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ORIGINAL PAPER

The efficacy of a novel antibacterial hydroxyapatite nanoparticle-coated indwelling urinary catheter in preventing biofilm formation and catheter-associated urinary tract infection in rabbits

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Abstract The aim of this study was to investigate of the efficacy and reliability of a novel antimicrobial hydroxyapatite (HA) nanoparticle coating of urethral catheters, in the prophylaxis of biofilm formation and bacteriuria in rabbits. A total of 60 male rabbits were randomized to the control and study groups and each group was divided into three subgroups depending on 3, 5 and 7 days of the urethral catheterization period. The rabbits in the study group were catheterized with Ag^{+}-incorporated nano-HA coated urethral catheters and those in the control group with standard silicon–latex urethral catheters. Urine and catheter surface smear samples were conducted for bacteriological analysis. Catheter cross-section samples were undergone measuring of biofilm thickness. Tissue samples of bladder and urethra were inspected for histological changes. The results indicate that at the end of 7 days of the catheterization period, the number of the rabbits with bacteriuria was significantly lower in the study group versus control group ($p^{\P} = 0.020$). The biofilm formation on luminal surface of the catheters was significantly thinner in the study group versus control group, at the end of 5 and 7 days of the catheterization period (0.035 and 0.035, respectively).No histological adverse change or particle penetration was detected in the urothelium. In conclusion, it was observed that Ag^{+} + HA nanoparticle coating significantly lowered the incidence of catheter-related bacteriuria and decreased biofilm formation, at the end of 7 days study period. The novel antimicrobial urethral catheter coating appeared to have a potential in the prophylaxis of catheter-induced urinary tract infections.

Keywords Catheter-associated urinary tract infection · Biofilm formation · Urethral catheter coating · Hydroxyapatite nanoparticles

Abbreviations and anonyms

SEM	Scanning electron microscopy
EDX	Energy dispersive X-ray spectrophotometer
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
HA	Hydroxyapatite

Introduction

Urethral catheterization-related urinary tract infections have been the most frequent cause of hospital acquired infections. Even short-term catheterization, up to 7 days will charge 10–30% of the patients with acute urinary infection [1]. Urinary tract infections acquired in health institutes not only increase the rate of complications such as pyelonephritis, urolithiasis or bacteremia, but also threefold the mortality rate [2].

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Biofilm formation, on luminal and extraluminal surfaces of indwelling urethral catheters, protects the residing bacteria from antimicrobial penetration and facilities an environment, in which, bacteria acquire genotypic resistance against antimicrobials and the ability to transfer this resistance into the adjacent cells. Biofilm formation also triggers encrustation and impaction of the catheters by adhering calcium, magnesium, ammonium and phosphate crystals on them. Preventive measures, ranging from irrigation of the catheters with antiseptic agents, producing catheters from smooth surfaced silicon instead of rough surfaced latex, to coating catheters with hydrophilic polymers, antimicrobial agents, silver salts and alloys, all, have yielded variable benefits. However, applying these preventive measures has been costly and the benefits have lasted for a short period of time [3–7].

In our experimental study, we aimed to investigate the efficacy and reliability of a novel biocompatible sustained silver ion liberating and nano-HA based coating of indwelling urethral catheter, in protection from biofilm formation and catheter-emergent urinary tract infection.

Materials and methods

Preparation of the coating material

Silver ion-incorporated calcium phosphate powder was prepared in Material Science and Engineering Department of Anatoly University Engineering and Architecture Faculty, using an aqueous chemical method. Silver ions were dissolved completely in equal amount of pure water and mixed with calcium hydroxide. Orthophosphoric acid was added to the chemical reaction, drop by drop, while stirring with a magnetic stick. In order to form a stoichiometric hydroxyapatite structure pH was strictly controlled. The precipitate thus formed during the reaction was filtered and dried at 80°C. By adding metal ions and adjusting the final pH to 5.5, powder form was produced. Ag^{+} ion containing powder was sequentially dried, blended and sieved to yield 38–78 μm particles.

