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# Bacterial adherence to silver nitrate coated poly-L-lactic acid urological stents in vitro

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**Abstract** The purpose of this study was to see whether it is possible to prevent bacterial adherence to bioabsorbable self-reinforced L-lactic acid polymer (SR-PLLA) urological stents. The SR-PLLA stents were coated with silver nitrate blended ε-caprolactone/L-lactide copolymer. The adherence of five bacterial strains (*Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Proteus mirabilis* and two strains of *Escherichia coli*) to coated and non-coated SR-PLLA wires were tested. It was found that silver nitrate coating prevented the adherence of bacteria (except *E. faecalis*) to SR-PLLA stents. The preventive effect correlated with the silver nitrate concentration. It was also found that silver nitrate coating reduced the amount of bacteria in ambient urine. In conclusion, silver nitrate coating may reduce stent-associated bacterial infections by preventing the adherence of bacteria. Further studies are needed to confirm its efficacy and safety in clinical practice.

**Key words** Biodegradation · Infection · Urology · Stents

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## Introduction

In 1980, Fabian [1] introduced a prostatic spiral stent, which kept prostatic urethra open and enabled voiding and continence. Since then, several transient, permanent and biodegradable prostatic stents have been used to relieve urinary obstruction. The most common complications of stents are migration, encrustation and infection [2, 3], which have significant morbidity rates. Infection rates between 7.5 and 27% have been reported in patients with postoperative ureteric double-J stents, in spite of antibiotic prophylaxis [4–6]. Urinary tract infection occurred in 14% of patients with a biodegradable prostatic stent after visual laser ablation of prostate (VLAP) [7]. However, all these patients also had a suprapubic catheter, which probably increased the infection rate. Petas and coworkers [8] showed later that the duration of suprapubic drainage correlated with the frequency of postoperative infection rate after VLAP.

The adherence of uropathogens to the uroepithelium or to the surface of prosthetic devices is the key event in the pathogenesis of urinary tract infection [9, 10]. It appears that bacterial properties are more important than stent type in determining bacterial adherence to biodegradable stents [11].

The adherence of uropathogens to a biodegradable stent surface can be prevented by immersion in a suitable antibiotic solution [12]. Silver coating of urinary catheters is known to inhibit adherence and growth of bacteria [13, 14]. Even salicylic acid impregnation and electrification are known to inhibit bacterial adherence to urethral catheters [13, 15]. The aim of this study was to see whether it is possible to prevent bacterial adhesion to a bioabsorbable stent surface by silver nitrate coating.

## Materials and methods

The bioabsorbable self-reinforced L-lactic acid polymer (SR-PLLA) wire was coated with four different concentrations of silver nitrate (10, 5, 2 and 0.5 wt.%) blended ε-caprolactone/L-lactide

copolymer. This coating consists of a semi-crystalline copolymer of ε-caprolactone and L-lactic acid (P(ε-CL/L-LA)). This material is rigid and hard below −60 °C but becomes soft and flexible above this temperature. The copolymer was developed and synthesized at the Helsinki University of Technology. The coating was blended by and the stent pieces were manufactured by Bionx Implants (Tampere, Finland). The 1.1-mm thick SR-PLLA wire was cut to exactly 5-mm pieces. The pieces were preincubated in artificial urine for 1 h, 24 h, 1 week and 2 weeks at 37 °C. Each assay was performed in duplicate and non-coated pieces were used as controls. The composition of artificial urine is given in Table 1.

Four bacterial strains had been isolated from patients with urinary tract infection: *Pseudomonas aeruginosa* (IH 50176), *Enterococcus faecalis* (IH 50167), *Proteus mirabilis* (IH 50163) and *Escherichia coli* (IH 13258). A strain of *E. coli* (IH 50797) was isolated from stools of a healthy subject. The strains were stored in skimmed milk at −70 °C until use.

