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Antibacterial coatings on haemodialysis catheters by photochemical deposition of silver nanoparticles

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Abstract Antibacterial coatings on catheters for acute dialysis were obtained by an innovative and patented silver deposition technique based on the photo-reduction of the silver solution on the surface of catheter, with consequent formation of antibacterial silver nanoparticles. Aim of this work is the structural and morphological characterization of these medical devices in order to analyze the distribution and the size of clusters on the polymeric surface, and to verify the antibacterial capability of the devices treated by this technique against bacterial proliferation. The structure and morphology of the silver nanoparticles were investigated by using scanning and transmission electron microscopy. The antimicrobial capability of the catheters after silver deposition was confirmed by antibacterial tests with *Escherichia coli*. Both scanning electron microscopy analysis and antibacterial tests were performed also after washing catheters for 30 days in deionized water at 37°C, relating these data to thermogravimetric analysis and to energy dispersive spectroscopy, in order to check the resistance of coating and its antimicrobial capability after the maximum time of life of these devices.

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1 Introduction

The use of implantable medical devices represents a common practice in medicine but infections associated to vascular access are reported as the major causes of morbidity and mortality. Silver and its antimicrobial activity are well known since antiquity, and its use in modern medicine is growing thanks to its strong biocide activity against a broad spectrum of bacteria and its good degree of biocompatibility [1]. In haemodialysis patients, the risk of bacteremia associated to the use of vascular access is very high; many reports count the use of both tunnelled and not tunnelled catheters as one of the major cause of infections [2]. The reason for the high risk of infection lies in the formation and growing of a bacterial biofilm on the surface of biomaterial devices [3]. Bacterial biofilm formation occurs in sequential steps [3]. Initially, free floating (*planktonic*) bacteria come into contact with the surface of the indwelling foreign device and, after an initial attachment, they adhere irreversibly to the catheter, forming the microbial infective biofilm [4, 5]. The growth of a bacterial biofilm is influenced by different variables, among which the physical and chemical properties of the device surface [5]. In order to reduce the bacterial adhesion to catheter surface, many strategies have been recently developed and tested [6], including physical–chemical modifications of the surface to prevent the bacterial adhesion on the substrate or incorporation of antimicrobial agents into polymer bulk [7]. Catheters have been coated with antibiotics, biocides such as chlorohexidine, heparin [8] and also with silver-based coatings, because these elements are able to fight the attachment and growth of the biofilm [9, 10].

It's known that silver ions induce the inhibition of bacterial DNA replication and to the deactivation of metabolic enzyme [10, 11]. In particular, silver nanoparticles (NP) have demonstrated the stronger antibacterial activity. Despite the mechanism of the inhibition of bacterial function by silver NP is not totally clear and it's still a matter of discussion it is undoubted that their large surface area have the advantage over other silver systems [12, 13].

The use of silver in medicine is growing, thanks to its "oligodynamic activity" against a broad spectrum of bacteria and fungi [14]. Among metals, it shows a good degree of biocompatibility demonstrated through in vivo animal study [15] and low human toxicity [16]. Silver can be used to prevent wound colonizations and for wound dressing [17], in textile fabrics [18] to improve health quality and welfare, for bacterial filter in water treatment [19], and to prevent bacterial colonization on medical devices [20].

Plastic devices, such as central venous catheters, are frequently colonized by bacteria on their outer or inner surface [21] with an incidence of infection that is related to the duration and the site of catheterization, the number of manipulations [22] and the number of lumina [21].

The impregnation of catheters with some types of antibiotics or antiseptics, such as a gentamycin/sodium citrate mixture or sodium citrate alone [23], or with minocycline and rifampin or chlorhexidine and silver sulfadiazin, appears promising for the reduction of bacterial colonization rate [24].

In this work, silver has been used for superficial treatment of catheters. The bactericidal effect is expected to be size-dependent, because the binding of the particles to the bacteria depends on the surface area available for interaction. Smaller particles having the larger surface area available for interaction will give more bactericidal effect than the larger particles [25].

