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Surface treatment of artificial implants with hybrid nanolayers: results of antibacterial tests, leachates and scanning electron microscope analysis

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Purpose: The aim of this study was to evaluate the antibacterial efficacy of surface-treated hernia implants modified by a hybrid nanolayer with incorporated Ag, Cu, and Zn cations using the sol-gel method.
Methods: The materials (polypropylene, polyester, and polyvinylidene difluoride) were activated by vacuum plasma treatment or UV C radiation, then modified and tested for bacterial strains of *Escherichia coli* (gram-negative) and *Staphylococcus aureus* (gram-positive). The AATCC 100 (2019) method for quantitative and the ISO 20645 agar plate propagation method for qualitative evaluation of microbiological efficacy were used. The gradual release of incorporated ions was monitored over time in simulated body fluids (blood plasma, peritoneal fluid) and physiological saline using an inductively coupled plasma mass spectrometer. The thickness and the homogeneity of the layers were measured for individual random samples with scanning electron microscope analysis (SEMA) and evaluated with an elemental analysis.
Results: Qualitative and quantitative microbiological tests clearly show the great suitability of vacuum plasma and UV C with sol AD30 (dilution 1:1) surface treatment of the implants. The absolute concentration of Ag, Cu, and Zn cations in leachates was very low. SEMA showed a high degree of homogeneity of the layer and only very rare nanocracks by all tested materials appear after mechanical stress.
Conclusion: This study confirms that surface treatment of meshes using the sol-gel method significantly increases the antibacterial properties. The nanolayers are sufficiently mechanically resistant and stable and pose no threat to health.
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Key Words: Antibacterial agent, Scanning electron microscopy, Sol gel, Surface, Surgical mesh

INTRODUCTION

In the surgical treatment of hernias, surgical mesh known as implants, are increasingly used for the augmentation, reconstruction, and replacement of soft (connective) tissues. Apropos materials, there is a relatively diverse group of implants [1]. Significantly better results are achieved by nonabsorbable mesh, in terms of a permanent solution where the chemical basis is one of the most commonly used synthetic polymers (polypropylene [PP], polyester [PE], polyvinylidene difluoride [PVDF]) [2].

However, a general problem with these implants is their relatively high susceptibility to contamination and following bacterial growth leading to infection of the prosthesis with

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a specific course and consequences [3,4]. The incidence of infections related to mesh implantation after hernia surgery is reported in various studies to be up to 8% [5]. Since these complications incur health risks, increased financial costs, and often complete failure of the implant, there are long-term efforts to develop such materials or modify existing ones in a way that retains their properties to support tissues and to be as resistant as possible to infection (including also multidrug-resistant strains).

In addition, research into preventive postoperative measures is ongoing. The antiseptics that most often impregnate suture material are triclosan (Ethicon) or chlorhexidine (B. Braun). One line of research and practice is the use of synthetic or biologically absorbable implants (the use of extracellular matrix), whose higher resistance to infection is undermined by a significantly greater hernia recurrence [6].

Another direction of research is the mesh that is impregnated with and releases antibiotics. However, concerns remain about the emergence of bacterial resistance with the topical use of antibiotics, their selectivity, and only temporary efficacy [7,8].

The most meaningful direction of research currently appears to be antiseptic surface treatment of commonly used nonabsorbable materials but the vast majority of partial successes of such modifications have so far ended in the research phase and have not been transferred into clinical practice [9].

A number of studies have been done on polycaprolactone, a synthetic hydrophobic polyester with a semi-crystalline structure, slow degradation rate, and good resistance to infections [10]. Its compounds and derivatives (e.g., polycaprolactone methacrylate fibers with gelatin [denatured collagen] metacryloyl) are also the subject of investigation. The problematic use of titanium dioxide nanoparticles is a separate chapter [11]. Chlorhexidine, cyanoacrylate tissue adhesives, and anti-staphylococcal endopeptidase lysostaphin are also in the crosshairs [12,13]. A very promising direction is the implementation of the known antimicrobial effect of metal ions Ag, Au, Cu, and Zn [14,15].

Metals interfere with microbial respiratory cytochromes, electron transport, and DNA replication. Their mechanism of action is effective not only against gram-positive and gram-negative, aerobic and anaerobic bacteria but also against yeasts, fungi, and viruses.

Antimicrobials (inorganic metal ions or organic molecules) can be incorporated into the surface using advanced deposition techniques such as steam deposition, implantation, sputtering, and electrochemical deposition from solution. However, these technologies can be costly and not easily applied to large or complex subjects or textured surfaces. A very promising alternative for coating surfaces is the sol-gel method which produces a coating of high purity and homogeneity and about which we have already published [11,16,17].

The sol-gel forms a glassy surface and offers the possibility of incorporating metal ions into the matrix in various ways such as simple capture, electrostatic forces, adsorption, and especially ionic bonding.

The resulting nanolayer is also resistant to chemical substances such as toluene, acetone, isopropyl alcohol, concentrated sulfuric acid, nitric acid, hydrochloric acid, and others. The functional antibacterial nanolayer has a thickness of 80–300 nm with a diameter of 150 nm. The basic organic-inorganic hybrid network of the nanolayer does not contain any substances that could be potentially hazardous to the human body from a biochemical viewpoint.

