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The Erlanger Silver Catheter: *In Vitro* Results for Antimicrobial Activity

Summary: The antimicrobial activity of a silver-impregnated polymer catheter (the Erlanger silver catheter) was demonstrated by determining the microbial adhesion to the surface of the catheter and by measuring the rate of proliferation (viability) of microorganisms at this site. On the surface of a catheter impregnated with silver, according to previously described methods, the bacterial adhesion of *Staphylococcus epidermidis* is reduced by 28–40%. Bacterial proliferation on the surface of the catheter and biofilm production are also substantially reduced by the elution of free silver ions from the catheter matrix. Bacteriostatic and bactericidal activities can be determined. The antimicrobial efficacy of the silver catheter is not reduced by blood components. There is no loss in antimicrobial activity for weeks after preincubation in water or phosphate buffered saline. The antimicrobial activity depends on the extent of the active silver surface.

Introduction

Standardized test methods allow the antimicrobial activity of various substances against microorganisms to be determined *in vitro*. These tests are performed either in liquid medium by microdilution and determination of the minimal inhibition concentration (MIC) or on solid media by the determination of the zone of inhibition around a filter paper impregnated with the antimicrobial substance (disc diffusion test). A major requirement for these test systems is the solubility of the antimicrobial agent in water and the diffusion of the agent on the agar plate.

The demonstration of antimicrobial activity on a surface (e. g. of polymers), which restricts biofilm formation and exhibits bactericidal activity, has not been defined and cannot be performed adequately with traditional test methods. The aim of antimicrobial activity testing of surfaces is to monitor the inhibition of microbial colonization directly on the surface itself, and not the analysis of antimicrobial activity elsewhere.

Traditional Test Methods

Measurement of the Inhibition Zone

The antimicrobial activity of polymers which are impregnated with antibiotics or disinfectants has been determined by traditional methods, e. g. the measurement of the zone of inhibition of growth [1]. These investigations show that the antimicrobial substance is eluted from the polymer and demonstrates activity against microorganisms even up to a distance of 14 mm. The presence of a zone of inhibition, however, also implies that antimicrobial agents are lost from the surface as they are continuously eluted from the polymer.

For silver-impregnated polymers the measurement of the zone of inhibition cannot be used with meaningful results. Silver is poorly water soluble and only a minimal diffusion rate of antimicrobially active silver ions can be observed. Activity at a distant site away from the surface is not detectable for the silver catheter. Thus, the lack of a zone of inhibition of growth does not imply that there is no antimicrobial activity and that the polymer could be colonized by microorganisms.

Flush Method

To detect antimicrobial activity in an antibiotic-impregnated catheter, *Jansen* used a system whereby catheter samples were flushed with bacteria [2]. Antimicrobial activity was determined by roll on cultures which showed a rapid bactericidal activity of antibiotics. The samples were then analyzed by electron microscopy, which allowed the determination of the number of bacteria/cm^2, but gave no information about the viability of the microorganisms.

The full assessment of antimicrobially-impregnated polymers also includes, in addition to bactericidal effects, an analysis of biofilm formation and the adhesion of microbes to the surface of the catheter. Silver ions demonstrated a slower antimicrobial activity which was dependent on concentration and time. For clinical purposes this slower eradication seems to be more effective in preventing the colonization of surfaces and foreign bodies. Since the kill kinetics of bacteria and fungi under the effect of silver ions are dependent on concentration and time, the time factor must also be included in the evaluation of antimicrobial efficacy [3]. This, however, is not sufficiently taken into account in the flush model.

Flask-Shake Model

Pieces of catheters are incubated in bacterial suspensions. The time period required for the eradication of the bacte-