An innovative and patented technology [26] for the surface treatment on different types of substrates was adopted and adapted to polyurethane haemodialysis catheters. The deposition of a silver based solution and the silver photo-reduction on the surface by UV irradiation was obtained. The amount of silver, deposited on the catheter surface, was evaluated by thermogravimetric analyses (TGA) and by energy-dispersive X-ray spectroscopy (EDS). The morphology and the distribution of the silver clusters were evaluated by SEM and TEM analysis. The antibacterial activity of the treated catheter was measured by Agar diffusion test according to Standard 'SNV 195920-1992'. Aim of this work is the characterization of these medical devices in order to analyze the distribution and the size of clusters on the polymeric surface, and to evaluate the antibacterial capability of the devices.

2 Materials and methods

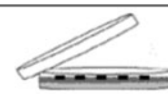
The antibacterial haemodialysis catheters have been developed by Silvertech Ltd. Such devices are temporary double lumen polyurethane "Carbothane" catheters 30 cm long and with an outer diameter of 4 mm. They penetrate into the jugular vein with a length of 10–15 cm and remain in contact with the blood stream both inside and outside the lumina for about 30 days for acute dialysis and blood filtration. Both the inner and outer surface of the catheters were silver treated by using an innovative and patented silver deposition technique extensively used to coat natural and syntethic fibers and adapted to this polymeric substrate [26]. Samples were obtained by dipping the catheters into a box containing the solution made of a silver salt dissolved in an alcohol and ensuring the treatment of the inner surface too, using a syringe trough which the solution is forced to flow inside the device. Then wet catheters were placed into a box containing UV lamps and exposed to UV irradiation in order to induce the photo-reduction of silver and the formation of silver NPs on the catheters surface.

The amount of silver deposited on the polyurethane catheter substrate was calculated by TGA using a NETZSCH STA 409 operating in air and by a heating rate of 10°C/min, from 20 to 900°C. The solid residue above 900°C is attributed to the incombustible silver coating. A quantitative analysis of silver amount was performed also by EDX analysis carried during SEM session.

The morphology of the silver coating deposited both on the internal and external surface of the catheters was observed by SEM using a JEOL JSM6500F. The analysis was focused on the evaluation of uniformity of the deposition and the dimension of silver NP. The measurements were carried out by using a low energy (1 keV) and low current electron beam in order to reduce the charging effects due to the insulating nature of the samples. Further TEM investigations were performed with a FEI TECNAI G2 300F instrument on the silver solute deposited onto a carbon-coated Cu grids in order to derive information about the composition on the solute, the size of the NPs, the possible presence of NP aggregates. The grids were coated with silver by the same technique adopted for catheters, that is by dipping into silver solution and UV irradiation after which silver NPs are formed and fixed on the substrate.

The antibacterial activity of the silver-treated samples was tested according to Standard 'SNV 195920-1992' (Fig. 1). It consists in a diffusion test in agar: an *E. coli* colony is put on top of an agar layer in a petri dish. Then, the catheter sample to be tested is placed over the bacteria and the dish is incubated in oven at 37°C for 24 h. After this time, if sample has an antibacterial capability, an inhibition area will be observed around the sample. The

Fig. 1 Evaluation of antibacterial capability through diffusion test in agar (Standard 'SNV 195920-1992')

(1) Escherichia coli incubated 24 h 37°C with nutrients	
(2) Nutrients addition (Agar) to the Petri dish	
(3) Addition 1 ml of bacterial suspension	
(4) Samples placement	
(5) Incubation 24 h at 37°C	
(6) Antibacterial properties evaluation	

dimensions of this area are related to the entity of the antibacterial capability of the coating, as shown in Fig. 2.

The morphology of the coating and its antibacterial capability were checked also after an intensive washing 30 days long, in order to verify if the mechanical action of a fluid flowing inside and outside the device could compromise the efficacy of the treatment. The washing system developed for this aging test provides a thermal bath set to 37°C in which deionized water flows in and out the catheter pushed by a pump with a capacity of 5 l/min.

