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Bactericidal Properties of the Sol-gel Layer on Polymer Substrates in Medical Applications

Paulina Armatys[a]*, Elżbieta Długoń[a], Wojciech Smółka[b], Robert Sobota[c], Aneta Frączek-Szczypta[a], Jarosław Markowski[b], Ewa Stodolak-Zych[a]

[a] AGH University of Krakow, Faculty of Materials Science and Ceramics, Mickiewicza 30, 30-059 Krakow, Poland
[b] Medical University of Silesia in Katowice, School of Medicine in Katowice, Laryngology Department, Poniatowskiego 15, 40-055 Katowice, Poland
[c] Medical Details Plant „DEMED" Sp. z o.o, Żwirki i Wigury 56 C, 43-190 Mikołów, Poland
e-mail: parmatys@agh.edu.pl

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Abstract

Polymeric biomaterials are a group of plastics used in medical devices, implants, and artificial organ components. In order to maintain the higher asepticity of the products, solutions based on the modification of the volume or surface of a plastic with biocidal agents, e.g. antibiotics, nanoparticles, are used. One of the methods used to impart biocidal properties to the material can be the use of layers applied by the sol-gel method. The aim of this study was to produce homogeneous and durable coatings on inert and hydrophobic silicone surfaces based on polysiloxane sols with biocidal activity against model bacteria: Gram negative (*Escherichia coli, Klebsiella pneumoniae*) *and* Gram positive (*Staphylococcus aureus, Enterococcus faecalis*). This paper presents results of investigations on a commercial biomedical silicone material (DEMED Sp. zoo) modified with eight sols; siloxane (TD), phenol-siloxane (FD), siloxane-aluminum (TD-Al), siloxane-titanium (TD-Ti), titanium (Ti), zinc (Zn), and TD and TD-Al sols modified with green tea extract (TD-GT, TD-Al-GT). The reference for the tested materials was unmodified silicone. In order to be prepared for coating, the siloxane samples were etched with hydrofluoric acid. Residual acid was removed from the silicone by washing and the substrates were then coated with sols by means of dip coating. The modified materials were then polymerized at 100°C for one week. The produced layered composites were subjected to microstructural, physicochemical, structural and microbiological analysis by contacting them with Gramm negative and Gramm positive bacteria. It was shown that the strongest biocidal properties were exhibited by samples modified with the sols based on - Zn, TD-Ti, Ti, TD-Al-GT and TD-GT.

Keywords:
sol-gel method, biocidal properties, implants, inert polymers, silicone

1. INTRODUCTION

Bacterial infections are a significant medical problem. The patient is exposed to the effects of bacterial infection both during standard treatment with medical devices and during implantation or therapies that support the treatment process [1–3]. An antibiotic therapies currently used often prove inadequate due to an increasing incidence of antibiotic resistance as well as the kinetics of a bacterial biofilm formation [4–7]. The current task is to develop methods to impart biocidal properties to biomaterials without the use of antibiotics. The first stage of biofilm formation is a surface. Its microstructural as well as physicochemical properties affect adhesion of bacteria to the surface layer of the material, and are crucial in this case [8, 9]. Many ceramics as well as polymeric materials show inertness to chemical and physicochemical methods of surface modification. A number of layering techniques on plastics (polymers) aim to alter the surface properties (e.g., wettability, surface energy) or modify the surface and make it bioactive or biocidal. Particularly well known in the literature are metallic layers, which have documented antibacterial properties [1, 10, 11].

A common method of achieving antibacterial properties is to apply metal nanoparticles to the surfaces of materials – in particular copper, zinc, silver, titanium, iron, magnesium and gold [12]. Metallic-coated implants have been proven to reduce biofilm while maintaining high surface biocompatibility [13]. Particularly noteworthy are nanoparticles of metal oxides of such elements as Zn, Mg, Cu, Ag and Ti. Metal oxides have biocidal properties towards both Gram Negative and Gram Positive bacteria, and their properties are already evident at relatively low concentrations [14, 15]. Unfortunately, their presence is time-limited, which strongly affects the hazard of the implant, hence the number of implants coated with biocidal metallic layers is relatively small [12].

Another method of imparting antibacterial properties to polymeric materials is to modify their surfaces using non-toxic

and biocompatible polysaccharides e.g., carboxymethyl cellulose (CMC), carboxymethyl-1,3-dextran (CMD), and alginic acid (AA) [16]. The presence of the polysaccharide affects the ability of bacterial proteins to adhere and aggregate to form a biofilm [12]. Extracellular polymeric substances (EPS) secreted by bacterial cells in biofilms are referred to as exopolymers and contribute to the permanent binding of the microorganism to the substrate. A highly hydrophilic or hydrophobic surface hinders EPS adsorption and thus significantly reduces the possibility of biofilm formation and growth. Layers similar to polysaccharide ones can be obtained by the chemical vapor deposition method (CVD), examples of which are layers with hexamethyldisilazane (HMDS) and perfluorodecyltrichlorosilane (FDTS). The mentioned modifications reduce the wetting angle of silicone surfaces from 20° to 70° [17].