For analytical tests, the purpose of which is to monitor the release of ions from the implant, simulated body fluids (blood plasma, peritoneal fluid) were used, which were prepared according to cited publications [18-20]. Distilled water and physiological saline were used as standard fluids. The selected simulated body fluids are those with which the mesh comes into direct contact most often during the implantation process. For example, in the case of pleural fluid, the literature reports a pH of around 7.6, while for peritoneal fluid, the values are more likely to vary between pH 7.5 and pH 8.0 [20]. In pathology (most often e.g. inflammation), the pH shifts towards acidic through to pH 7.3 in both cases. In practice, for several reasons, these fluids are considered identical to their composition in the physiological state.

The aim of this step was to obtain comprehensive information about the duration of the effect on potential pathogens that can colonize the wound during surgery. For surface treatment of biomedical devices, a high initial release rate of antibacterial agents is desirable to prevent biofilm formation. Studies have shown that when silver ions are incorporated into the sol-gel layer, their high initial release is followed by a more gradual and prolonged release profile. The release of ions from such biomaterials is governed by the amount of available reacting metal, the area exposed by the measured area, the degree of hydrophobicity of the interface of the coated layer, and the volume of contact fluid. There are silver nitrate ions in the layer, and their release is faster than the release of copper and zinc in their more stable oxide forms.

The initial intensive release of a broad-spectrum antibacterial agent is particularly desirable for the first 6 hours after implant insertion, and critical for bacterial colonization. The examined surface treatment with a hybrid nanolayer is assumed to have sufficient resistance to mechanical damage as the implant is manipulated to a greater or lesser extent during the operation in terms of bending and/or stretching [21,22]. This resistance has already been proven in the treatment of solid surfaces and textiles (a few dozen washes will not significantly reduce the efficacy). The antibacterial layer was also previously repeatedly

tested for cytotoxicity on Vero cells and HeLa cells [17]. Results showed no cytotoxicity of the investigated surface to any of the tested cells.

The aim of this part of the study was to evaluate the antibacterial effects of surface modification of different artificial implants with the sol-gel method, as well as to prove good mechanical resistance and chemical stability of such nanolayers, suggesting their safety for organisms.

METHODS

Ethics statement

This study was deemed unnecessary for ethics approval according to local and national guidelines, as no human or animal subjects took part in the study (Ethics Committee opinion reference No. EK/1/2024).

Mesh materials

Commonly used implants were used for testing as follows:

1) Bard Mesh (C.R. Bard Davol Inc.)
2) Bard Soft Mesh (C.R. Bard Davol Inc.): polypropylene knitted monofilament mesh is a representative of the most widespread type of meshes in the materially lightweight-soft-version (lightweight mesh) and in the classic version (heavyweight mesh). Dimensions 15 × 10 cm (light; 44 g/m^2 with macropores of 2.5 mm) and 15 × 15 cm (heavy; 90 g/m^2 with micropores of 0.46 mm).
3) Premilene Mesh (B. Braun): polypropylene microporous (pore size, 0.8 mm) heavy nonabsorbable mesh (82 g/m^2); dimensions 15 × 15 cm.
4) Parietex Hydrophilic 3-Dimensional Mesh (Medtronic): polyester very elastic hydrophilic, multifilamentous, large-porous (1.0–1.6 mm) medium-weight (78 g/m^2) mesh for open surgery as a 3-dimensional hexagonal knitwear; dimensions 15 × 10 cm.
5) DynaMesh Endolap (FEG Textiltechnik): PVDF monofilament mesh with good shape memory, causing a lower creation of granuloma tissue and thus allowing the use of knitted fabric with lower porosity (with very good biological stability). For handling and positional recognition, some fibers are colored green or black; dimensions 15 × 10 cm.

Mesh pretreatment/activation of the surface by physical plasma treatment and UV C radiation

Due to the activation of functional/bonding groups and thus good adhesion of the sol to the material of the mesh, the surfaces were activated before the application of the antibacterial sol. Three methods of activation were chosen for comparison: vacuum plasma, atmospheric plasma, and irradiation using UV C radiation. Initial tests proved the unsuitability of atmospheric plasma (too drastic as it "burns" and tears the mesh) and, hence, samples modified only with vacuum plasma were subjected to further testing [11].

Vacuum plasma activation

Thus, only vacuum plasma was used for physical plasma activation. The treatment was carried out in the pilot laboratory of SurfaceTreat Turnov, Czech Republic, on an LA_400 apparatus developed by them. Oxygen was added under low pressure for increased efficacy. The flow rate of the oxygen was 2.5 × 10^{-8} kg/sec^{-1} (200 standard cubic centimeters per minute) under a pressure of 100 Pa. The height of the table was 240 mm, rotation of 7 revolutions/min was left standard, and the duration of plasma treatment was set to 5, 10, 30, or 60 seconds. Consequently, sol AD30 in 2 dilutions of 1:1 and 1:3 was applied to the prepared samples. The application of the sol must be carried out immediately after the activation of the surface, as the activation wears off within minutes. Thermal polymerization of the hybrid nanolayer was finished in a dryer at 85 °C for 5 hours after 30 minutes of evaporating of the solvent and final hydrolysis in a laboratory environment.

UV C activation

Before activating the surface using UV C radiation, the samples were pretreated and cleaned. They were immersed in pure isopropyl alcohol for 10 minutes, and then left aside to evaporate all the solvent at room temperature. After drying, the sample was irradiated with UV C radiation (UV-C Hg lamps TUV 15W G15T8 UV-C long-life; Philips) on each side for 1 minute.

Applied sol: dilution, polymerization

Immediately after surface activation (plasma, UV C radiation), an antibacterial sol in 2 dilutions was applied to the samples. Dilutions of 1:1 and 1:3 were chosen (the sol was diluted with isopropyl alcohol p.a.).