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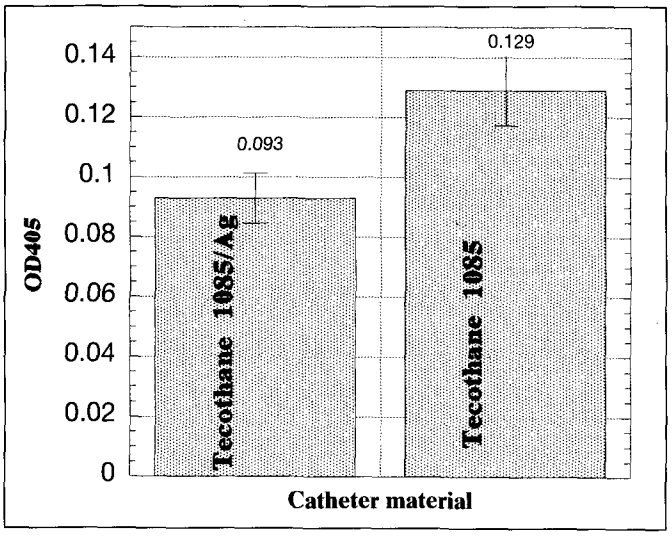

Figure 1: Adherence of *Staphylococcus epidermidis*. On the silver material (Tecothane 1085/Ag), the adherence of coagulase-negative bacteria is reduced by 28% compared to the source material (Tecothane 1085). The adherence is quantified by immunologic methods (ELISA). The optical density (OD at 405 nm) reflects the activity of alkaline phosphatase, a secondary antibody conjugate that specifically binds to polyclonal anti-*Staphylococcus* serum. The plot shows the mean value of 16 measurements for each material. The error bars indicate the coefficients of variation (7.8% for the silver material, 9.3% for the source material).

ria is monitored. This method is a modification of the disc diffusion test, and determines the antimicrobial activity eluted from the polymer. The antimicrobial activity of silver also depends on the inoculum size and on the nutrient medium. The flask-shake method is not suitable for the analysis of microbial colonization of surfaces and biomaterials.

Perfusion Model

The simulation of the clinical situation that gives rise to catheter-related infections led to the development of the perfusion model [4]: physiological saline or a 1:1 mixture of physiological saline and 5% glucose were inoculated with 5 x 10^7 CFU/ml. The antimicrobial activity against different test strains (*Staphylococcus epidermidis, Staphylococcus aureus*) and clinical isolates (enterococci, *Escherichia coli, Pseudomonas aeruginosa, Candida albicans*) was investigated. The test catheters were perfused with the bacterial suspension at a rate of 10 ml/h. Every 12 and 24 h a piece (1 cm) of the catheter was cut off after flushing with sterile physiological saline and incubated in 2 ml sterile trypticase soy broth. The interpretation of bacterial colonization is related to the degree of turbidity of the medium within 48 h. Contamination of the catheter was defined by the detection within 48 h of broth turbidity, indicating microbial growth. Only growth of the same organisms as those with which the catheters were initially perfused was considered as contamination. Colonization rates of silver catheters and control catheters were compared. At least a 4-fold, on average 8-12-fold and in some cases a 20-fold or longer period of sterility was observed for the silver-impregnated catheters. Signs of regeneration of the system, i. e. silver-impregnated catheters becoming sterile after 24 h of perfusion with sterile physiological saline, were observed in some experiments; control catheters never regenerated. At present, a modification of the model is used in which the catheter is perfused with the bacteria for 6 h and subsequently perfused with sterile physiological saline for 18 h.

One of the major disadvantages of the perfusion system is that accidental contamination could give false-positive results. Moreover, the method is time consuming and not suitable for mass screening of larger numbers of prototypes. There have also been concerns with statistical reliability and reproducibility.

The problems with the traditional test systems show that analysis of the microbial colonization of polymers is still unsatisfactory. A new test system which is reliable, reproducible, cost-effective and allows the simultaneous investigation of different prototypes was therefore urgently needed.

Materials and Methods

Test materials: Different catheter prototypes made of polyurethane (Tecothane 85A and 95A, provided by Thermedics Inc. Woburn, USA) with the incorporation of different amounts of silver, different technologies for silver impregnation (e. g. silver particles and active surfaces in different sizes) and different procedures for extrusion were compared with commercial catheters (Arrow, USA, CS-04701).

Microorganisms: S. epidermidis 9142 and 047 provided by *D. Mack* [5] and *F. Götz* [6]; *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 29213 were investigated. Organisms were grown in trypticase soy broth and transfered to agar plates with trypticase soy with 5% defibrinated horse blood (Biomerieux).