3 Results and discussion

The evidence of metal Ag deposition on the catheters was the strong color change of polyurethane surfaces, as showed in Fig. 3. The substrates treated with this technology, in fact, turned to brown color due to the photoreduction of the silver salt solution on the polymeric surface. The color intensity of the coating depends on silver concentration on the treated surface and on the UV exposure time. The amount of silver deposited on the polymeric substrate was calculated by TGA, by subtracting at the value of the residue for the silver-treated sample the same contribution obtained for the untreated sample. Silver amount deposited on PU substrate was equal to 0.64 wt%. Figure 4 shows the loss of weight for the silver-treated and untreated catheters. TGA analysis carried on the sample subjected to aging treatment reveals a slightly smaller amount of silver coverage, that corresponds to 0.49 wt%. EDX analysis performed on the outer surface of silver treated catheters has revealed a superficial amount of silver equal to 0.54 wt% on the sample unwashed and equal to 0.41 wt% on the sample after 30 days of washing. Both EDX spectra are shown in Fig. 5, in comparison with untreated sample. The presence of barium, added as barium sulphate to make radio-opaque the material, is revealed too. No peaks for silver are visible in untreated sample, as expected.

Scanning electron microscope analyses and transmission electron microscope analyses were carried out to study the distribution and the size of silver NPs and the morphology of silver coatings.

Figure 6 shows the secondary (SE) and backscattered (BSE) electron images of the untreated and Ag-treated internal and external surfaces of the catheters. The SE image of the untreated sample, reported in Fig. 6a, shows a moderate roughness which should guarantee a good adhesion of the Ag coating on the polyurethane surface. The SEM analysis of the external silver-treated surface (Fig. 6b) shows a quite uniform distribution of silver NPs, resulting in a smoothing of the initial surface roughness. Due to the sensitivity of the backscattered electrons to the chemistry of the sample, the BSE images (Fig. 6a′, b′) show a contrast which is mainly related to composition, thus flattening the topography effects; thus, the treated sample clearly shows the Ag coverage of the surface, being the NPs brighter than the background, whereas the untreated sample exhibits quite a flat contrast, due to the homogeneous composition of the sample. The weak variations in the contrast are in this case related to a residual topographical component which is still present in the image.

The comparison between the SE images of the internal surfaces of the catheters (Fig. 6c, d) shows that the Ag treatment produces a less evident coverage of this part of

Presence of inhibition zone > 1mm		**Good**
Presence of inhibition zone < 1mm		**Fairly good**
Absence of bacterial growth on the sample		**Sufficient**
Limited bacterial growth on the sample		**Limited**
Sample is partially (50%) rehabitated by the bacteria		**Not sufficient**
Sample is totally rehabitated by the bacteria		**Not sufficient**

Fig. 2 Different grades of the antibacterial capability for the analyzed samples (Standard 'SNV 195920-1992')

Fig. 3 Visual comparison of **a** untreated catheter **b** silver-treated catheter

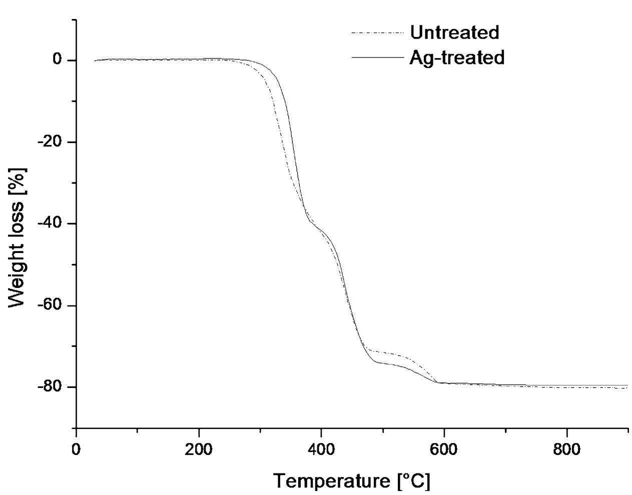


Fig. 4 Results of TGA carried out on samples before and after silver deposition

the substrate, probably due the major difficulty of the UV irradiation to reach the inner zone of the catheter.

Figure 7 shows a SEM image at high magnification (50,000×) of the external surface of the silver treated catheter. Silver particles with size of about 20 nm locally aggregate in bigger clusters of 100–120 are visible.

