Materials Science & Engineering C 93 (2018) 782–789

Contents lists available at ScienceDirect

Materials Science & Engineering C

journal homepage: www.elsevier.com/locate/msec

A novel cysteine-linked antibacterial surface coating significantly inhibits bacterial colonization of nasal silicone prongs in a phase one pre-clinical trial

Jacob Odeberg[a,b,c,d,*], Anders Wirsén[b], Åke Norberg[b], Jakob Frie[e], Gordana Printz[e], Hugo Lagercrantz[e], Gudmundur H. Gudmundsson[f], Birgitta Agerberth[b,g], Baldvin Jonsson[e]

[a] School of Biotechnology, Royal Institute of Technology (KTH), Science for Life Laboratory (SciLifeLab) Stockholm, Sweden
[b] CytaCoat AB, Stockholm, Sweden
[c] Department of Medicine, Karolinska Institutet, Stockholm, Sweden
[d] Centre for Hematology, Karolinska University Hospital, Stockholm, Sweden
[e] Department of Women's and Children's Health Karolinska Institutet, Sweden
[f] The BioMedical Center of the University of Iceland, Iceland
[g] Department of Laboratory Medicine, Karolinska Institutet, Huddinge, Sweden

ARTICLE INFO

Keywords:
Antibacterial coating
Cysteine ligand
Covalent binding
Phase one trial

ABSTRACT

Ventilator associated pneumonia and sepsis are frequent complications in neonatal care. Bacterial colonization of medical devices and interfaces used for respiratory support may contribute by functioning as a bacterial reservoir seeding bacteria into airways. We have developed an antibacterial surface coating based on a cysteine ligand covalently coupled via a spacer to a carboxylic backbone layer on an acrylic acid grafted silicone surface. This coating was applied on a commercially available nasal prong and the antibacterial effect was evaluated both in vitro and in vivo in a first-in-human phase 1 trial. The coated nasal prongs had strong antibacterial activity against both Gram-negative and Gram-positive bacteria in vitro. In a randomized pre-clinical trial study of 24 + 24 healthy adult volunteers who carried coated or non-coated nasal prongs for 18 h, a ^{10}log difference in mean bacterial colonization of 5.82 ($p < 0.0001$) was observed. These results show that this coating technique can prevent colonization by the normal skin and mucosal flora, and thus represent a promising novel technology for reduction of medical device-associated hospital acquired infections.

1. Introduction

The European Centre for Disease Prevention and Control (ECDC) estimate that hospital acquired infections (HAIs) affect > 4 million patients each year in Europe and at least 37,000 die as a direct result thereof [1]. The problem of HAI is accentuated by the increased prevalence of multi-resistant bacteria [2,3]. Despite implementation of stricter hygiene routines with the aim to reduce HAIs below 5%, the average prevalence of HAI was still over 8% in Sweden in 2014 [4].

Many of the HAIs can be directly linked to the use of different medical devices in contact with patients, and the sequent bacterial colonization on the surface of the device. Such HAIs can include blood stream infections originating from central venous catheters, catheter-associated urinary tract infections, and ventilator-associated pneumonia (VAP). The bacteria can originate from the patient, from health care workers in close contact with the patient, and from the immediate surroundings.

Intubated patients on respirator support in an intensive care unit are at high risk of VAP with increasing risk depending on the duration of the use [5]. Premature babies are especially vulnerable to HAI/VAP because of their immature immune system and immature and/or compromised natural protective barriers (skin, gut, lungs) [6]. Many infectious episodes and cases of sepsis in neonates are related to the use of intubation and respirator support, starting with a VAP that rapidly spreads over the immature lung barrier and into the blood stream. The most common mode of respiratory support for preterm infants is, however, non-invasive. Most neonates on non-invasive respiratory support are connected to the gas delivery system (ventilator or dedicated CPAP driver) via nasal prongs. Only a small proportion is treated with invasive ventilation via an endotracheal tube connected to the

* Corresponding author at: School of Engineering Sciences in Chemistry, Biotechnology and Health, Royal Institute of Technology (KTH), SciLifeLab, Box 1031, 171 27 Solna Sweden
E-mail address: Jacob.Odeberg@scilifelab.se (J. Odeberg).

https://doi.org/10.1016/j.msec.2018.08.040
Received 16 October 2017; Received in revised form 9 July 2018; Accepted 18 August 2018
Available online 23 August 2018
0928-4931/

ventilator. In the 2015 yearly report from the Swedish Neonatal Quality Register (SNQ) [7], > 80% of all babies born before gestational week 32 were on CPAP support, with a median duration of 35 days. In Sweden, > 20,000 accumulated days of respiratory support via a nasal prong were reported in 2015. The proportion of children that acquired a nosocomial 'late onset' infection (debut ≥3 day in the unit) was 5–17% for infants born before 32 weeks of gestational age.