Blood proteins and antibodies: Human plasma and serum were freshly prepared. Human albumin (5%) was obtained from Biotest Isoton. Polyclonal rabbit sera against *S. epidermidis* were prepared by *D. Mack* [5]. A commercially available alkaline phosphatase conjugated secondary anti-rabbit IgG antibody (Boehringer Mannheim) was used.

Chemicals: P-nitrophenylphosphate (Sigma), Tween 20 (Sigma), phosphate-buffered saline (Gibco-BRL), Na_2S and Ethanol (Merck) were obtained from commercial sources.

96-well microplates: Standard microplates (Greiner, 655101) were read in an automated plate reader (Spectramax 250, Molecular Devices). Endpoint measurements (ELISA) were carried out at 405 nm. Proliferation kinetics of bacteria (at 578 nm) were monitored. Data were processed with Softmax Pro 2.0 (Molecular Devices).

Quantification of bacterial adherence on the surface of catheters: The adherance of bacteria was quantified by an enzyme-linked immunosorbent assay (ELISA): A microplate system (96-well) was developed. Catheter samples, 1 cm in length, were attached to microplate lids (Greiner, 656101). Catheter samples were incubated with bacteria (5 x 10^7 CFU/ml in PBS, 37°C) for 2 h. After incubation the bacterial suspension was discarded. The

catheter samples were washed 5 times in PBS/0.05% Tween 20. Subsequently, the probes were incubated with polyclonal (rabbit) anti-*S. epidermidis* antibodies (2 h, 25°C). The antibody concentration, which corresponds to the number of bacteria bound on the catheter, was determined at 405 nm by secondary anti-rabbit IgG conjugated antibodies.

Quantification of bacterial proliferation on the catheter surface: The antimicrobial activity of the catheters was monitored by the analysis of the viability of the microorganism adherent on the surface. Bacteria bound to the surface of a polymer show the tendency to proliferate and to form biofilms. The polymer is therefore rapidly covered with a thick layer of bacteria. The inhibition of proliferation leads to a bacteriostatic effect and the prevention of biofilm formation.

Our test system was modified in such a way that only a small defined area of the catheter sample was contaminated with bacteria. The colonization was determined both within and outside the area of contamination. The analysis was carried out by the same ELISA method as described above or after incubation of the samples in liquid medium (trypticase soy) by determination of bacterial proliferation by continuous photometry (kinetics at 578 nm). Silver catheters were compared with commercial catheters.

Documentation and quantification of bacteriostatic and bactericidal activity on polymer surfaces: It is well known that daughter cells are released from the surface of catheters colonized by bacteria. These daughter cells are capable of proliferation if transferred into appropriate media. Silver ions reduce the proliferative activity of bacteria. Low concentrations of silver ions released from the surface for a limited period of time induce a reversible antimicrobial activity, which means that for a certain time period the proliferation is stopped. The time period until logarithmic growth is observed is directly proportional to the degree of damage provided by the amount and activity of released silver ions [3]. At higher silver concentrations, bactericidal activity is found and confirmed by the observation that bacterial growth is not detectable after a 120 h incubation of samples in trypticase soy medium. Depending on the incubation period, and the concentration of free silver ions eluting from the catheter which in turn corresponds to the size of the active surface of silver particles inside the material, the antimicrobial activity can be described as either bacteriostatic or bactericidal. These effects can be measured in a microplate system.

Catheter samples were attached to microplate lids and subsequently contaminated with 5 x 10^7 CFU/ml PBS (60 min, 37°C). After this incubation step the samples were transferred to a minimal medium (PBS plus 1% trypticase soy broth, 24 h, 37°C). The number of vital daughter cells released into the surrounding medium was dependent on the number of adherent bacteria and the proliferation rate. After 24 h 50 µl of full concentration trypticase soy broth was added. The number of vital bacteria and their proliferation kinetics were monitored on-line by photometry (578 nm). Generally, an average of 96 measurements were recorded during a 48 h time course (30 min interval). After a further 30, 60, 90 and 120 h the samples were analyzed for sterility.

Potential effect of surface changes on the antimicrobial efficacy of silver catheters: During insertion and in the human body, the catheters come into contact with amino acids, fats, blood and blood components, lymph and pus. It is essential to assess whether these factors lead to a reduction or even abolition of antimicrobial activity. For this purpose, the catheter samples were precoated with the relevant substances and subsequently analyzed according to our test methods, as described above. It was also of interest to establish whether a 4-week preincubation of catheter samples in physiological saline produced any reduction of antimicrobial activity.