Surface modification methods involving the grafting of more complex molecules to the polymer surface are also explored. Also, grafting the surface of implants with antimicrobial peptides provides bactericidal activity, which is an effective tool against antibiotic resistance [18–20]. Short peptide chains containing specific amino acids such as tryptophan, arginine, and lysine are among the most promising biocidal peptides [13]. The most commonly modified polymers are polyethylene glycol (PEG) and its derivatives, due to their high biocompatibility, high safety, hydrophilic properties and high impact on bacterial adhesion [21, 22]. Disadvantages of this process are the long time required and its complicated procedure [12].

Another interesting way of modifying the surface is the use of polyphenols i.e. organic compounds that occur naturally in green tea and easily form layers, increasing the hydrophilicity of the surface [23]. Polyphenols possess a number of mechanisms that interfere with a normal biofilm function, which are; inhibition of the EPS formation and reorganization, disruption of the biofilm's quorum sensing system, disruption and inhibition of enzymes involved in DNA synthesis or bacterial metabolic processes, or binding to the bacterial cell membrane causing disruption of its continuity. The antibacterial action of polyphenols is lower than that of antibiotics, but due to their unique and multidirectional mechanisms of action synergistically supporting the action of antibiotics [24] and their natural occurrence in the human diet, they are a potential complement to the antibiotic therapy [25]. Unfortunately, they are exposed to various factors that cause their deactivation. These include temperature, radiation (e.g. UV) or the need to use high concentrations to achieve a biocidal effect [26, 27]. At the same time, this is associated with the limited solubility of these compounds. In addition, the best solvent for many of them is alcohol – so testing the biocidality of pure extracts is very difficult and often done indirectly. Using solvents based on alcoholate derivatives, we do not eliminate the problem of solubility of the extract, in addition, we can introduce it in quantities that guarantee the concentration of MIC [28]. Solidification of such a layer is carried out at temperatures not exceeding 70–80°C because it guarantees the stability of the extract [29].

A sol-gel technique is used to obtain continuous layers with the possibility of even introduction of various agents, including those of a biocidal nature. The application of sol-gel coatings containing nanoparticles TiO_2 and ZrO_2 makes it possible to obtain durable coatings with strong hydrophilic properties. In addition, these layers can promote the reduction of bacterial growth and adhesion on the surface of materials [30, 31]. On the other hand, layers based on nanometric SiO_2 effectively reduce the adhesion of fine particles to the surface by increasing the hydrophilicity of the substrate [17]. The addition of TiO_2 helps control the adhesion of bacteria to the sample surface [32]. Metal oxides and silicon oxide exhibit anti-adhesion properties by retarding the adhesion and adsorption of bacterial proteins (EPS).

As the methods of grafting on polymeric surfaces are often complicated and the use of nanoparticles raises some concerns about their behavior in the body, it was decided to test the effectiveness of antibacterial properties of films obtained by a simple and inexpensive sol-gel method, using elements with documented antibacterial properties to produce the sol. In addition, a microbiologically active addition of green tea extract to polysiloxal in the sol was used and which, thanks to the low process temperature, allows the preservation of the antibacterial properties of the extract.

The aim of the presented work was to obtain continuous sol-gel coatings on the surface of silicone, i.e. on the surface of a material difficult to modify, which would have bactericidal properties. In addition, the work aims to conduct comparative studies of the effectiveness of using various additives with documented bactericidal effects. In this study, modified, commercially available, medical silicone materials were investigated. The surface of the silicone materials was etched with hydrofluoric acid and then layers of materials with known antimicrobial properties or enriched with antimicrobial materials were applied to the prepared surface using the sol-gel method. Layers containing TiO_2, ZnO, Al_2O_3 particles but also modified with green tea extract were tested to compare the effect of the antibacterial action of polyphenols with that of the free radical oxides.

2. MATERIALS

Commercial medical silicone (DEMED SP zoo) was used in this study – samples of 5 mm × 5 mm were cut hydrofluoric acid (HF, 70%), zinc acetate dihydrate (97%), monoetanoloamine ($HOC_2H_4NH_{3+}$, 1000 mg/l in H_2O), phenyltriethoxysilane (98%,), aluminum nitrate ($Al(NO_3)_3$, 99%) was purchased from Pol-Aura. Powdered green tea extract (*Camellia sinensis*, green tea extract 98%), dimethyldiethoxysilane (DEDMS, 97%), titanium (IV) isopropoxide ($Ti[OCH(CH_3)_2]_4$, 98%) were provided by Sigma-Aldrich. Methyltriethoxysilane (TEOS, ≥98%) were acquired from Merck. Phenol red ($C_{19}H_{13}NaO_5S$) were purchased from ChemiLab. hydrochloric acid (HCl, 35–38%) were provided by Chempur glacial acetic acid (CH_3COOH, 99.8%) were acquired from Lach-Ner. Diiodomethane (98%) were acquired from Chemland.