Devices that are colonized by bacteria will be a bacterial reservoir that continuously can seed bacteria into the airways. Preventing bacterial colonization and biofilm formation on the surface of devices used for respiratory support in neonatal units could significantly reduce the risk of VAP and VAP-associated sepsis. However, no technical solution for preventing colonization of devices has successfully been introduced in this setting, and thus new technologies are urgently required to address this problem.

The function of antibacterial surfaces that are currently in use on medical devices is commonly based on the release of bactericidal agents such as silver ions, chlorohexidine, sulfadiazine, triclosan, and nitrofurazone alone [8], or in combinations [9], and to some extent antibiotics [10]. These surfaces usually have a decreasing effect over time and the release of antibacterial agents represent a potential risk for creating bacterial resistance. Therefore it is of great interest to develop antibacterial surfaces with non-releasing antibacterial layers, with longer lifetime and minimized or eliminated leakage [11,12]. Recent developments in this field have focused on the attachment of biocidal polymers carrying cationic groups, mostly quaternary amines such as poly(alkylpyridine) [13], quarternized polyethyleneimine [14], and alkylated amino functional polyacrylates [15]. In addition, an inorganic concept has been used on urinary catheters consisting of an alloy of noble metals applied as a very thin layer on the catheter surface [16] [17].

The aim of our research was to develop a surface with a stable leakage-free antibacterial coating based on nontoxic, biocompatible organic components forming a molecular structure that prevents colonization of bacteria. In this study we have identified promising structures containing a ligand constituted by cysteine or cysteine derivatives covalently bound via a disulfide bond to an aliphatic spacer. The spacer is bound by an amide bond to a carboxylic backbone layer generated on the substrate via electron beam initiated grafting of acrylic acid. In this investigation we applied the coating onto nasal prongs of silicone and evaluated the antibacterial effect with respect to stability, biocompatibility, toxicity and efficacy when exposed to a normal skin flora in a first-in-human phase one trial.

2. Materials and methods

2.1. Medical device

The nasal prong (NP) used (nCPAP NP, Inspiration Healthcare, Crawley, UK) is a commercially available medical grade silicone device intended to provide an interface for delivery of air supplemented with oxygen, aerosols, and nebulizers in non-invasive ventilation (Fig. 1A and B). The NP is intended for single-use on neonatal children in a hospital setting.

2.2. Reagents

Acrylic acid, L-cysteine monohydrochloride, 2,2′-Dipyridyldisulfide, 2-aminoethanethiol, *N*-(3-dimethylamino-propyl)-*N*-ethylcarbodiimide HCl (EDC), *N*-hydroxysuccinimide (NHS) and Crystal Violet were all from Acros (Fair Lawn, USA) and ascorbic acid from Scharlau (Barcelona Spain). All chemicals were of analytical grade and used as received. Deionized water was used in all aqueous solutions and in the washing steps unless otherwise noted.

Synthesis of 2-pyridyl-2-aminoethanedisulfide monohydrochloride (PDEA) vas made by reacting aminoethanethiol with 2,2′-Dipyridyldisulfide as previously described [18]. Briefly, 4.29 g of mercaptoethylamine hydrochloride was dissolved in a mixture of 4.54 mL of methanol and 3.0 mL of acetic acid and was then added dropwise to a stirred solution of 25 g of 2,2′- dithiopyridine in 113 mL of methanol during 10 min. The yellow reaction mixture was stirred for 60 min at room temperature and was then slowly poured into a beaker with 800 mL of stirred ether, where it precipitated. The product that was separated from the ether phase was dissolved in a small volume of methanol and was again precipitated by ether. This procedure was repeated until the crystals appeared white, and the product was then dried under vacuum. The synthesised PDEA structure was verified by FTIR-ATR analysis using a Perkin Elmer Spectrum Two instrument by comparing with a reference PDEA obtained from a commercial source (Bioacore, Uppsala, Sweden). (Supplementary Fig. 1).

2.3. Electron beam (EB) grafting

The grafting of nasal prongs was initiated to a dose of 10 kGy using a 6.5 MeV pulsed electron accelerator (Microtrone, Acceleratorteknik, Royal Institute of Technology, Stockholm, Sweden) with the nasal prongs immersed in an aqueous grafting solution containing acrylic acid (10%) and a homopolymerisation inhibitor (0.001 M $Cu(NO_3)^2$) together with a small amount of detergent (0.3‰) TritonX-114 to increase wetting.