Bacteria were grown overnight at 37 °C in ambient air under gentle shaking in broth culture. They were first diluted 1:10 and then grown in similar conditions for 2 h. A 50-µl aliquot of bacterial suspension was added to a test tube containing 1 ml of artificial urine and two stent pieces. To calculate the amount of bacteria added (inoculum dose), 100-µl samples of bacterial suspension were plated on cystine-lactose-electrolyte-deficient (CLED) agar plates after serial 1:10 dilutions of bacterial suspensions. The amount was expressed as colony forming units (CFU) per millilitre. After 3 h of incubation with mild shaking (50 rpm), the stent pieces were removed from the first test tube and rinsed in a test tube containing 4 ml of phosphate-buffered saline (PBS). Washing was repeated four times. After that, adherent bacteria were detached with a water-bath sonicator. The amount of detached bacteria was calculated as CFU/ml after the 1:10 dilution series were plated onto CLED agar plates. A control series without bacterial inoculation was performed in parallel.

The effect of inoculum dose was taken into account by dividing CFU of detached bacteria by CFU of inoculum dose. To get "easier" numbers, the ratio was multiplied by 10,000, and the result is defined as bacterial adherence later in text. For the same reason, only values to two decimal places are shown in the tables. However, when counting the means and percentages, five values to more than two decimal places were used.

To see whether the stent pieces could inhibit bacterial growth during the 3 h incubation period, remaining bacteria were calculated as CFU/ml after a tenfold dilution series. The ratio of CFU of remaining bacteria to CFU of inoculum dose was calculated.

The statistical package used was SPSS for Windows 8.0. Friedman's test was used to estimate the differences in bacterial adherence, with statistical significance level at $P < 0.05$.

**Table 1** The composition of artificial urine (A:B = 1:1)

| A solution (g/l) | | B solution (g/l) | |
|---|---|---|---|
| $CaCl_2 \times 2\ H_2O$ | 1.765 | $NaHPO \times H_2O$ | 6.800 |
| $Na_2SO_4$ | 4.862 | $NaHPO_4$ | 0.869 |
| $MgSO_4 \times 7\ H_2O$ | 1.143 | $Na_3Cit \times 2\ H_2O$ | 1.168 |
| $NH_4Cl$ | 4.643 | NaCl | 13.545 |
| KCl | 12.130 | | |

## Results

The adherence of different bacteria to stent pieces after various incubation periods is shown in Table 2. With the

**Table 2** Bacterial adherence to stent pieces after various incubation periods in artificial urine. *Bacterial adherence* CFU of detached bacteria to CFU of inoculum dose × 10,000. *h1* 10 wt.% $AgNO_3$, *h2* 5 wt.% $AgNO_3$, *h3* 2 wt.% $AgNO_3$, *h4* 0.5 wt.% $AgNO_3$, *p* non coated. *Mean* mean of bacterial adherence after various incubation periods. *%* bacterial adherence in percent compared with adherence to non-coated SR-PLLA stent

| Bacterial strain | Incubation time | SR-PLLA coating | | | | |
|---|---|---|---|---|---|---|
| | | h1 | h2 | h3 | h4 | p |
| *P. aeruginosa* (IH 50176) | 1 h | 0 | 0.01 | 0.01 | 250 | 40 |
| | 24 h | 0 | 0 | 0.07 | 15 | 3.5 |
| | 1 week | 0 | 0 | 0 | 0.01 | 50 |
| | 2 week | 0 | 0 | 0 | 0.5 | 0.2 |
| | Mean | 0 | 0.00 | 0.02 | 66.38 | 23.43 |
| | % | 0 | 0.01 | 0.08 | 283 | 100 |
| *E. faecalis* (IH 50167) | 1 h | 0.03 | 0.2 | 0.04 | 0.45 | 0.3 |
| | 24 h | 0.06 | 0.07 | 0.02 | 0.4 | 0.07 |
| | 1 week | 0.03 | 0.1 | 0.02 | 0.08 | 0.1 |
| | 2 week | 0.4 | 0.05 | 0.15 | 0.05 | 0.15 |
| | Mean | 0.13 | 0.10 | 0.05 | 0.24 | 0.15 |
| | % | 83 | 64.1 | 32.1 | 156 | 100 |
| *P. mirabilis* (IH 50163) | 1 h | 0 | 0.03 | 1.14 | 0.86 | 2.57 |
| | 24 h | 0.01 | 0.05 | 3.0 | 2.0 | 5.0 |
| | 1 week | 0.01 | 0.2 | 0.02 | 0.1 | 0.8 |
| | 2 week | 0.02 | 0.04 | 0.2 | 0.2 | 0.2 |
| | Mean | 0.01 | 0.08 | 1.09 | 0.79 | 2.14 |
| | % | 0.46 | 3.7 | 50.9 | 36.9 | 100 |
| *E. coli* (IH 50797) | 1 h | 0 | 0.01 | 0.15 | 0.5 | 0.03 |
| | 24 h | 0 | 0 | 0 | 0.09 | 0.1 |
| | 1 week | 0 | 0 | 0 | 0 | 0.02 |
| | 2 week | 0.00 | 0 | 0 | 0.2 | 0.03 |
| | Mean | 0.00 | 0.00 | 0.04 | 0.2 | 0.04 |
| | % | 0.57 | 6.8 | 85.2 | 454 | 100 |
| *E. coli* (IH 13258) | 1 h | 0.00 | 0.14 | 0.01 | 0.04 | 0.08 |
| | 24 h | 0 | 0 | 0.01 | 0.01 | 0.1 |
| | 1 week | 0 | 0.00 | 0.2 | 0 | 1.6 |
| | 2 week | 0 | 0 | 0.1 | 0.9 | 1.0 |
| | Mean | 0.00 | 0.04 | 0.08 | 0.24 | 0.7 |
| | % | 0.04 | 5.2 | 11.5 | 34.1 | 100 |