Reagents necessary for biological tests and bacterial strains, i.e. MUELLER HINTON LAB-AGAR™, Nutrient Broth, *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6538P), *Enterococcus faecalis* (ATCC 19433), *Klebsiella pneumoniae* (NCTC 13443) was purchased from Biomaxima.

3. METHODS

The experiment conducted of two parts; in the first, sol-gel layers were applied by immersion, and in the second, they were characterized by various techniques to determine their microbiological activity in the last test. The preliminary stage was the preparation of substrates; degreasing, etching. All sols were prepared as described below.

3.1. Coating preparation

Medical silicone was etched in hydrofluoric acid for 30 minutes to modify its microstructure and alter its surface properties. The samples were then thoroughly rinsed with deionised water (UHQ).

The sols were prepared from organic precursors and suitable catalysts (Table 1). Layers prepared by the sol gel method were made according to the following scheme: a sol solution (solution 1) was mixed with a catalyst water solution (solution 2). The precursor was dissolved in ethanol (proportions depending on the sol type). The precursor solution prepared in this way (solution 1) was homogenized on a magnetic stirrer (30 min/25°C), and then the catalyst water solution (solution 2) was successively dropped into it. The slow dropping of the catalyst solution did not cause the sol solution to become cloudy. The sol obtained in this process was tightly closed in a bottle and stirred on a magnetic stirrer for another 24 hours at room temperature. Then the obtained sol was stored in the refrigerator at 3°C until the coating procedure [33].

Eight different sols were prepared: zinc (Zn), phenol derivative modified siloxane (FD), aluminum modified siloxane (TD-Al), siloxane (TD), titanium oxide modified siloxane (TD-Ti), titanium (Ti), aluminum modified siloxane with green tea (TD-Al-GT) and green tea modified siloxane (TD-GT). The addition of green tea consisted in introducing of a powdered green tea extract (GT) at a mass concentration of 1% into the sol (prior to its transition to a gel) and undergoing homogenization using a magnetic stirrer and an ultrasonic scrubber.

The coatings were deposited on the silicone substrate using the dip coating method [28] – the immersion and the elevation rate was 30 cm/min, the immersion time was 30 s, and the process was carried out at room temperature and atmosphere. After application, the samples were placed in an oven (MEMMERT) at 100°C for 7 days for the layer polymerization. As reference materials, an unmodified silicone S_Ref and a modified silicone S_HF were used.

3.2. Coating characterization

Microscopic observations were performed with an optical microscope (KEYENCE) using 500 × magnification and a depth-of-field measurements. The reference silicone (S_Ref) and the silicone samples after the etching process (S_HF) were examined to compare changes in surface microstructure occurring during the etching process.

The wetting angle and the surface free energy tests were carried out on a DSA 25 Kruss goniometer. The purpose of the study was to check effects of the etching and the coatings on the physicochemical properties of the surface. Distilled water (UHQ) was used as the polar liquid and diiodomethane was the non-polar liquid. The wetting angle was determined by the sessile drop method, while the surface energy was determined by the Owens–Wendt method [34]. A droplet of 1 ml volume was applied to the surface of the material. Average values of the wetting angle were determined from 25 measurements. The measurement error was determined from the standard deviation of the measurements. The test was conducted at room temperature.

Table 1
The designations of the samples (materials) and the precursors and catalysts used to produce them

Sol/samples (numbers)	Sol description	Precursor (source)	Catalyzer/ catalyst
Zn (1)	Zinc sol	Zinc acetate dihydrate	Monoetanoloamine
FD (2)	Phenolic modified siloxane sol	Methyltriethoxysilane Phenyltriethoxysilane	Phenol red
TD-Al (3)	Aluminium modified siloxane sol	Aluminum nitrate Dimethyldiethoxysilane Methyltriethoxysilane	Hydrochloric acid
TD (4)	Siloxane sols	Dimethyldiethoxysilane Methyltriethoxysilane	Hydrochloric acid
TD-Ti (5)	Titanium-modified siloxane sol	Titanium(IV) isopropoxide Dimethyldiethoxysilane Methyltriethoxysilane	Hydrochloric acid Glacial acetic acid
Ti (6)	Titanium sols	Titanium(IV) isopropoxide	Glacial acetic acid
TD-Al-GT (7)	Aluminium modified siloxane sols with green tea added	Aluminum nitrate Dimethyldiethoxysilane Methyltriethoxysilane	HCl, Hydrochloric acid
TD-GT (8)	Green tea modified siloxane sols	Dimethyldiethoxysilane Methyltriethoxysilane	HCl, Hydrochloric acid