The samples were immersed in the sol of the appropriate dilution for 30 seconds and then withdrawn. After evaporation of the solvent residues from the applied layer at room temperature (for about 30–60 minutes), the nanolayer was polymerized in a preheated oven at 85 °C for 3 hours. Higher temperatures could not be applied due to the temperature resistance of the materials used. For this reason, the polymerization time was extended.

Microbiological tests

The microbiological efficacy was tested using qualitative and quantitative methods. All samples were tested in a triplet 3 times (3 × 3).

Bacterial strains

Pathogenic bacterial strains from the Czech Collection of Microorganisms of Masaryk University in Brno were used for the tests. *Escherichia coli* (Czech Collection of Microorganisms [CCM] 2024, American Association of Textile Chemists and Colorists [AATCC] 9637) was used as a representative of the gram-negative rod-shaped bacterium, and *Staphylococcus aureus* (CCM 2260, AATCC 1260) as a typical gram-positive coccal bacterium. Nutrient agar and blood agar (both from BioRad s.r.o.) were used for cultivation.

Qualitative evaluation of efficacy

For qualitative evaluation, the International Organization for Standardization (ISO) 20645 agar plate propagation method was used, where the zone of inhibition and bacterial growth under the sample were evaluated [23]. First, a bacterial suspension was prepared (*E. coli*, 5.1×10^8 CFU/mL; *S. aureus*, 5.7×10^8 CFU/mL; measured on the McFarland scale). Inoculum in 1 mL was transferred to 150 mL of agar medium cooled to 45 °C. The inoculated medium was thoroughly mixed (the concentration of bacteria in the medium in *E. coli* was 5.1×10^6 CFU/mL, for *S. aureus* 5.7×10^6 CFU/mL). Inoculated bacterial agar medium in 5 mL was transferred to a sterile agar plate on a Petri dish so that it was spilled over the entire surface of the plate. After solidification, a tested sample of 2.5 × 2.5 cm was placed on the plate. Incubation took place in a thermostat cabinet for 24 hours at 37 °C. The results were evaluated according to Table 1.

Quantitative evaluation of efficacy

AATCC 100 (2019) [24] was used to evaluate quantitative microbiological efficacy. First, a bacterial suspension was prepared (*E. coli*, 2.1×10^5 CFU/mL; *S. aureus*, 2.0×10^5 CFU/mL; measured on the McFarland scale). The test sample weighing 1 g was placed in a sterile container. Bacterial inoculum of 100 μL was carried onto the tested samples. The whole sample had to be moistened before that. The closed container was placed in a thermostat cabinet where it was incubated for 24 hours at 37 °C. Shaker medium (saline) of 10 mL was then pipetted into the sample and vortexed for 1 minute. After that, 1 mL of the suspension was inoculated on an agar plate (blood agar). The plates were incubated for 24 hours at 37 °C. The tests were always repeated and in triplicate. It is a quantitative method in which the reduction factor (R) is evaluated that indicates the percentage by which the concentration of inoculated bacteria has been reduced.

Statistical evaluation

Statistical processing of the results in the number of colonies obtained from repeated determinations (n = 3) was carried out in a standard manner, assuming a normal distribution of deviation of repeated determinations. From the values of the number of colonies, the arithmetic mean, the standard error, and the standard error of the mean (SEM) were calculated.

For the confidence interval of the probable value of the mean determination, a probability of 99% (2.58 SEM) was chosen. Due to the specificity of the processed values (colony numbers), the resulting statistical results were rounded to integers.

Testing the release of metal cations from modified meshes in simulated body fluids

These tests are intended to provide information on how quickly the used cations of the metals Ag, Cu, and Zn, which are part of the antibacterial layer, are released into body fluids and, in this way, obtain comprehensive information about the duration of effect on potential pathogens that may get into the wound during surgery. They should also determine the maximum concentrations of metal cations in close proximity to these materials after implantation into the human body.

Simulated blood plasma (fluid A), simulated peritoneal fluid (fluid B), and physiological saline (8.5-g NaCl/1,000 mL, fluid C) were used as model fluids. Simulated liquids for extracts were prepared according to standards [18].

For the purpose of these tests, 2 types of modified mesh were selected: polypropylene mesh of Bard Mesh and polyester mesh of Parietex Hydrophilic 3-Dimensional Mesh. Since leaching should not depend on the substrate (mesh), but only on the specific surface of the mesh, the results should be representative.

Samples measuring 3 × 3 cm were prepared from the modified implants. These were first rinsed thoroughly in warm distilled water and then put into a 250-mL bottle containing 100 mL of pre-tempered (37 °C) simulated fluid A, B, or C. The sample vials were then placed in a tempered water bath (37 °C). At predefined intervals (0, 1, 2, 4, 6, 8, 24, 26, 28, 30, 32, 48,

Table 1. Evaluation of results according to ISO 20645

Five degrees of inhibition	Evaluation
Inhibitory zone present, nothing grows under the sample	Good effectiveness
Without an inhibitory zone, nothing grows under the sample	Good effectiveness
Without an inhibitory zone, growth under the sample is rare	Borderline effectiveness
Without an inhibitory zone, growth under the sample is about half	Insufficient effectiveness
Without an inhibitory zone, growth under the sample is strong	Insufficient effectiveness

and 72 hours), 500 μL of leachates were then collected from the vials into plastic tubes for analytical tests using an automatic pipette. The contents of metals (in the form of cations) in leachates were determined by inductively coupled plasma mass spectrometer in the Laboratory of Chemical Remediation Processes at the Institute for Nanomaterials, Advanced Technologies and Innovations, in the Technical University of Liberec. The uncertainty of the determined concentration value of metal cations Ag, Cu, and Zn can be estimated at 30 parts per billion (ppb) based on experience with routine analysis of similar materials with low and very low concentrations of these metals.