exception of *Enterococcus faecalis*, silver nitrate blended ε-caprolactone/L-lactide copolymer coating reduced bacterial adherence to SR-PLLA stent pieces. The prevention of bacterial adherence correlated with the silver nitrate concentration of the coating. The 10 and 5 wt.% coatings could nearly totally prevent the adhesion of the tested bacteria (except *E. faecalis*) to stent material; whereas the weakest coating in fact promoted bacterial adherence in some cases (*P. aeruginosa* and *E. coli* IH 50797).

The ranks of various coatings in preventing bacterial adherence according to Friedman's test are shown in Table 3. When all five bacteria are taken into account as a group, the preventing effect of the silver nitrate coating was highly significant ($P < 0.001$). When looking at a single bacterial strain, silver nitrate coating again reduced bacterial adherence, with the exception of *E. faecalis*. The prevention of bacterial adherence significantly correlated to silver nitrate concentration in ε-caprolactone/L-lactide copolymer coating ($P = 0.007$–0.049).

The bacterial adherence preventing effect was stable and the incubation time in artificial urine of between 1 h and 2 weeks did not appear to have any substantial effect on bacterial adherence. The values are presented in Table 2. The amount of living bacteria in the incubation solution was analysed after the 3 h incubation period, when the stent pieces were taken away. The findings are

**Table 3** The ranks of various coatings in preventing bacterial adherence according to Friedman's test

| Bacterial strain | Coating | Rank | Significance |
|---|---|---|---|
| All bacteria | 10 wt.% $AgNO_3$ | 1.73 | |
| | 5 | 2.50 | |
| | 2 | 2.67 | |
| | 0.5 | 3.72 | |
| | Non-coated | 4.38 | $P < 0.001$ |
| *P. aeruginosa* (IH 50176) | 10 wt.% $AgNO_3$ | 1.00 | |
| | 5 | 2.50 | |
| | 2 | 3.50 | |
| | 0.5 | 3.25 | |
| | Non-coated | 4.75 | $P = 0.012$ |
| *E. faecalis* (IH 50167) | 10 wt.% $AgNO_3$ | 2.50 | |
| | 5 | 3.13 | |
| | 2 | 1.88 | |
| | 0.5 | 3.63 | |
| | Non-coated | 3.88 | $P = 0.339$ |
| *P. mirabilis* (IH 50163) | 10 wt.% | 1.63 | |
| | 5 | 2.12 | |
| | 2 | 2.25 | |
| | 0.5 | 4.75 | |
| | Non-coated | 4.25 | $P = 0.007$ |
| *E. coli* (IH 50797) | 10 wt.% | 2.13 | |
| | 5 | 2.00 | |
| | 2 | 2.50 | |
| | 0.5 | 4.13 | |
| | Non-coated | 4.25 | $P = 0.049$ |
| *E. coli* (IH 13258) | 10 wt.% | 1.38 | |
| | 5 | 2.75 | |
| | 2 | 3.25 | |
| | 0.5 | 2.88 | |
| | Non-coated | 4.75 | $P = 0.046$ |