2.4. Characterisation of carboxylated surfaces

After EB grafting with acrylic acid, quantification of the carboxylic surfaces was performed by titration with 0.01 M HCl after exposure to a predetermined amount of 0.01 M NaOH for 6 h. The result was compared to titration of the same amount of NaOH without a sample using a Metrohm Titrino Plus 848. The uniformity of the acrylic acid grafted surfaces of the NPs was analysed by staining in an aqueous solution containing 25% by weight of methanol and 0.5% by weight of Crystal Violet for 10 min. After thorough washing in water the coloring of the coated NPs was compared with a stained ungrafted silicone NP.

2.5. Ligand coupling

The ligand was attached to the carboxylated surface by chemical coupling reactions essentially as reported in the literature [19]. In short the carboxylic groups in the grafted layer were activated with an aqueous solution of EDC/NHS and then reacted with PDEA. Finally the surface bound PDEA was reacted with L-cysteine. The sequential reactions with EDC/NHS, PDEA and L-cysteine were performed at room temperature (RT) during 10 min for each reaction with intermittent washing in deionized water. Formation of the disulfide bond was analysed by measuring the leaving group 2-mercaptopyridine by UV at 343 with a Liber Biochrome spectrometer. The amount of L-cysteine coupled via disulfide binding was calculated from the amount of released 2-mercaptopyridine using an extinction coefficient of 8060 $cm^{-1} M^{-1}$ [18]. The coated NPs were shaken for 20 h in 60 mM phosphate buffer, pH 7.4 at RT and washed in deionized water and dried. After coupling of the ligands the surfaces were equilibrated to pH 7.4 in 60 mM phosphate buffer over night for 20 h and thoroughly washed in deionized water. For comparison the phosphate buffer was occasionally replaced by Phosphate Buffer Saline (PBS) for 20 h at 37 °C. No signs of PDEA or 2-mercaptopyridine could be seen in the washing solutions on UV on a Liber Biochrome spectrometer at 343 and 282 nm respectively. Analyses of solid reactants were made by FTIR-ATR using a Perkin Elmer Spectrum Two instrument. After coupling of the ligand to the polyacrylic acid surface, the final 'CytaCoat' surface coating was analysed by XPS on a Kratos AXIS $Ultra^{DLD}$ x-ray photoelectron spectrometer using a monochromatic Al x-ray source, using a non-coated commercial prong for comparison of spectras.

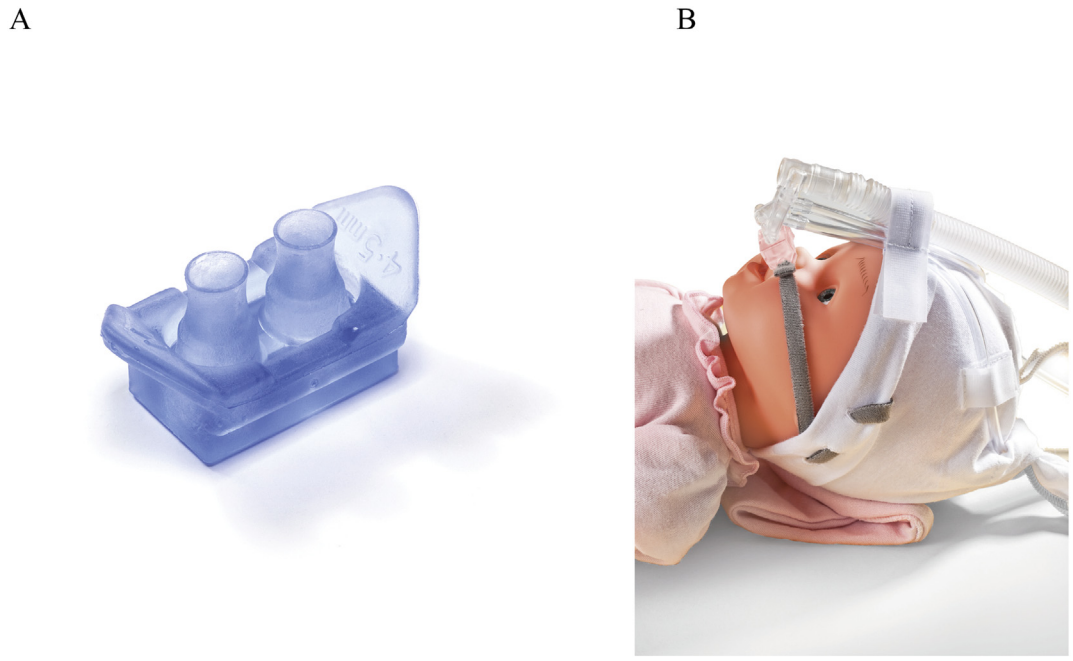

Fig. 1. Nasal prong.
The nasal prong (Inspiration Healthcare Ltd) used for coating (A). The nasal prong when mounted with tubing on a model (B). (Image of the Inspire nCPAP reproduced with permission from Inspiration Healthcare Ltd. (https://www.inspiration-healthcare.com/).