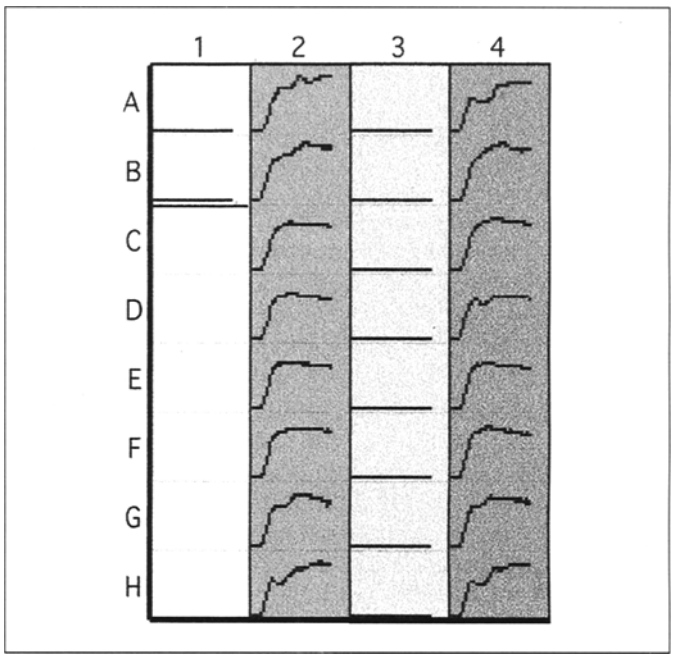

Figure 2: Proliferation of *Staphylococcus epidermidis* on catheter surfaces. The antimicrobial activity was investigated by monitoring the proliferation rate of bacteria that are adherent to catheters. Growth curves of *S. epidermidis* on three different catheters are shown. Lane 1, blank (medium); lane 2, Arrow CS-04701; lane 3, Erlanger silver catheter C703111 (containing 20% $BaSO_4$, 0.35% silver [w/v], 1 x 10^{15} particles, active surface 450 cm^2); lane 4; Tecothane 1095 basic material. Twenty-four samples, 8 samples of each material, were colonized with bacteria under simultaneous conditions. Samples with adherent bacteria were transferred to a minimal medium (PBS plus 1% trypticase soy broth, 24 h, 37°C). A minor population of daughter cells was released into the surrounding medium. After consuming the available nutrients, the bacteria reverted to the resting state. After incubation for 24 h the catheter samples were discarded. When sterile trypticase soy broth was added, vital bacteria resumed growth. A kinetic course (X-axis) of the proliferation of vital bacteria was recorded by optical density measurements (Y-axis) at 578 nm. For the control catheters (lanes 2 and 4) no antimicrobial activity was seen: In all 16 measurements, a logarithmic growth was detected. In contrast, the silver catheter samples remained sterile. Eighty-three measurements were recorded (30 min interval) over a time period of 41.5 h. After an additional 120 h the silver-catheter samples remained sterile. The concentration of free silver ions eluting from the surface completely killed the layer of adherent bacteria.

Blocking the antimicrobial activity of silver ions: In order to prove that antimicrobial activity is related to the diffusion of silver ions, sulphur compounds with a high affinity for silver were added. Polyurethane was soaked in ethanol (7.6%, 37°C, 24 h) supplemented with Na_2S (2.5%, 37°C, 24 h) to induce the formation of water insoluble Ag_2S.

Results

This paper quantitatively differentiates the adhesion and proliferation of microorganisms on the surface of catheters. Quantitative results concerning the antimicrobial activity of the Erlanger silver catheter are demonstrated. The antimicrobial activity of a surface is related to mechanisms that reduce bacterial adhesion and also to the active and continuous release of antimicrobial agents so that adherent microbes are killed. Our investigations show that both the adherence and the proliferation of bacteria are substantially reduced on the silver surface.