Coating procedure of the catheters

The silico-latex two-way indwelling catheters were soaked to a bath of toluene–isopropyl alcohol solution aiming to clear the surfaces from antioxidative and catalyzing residual compounds. Silver ion-incorporated hydroxyapatite powder was mixed with silicon elastomers and a catalyzer was added to the formed suspension. The catheters were soaked in the suspension and incubated at 50°C for 3 h to have the coating material adhere to both the luminal and extraluminal surfaces. In this step, polymeric chains, formed by cross-binding among the acetoxy groups enhanced the adhesion of the material. The antimicrobial efficacy of the coating was checked using the in vitro Halo and contact test methods. The coated catheters were packed individually and sterilized using ethylene oxide.

Animals and experiment design

The study was approved by the Ethics Committee of the Çukurova University Medical Sciences Experimental Research and Application Center where the experiment would be proceeded. A total of 60 male New Zealand rabbits, weighing 3–4 kg, were randomly divided into the study and control groups following an acclimation period of 7 days. The rabbits were housed individually in stainless steel cages. A special wooden collar resembling a headlock was designed and fixed at the front part of the bottom of the cage to stabilize the head and two soft leather belts were used to fix the rear legs to the bottom of the back part of the cage. These fixation aids permitted free access to food and water, but prevented disturbances to catheter, penis and the closed drainage system. All the rabbits were fed with hay-based pellets with an aim to lower the dietary calcium.

The rabbits were anesthetized with im. injection of 25 mg/ml ketamine and 20 mg/ml xylazin mixture at a dose of 0.7 ml/kg. The perineal fur was cleansed with 10% povidone-iodine solution. Following appropriate covering and lubrication, under strict sterility precautions, the rabbits of the control group were catheterized with non-coated 8 Fr silico-latex two-way Foley (Rüsch® ™) urinary catheters. The rabbits of the study group were catheterized with antimicrobial nano-HA coated 8 Fr silico-latex Foley (Rüsch® ™) catheters. Urinary outlet of the urethral catheters were tightly connected to collecting urinary bags and by passing the tube through the bars of the cage the bags were suspended from the bottom to achieve an instant downward flow through each.

Procedure

A total of 60 rabbits were randomly allocated to the nano-HA coated and non-coated catheter recipient groups, in equal numbers. Both of the two groups were, then, randomly and equally subdivided into three groups according to 3, 5 and 7 days of the catheterization period. At the end of each catheterization period, urine specimens, taken from each rabbit by mid-catheter aspiration, were sent for bacteriological analysis. The bacterial growth over 10^2 cfu/ml in each aspirated urine specimen was defined as bacteriuria. Subsequently, catheters were withdrawn and at a point 1 cm proximal from the tip, including the drainage eyes, catheters were transected. Cotton swab specimens of the

luminal surfaces taken from 1 cm depth of the proximal part of the severed section were sent for bacteriological analysis The bacterial growth over 10^2 cfu/ml was considered as positive. The distal 1 cm long tip segment of each catheter was individually immersed in a transferable culture media and sent to Anatoly University where scanning electron microscopic (SEM) evaluations and measurements would be carried out. For the SEM analysis, each catheter specimen was fixed in 5% glutaraldehyde buffered with (0.1 M/L, pH 7.2) cacodylate at 4°C for 24 h. Following the post-fixation procedure with 1% osmium tetra oxide, the catheter specimens were dehydrated in ethylene alcohol and trichlorotrifluoroethane, and dried in a CO^2 dryer. The dried specimens were then taken on cupper stick and coated with a thin gold–palladium layer. Inspections were done using SEM (Zeiss Supra 50 VP) under 15 kV. A cross section from each catheter specimen was taken at the same distance from the severed tip and the thickness of the most prominent biofilm layering was measured and recorded.

At the end of each study period, the rabbits were killed by lethal anesthesia. Urethra and bladder specimen from each rabbit was excised en block and preserved in 4% paraformaldehyde phosphate buffer until histopathological evaluation was undertaken. Tissue sections were stained with haematoxylene eosin and inspected at 40× magnification.