2.6. In vitro assay of antibacterial coating on the Nasal Prong (NP)

Colonies of *S. aureus* B5381 or *E. coli* D21 were transferred from a frozen stock culture to a respective Luria-Bertani (LB) agar plate and incubated at 37 °C for 20 h. From the respective agar plates, approximately 10 colonies were picked and transferred to LB broth in a culture flask and incubated at 37 °C, while shaking at 170 rpm, until $OD^{590} = 0.40$. This OD corresponded to a bacterial count of approximately 10^8 cfu/mL of *S. aureus* and 10^9 cfu/mL for *E. coli,* which was diluted to a bacterial concentration of 2×10^4 cfu/mL for *S. aureus* and 5×10^5 cfu/mL for *E. coli*. Of this final dilution 10 mL of the bacterial suspension was added to a 50 mL test tube containing the NP and incubated at 37 °C. After 3 h incubation, 100 μL of the respective bacterial suspensions were aliquoted for serial dilutions, which were spread on agar plates and incubated 20 h at 37 °C with subsequent cfu counting. The NP was removed from the test tube with flamed forceps, vortexed 1–2 s in 10 mL PBS, pH 7.4. This procedure was repeated 3 times and followed by a vortex for 1–2 s in 10 mL LB broth. From this LB solution 100 μL were taken for serial dilutions, which were spread onto agar plates and incubated at 37 °C for 18 h and 22 h for *S. aureus* and *E. coli*, respectively. The NPs from the respective bacterial cultures were then vortexed 1–2 s in 10 mL PBS. Between each washing step, each NP was carefully put onto sterile medical wipes to remove excess fluid. Finally, each NP was placed in 10 mL sterile LB and incubated for 20 h at 37 °C, while shaking at 170 rpm. From this solution 100 μL aliquots were removed for serial dilution, spread on agar plates and incubated overnight at 37 °C with subsequent cfu counting.

2.7. Biocompatibility and toxicity tests

Biocompatibility and toxicity tests were performed by Envigo Research Ltd. (Shardlow, UK), according to requirements for a class 2a medical device [20]. The tests included extraction assay, lymph node assay, skin irritation assay, and in vitro cytotoxicity (ISO10993-10:2010, ISO10993-12:2012, ISO 10993-5:2009).

2.8. Study subjects

In a prospective double-blinded phase 1 study, 48 healthy adult volunteers aged 18–60 years were included after receiving verbal and written information and having signed informed consent. Each subject was evaluated for eligibility by a physical examination, review of medications and confirmation of the inclusion and exclusion criteria. Included subjects had intact skin at the NP application site with no wounds or scars. Furthermore, the following exclusion criteria were applied: 1) Pregnant or nursing women, 2) active smokers or snuff users, 3) subjects suffering from and/or had experienced any nosebleeding within 1 month prior to entering the study, 4) usage of oral or topical antibiotics within 2 weeks prior to entering the study, 5) usage of oral or topical anti-inflammatory drugs within 1 week prior to entering the study, 6) participation in any other clinical study, 7) a diagnosis of any type of skin infection (bacterial, viral or fungal) or inflammatory skin diseases including psoriasis, eczema or severe acne, 8) a diagnosis of any type of disease affecting mucus membranes, 9) subjects suffering from any other condition or symptoms preventing the subject from entering the study, according to the investigator's judgment, 10) having a lesion (including lymphadenopathy), dysaesthesia, previous surgery or abnormal anatomy at the NP target site, and 11) deemed unsuitable for study enrolment according to the Declaration of Helsinki.