Reduction of Bacterial Adherence on the Silver Catheter

Polyurethane impregnated (0.7% w/v) with submicronic particles of metallic silver (particle size 20 nm x 20 nm) shows, in contrast to polyurethane alone and control catheters, hydrophilic properties. Bacterial adhesion is reduced. Figure 1 shows the adherence of coagulase-negative staphylococci onto the silver-impregnated material in comparison to the identical silver-free polyurethane.

On the surface of the catheter impregnated with submicronic particles of silver a 28% reduction in bacterial adherence compared to the basic material and a 40% reduction of adherence in comparison to conventional catheters was observed. This result was reproduced in several experiments performed independently. The reduction of adherence was shown for a variety of isolates of *S. epidermidis*. Catheter samples incorporating metallic silver (2% w/v) in a particle size of 1.2–2.5 µm were not hydrophilic and did not reduce adherence.

Bacterial Killing by the Silver-Impregnated Polyurethane

The viability and growth characteristics (proliferation) of bacteria adherent to catheters were studied quantitatively. After the simultaneous contamination of control and silver catheters, bacterial proliferation was only found on silver-free controls (Arrow and Tecothane 1095). In contrast, the surface of the silver catheter was bactericidal: proliferation was stopped and adherent bacteria were killed. The spectrum of antimicrobial efficacy ranges from bacteriostatic to bactericidal effects and was dependent on the initial inoculum and the contact period of adherent microbes. The results were:

1) On the surface of the Erlanger silver catheter, adherent bacteria were unable to proliferate and bacterial accumulation did not occur. Bacterial proliferation was, however, observed on the control catheters (Figure 2).

2) After coating the surface with human albumin, the antimicrobial activity of the Erlanger silver catheter was not reduced (Figure 3).

3) In contrast to the control catheters, the silver catheter was not colonized by bacteria spreading on the surface (Figure 4).

4) After a 4-week preincubation of the silver catheter in phosphate buffered saline (PBS, pH 7.3) or bidistilled water, the antimicrobial activity remained unaffected.

5) The antimicrobial activity of the silver catheter was abolished by Na_2S because of formation of water insoluble Ag_2S. Biologically active silver ions were bound (negative control, Figure 5).

6) The antimicrobial efficacy of the Erlanger silver catheter was related to the highly active surface (Figure 6).

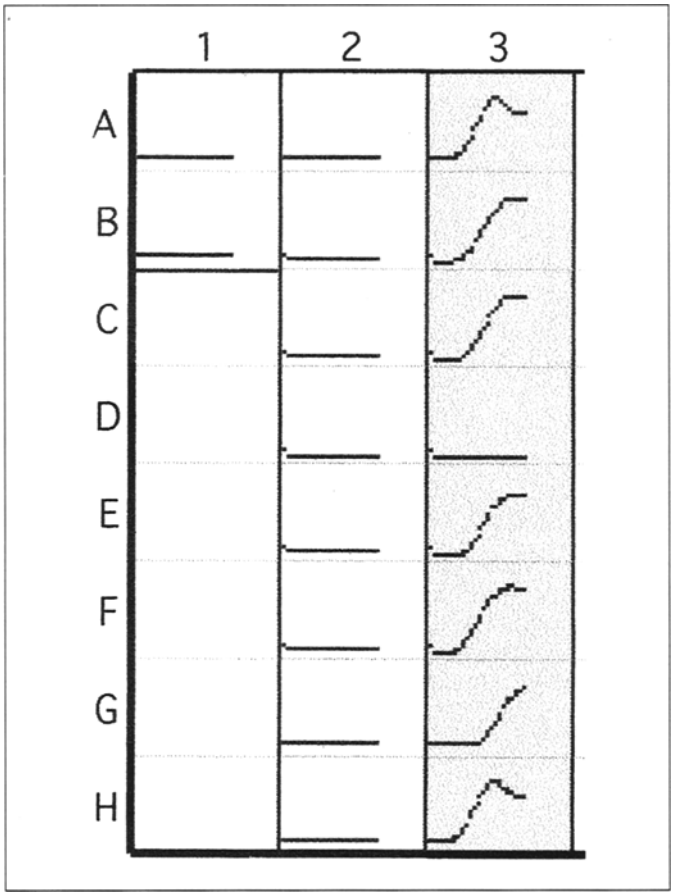

Figure 3: Proliferation of *Staphylococcus epidermidis* on albumin-coated catheters. Experiment corresponding to Figure 2 on albumin-coated catheter samples. The antimicrobial efficacy of the Erlanger silver catheter was not reduced. Similar results were obtained with samples that had been pre-incubated with human blood plasma. Lane 1, blank; lane 2, silver catheter C703111; lane 3, Arrow CS-04701.