Statistical analysis

Categorical variables such as the bacterial growth and the bacterial species that grew were summarized as the number of cases and proportions. Continuous variables such as biofilm thickness were summarized as the mean ± standard deviation (SD). Bacterial growths with respect to the catheterization period between the control and the study groups were compared using the Pearson's Chi-square test. Variables about the number of growing bacteria and biofilm thickness in these groups were compared using the nonparametric Mann–Whitney *U* test, since parametric test assumptions were not accomplished. All the analyses were conducted using the Statistical Package for Social Sciences, for Windows, version 16 (SPSS, Chicago, IL). In all tests, $p < 0.05$ was considered statistically significant.

Results

Bacteriological outcome

All the animals in each group completed the study. Catheter-associated bacteriuria developed in 14 of the 60 catheterized rabbits (23.3%). The number of bacterial growth on cultures from mid-catheter urine aspirates and from cotton swabs of luminal surfaces with respect to the catheterized period of the groups were listed in Table 1. Overall, bacteriuria developed in 10% of the rabbits in the study group, in contrast to 36.7% in the control group; the difference being significant ($p^{\P} = 0.030$). The swab cultures of the luminal surfaces were negative in all of the rabbits in the study group; however, were positive in 18% of the rabbits in the control group and the difference appeared significant ($p^{\P} < 0.01$).

The number of rabbits with positive culture on urine samples, at the end of 3 and 5 days of the catheterization period, were not significantly different between the study and control groups ($p^{\P} = 0.999$, for each). However, at the end of the 7-days catheterization period the rate of acquired bacteriuria was significantly lower in the study group compared with the control group ($p^{\P} = 0.020$). Although, at the end of 3 days of the catheterization period, the number of catheter surface swab culture positive rabbits was not significantly different between the two groups, ($p^{\P} = 0.087$), at the end of 5 and 7 days of the catheterized periods, it was significantly lower in the study group versus the control group ($p^{\P} = 0.033$ and $p^{\P} < 0.001$, respectively).

Within each catheterization period, the number of urine and catheter surface culture positive rabbits did not differ significantly in both of the groups ($p^{\S}$ values in Table 1). Polymicrobial bacteria was detected in cultures of nine rabbits, however, neither growth occurred over 10^2 colonies. The species of bacteria, recovered in urine and on catheter surface samples of each rabbit, were also in harmony. The distributions of the number of bacteria species that positively grew in urine and on catheter surface samples, with respect to indwelling time, were shown in Table 2.

E. coli was the most frequent bacteria recovered in urine and on catheter surface in the control group, followed by, in a decreasing frequency, *Staphylococcus* species, *Proteus mirabilis* and *Enterobacter cloacae*.

Effect on biofilm formation

The SEM examination revealed a smoother surface on the nano-HA coated silico-latex catheters compared with non-coated catheters (Fig. 1). Biofilm node thickness did not differ significantly between the luminal surfaces of the coated and non-coated catheters that remained 3 days in situ ($p = 0.579$) (Table 3). However, node thickness was significantly higher on luminal surfaces of the non-coated catheters compared with the nanoparticle-coated catheters that remained 5 and 7 days in situ ($p = 0.035$, each). Figure 2 represents cross section SEM view of samples from 7-day retained coated and non-coated catheter.

Table 1 Bacterial growth in urine and on catheter surface

	Nano-HA coated group				Non-coated group							
	Catheterization period (days)				Catheterization period (days)							
	3	5	7	Total *n* (%)	3	$p^{¶}$	5	$p^{¶}$	7	$p^{¶}$	Total *n* (%)	$p^{¶}$
Urine	0	2	1	3 (10)	1	0.999	3	0.999	7	0.020	11 (36.7)	0.030
Surface	0	0	0	0 (0)	4	0.087	5	0.033	9	<0.001	18 (60)	<0.01
$p^{§}$	0.999	0.474	0.999	0.237	0.303		0.650		0.582		0.120	

$p^{§}$ In urine versus on catheter surface growth within the same catheterization period

