities of the shared research center "National Center of Investigation of Catalysts" at Boreskov Institute of Catalysis.

Conflicts of interest statement

The authors declare that they have no conflict of interest.

Author contributions

Conceptualization: VS and SE; *Data curation*: SE, YP, EA and VS; *Formal analysis*: SE and VS; *Investigation*: SE, YP, EA, DM, and VS; *Methodology*: SE, YP and AK; *Project administration*: VS; *Resources*: YP, AK and VS; *Supervision*: VS; *Validation*: SE; *Writing-original draft*: SE, VS and AK; *Writing-review & editing*: VS and SE.

Ethics approval and consent to participate

The experiment has received informed consent from this volunteer and approval from the relevant ethics committees.

Consent for publication

Informed consent was obtained from the volunteer.

Availability of data

Not applicable.

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Received: October 20, 2024

Revised: January 21, 2025

Accepted: February 14, 2025

Available online: April 24, 2025