Discussion

The microbial colonization and antimicrobial activity of polymer surfaces cannot be studied with traditional methods. There are too many different considerations: changes in the hydrophilic properties of the catheter surface which affect bacterial adhesion, the inhibition of proliferation and biofilm formation, which stops the spread of bacteria over the catheter surface, and finally the release of active agents.

The silver impregnation of catheter materials provides an opportunity to utilize the oligodynamic action of metal ions. The release of free silver ions is essential and depends on the extent of the active surface. Because the mechanical properties of catheters impregnated with sil-

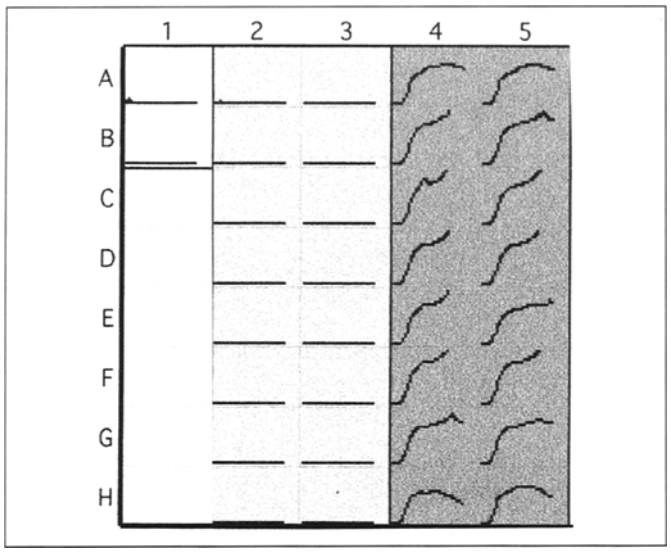

Figure 4: Spreading of *Staphyloccus epidermidis* over catheter surfaces. Experiment corresponding to Figure 2 for the evaluation of bacterial spread (biofilm formation) over catheter surfaces. After the initial bacterial contamination of a defined sample area, the bacterial proliferatory behavior is analyzed outside the area of contamination. Lane 1, blank; lanes 2 and 3, silver catheter C703111; lanes 4 and 5, Tecothane 1095 without silver.

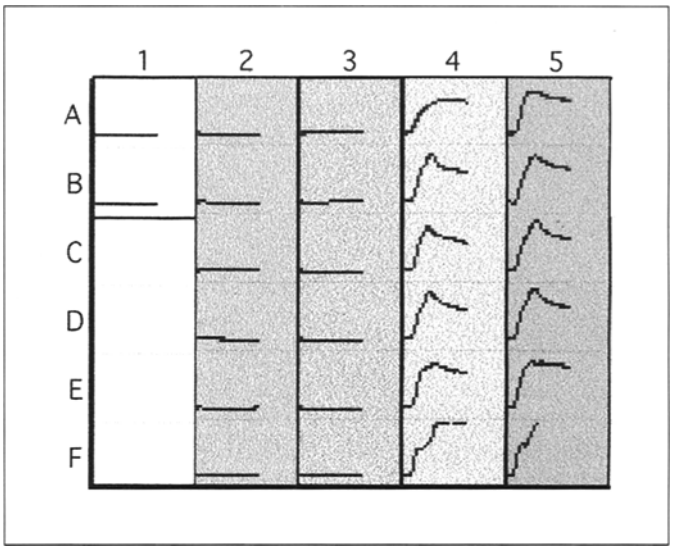

Figure 5: Control of the silver activity. Experiment corresponding to Figure 2 with silver catheter samples preincubated in Na_2S. Silver ions which are released from the surface are complexed and form water insoluble Ag_2S. Lane 1, blank; lane 2, silver catheter C703111 without Na_2S pretreatment; lane 3, silver catheter prototype incorporating 1.5% silver (w/v) without Na_2S pretreatment; lane 4 = lane 2 with Na_2S pretreatment; lane 5 = lane 3 with Na_2S pretreatment.

ver in concentrations of more than 4–5% (w/v) deteriorate rapidly, the amount of silver which can be incorporated into a polymer is limited. An increase in the release of free silver ions from the catheter can therefore only be achieved by an increase in the size of the active surface. This increase in surface can only be achieved – with identical concentrations of silver – by a substantial decrease in silver particle size. Only with an active surface of 450 cm^2, which can be obtained with 10^{15} particles per g polyurethane with a size of 20 x 50 nm, can bactericidal activity be achieved. A smaller surface only has a reversible, bacteriostatic mode of action.