Scanning electron microscope analysis

To check the quality of nanolayers on the mesh surface and to confirm their chemical composition, a Carl Zeiss ULTRA Plus scanning electron microscope from the Technical University of Liberec was used.

The research is thus supplemented with information from the scanning electron microscope analysis (SEMA), where the thickness of the surface treatment was measured for individual samples, the homogeneity of the applied layer was evaluated, and an element analysis was carried out.

An ultra-high resolution field emission scanning electron microscope (Carl Zeiss ULTRA Plus) equipped with an energy dispersive spectroscopy detector (Oxford X-Max20, Oxford Instruments), was used to check the quality of the nanolayers on the surface of the meshes and to confirm their chemical composition. Before evaluation of the quality of layers, the samples' surface was conducted by deposition of 1 nm of platinum using sputter coater Quorum Q150R ES (Quorum Technologies). The samples were observed at an accelerating voltage of 2 kV, an aperture of 10 μm, an extractor voltage of 3 kV (probe current of approximately 1–2 pA), and a working distance of approximately 2.5–3.0 mm; the topographic signal was collected using highly sensitive integrated in-lens secondary electron detector. For the local chemical analysis, the following parameters were set: accelerating voltage of 10 kV, aperture of 60 μm, extractor voltage of 4.26 kV (probe current of approximately 1 nA), working distance of 8.5 mm, and activated platinum coating correction. The quality of the prepared nanolayers was monitored for all prepared samples (5 mesh materials, various surface modifications) in comparison with the original samples (without the application of nanolayers).

At the end of the experiment, after imitation/reconstruction of perioperative extensive treatment of the implant (repeated

Table 2. The results of qualitative antibacterial tests (ISO20645) for *Escherichia coli* and *Staphylococcus aureus*

Sample	Treatment time (sec)	Sol dilution ratio	
		1:1	1:3
Bard Mesh	5	Insufficient effect	Insufficient effect
	10	Good effect	Insufficient effect
	30	Good effect	Insufficient effect
	60	Good effect	Insufficient effect
Bard Soft Mesh	5	Insufficient effect	Insufficient effect
	10	Insufficient effect	Insufficient effect
	30	Insufficient effect	Insufficient effect
	60	Good effect	Insufficient effect
Premilene Mesh	5	Borderline effect	Insufficient effect
	10	Good effect	Insufficient effect
	30	Good effect	Insufficient effect
	60	Good effect	Insufficient effect
Parietex Mesh	5	Good effect	Insufficient effect
	10	Good effect	Insufficient effect
	30	Good effect	Insufficient effect
	60	Good effect	Insufficient effect
DynaMesh Endolap	5	Borderline effect	Insufficient effect
	10	Good effect	Insufficient effect
	30	Good effect	Insufficient effect
	60	Good effect	Insufficient effect

The samples after treatment with vacuum plasma applied for 0, 5, 10, 30, and 60 seconds and with 1:1 and 1:3 sol dilution with results identical for *E. coli* and *S. aureus*. The comparative standards without treatment were in all products with insufficient effect and are not mentioned in the table.
Bard Mesh, C.R. Bard Davol Inc.; Bard Soft Mesh, C.R. Bard Davol Inc.; Premilene Mesh, B. Braun; Parietex Hydrophilic 3-Dimensional Mesh, Medtronic; DynaMesh Endolap, FEG Textiltechnik.

Table 3. The results of the AATCC 100 quantitative tests

Sample	Treatment time (sec)	Factor R (%)			
		Sol dilution ratio 1:1		Sol dilution ratio 1:3	
		E. coli	*S. aureus*	*E. coli*	*S. aureus*
Bard Mesh	5	99.9	79.0	66.0	15.0
	10	99.2	99.1	81.0	50.0
	30	99.5	99.9	74.0	0
	60	99.5	99.9	69.0	9.0
Bard Soft Mesh	5	99.5	91.0	95.6	20.0
	10	99.9	99.5	97.4	45.0
	30	99.9	99.5	96.6	25.0
	60	99.9	99.5	96.9	42.0
Premilene Mesh	5	99.7	92.5	92.9	28.0
	10	99.9	99.3	95.5	10.0
	30	99.9	97.5	94.4	18.0
	60	99.9	99.3	93.1	33.0
Parietex Mesh	5	99.9	93.5	98.6	68.0
	10	100	100	98.3	74.0
	30	100	100	98.4	39.0
	60	100	100	98.5	29.0
DynaMesh Endolap	5	99.8	93.0	96.9	0
	10	99.9	98.8	98.6	25.0
	30	99.9	97.1	97.3	0
	60	99.9	98.8	98.6	21.0

Escherichia coli and *Staphylococcus aureus* strains with mesh samples after treatment by vacuum plasma applied for 5/10/30 and 60 seconds and sol dilution 1:1 and 1:3. The comparative standards without treatment were in all products with insufficient effect and are not mentioned in the table.
AATCC, American Association of Textile Chemists and Colorists.
Bard Mesh, C.R. Bard Davol Inc.; Bard Soft Mesh, C.R. Bard Davol Inc.; Premilene Mesh, B. Braun; Parietex Hydrophilic 3-Dimensional Mesh, Medtronic; DynaMesh Endolap, FEG Textiltechnik.