FTIR spectroscopic analysis was carried out on Tensor 27 (Bruker) spectrophotometer with the OPUS software in transmission measurement mode, where potassium bromide (KBr) was the carrier of the studied materials. The measurements were performed in the air atmosphere at 400–4000 cm^{-1} wavenumber range with a 4 cm^{-1} resolution and accumulation of 64 spectra. The purpose of the study was to verify the effectiveness of the method of applying sols doped with the bactericidal elements. The study was conducted at room temperature.

Two types of microbiological tests were carried out: disk diffusion testing and live-dead survival staining. The tests were to confirm the biological properties of the obtained layers. The disk diffusion test was performed for selected materials in accordance with EUCAST recommendations [35]. The powder formed after grinding the cured gel/coating was used for the test. Standardized petri dishes with agar medium were used as the substrate, onto which a bacterial suspension in saline with a density of 0.5 McFarland scale was applied. Four bacterial strains were used for the study: *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*. Agar media with applied bacterial solutions were loaded with small piles of the held powders to form approximately equal diameter piles. The dishes were then placed in an oven (UF55, Memmert) at 37°C for 24 hours, after which images were taken to allow measurement of the diameter of the zone of inhibition were made by using the ImageJ software. For each test material, the test was repeated three times. The result was presented as a mean value with standard deviation. In addition, for reference strains of Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria, a test of control materials was carried out. The negative control was a sterile filter paper disc soaked in saline (N – negative sample). The positive control (P – positive control sample) was a sterile filter paper disc soaked in green tea extract with a concentration equal to the MIC determined experimentally (100 mg/ml). The behavior of silicone-based materials treated as references was also investigated: S_Ref (base silicone, no modifications) and S_HF (HF acid-etched silicone, rinsed to constant pH, base material for other modifications) to test their behavior in contact with bacteria.

The viability staining was performed using the LIVE/DEADTM BacLightTM Bacterial Viability Kit (Thermo Fisher) according to the manufacturer's recommendations [36]. Into the wells of a 24-well plate, the silicone materials with the applied coatings were inserted and then poured with a bacterial suspension of *Escherichia coli* in 0.5 McFarland scale saline enriched with nutrient solution. The test materials were inoculated in a UF55 (Memmert) oven at 37°C for 6 hours. A mixture of SYTO9 and propidium iodide dyes was then added to the wells at a ratio of 1:1. The samples were inoculated for another 15 minutes to stain the bacterial cells. Using an Axiovert 40 microplate reader (Carl Zeiss AG), the fluorescence of the supernatant from above the samples was measured. The determination performed concerned the filtrate, in which the bacteria floating in the filtrate were detached from the surface of the test layer and giving a fluorescent signal. The obtained fluorescence results were compared with one another: treating the measurement result for the S_Ref as the reference result, from it the percentage reduction of the bacteria was calculated based on the Formula (1):

$$\%\ reduction = \frac{fluerescence\ measurement}{reference\ measurement} \times 100\% \qquad (1)$$

where:

fluorescence measurement – fluorescence measurement of a given sample,
reference measurement – fluorescence measurement of the reference sample S_Ref.

4. RESULTS ANALYSIS AND DISCUSSION

The etching process significantly affected the surface microstructure of the commercial silicone material (S_Ref) (Fig. 1). The number and scale of irregularities present on the surface of the material was noticeably greater after the etching process. This result was expected and consistent with the literature reports. The studies reported that etching of silicone surfaces in hydrofluoric acid results in a significant increase in their porosity [37], which was also the case in the current study. Therefore, it can be assumed that the etching process was successful, and that etching of silicone surfaces in HF is an effective method for increasing their roughness and porosity. This is related to the fact that HF is commonly used to etch silicon-containing materials [38, 39]. In addition, the transparency of the material decreased – the transparent silicone substrate became milky.