The results of the TEM investigations are reported in Fig. 8, which shows a typical bright field image of a wide area of the sample. Two types of structures can be observed on the grid: a bigger structure, which is likely to be related to a residual presence of Ag salt, and a uniform distribution of NPs. The high resolution image obtained from the NPs (inset of Fig. 8) shows that they consist of either single nanocrystals, with a diameter below 2 nm (particle labelled 1 in the inset), or aggregates of few nanocrystals (particle labelled 2). The nanocrystals exhibit lattice fringes whose distance is compatible with the 111 lattice planes of the Ag crystal.

The results of the antibacterial tests, performed by incubation in oven at 37°C for 24 h on untreated and silver-treated catheters, are reported in Fig. 9. The presence of a clearly visible bacteria-free area around the silver-treated sample is evident, confirming the bacterial growth inhibition effect induced by silver ions (Fig. 9b). As expected, the untreated catheter does not produce any inhibition to the bacteria growth (Fig. 9a).

SEM analysis on external surface of the sample subjected to washing for 30 days are reported in Fig. 10 in which secondary (a–b) and backscattered (c) electron images of the external untreated (a) and Ag-treated (b–c) surfaces of the catheters after aging treatment are shown. A

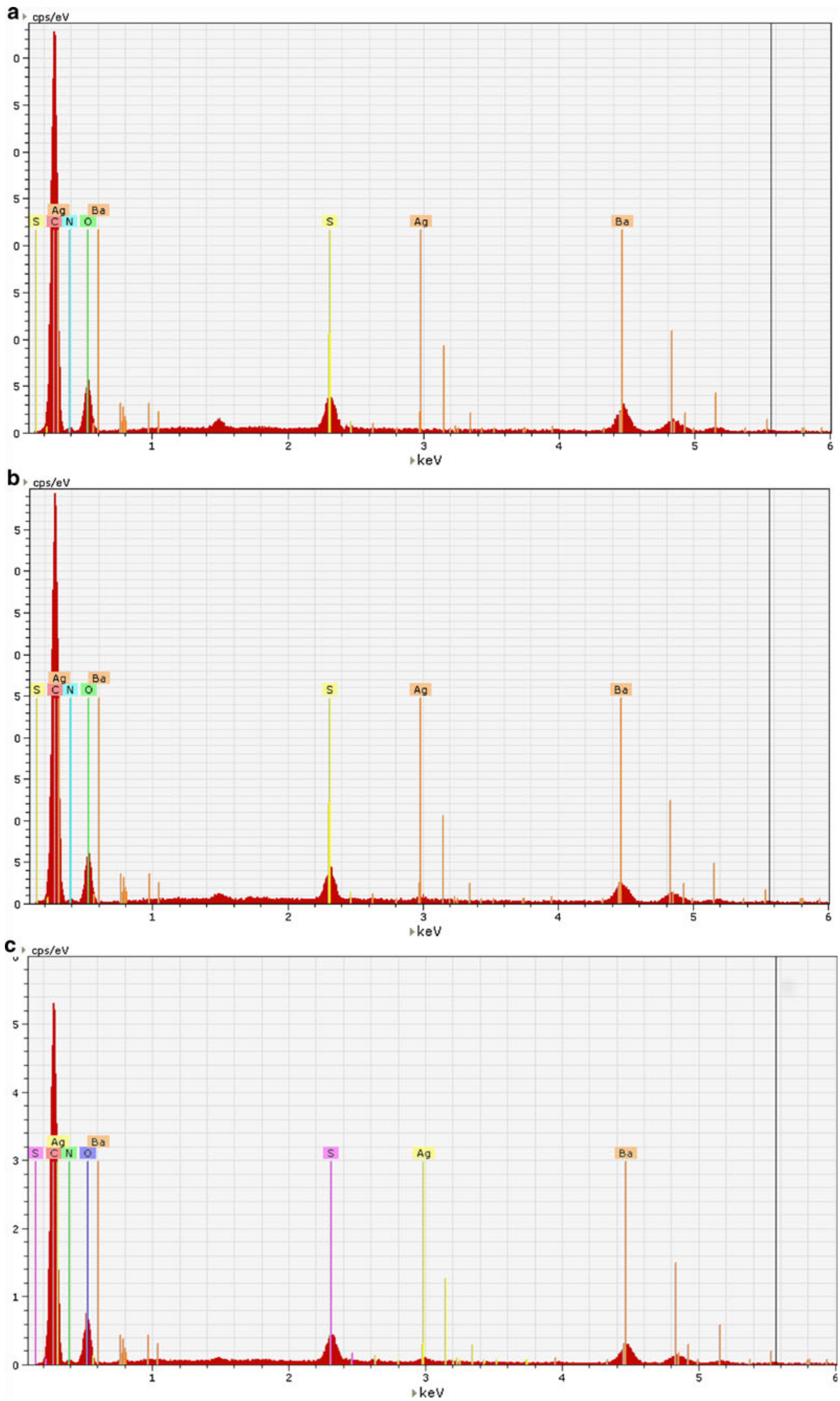


Fig. 5 EDX analysis on silver treated catheter surface before washings (**b**), after 30 days washings (**c**), in comparison with untreated sample (**a**)

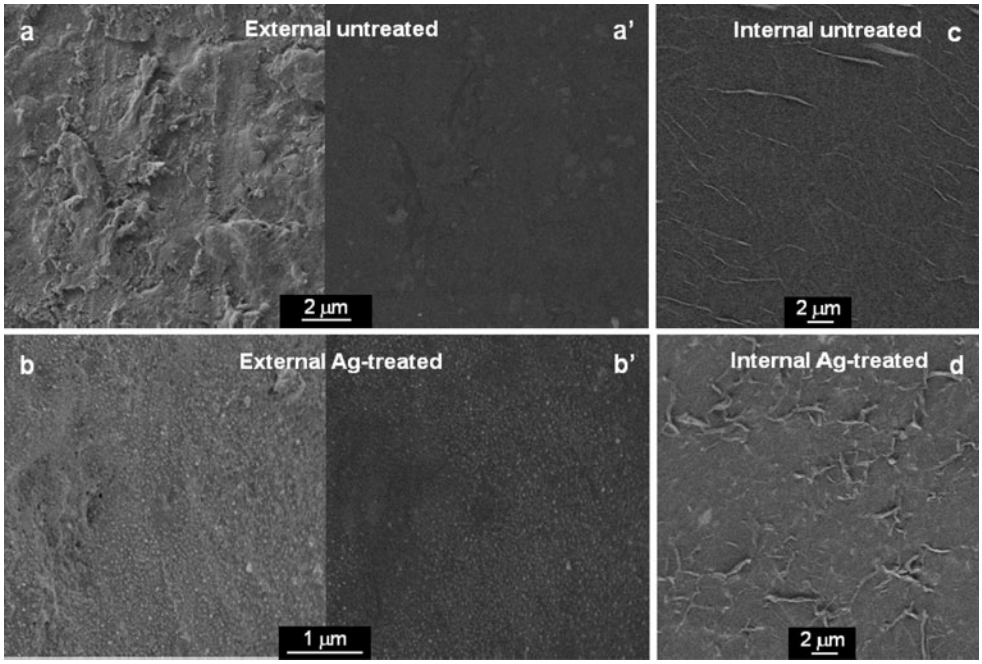


Fig. 6 SEM images of the external (**a**–**a**′, **b**–**b**′) and internal (**c**, **d**) catheter surface which compare the morphology effects of the Ag NP treatment. Secondary and backscattered (**a**′, **b**′) electron images of the same region of the untreated (**a**, **a**′) and Ag-treated (**b**–**b**′) external catheter surfaces; Secondary electron image of the untreated (**c**) and Ag-treated (**d**) internal surface of the same catheters