Table 4. Results of qualitative antibacterial tests (ISO 20645) after UV C sample treatment

Sample	Sol dilution ratio	*Escherichia coli*	*Staphylococcus aureus*
Bard Mesh	1:1	Good effect	Good effect
Bard Soft Mesh		Good effect	Good effect
Premilene Mesh		Good effect	Good effect
Parietex Mesh		Good effect	Good effect
DynaMesh Endolap		Good effect	Good effect
Bard Mesh	1:3	Insufficient effect	Borderline effect
Bard Soft Mesh		Insufficient effect	Borderline effect
Premilene Mesh		Insufficient effect	Borderline effect
Parietex Mesh		Insufficient effect	Borderline effect
DynaMesh Endolap		Insufficient effect	Borderline effect

ISO, International Organization for Standardization.
Bard Mesh, C.R. Bard Davol Inc.; Bard Soft Mesh, C.R. Bard Davol Inc.; Premilene Mesh, B. Braun; Parietex Hydrophilic 3-Dimensional Mesh, Medtronic; DynaMesh Endolap, FEG Textiltechnik.

creasing and stretching), the modified samples were examined by an experienced surgeon with a scanning electron microscope to exclude any significant damage to these layers.

RESULTS

Antibacterial tests after physical plasma treatment

Pilot microbiological tests (qualitative and quantitative) clearly demonstrated the suitability of only vacuum plasma compared

Table 5. Results of AATCC 100 quantitative tests

Sample	Factor R (%) after UV C and 1:1 sol dilution modification	
	Escherichia coli	*Staphylococcus aureus*
Bard Mesh	96.0	100
Bard Soft Mesh	100	100
Premilene Mesh	99.9	97.5
Parietex Mesh	99.7	96.8
DynaMesh Endolap	100	95.8

AATCC, American Association of Textile Chemists and Colorists.
Bard Mesh, C.R. Bard Davol Inc.; Bard Soft Mesh, C.R. Bard Davol Inc.; Premilene Mesh, B. Braun; Parietex Hydrophilic 3-Dimensional Mesh, Medtronic; DynaMesh Endolap, FEG Textiltechnik.

to atmospheric plasma, where the surface was not treated in such a way that the applied sol could show an antibacterial effect.

After vacuum plasma of the surface of the tested samples and subsequent microbiological evaluation according to ISO 20645, the dilution of the sol in the ratio of 1:1 was clearly the most appropriate. Dilution of the sol in a ratio of 1:3 showed insufficient efficacy compared to both tested bacterial strains according to the above standard (Table 2). In the bacterial strain *S. aureus*, the efficacy in terms of R factor ranged from 79% to 100% in the tested samples. In the bacterial strain *E. coli*, the efficacy ranged from 99.5% to 100%. The AATCC 100 antibacterial efficacy rating also confirmed the appropriateness of a 1:1 dilution of the antibacterial sol. When diluted 1:3, the antibacterial effect is very reduced, especially against *S. aureus*. Very interesting results were achieved for Parietex Hydrophilic 3-Dimensional Mesh, where there was no difference between 10, 30, and 60 seconds of plasma treatment, but insufficient time was 5 seconds. It follows that a minimum of 10 seconds of physical plasma treatment is sufficient to achieve an antibacterial effect in general for all samples (Table 3).

Antibacterial tests after UV C radiation treatment

The samples were tested even after activating the surface of the samples before the application of the antibacterial sol by UV C radiation (as an alternative to physical plasma). The results according to ISO 20645 showed good efficacy on both tested bacterial strains in samples labeled (Table 4). The AATCC 100 antibacterial efficacy rating also confirmed the appropriateness of a 1:1 dilution of the antibacterial sol (Table 5). In contrast, the dilution of the sol in a ratio of 1:3 was absolutely insufficient. More detailed statistics of quantitative tests are in Supplementary Material 1.

Results of leachate materials in body fluids

The results for the determination of metal cations in leachates are clearly shown in Fig. 1. It should be noted that there is uncertainty in the determination (due to low to very low concentrations) for 30 ppb. According to the results of the analyses, within a few hours to tens of hours in the solution the equilibrium is stabilized and further transition of cations into the solution is minimal. It may also be related to the possibility of local formation of poorly soluble compounds between metal cations and anions in leaching solutions (precipitation of poorly soluble zinc and copper phosphates or silver chloride on the surface of the antibacterial layer), which limit the concentration of these cations in the leachates. However, the absolute concentration of all 3 cations in leachates is very low (10–350 ppb, only in model solution with physiological saline up to 500 ppb) and should not negatively affect living organisms.

Scanning electron microscope analysis

A method that tracked samples before and after application and subsequently element analysis was SEMA (Fig. 2). Antibacterial layers were present and homogeneous on all samples tested.

Elemental sample analysis

Elemental analysis of all standard samples confirmed that the materials do not contain contamination by any element other than expected for a chemically pure material as stated by the manufacturers. The elemental analysis also confirmed that all modified samples of the hybrid nanolayer contain all the expected elements of the AD30 sol type used (Fig. 3, Supplementary Material 2).