The oligodynamic activity of silver ions, incorporated into polyurethane by a special manufacturing technique, is not reduced by preincubation with albumin and fibrinogen. Also, a 4-week preincubation of catheters with physiological saline results in no measurable loss of activity.

Complexing silver ions with sulfur, which results in the formation of water insoluble Ag_2S, abolishes the activity of silver ions. We consider this phenomenon to be additional evidence for the antimicrobial activity of silver ions.

In view of a growing number for foreign-body-associated nosocomial infections [7], the results of these investigations are of significance in two respects: on the one hand the results reveal an evident quantitative proof of the antimicrobial activity of the Erlanger silver catheter, and on the other the results are of general interest in the investigation of the antimicrobial activity of biomaterials with regard to microbial adherence and bacteriostatic and bactericidal properties.

As far as we are aware from the literature and from our own investigations, the continuous release of silver ions is a critical factor in the prevention of foreign-body-associated infections. The maintenance of surface bactericidal activity is more effective than a surface that merely reduces bacterial adherence. Investigations in our laboratory show that hydrophilic surfaces reduce bacterial adhesion but do not influence bacterial proliferation. In addition, the efficacy of a reduction of bacterial adherence is abolished as soon as the surface is coated by blood components. The influence of blood proteins has been described by *Vaudaux* [8]. Because central venous catheters come into immediate contact with blood, it is particularly important that the antimicrobial activity of silver is not reduced after the surface is coated by blood components. Our results are in agreement with other laboratories which analyzed the influence of albumin on the diffusion of silver. *Jansen* and coworkers have shown that silver diffusion is increased for a silver catheter coated with albumin [2]. Our data show a similar relationship: silver activity is not reduced by human albumin or blood plasma, but may even be moderately increased.

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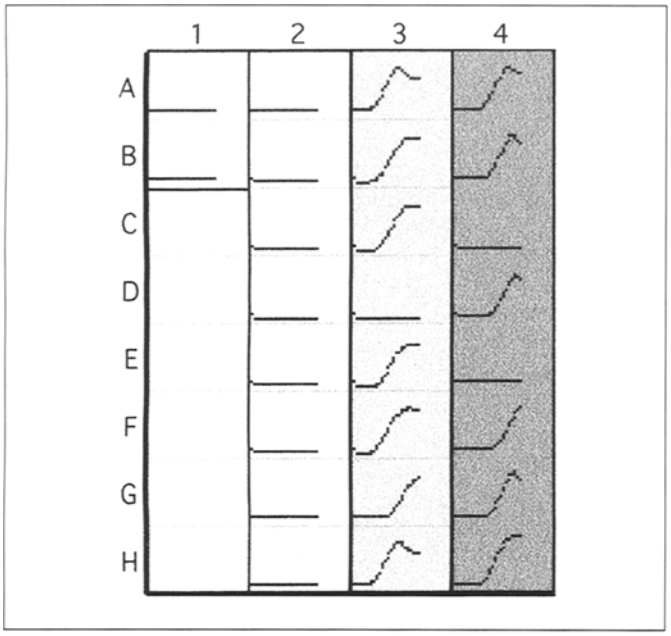

Figure 6: Proliferation of *Staphylococcus epidermidis* on catheters with different active silver surfaces. Lane 1, blank; lane 2, Erlanger silver catheter C703111 (20% $BaSO_4$, 0.35 [w/v] silver, 1 x 10^{15} particles, active surface 450 cm^2; lane 3, Arrow CS-04701; lane 4, polyurethane incorporating 2% silver powder, particle size 1.2–2.5µm, active surface 40 cm^2).

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