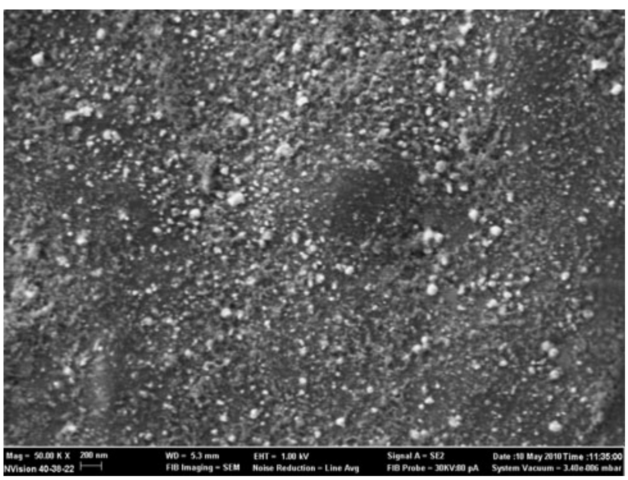


Fig. 7 SEM image of the external surface of the silver treated catheter at high magnification (50,000×)

flattening of the surfaces is evident for Ag-treated samples, showing also the appearance of holes in the Ag coverage. The high magnification backscattered electron image in Fig. 10c shows the absence of Ag NPs in the holes, and the presence of Ag NPs in the other regions, where comparable morphological features with the unaged sample are visible. The presence of holes cannot be attributed to silver treatment but to the loss of barium sulphate that usually occurs in conventional catheters under conditions of use, i.e. in contact with water solutions, as results from in vivo and in vitro tests reported in literature [27].

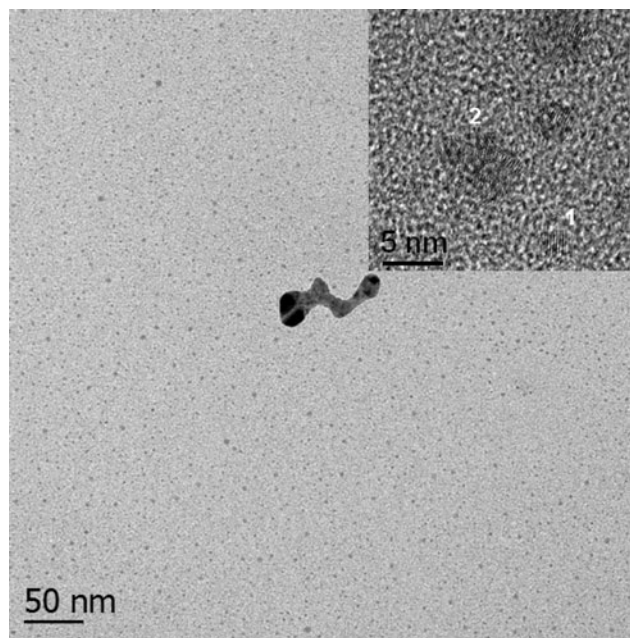


Fig. 8 BF TEM image of a wide area of the sample, showing a silver salt structure at the center and a uniform distribution of small particles in the surrounding area; (*inset*) high resolution image of the Ag nanoparticles showing 111 lattice fringes

Despite washing, the antibacterial activity of silver treated catheters is confirmed, as shown in Fig. 11. A smaller zone of inhibition to bacterial growth is present around the sample, confirming data obtained by TGA and

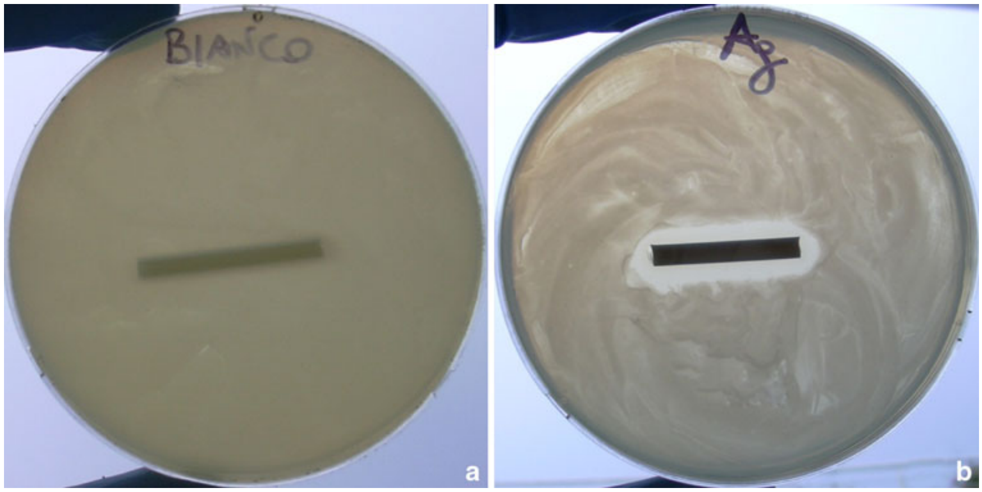


Fig. 9 Test of *E. coli* growth on **a** untreated samples **b** silver-treated samples