The thickness of the antimicrobial layer (SEMA) and visual evaluation of the degree of disruption after mechanical handling

The measurements are as follows: Bard Mesh, 192.4 nm; Bard Soft Mesh, 92.58 nm; Premilene Mesh, 84.49 nm; Parietex Hydrophilic 3-Dimensional Mesh, 98.42 nm; and DynaMesh Endolap, 99.10 nm. The average layer thickness of these measured random samples was therefore 113 nm. The median of the larger number of samples examined was just below 100 nm. Layer disruption was very rare after mechanical stress and

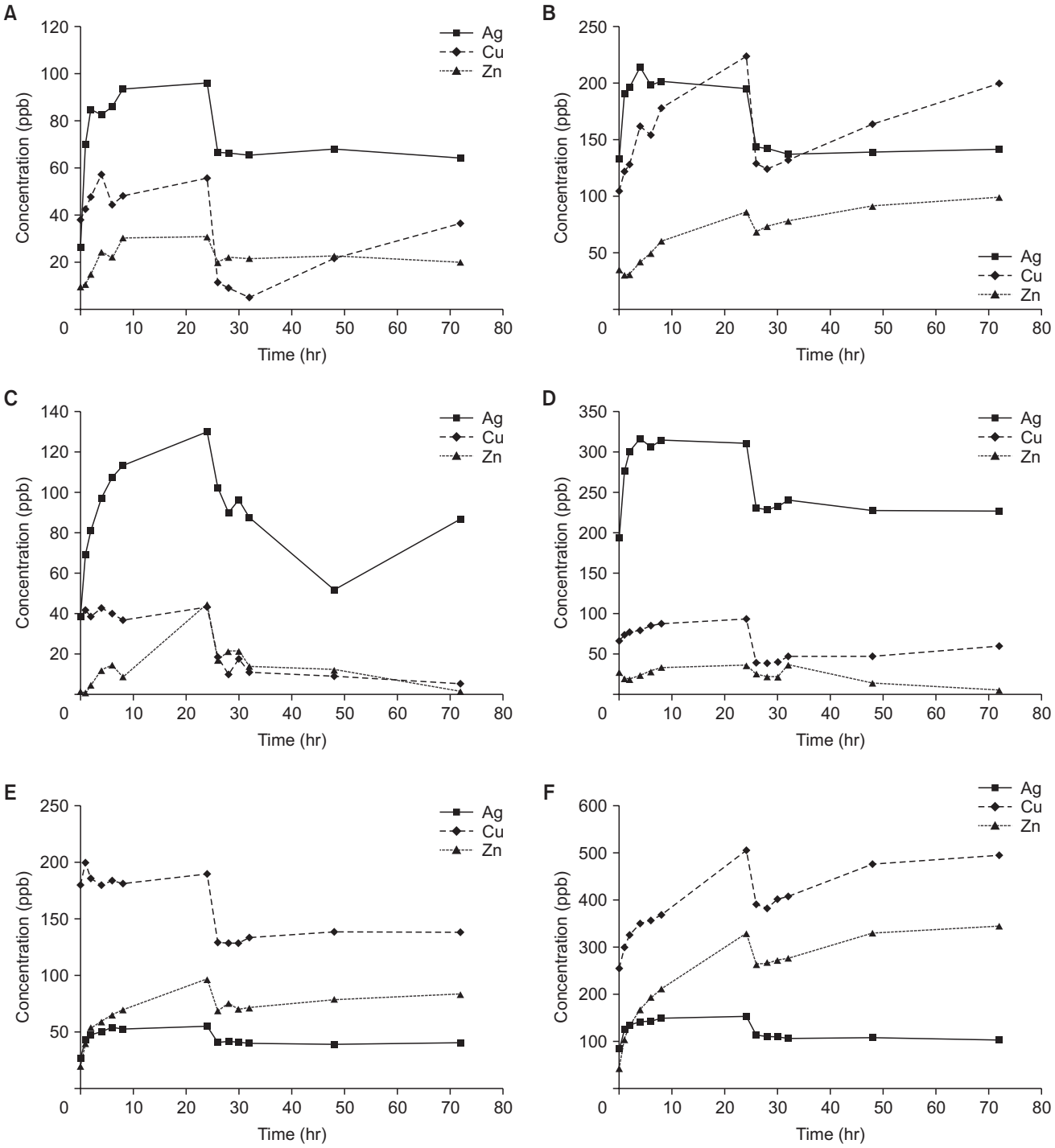

Fig. 1. Results of determination of Ag, Cu and Zn metals (as cations) in the leachate of the mesh modified by the antibacterial layer. (A) Polypropylene (PP) mesh (Bard Mesh, C.R. Bard Davol Inc.) in simulated blood plasma. (B) Polyester (PE) mesh (Parietex Hydrophilic 3-Dimensional Mesh, Medtronic) in simulated blood plasma. (C) PP mesh leachate in simulated peritoneal fluid. (D) PE mesh in simulated peritoneal fluid. (E) PP mesh in physiological saline. (F) PE mesh in physiological saline, as a function of sampling time (time dependence for all 3 metal cations, uncertainty of metal determination 30 parts per billion [ppb]).

almost difficult to find (Figs. 4, 5).

DISCUSSION

Qualitative and quantitative microbiological tests clearly proved the suitability of only vacuum plasma compared to