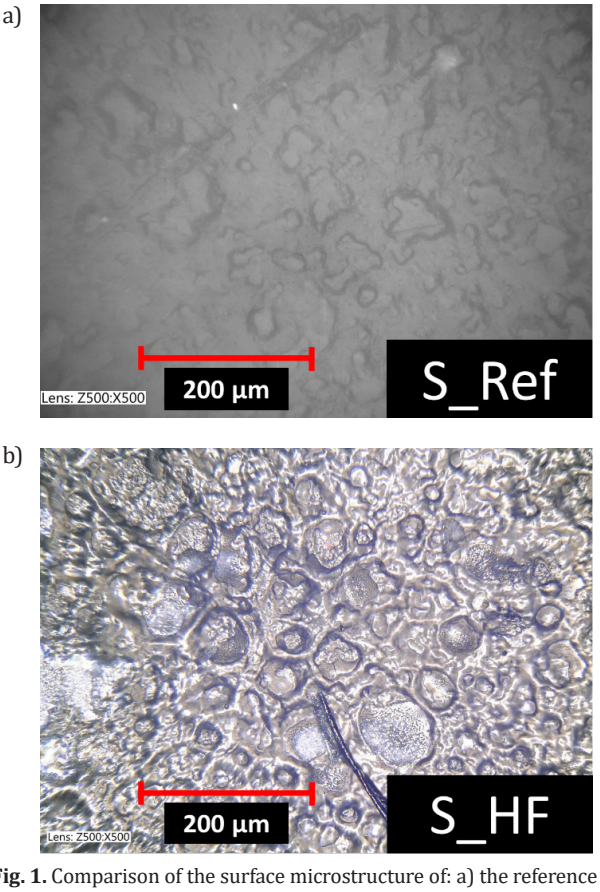

Fig. 1. Comparison of the surface microstructure of: a) the reference i.e. non-etched (S_Ref); b) the etched (S_HF) silicone

The results of the wetting angle and surface free energy studies are shown in Figure 2. The etching of silicone increases its surface wetting angle by about 12°, while simultaneously lowering the polar component of the surface free energy by more than 15%. This means that etching of the silicone surface in HF contributed to a significant change in the physicochemical properties of the surface. On the other hand, application of the coatings on the etched surfaces did not significantly affect the hysicochemical properties of the etched surface. The highest material hydrophobicity was obtained for the materials coated with titanium-containing layers (wetting angle of 120°), while the lowest was obtained for the surfaces modified with green tea (wetting angle of 110°). Nevertheless, all of the surface modifications obtained had greater hydrophobicity than the unmodified surface (a wetting angle larger at least by 10°). In addition, the application of the coatings to the etched surface resulted in a decrease in fraction of polar component of the total surface energy. In any case, the polar component reached or exceed a value similar to that of the untreated sample (S_Ref). Application of the sol-gel layers to the silicone generally resulted in an increase in the value of the surface free energy relative to the etched surface (S_HF). The exception was the Zn-base sol coating, whose surface energy decreased by about 2 mN/m. In addition, samples containing sol TD-Al increased the value of surface free energy by about 3 mN/m relative to the untreated sample (S_Ref) and 6 mN/m relative to the etched sample (S_HF).

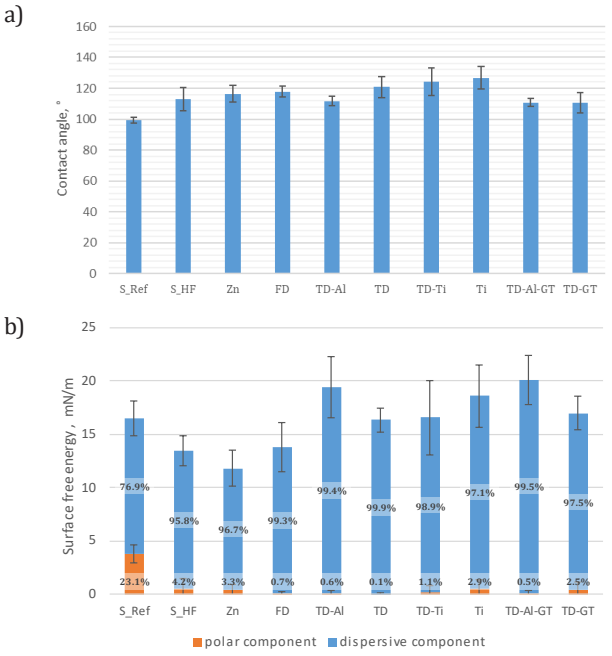

Fig. 2. Surface properties of the materials: a) water contact angle of the materials; b) surface free energy of the materials with division into dispersive and polar components

Thus, as can be seen, the differences in the physicochemical properties of the surface, although noticeable, were quite subtle. In order for a material to exhibit antifouling and thus antibacterial properties, it must exhibit superhydrophobicity i.e. a wetting angle higher than 150°. To achieve this, it is necessary to achieve both low surface free energy values and high surface roughness [40–42]. In the present studies, despite low values of the surface free energy, the wetting angles did not exceed 130° – so one can speak of clearly hydrophobic surface properties. In addition, the etched material having porosity/roughness on the micrometer scale can hardly be considered to be eminently rough. This also implies that the potential antibacterial properties are related to effect of chemical composition of the coatings on bacteria, and not to the change in the surface properties of the materials.