$p^{¶}$ Coated versus control group within the same catheterization period

n Number of rabbits with positive growth on cultures

Table 2 Number of bacterial species grew in urine and on catheter surface with respect to the indwelling time

Bacterial species	Nano-HA coated group								Non-coated group							
	Urine (days)				Cath. surface (days)				Urine (days)				Cath. surface (days)			
	3	5	7	Total	3	5	7	Total	3	5	7	Total	3	5	7	Total
Escherichia coli									1	2	2	5	2	2	3	7
Staphylococcus species		1		1							4	4			4	4
Proteus mirabilis										1		1	1	3		4
Enterobacter cloacae											1	1	1		1	2
Pseudomonas aeruginosa															1	1
Pseudomonas alcaligenes		1		1												
Acinetobacter haemolyticus			1	1												
Total		2	1	3				0	1	3	7	11	4	5	9	18

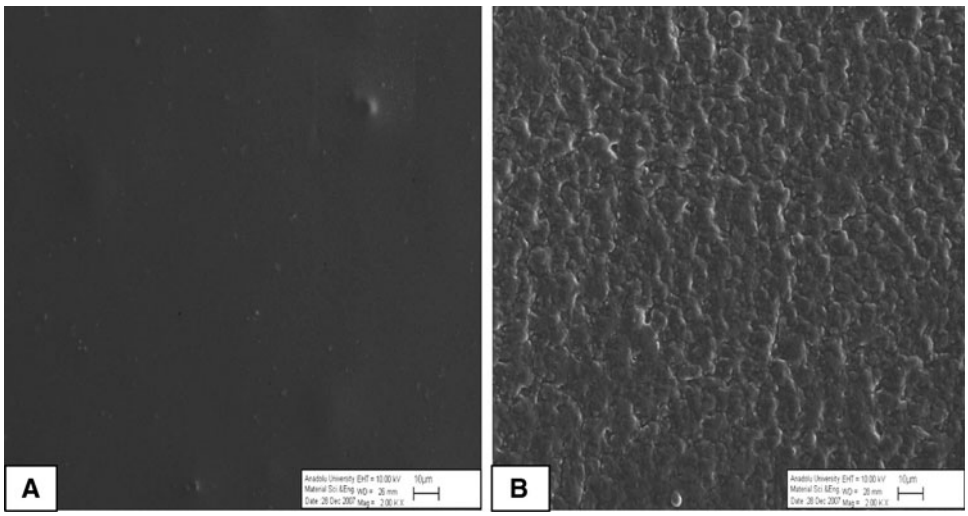

Fig. 1 Scanning electron micrographs of extraluminal surfaces from samples of unused nano-HA coated (**a**) and non-coated (**b**) indwelling urethral catheters (×2,000)

Safety

Histopathological evaluation of the urethra and bladder specimens removed from coated catheter recipient rabbits did not reveal any particle migration into the mucosa and submucosa or any sign of atypia.

Discussion

Since 20 years, hydroxyapatite based bioceramics have widely been used as surface covering of dental implants, bone cement and bone supplement. The porous structure provided by nano-HA microspheres facilitated sustained

Table 3 Average biofilm thickness on luminal surface of the nano-HA coated and uncoated catheters with respect to the indwelling time

Catheterization period (days)	Coated group (μm)	Non-coated group (μm)	*p* value
3	9.50 ± 6.04	21.30 ± 29.57	0.579
5	9.50 ± 6.45	56.50 ± 60.05	0.035
7	9.90 ± 7.80	47.50 ± 61.38	0.035