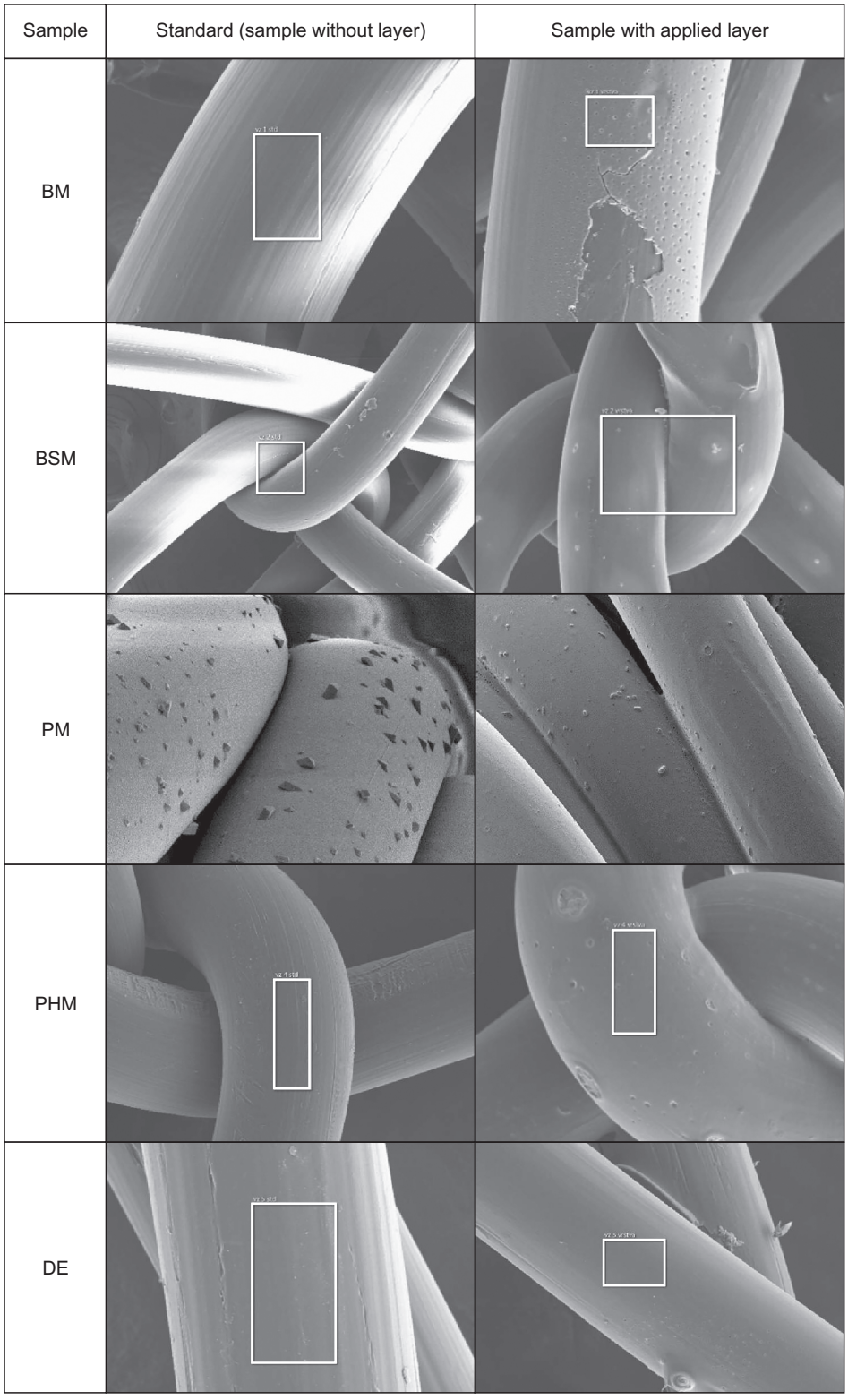

Fig. 2. Comparison of materials without/with antibacterial layer by electron microscope. Sample identification: BM, Bard Mesh (C.R. Bard Davol Inc.); BSM, Bard Soft Mesh (C.R. Bard Davol Inc.); PM, Premilene Mesh (B. Braun); PHM, Parietex Hydrophilic 3-Dimensional Mesh (Medtronic); DE, DynaMesh Endolap (FEG Textiltechnik).

atmospheric plasma, where, as expected, the surface was not treated so that the adhesion of the applied sol showed the desired antibacterial effect. Good efficacy at 1:1 dilution was demonstrated in all samples for both bacterial strains tested. In conclusion, the minimum sufficient plasma time was 10 seconds, and the most suitable dilution of the sol was a 1:1 ratio.

From the results of leachates, it can be stated that within

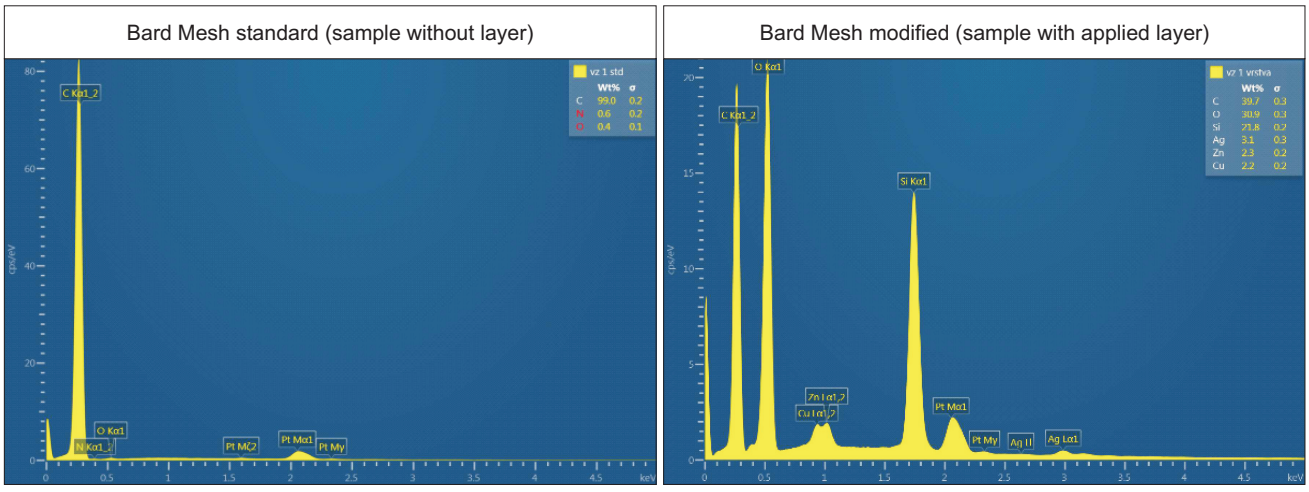

Fig. 3. Example of elemental analysis in graphs of Bard Mesh (C.R. Bard Davol Inc.) samples.