2.9. Study design

The study consisted of two visits: One screening/baseline visit and one follow-up visit after 18 h. An eligible subject were randomized (1:1) to either the treatment group (wearing the coated NP) or to the control group (wearing the reference non-coated NP). The corresponding NP was fitted onto the nose of the subject, protruding partly into nostrils and secured using ViTri™ suture tape (ViTri Medical, Stockholm). The subject received instructions on how to refit the NP if necessary. The subject was wearing the NP continuously for 18 h until the follow–up visit the day after. At the follow-up visit, the NP was removed by the subject and placed in a sterile container, which was covered with sterile PBS and kept at +4 °C for maximum 4.5 h including the time of

shipment to the external accredited contract laboratory (Mikrolab AB, Sollentuna, Sweden) for analysis of bacterial colonization by a Colony Forming Unit (cfu) based assay (see below). The subjects were asked about any medications used during the study and if any adverse events (AEs), discomfort, or need to refit the device during the 18 h had occurred. A physical examination of the nasal prong target site, i.e. the area in and around the nose, was also performed by a physician. If no severe adverse event (SAE) was reported the subject was considered as having completed the participation in the study, with no further follow up.

2.10. Colony forming unit (cfu) assay

At the contract laboratory, the NPs were removed from the storage buffer and washed in PBS twice and placed in a nutrient solution of Tryptic Soy Broth (TSB) and incubated at 37 °C. Aliquots of 100 μL were taken from the nutrient solution at 4 h and 18 h of incubation and serially diluted and plated on blood agar plates. The plates were incubated at 37 °C for 20 h and plates with dilution giving 30–300 colonies/plate were selected for CFU counting to calculate cfu/mL of the non-diluted growth media at the corresponding time point.

2.11. Statistical analysis

Summary statistics of data collected included, when applicable, number of subjects, mean, standard deviation, median, minimum, maximum, and any missing observations for continuous data and frequency and percentage for categorical data. Summary statistics were divided by investigational product and visit, where applicable. The data lists of the subjects were sorted by investigational product, subject and timing of assessments. Two models were used: 1) Mixed model using 'proc mixed' in SAS version 9.4, and 2) Wilcoxon rank sum test. The primary performance was analysed using the log cfu values with a mixed model analysis with number of hours with the device as covariate. The 4-hour analysis did not have any covariate included in the model. In a secondary performance analysis, the actual cfu values were analysed with a non-parametric approach using the Wilcoxon rank sum test.

3. Institutional ethics review

The study was approved by the Regional Ethics committee in Stockholm (DNR 2015/2160–31/4).

4. Results

4.1. Application of the antibacterial surface coating on to silicone NPs

The antibacterial coating was applied on commercially available medical grade silicone nasal prongs (NPs) of the type used for non-invasive ventilation at neonatal units (Fig. 1A and B). In order to activate the silicon surface of the NP, EB initiated grafting was used to obtain a carboxylic layer. The yields of grafting of acrylic acid as obtained by titration were reproducible between batches and in the range of 87 ± 0.5 μmol/cm^2. Coloring with crystal violet demonstrated a complete grafting of all surfaces of the prong with intense blue color evenly distributed over the grafted surfaces. Silicone NPs stained before and after grafting are shown in (Fig. 2). After coupling of the ligand, comprising an ethylenic spacer and the disulfide bound terminal cysteine component, the coated NPs were shaken for 20 h in PBS at 37 °C, or in 60 mM phosphate buffer at room temperature, and analysed by XPS. The result showed coupling of the ligand with the sulfur (S) 2p peak at 163.8 eV and the nitrogen (N) 1 s peak at 400 eV (Fig. 3). The concentrations measured as atomic percent (excluding hydrogen) ranged from 1.3 to 2.2. There was also a contribution of (Si) 2p at 101.8 eV of 12.8%, which emanated from the silicone substrate that compared to 25% in pure silicone substrate. This indicated that the surface coating was very thin with the ligands bound to the outermost surface of the grafted layer.