**Table 4** The amount of bacteria in artificial urine (CFU) to CFU of inoculum dose after bacterial inoculation and 3-h incubation with stent material. *h1* 10 wt.% $AgNO_3$ blended ε-caprolactone, *h2* 5 wt.%, *h3* 2 wt.%; *h4* 0.5 wt.%, *p* non coated

| Bacterial strain | Preincubation | Stent coating | | | | |
|---|---|---|---|---|---|---|
| | | h1 | h2 | h3 | h4 | p |
| *P. aeruginosa* (IH 50176) | 1 h | 0.03 | 0.03 | 0.03 | 0.3 | 1.5 |
| | 24 h | 0.00 | 0.00 | 0.02 | 0.1 | 2.0 |
| | 1 week | 0.00 | 0.00 | 0.00 | 0.00 | 1.0 |
| | 2 week | 0.00 | 0 | 0.00 | 0.01 | 0.15 |
| | Mean | < 0.01 | < 0.01 | 0.01 | 0.10 | 1.16 |
| | % | 0.58 | 0.58 | 0.97 | 8.8 | 100 |
| *E. faecalis* (IH 50167) | 1 h | 0.04 | 1.0 | 0.47 | 1.0 | 1.0 |
| | 24 h | 0.13 | 0.47 | 0.01 | 0.53 | 1.67 |
| | 1 week | 0.13 | 0.17 | 0.2 | 0.33 | 0.67 |
| | 2 week | 1.5 | 2.0 | 1.5 | 2.5 | 2.5 |
| | Mean | 0.45 | 0.91 | 0.55 | 1.09 | 1.46 |
| | % | 30.8 | 62.3 | 37.3 | 74.7 | 100 |
| *P. mirabilis* (IH 50163) | 1 h | 0.09 | 2.85 | 2.85 | 2.85 | 2.85 |
| | 24 h | 0.05 | 0.25 | 0.5 | 2.0 | 1.5 |
| | 1 week | 0.2 | 0.3 | 0.5 | 1.0 | 4.0 |
| | 2 week | 0.1 | 0.14 | 2.0 | 2.0 | 2.0 |
| | Mean | 0.11 | 0.89 | 1.46 | 1.96 | 2.58 |
| | % | 4.2 | 34.2 | 56.5 | 75.8 | 100 |
| *E. coli* (IH 50797) | 1 h | 0 | 0.5 | 1.5 | 1.0 | 1.5 |
| | 24 h | 0 | 0.00 | 0.02 | 3.3 | 5.0 |
| | 1 week | 0 | 0 | 0.00 | 0.00 | 1.5 |
| | 2 week | 0.00 | 0.00 | 0.00 | 3.0 | 5.0 |
| | Mean | 0 | 0.13 | 0.38 | 1.83 | 3.25 |
| | % | 0 | 3.8 | 11.7 | 56.2 | 100 |
| *E. coli* (IH 13258) | 1 h | 0.6 | 1.4 | 1.4 | 2.0 | 2.0 |
| | 24 h | 0.00 | 0.00 | 1.0 | 0.75 | 5.0 |
| | 1 week | 0.00 | 0.00 | 2.0 | 0.00 | 10 |
| | 2 week | 0.00 | 0.00 | 5.0 | 2.0 | 5.0 |
| | Mean | 0.15 | 0.35 | 2.35 | 1.19 | 5.5 |
| | % | 2.7 | 6.4 | 42.7 | 21.6 | 100 |