Values are mean ± SD

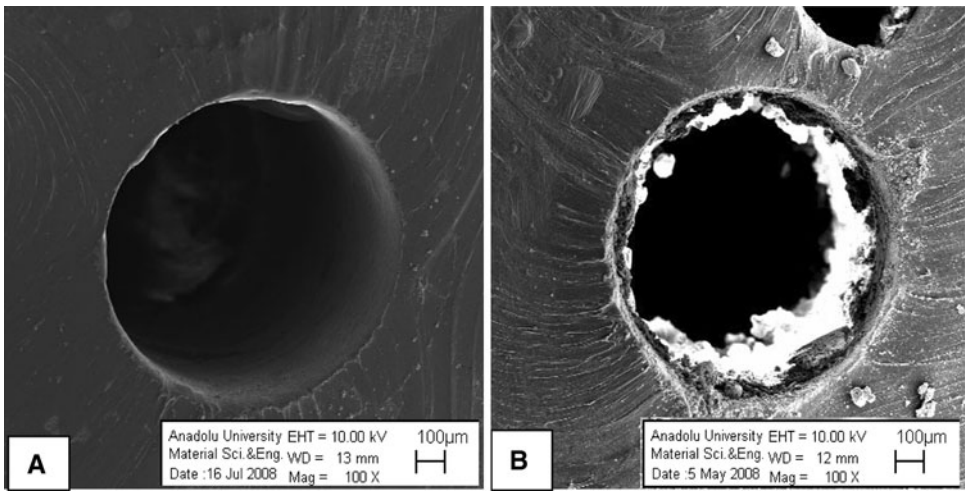

Fig. 2 Scanning electron micrographs of cross sections from samples of 7-day in situ remained nano-HA coated (**a**) and non-coated (**b**) indwelling urethral catheters (100×)

release of various enzymes and antibiotics. Our previous in vitro experiments evaluating the antimicrobial activity of a novel sustained Ag^+ ion liberating nano-HA based bioceramic powder revealed significant activity against bacterial species including *E. coli*, *P. aeruginosa*, *S. aureus* and *C. albicans* [8]. This outcome encouraged us to use the powder as urinary catheter coating. Our current trial examining the in vivo efficacy of nanoparticle coating of indwelling urethral catheter in a rabbit model revealed an overall 3.6-fold difference in the number of participants with bacteriuria between the coated and non-coated catheter recipient groups, in favor of the former. Only one rabbit was with bacteriuria in the study group, in contrast to seven in the control group, at the end of 7 days of catheterization. The nano-HA coating provided antimicrobial activity against biofilm-resident bacteria in the same extend as did to the planktonic bacteria. Of the 30 smear cultures obtained from the biofilm deposits on luminal surface of the non-coated catheters at the end of 7-days catheterization, nine were positive, while, none were positive obtained from those on luminal surface of the coated catheters. A striking finding was that antimicrobial activity against both planktonic and biofilm-resident bacteria were equivalently provided, regardless of the bacterial species. The infections due to the most common microorganisms recovered in urine and on catheter surface of the control group, being as *E. coli*, Staphylococcal species including *S. aureus*, *P. mirabilis* and *E. cloacae*, were significantly avoided using the coated catheter. Data from the clinical studies have demonstrated that among the range of species commonly isolated from catheter-associated urinary tract infections, *P. mirabilis*, a urease splitting bacteria, was the most effective at producing catheter blocking crystalline encrustation and at triggering episodes of bacteremia and septic shock [9]. Ag^+ enriched nano-HA coating in our rabbit model provided significant protection against *P. mirabilis* in as much as did against *E. coli* and Staphylococcal species, which, were the most common cause of catheter-emergent urinary tract infection in human. *E. coli*, although do not have the ability to hydrolyze urea, participate in biofilm formation by readily attaching to surfaces using pili and motility [10].

Clinical studies have revealed that closed drainage use and avoidance of catheter care violations lowered catheter-associated infections to 3–10%/catheter-day [11]. In our study, following 3 and 5 days of catheterization period, bacteriuria rates in the non-coated catheter recipient rabbits appeared to be 10 and 30%, respectively. Additionally, polymicrobial growth was recorded in none of the rabbits throughout the 7-day catheterization period. These rates were almost near to the rates reported for human in clinical studies. Hence, these findings confirm that meticulous care and precaution in insertion and during maintenance of the catheters were fulfilled in our rabbit model and thus help to emphasize the interpretation of our results. In most of the studies in the literature, including those that were reviewed in Cochrane database, urinary tract infection or bacteriuria was defined as growth over 10^5 cfu/ml [12]. The lower threshold applied in defining the bacteriuria, as growth over

10^2 cfu/ml, in our study provided an additional support in interpretation of the favorable outcome.