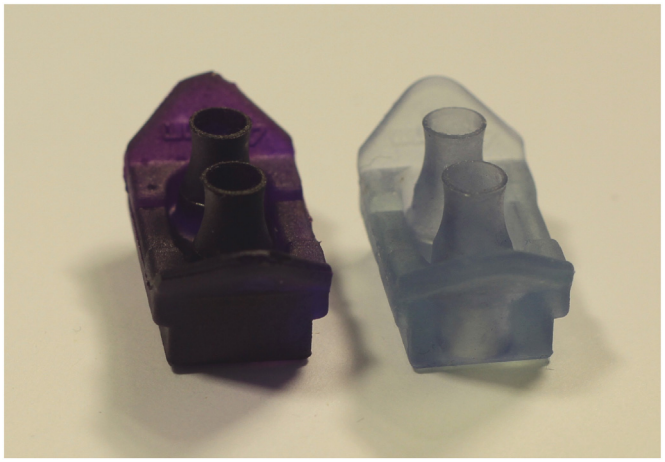

Fig. 2. Stained nasal prongs.
Silicone nasal prongs stained with crystal violet after (left) and before (right) grafting. An evenly distributed coloring on all surfaces of the device was observed on the grafted nasal prong (left) compared to the non-grafted nasal prong (right). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The NPs were evaluated in vitro for antibacterial activity using one Gram negative reference strain (*E. coli*) and one Gram positive strain (*S. aureus*). The results showed a total inhibition of colonization of the coated NP when exposed to both bacterial strains, measured as a titer of 0 cfu/mL in nutrient broth, compared to the non-coated NPs, where a titer > 10^8 cfu/mL in the culture media of the two bacterial strains was measured (Table 1).

4.2. Stability and biocompatibility of coated silicone NP

Coated silicone NPs conditioned during 20 h in phosphate buffer at room temperature or in PBS at 37 °C, showed retained antibacterial capacity against *E. coli* when evaluated with the same in vitro assay. Furthermore, coated NPs stored for > 21 months in a dry state during ambient conditions exhibited unchanged antibacterial capacity towards *E. coli* in the in vitro assay (Supplementary table S1). The antibacterial activity was also intact after sterilization of the NP by EB irradiation to a dose of 25 kGy as evaluated in vitro using *E. coli* (data not shown). Biocompatibility and toxicity tests (local lymph node assay, tissue culture cytotoxicity assay, skin irritation and extractivity test) performed by Envigo Research Ltd. according to standardized protocols for medical device class 2a (see materials and methods) concluded that the coated NPs were with low risk of adverse reaction and thus safe to use in a clinical trial (Supplementary File F1).

4.3. Clinical trial on healthy adult subjects

The silicone NPs were evaluated in a prospective double-blinded clinical pilot investigation, enrolling 48 healthy adult volunteers from one clinical investigation site in Sweden. The study consisted of two visits; one screening/baseline visit and one follow-up visit after 18 h (Fig. 4). The characteristics of the study subjects are shown in Table 2a and b. The primary endpoint of this study was the mean difference in bacterial colonization of the NPs after 18 h of carrying the device, when comparing the coated antibacterial NPs to the non-coated reference NPs, as described in the method section. The cfu counts in growth media incubated with the coated or the reference NPs were measured by plating assay at two time points, 4 h and 18 h, in order to ensure that bacterial growth was measured in the exponential growth phase.