The reference materials before and after etching (Fig. 3a) show bands characteristic of siloxanes: a Si–O–Si bridge in the 1000–1300 cm^{-1} range, with a distinct band of 1008 cm^{-1} and an arm of 1076 cm^{-1} corresponding to stretching vibrations in Si–O–Si [43]. The pronounced 2963 cm^{-1} band corresponds to stretching vibrations of the CH_3 group, while 1258 and 785 cm^{-1} bands are attributed to bending and stretching vibrations in Si–O–Si bridges. The 1258 and 1076 cm^{-1} bands are characteristic of silicon coatings of larger thickness [13]. Bands 1007 and 785 cm^{-1} as a result of the etching change their band intensity ratio from 1:1 for the non-etched material (S_Ref) to a value close to 1:2 for the etched surface (S_HF). As a result of the etching, 1258 cm^{-1} band also gains in intensity, and the area around 1220 cm^{-1} decreased in intensity, suggesting that the HF etching caused a loss in amorphous SiO_2, and made siloxane structures visible [44].

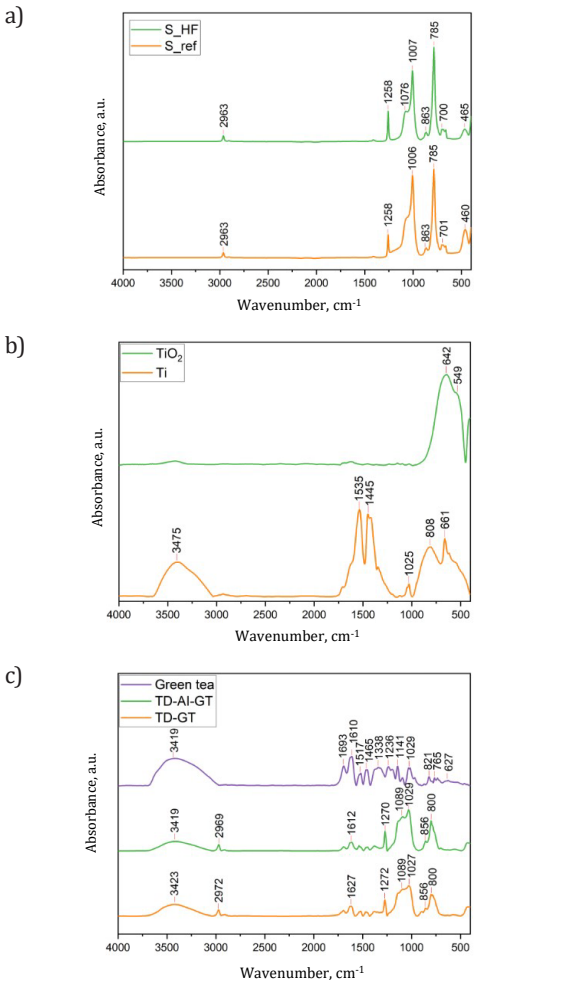

Fig. 3. FT-IR spectra of the selected materials: a) comparison of silicon before and after the HF etching; b) comparison of a pure titanium oxide with a Ti-containing gel layer; c) comparison of the pure green tea extract with layers enriched with the extract

In the case of the Ti-base sol derived layer (Fig. 3b), characteristic bands for water adsorbed on the oxide surface, i.e.: 3475 cm^{-1} band are visible. Also clearly visible are bands characteristic of Ti–CH_3 and TiO_2 bonds superimposed with bands characteristic for siloxane bonds. In the case of Ti, there is also a discernible band, 1535 cm^{-1}, which could be attributed to the N-O stretches. The 1445 cm^{-1} band could be an O–H bend, with a corresponding shoulder at 950–910 cm^{-1}. Figure 3c shows that the introduction of polyphenol-green tea extract into the sol environment leads to changes in the spectrum of the underlying sol named TD and TD-Al, in which characteristic responding bands appear: large number of phenolic groups: –OH aromatic ring, which can be observed on the spectra. These include the 3400–3200 cm^{-1} bands corresponding to symmetric and asymmetric stretches of OH hydroxyl groups, while a number of bands in the 1620–1580 cm^{-1} range confirm the presence of C–H and C=C– groups, which are elements of aromatic rings. An elevated region of 1200 cm^{-1} testifies to the presence of C–O group stretching, also present in phenolic groups [45, 46].

The microbiological activity tests against *Escherichia coli* and *Staphylococcus aureus* were carried out as a proof of concept (Fig. 4e, f) – the negative sample has no observable inhibition zone, and the positive sample, as expected, has the best inhibition zone observed equal 36 ±0,7 mm for *Staphylococcus aureus* and 21 ±0.9 mm for *Escherichia coli*. Both S_Ref and S_HF samples has no observable inhibition zone. This proves the lack of antibacterial properties of unmodified silicone and the proper cleaning of the S_HF sample during its etching process. The disk diffusion test showed that only sample no. 1, 6 and 8 (Fig. 4a–d) i.e. derived from sols containing: zinc, titanium and green tea-modified siloxane, exhibited bactericidal properties. The largest zone of bacterial growth inhibition was seen in the case of zinc-containing samples (Fig. 4a, sample no. 1) and was as large as 26 mm in diameter for *Staphylococcus aureus* bacteria (Tab. 2). In contrast, the worst bactericidal effect was shown by the titanium-containing sample (Fig. 4c, sample no. 6), which for the *Enterococcus faecalis* did not produce a zone of growth inhibition.