Researchers have shown that biofilm colonization maturated as early as 12–24 h following insertion of urinary catheters [13]. We measured biofilm thickness starting 72 h after insertion. In accordance with the finding of Stickler that encrustated biofilms extensively develop in the eye-hole region, in our study measurements included the eye-hole region [13].The SEM examination revealed that the surfaces of the catheters coated with HA nanoparticles appeared to be smoother than the non-coated latex catheters, contradicting the well-known fact that HA constitutes the major component of the crystalline deposits on biofilm formations. This outcome can be explained by the fine, tight web-like layering of the 200 nm particles and, mixture with silicone elastomers. Mean thickness of the biofilm nodes formed on luminal surfaces of the coated catheters at the end of 3 days of catheterization, although, were lower than those formed on the non-coated catheters; 9.50 ± 6.04 μm versus 21.30 ± 29.57 μm; this more than twofold difference did not appear to be significant owing to the extreme values dispersing the distribution in a relatively small sample size. However, at the end of 5 and 7 days of the catheterization periods the mean biofilm thickness in the coated catheters was significantly lower compared with the non-coated counterparts. More than fivefold difference in the median thickness of the biofilm formation in 7 day catheterized rabbits predicted that the efficacy of the nanoparticle coating would have been preserved for a longer catheterization period. Unfortunately, relatively low number of the participated animals and the short-term catheterization, in a retrospective review, constituted a limitation for our study. Since Akiyama and Okamoto, who were the first to describe the use of silver coated urinary catheters, a great deal of studies have investigated the protective advantage of urinary tract catheters coated with silver salts and alloys [14]. Silver oxide catheters were not found to prevent bacteriuria in short-term catheterized hospitalized adults in the three trials included in the analysis of Cochrane Database Systematic Review in 2008 [11]. However, from the nine trials collated in this review, silver alloy coated catheters, were found to be effective at reducing the incidence of asymptomatic bacteriuria at both less and more than 1 week of catheterization. Our coating material differs from silver alloy that Ag^+ ions are incorporated in small proportions being as low as 2%, while providing a benefit of sustained release within the nano-HA layout. Our previous in vitro pilot study, subjecting 2% Ag^+ containing powder to Energy Dispersive X-ray (EDX) Spectrophotometer analysis of liberation in aqueous media, revealed only 1.62% Ag^+ loss throughout a period of 10 days [8]. Significant difference, achieved in favor of the nano-HA coated catheters at the end of 7-day catheterization in the current study on animal model, in consistent with our former in vitro experiment, supported the likelihood of longer term efficacy. Additionally, small amount of silver incorporated in the HA powder actually translated into lower production cost of the coating, which amounted to only an additional 25% cost per catheter in the experimental setting.

The majority of the studies on human reported no toxicity for silver coated urinary catheters except black discharge and irritation which affected as low as 10% of the recipients [15]. In our rabbit model, we did not notice any black discharge, owing to the low amount of the incorporated Ag^+. Ag^+ incorporated HA nanoparticle-coated catheters were not black in color but rather a smoky form of the original color. Histopathological evaluation of the urethra and bladder specimens removed from the coated catheter recipient rabbits did not reveal any sign of disturbed or altered uroepithelial integrity. Neither sub or intra epithelial particle migration nor any sign of atypia was detected in any of the specimen. Thus, histological stability preserved in our animal model appeared to be in consistent with our former study in which 10 μg/ml aliquot of 0.5–2% Ag^+ containing coating powder specimens were subjected to in vitro cytotoxicity tests based on V79 cell cultures containing MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] and that revealed no toxicity [8].

Conclusion

A novel sustained silver ion liberating nano-HA particle based coating for the indwelling urinary catheters provided strong evidence in prevention of bacteriuria, in our pilot study on animal model. The nano-HA coating appeared to be safe and cost-effective. A clinical trial, addressing the efficacy and safety of the nano-HA coating of urinary catheters in human, throughout a longer catheterization period and concerning the aspect of comfort, is in progress.

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