**Table 5** Friedman ranks of various coatings in inhibiting bacterial growth in ambient artificial urine

| Bacterial strain | Stent coating | Rank | Significance |
|---|---|---|---|
| All bacteria | 10 wt.% $AgNO_3$ | 1.40 | |
| | 5 | 2.15 | |
| | 2 | 3.03 | |
| | 0.5 | 3.75 | |
| | Non-coated | 4.68 | $P < 0.001$ |
| *P. aeruginosa* (IH 50176) | 10 wt.% $AgNO_3$ | 1.88 | |
| | 5 | 1.38 | |
| | 2 | 3.00 | |
| | 0.5 | 3.75 | |
| | Non-coated | 5.00 | $P = 0.006$ |
| *E. faecalis* (IH 50167) | 10 wt.% $AgNO_3$ | 1.38 | |
| | 5 | 3.00 | |
| | 2 | 1.88 | |
| | 0.5 | 4.13 | |
| | Non-coated | 4.63 | $P = 0.009$ |
| *P. mirabilis* (IH 50163) | 10 wt.% | 1.00 | |
| | 5 | 2.38 | |
| | 2 | 3.38 | |
| | 0.5 | 4.13 | |
| | Non-coated | 4.13 | $P = 0.008$ |
| *E. coli* (IH 50797) | 10 wt.% | 1.38 | |
| | 5 | 2.13 | |
| | 2 | 3.13 | |
| | 0.5 | 3.50 | |
| | Non-coated | 4.88 | $P = 0.019$ |
| *E. coli* (IH 13258) | 10 wt.% | 1.38 | |
| | 5 | 1.88 | |
| | 2 | 3.75 | |
| | 0.5 | 3.25 | |
| | Non-coated | 4.75 | $P = 0.011$ |

expressed as a ratio of remaining bacteria in the incubation solution (CFU) to inoculated bacteria (CFU) in Table 4. Silver nitrate blended ε-caprolactone/L-lactide copolymer coating inhibited the growth of bacteria in the ambient artificial urine. The inhibitory effect increased as a function of silver nitrate concentration and preincubation time (from 1 h to 2 weeks). In Friedman ranks, the inhibitory effect on bacterial growth in ambient artificial urine significantly correlated to silver nitrate concentration in the coating ($P < 0.001$) when bacteria were analysed as a group. The same tendency applied with a few exceptions (*E. faecalis*) when each strain was looked at singularly ($P = 0.006$–$0.019$; Table 5).

## Discussion

It is known that the ability of uropathogens to adhere to the uroepithelium or to the surface of prosthetic devices is the key event in the pathogenesis of urinary tract infection. Factors influencing the initial attachment of bacteria to a biomaterial include electrostatic and hydrophobic interactions; ionic strength, osmolality and pH of urine; urinary concentration of urea, creatinine and proteins; surface properties of biomaterial and bacterial surface components [16]. Within a few hours, adherent bacteria can aggregate, multiply and form biofilm matrices, which, once surrounded by a dense glycocalyx, may become resistant to antimicrobial agents and constitute a reservoir of viable microorganisms [11–13, 16, 17].

Biofilms can be mineralized with struvite and hydroxyapatite and can facilitate the encrustation of a stent [14, 16, 18]. Encrustation may occur in both infected and sterile urine. Thus, by preventing bacterial adhesion to stent surface, the risk of infection and encrustation can probably be reduced.

Silver nitrate blended ε-caprolactone/L-lactide copolymer coating was effective in inhibiting bacterial adherence to a SR-PLLA stent in vitro. However, weak concentrations, like the 0.5 wt.% sample, paradoxically enhanced bacterial adherence in some cases when compared with uncoated SR-PLLA (*P. aeruginosa* and *E. coli* IH 50797). This may be partly due to the fact that the surface of the coating is rougher than the surface of the non-coated SR-PLLA stent. *Enterococcus faecalis* remains problematic, because even high concentrations of silver nitrate were unable to prevent the adherence of this bacterial strain.

It is very interesting that silver nitrate coating had the ability to reduce bacterial growth in ambient artificial urine. This is also likely to happen in vivo, because there is a microenvironment of urine when the stent pushes prostatic lobes apart. The inhibitory effect was significantly better with higher concentrations (10 and 5 wt.%) and improved further during preincubation of up to a fortnight. *Enterococcus faecalis* was problematic also in this respect, as even the strongest concentrations did not have much effect on the growth of this strain in ambient urine. According to our findings, it is possible to blend silver nitrate into ε-caprolactone/L-lactide copolymer and the product is a suitable material for coating of bioabsorbable SR-PLLA urinary stents. As a result, stents gain bacterial adhesion preventing properties and also a bacteriosidic microenvironment is formed around them. This may reduce the amount of stent-associated urinary tract infections. Before clinical applications of this new material, studies are needed to discover its biocompatibility properties to guarantee its safety to humans.

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