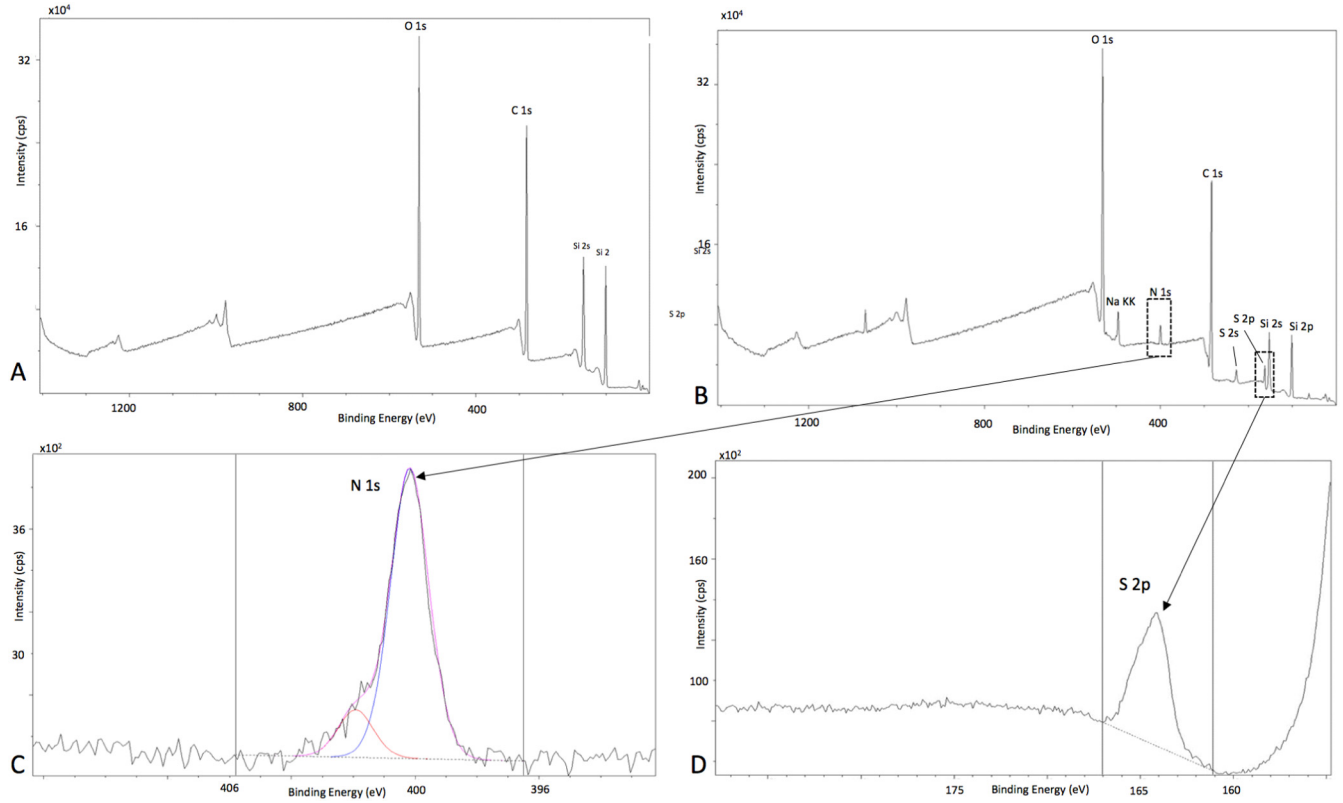

Fig. 3. XPS spectra.
The silicone surface of a nasal prong was analysed by XPS (A) before application of the CytaCoat technology and (B) after application of the CytaCoat technology comprising surface grafting with polyacrylic acid and coupling of a cysteine containing ligand. The result showed coupling of the ligand with the nitrogen (N) 1 s peak at 400 eV (enlarged in (C)) and the sulfur (S) 2p peak at 163.8 eV (enlarged in (D)). The concentrations measured as atomic percent (excluding hydrogen) ranged from 1.3 (S) to 2.2 (N). There was also a contribution of (Si) 2p at 101.8 eV of 12.8%, which emanated from the silicone substrate that compared to 25% in pure silicone substrate. This indicates that the surface coating is very thin with the ligands bound to the outermost surface of the grafted layer.

The results with respect to primary endpoint are shown in Fig. 5A and B and Tables 3–5. After 4 h incubation a difference of 2.48 in ^{10}log mean value between cfu counts of coated NPs (2.06) and non-coated reference NPs (4.54) was observed (Fig. 5A and Table 3). After 18 h of incubation, the difference in ^{10}log mean values as 5.82 between coated NPs (^{10}log 3.60) and non-coated reference NPs (^{10}log 9.42) (Fig. 5B and Table 3. The differences in ^{10}log mean values were statistically significant at both 4 h ($p < 0.0001$) and 18 h ($p < 0.0001$) using either a mixed model analysis (Table 4) or non-parametric analysis (Table 5). With respect to the secondary endpoint, no severe adverse events were recorded (Supplementary table S2). There were 21 minor adverse events recorded in the group with coated NPs compared to 22 in the group carrying the reference NPs. There were 3 moderate adverse events recorded in the group with coated NPs compared to 1 in the group carrying the reference NPs.

5. Discussion

We have here demonstrated that we could successfully coat a commercially available silicone nasal prong (NP) with a novel antibacterial technology, where a covalently attached ligand construct generates a stable antibacterial surface. In vitro this antibacterial ('CytaCoat') coating resulted in total inhibition of bacterial colonization using a clinical isolate of *S. aureus* and a reference strain of *E. coli,* thus being effective against both Gram positive and Gram negative bacteria. Furthermore, as a proof-of-concept first-in-human study on healthy adult volunteers we found a highly significant difference in colonization between coated and non-coated NPs ($p < 0.0001$) after being exposed

Table 1
In vitro evaluation of antibacterial activity.

Table 1	*E. coli* incubated 22-h in LB at 37 °C					*S. aureus* incubated 18-h in LB at 37 °C				
Dilution	N1	N2	C1	C2	C3	N3	N4	C5	C5	C7
10^{-0}			0	0	0			0	0	0
10^{-1}										
10^{-2}			0	0	0			0	0	0
10^{-3}										
10^{-4}										
10^{-5}	166	180								
10^{-6}						33	67			
cfu/mL	1.73×10^8		0			5.0×10^8		0		

Bacterial counts of *E. coli* (cfu/mL, 22 h incubation) and *S. aureus* (cfu/mL, 18 h incubation) on non-coated nasal prongs and coated nasal prongs after incubation in Luria Bertani broth at 37 °C (empty space not counted). C = coated nasal prongs, N = non-coated nasal prongs.