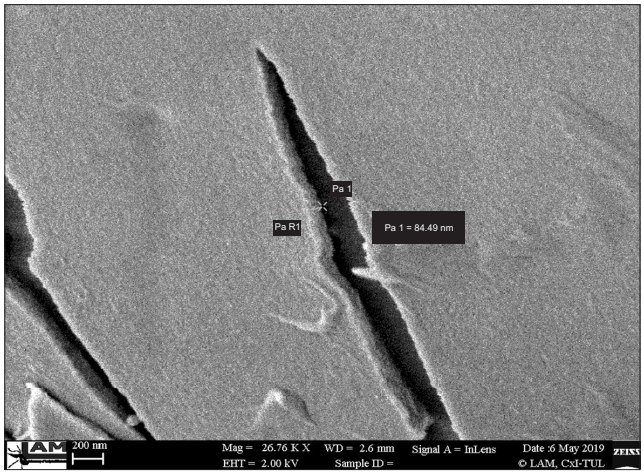

Fig. 4. Measurement of the thickness of the antimicrobial layer (scanning electron microscope) and visual evaluation of the degree of disruption after mechanical handling. Premilene Mesh (B. Braun): 84.49 nm.

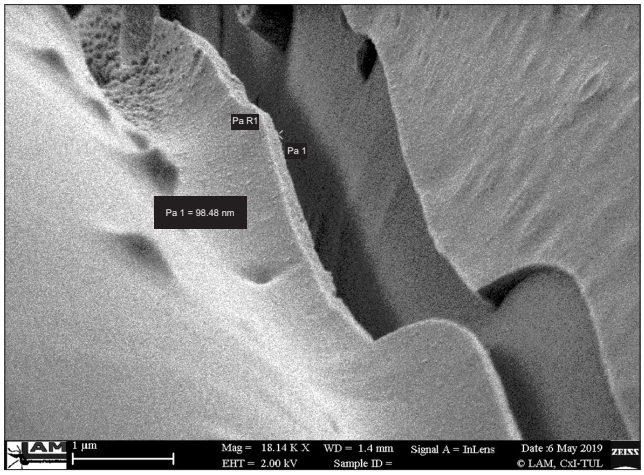

Fig. 5. Measurement of the thickness of the antimicrobial layer (scanning electron microscope) and visual evaluation of the degree of disruption after mechanical handling. Parietex Hydrophilic 3-Dimensional Mesh (Medtronic): 98.42 nm.

a few hours in solution (i.e., simulated body fluids) the equilibrium stabilized, and further transition of cations into solution was minimal. However, the absolute concentration of all 3 cations in the leachates was very low, which confirmed that the treatment cannot negatively affect the living organisms into which the mesh is implanted, but at the same time, sufficient antimicrobial activity is preserved. Modification of the mesh surface by a hybrid nanolayer with immobilized antiseptic substances (cations of Ag, Cu, and Zn metals) by the sol-gel method appears to be safe for confirmation of persistently low absolute concentrations in extracts of simulated body fluids. Its antibacterial effect is optimal for the rapid onset of ion concentration and tens of hours of persisting practically stationary sufficient concentration of these metal ions in the leachate.

To imitate/reconstruct perioperative extensive implant handling, repeated creasing, squeezing, and stretching by an experienced surgeon were performed. The modified samples were then examined with a scanning electron microscope to exclude major damage to these layers. Even after such manipulation, only isolated microscopic cracks in the nanolayer (again, clinically insignificant) occur in all materials without much difference. This indicates significant adhesion and mechanical resistance of these layers.

The thickness of the applied layer was measured on several randomly selected samples at different points of the mesh. The width of the layers always ranged from 80 to 200 nm, which demonstrates a high degree of homogeneity. Electron microscopy measurements on all samples showed the formation of a continuous layer on the original material, whose

thickness meets the criteria for nomenclature of the nanolayer.

Since the production of the initial sol and its application is not technologically, economically, or environmentally demanding and is a typical example of a technical solution with a high requirement for know-how, the result is a product with high added value.

Since the technology of hybrid nanolayers based on sol-gel has previously been successfully investigated, antibacterials on other types of surfaces and materials, as well as on multiresistant bacterial strains (e.g. methicillin-resistant *S. aureus*) and viruses (e.g. human immunodeficiency virus) [15,17]. The virucidal and fungicidal effect of silver ions have been known for a long time. Therefore, similarly promising results of such modified implants can be highly expected in tests for microorganisms other than those selected for our research.

Mesh infection is currently still a concern [25-27]. With this technology, virtually any commercially produced hernia implant could be modified relatively simply and cheaply, and gain new, invaluable properties that would lead to a significant reduction in infectious complications after surgery. Not only that, our research confirms that nanotechnology and antimicrobial coating are the key aspects of the surface modification of implants in the fight against infection [10,28-30].

For the clinical application of such a surface treatment, it would be necessary to study compatibility with human tissue and the long-term results. Testing in an animal model is therefore expected as the next step.

SUPPLEMENTARY MATERIALS

Supplementary Materials 1 and 2 can be found via https://doi.org/10.4174/astr.2024.107.2.108.

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Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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