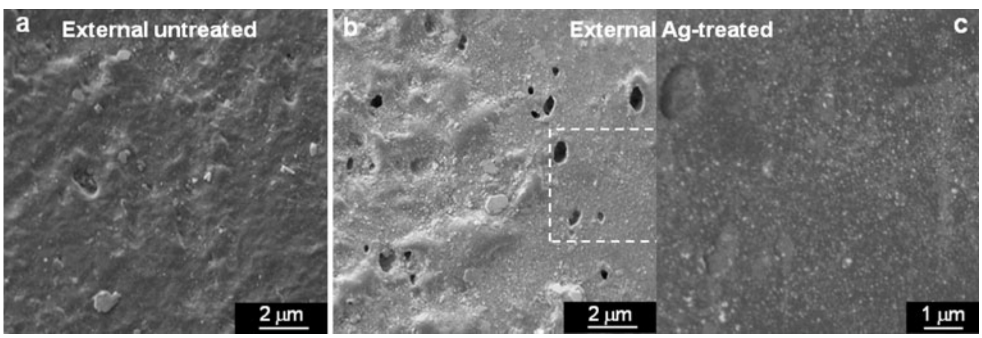


Fig. 10 Secondary (**a**–**b**) and backscattered (**c**) electron images of the external untreated (**a**) and Ag-treated (**b**–**c**) surfaces of the catheters after aging treatments

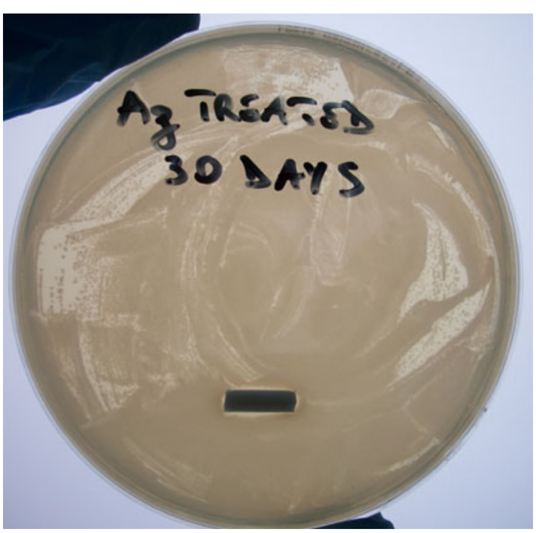


Fig. 11 Test of *E. coli* growth on the sample of catheter soaked in deionized water for 30 days at 37°C

EDX, that have revealed a small loss of silver coating due to washings and, hence, a reduced antibacterial capability.

4 Conclusions

In this paper, haemodialysis polyurethane catheters for vascular access and filtration of blood, that are often cause of serious infections and mortality among hospitalized patients, were treated with silver by an innovative technology based on the photochemical deposition of silver NPs.

After dipping the catheters in the silver solution and UV irradiation of the coating, firmly bonded silver NPs are formed on the substrate.

The morphology of silver coating deposited on catheters and the size of Ag particles were observed by SEM analysis that have revealed on samples a quite uniform distribution of silver particles with dimension of about 20 nm, locally aggregate in bigger clusters with size of about

100 nm. TEM analysis on silver coated copper grid revealed the presence of small nanocrystals with the lattice fringes compatible with 111 lattice planes of Ag crystals. Antibacterial tests performed against *E. coli* have revealed a strong antibacterial activity of these silver coated catheters. After 30 days of soaking in high water flow at 37°C the antibacterial activity is slightly decreased. Hence it is proved that the developed surface engineering process is very promising for the production of safe catheters with minimized infection risk for their entire work life.

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