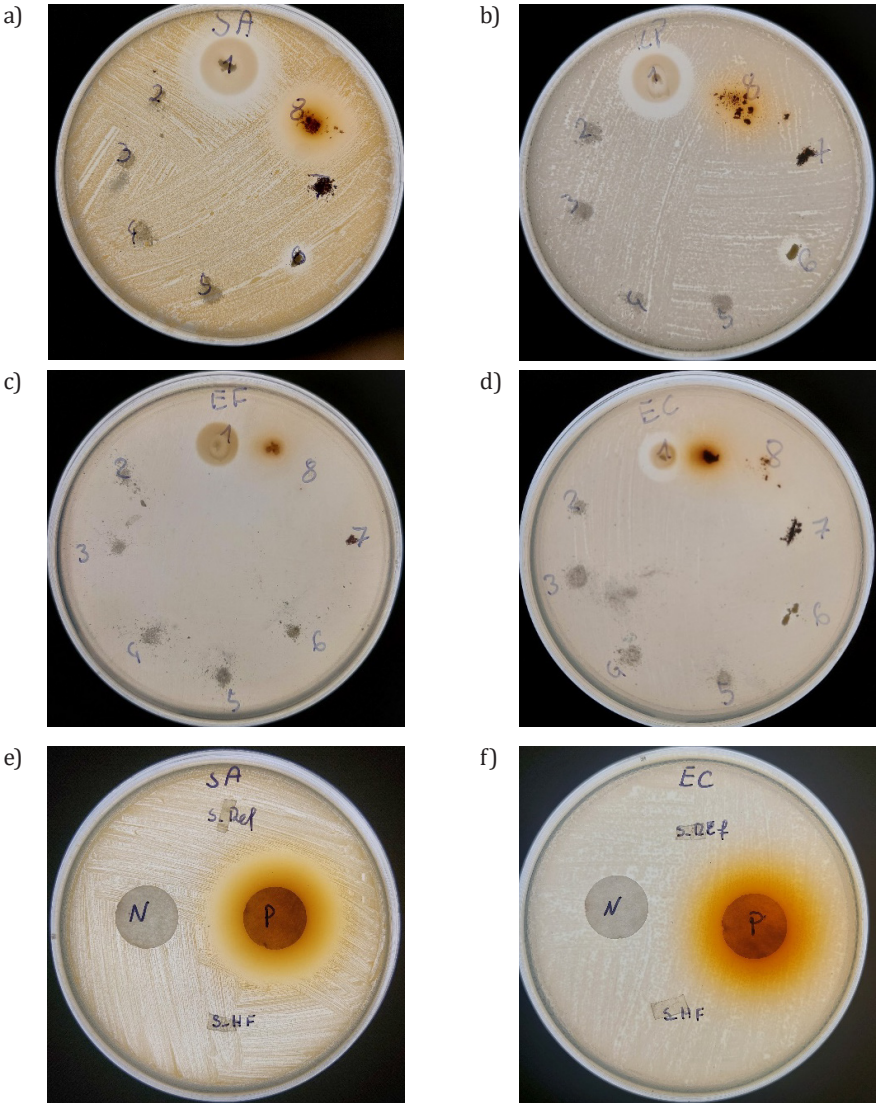

Fig. 4. Results of the disk diffusion method: a) *Staphylococcus aureus* (SA, Gram-positive bacteria); b) the *Enterococcus faecalis* (EF, gram-positive bacteria); c) *Klebsiella pneumoniae* (KP, Gram-negative bacteria); d) *Escherichia coli* (EC, Gram-negative bacteria); e) control positive, negative and results of the disk diffusion method for S_Ref and S_HF for Gram-positive bacteria; f) control positive, negative and results of the disk diffusion method for S_Ref and S_HF for Gram-negative bacteria

Table 2
A diameter of the inhibition zone for Zn, Ti and TD-GT materials, depending on the strains of bacteria

Strains of bacteria	Diameter [mm]		
	TD-Zn	TD-Ti	TD-GT
Klebsiella pneumoniae	19 ±0.95	7 ±0.35	9 ±0.45
Escherichia coli	12 ±0.6	3 ±0.15	5 ±0.25
Staphylococcus aureus	26 ±1.3	7 ±0.35	20 ±0.9
Enterococcus faecalis	10 ±0.51	–	4 ±0.2

The series of Zn-containing samples (Fig. 4a–d, sample no. 1) showed the greatest bactericidal effect, followed by the series of TD-GT (Fig. 4a–d, sample no. 8) and Ti (Fig. 4a–d, sample no. 6) samples, particularly against *Klebsiella pneumoniae* and *Escherichia coli* i.e. Gram-negative bacteria. For most of the samples tested, no zone of bacterial inhibition was observed, which may have been partly caused by the form of the samples – a powder form, which has a smaller surface area in contact with the substrate. In the case of EF bacteria, the zone of inhibition is seen only on the TD-GT sample (Fig. 4c, sample no. 7).

The fluorescence measurement of the supernatant from the tested samples allowed us to conclude that there was a reduction in the percentage of bacteria in all types of the modified samples. In the case of the samples with green tea, the amount of *Escherichia coli* bacteria was about 80% smaller than in the case of the untreated silicone (S_Ref). This demonstrates the excellent antibacterial abilities of the green tea additive. Satisfactory results were also obtained for the Ti sol, achieving a reduction in bacteria amount by about 40%, as can be seen in Figure 5.

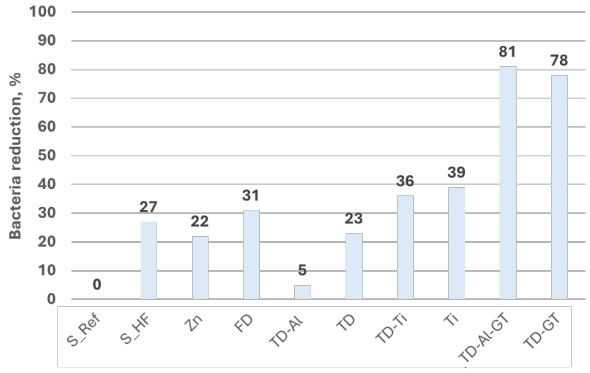

Fig. 5. Fig. 5. Reduction of *Escherichia coli* by the material determined by the fluorescence method

To summarize the microbiological studies, good antibacterial properties were achieved using sols containing additives of titanium, green tea, and zinc. All these compounds have literature confirmation of their antibacterial properties [25, 47–51] Using them in the process of making sol-gel coatings would allow for a low-cost and highly effective method of providing antibacterial properties to polymeric materials. However, it would be necessary to obtain a durable and homogeneous coating, which is a common problem in coating of polymer surfaces. It is very difficult to control the thickness of the coating and thus its durability may be limited [12]. In the case of the coatings investigated in this paper, it would be necessary to conduct a thorough study of the durability and thickness of the coatings and develop a highly reproductible method of their production.

It is also worth noting that etching the silicone surface with HF alone promoted the reduction of bacteria in the supernatant from the incubated samples. Cases of positive effects stemming from the etching of the surfaces of materials on their biological response are known in the literature. HF acid etching of titanium surfaces promotes early osteointegration, while in the case of silicon (silicon nitride) surfaces it not only increases osteoblast proliferation, but also reduces bacterial adhesion [38]. The presented studies also testify to the positive effect of this type of treatment on the bacterial response of silicone materials.

5. SUMMARY AND CONCLUSIONS

The study showed that etching the silicone changed its microstructure and increased the hydrophobicity of the material. This means that etching the silicone material using the proposed method can be a good first step, facilitating subsequent modifications and thus contributing to finding the ideal method for modifying this material. Unfortunately, the obtained coatings were not continuous and did not significantly affect the microstructure and physicochemical properties of the etched silicone surface. It would therefore be necessary to conduct a series of tests aimed at improving the application method – e.g. using the spin coating method or subjecting the sols to an ageing process. It also seems necessary to conduct additional measurements of the durability and thickness of the obtained coatings. In addition, the drying process of the obtained layers can also be improved.

As for the antibacterial properties of the obtained materials, among all the materials tested, Zn, TD-Ti, Ti, TD-GT and TD-Al-GT showed noticeable antibacterial properties. Particularly high potential was demonstrated by the addition of green tea extract, which provided a significant degree of bacterial reduction compared to the unmodified material. The tested sol-gel derived layers show antibacterial properties due to their composition rather than the effect on surface properties. It is therefore necessary to determine the MIC (*Minimum Inhibitory Concentration*) for green tea, zinc, and titanium and to estimate the amount of bactericidal substances included in the surface layer. It is also necessary to determine the content of green tea, zinc and titanium – the content of the compound in the produced coatings should be compared with the MIC, and

a method should be developed that allows for full control over the amount of the compound contained in the produced coatings. The discrepancies in the results of bacterial tests included in the article may have their basis in different amounts of biocidal additives depending on the tested fragment of the material surface

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