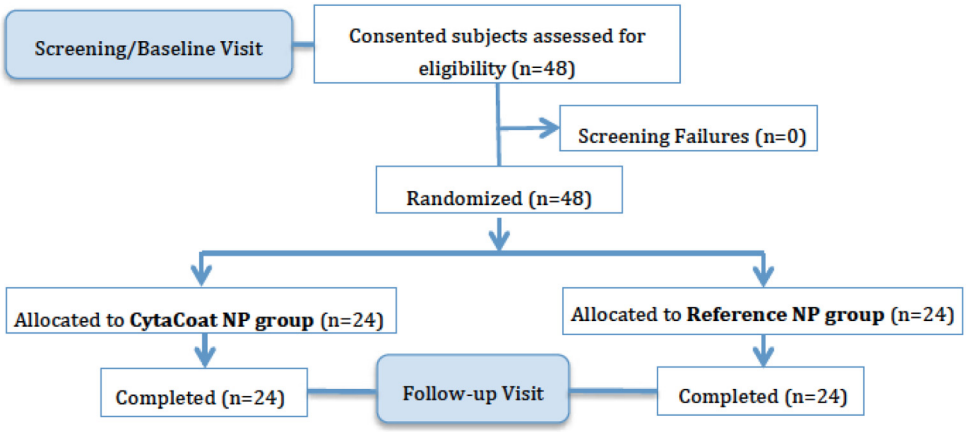

Fig. 4. Flow chart of the study design.
Forty-eight healthy adult volunteers were included in the trial and randomized in two groups, with two visits. All subjects completed the study.

Table 2
Demographic characteristics of included subjects.

A) Gender distribution

	CytaCoat NP		Reference NP		Total	
Gender	n	%	n	%	n	%
Male	6	25	3	12.5	9	18.25
Female	18	75	21	87.5	39	81.25
Total	24	100	24	100	48	100

B) Age distribution

Treatment group	n	Mean	SD	Min	Q1	Median	Q3	Max
CytaCoat NP	24	33.96	14.22	18.00	20.50	29.00	44.00	60.00
Reference NP	24	31.58	13.44	18.00	19.00	25.50	42.50	57.00

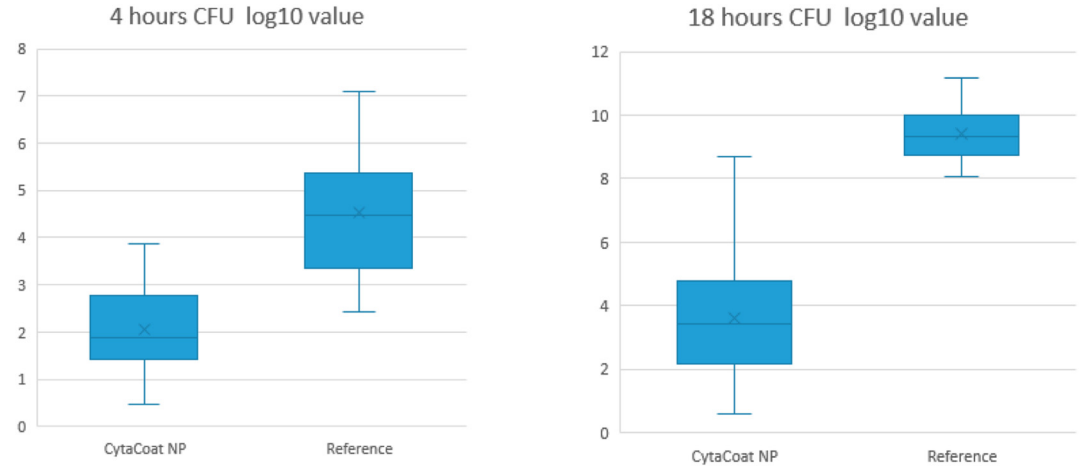

Fig. 5. Primary endpoint of bacterial colonization on the nasal prongs.
A. After 4 h incubation of used NPs in a nutrient growth medium, a difference of 2.48 in ^{10}log mean value between cfu counts for coated nasal prongs (2.06) and non-coated reference nasal prongs (4.54) was observed.
B. After 18 h of incubation, the difference in ^{10}log mean values was 5.82 between non-coated nasal prongs (9.42) and coated nasal prongs (3.60).

for 18 h to the mixed normal bacterial flora on skin and mucosa.

We selected to demonstrate the technology on NPs, a medical device frequently used in neonatal care, which is a clinical setting where the premature infant population is particulary vulnerable to HAI [7]. The use of CPAP via NPs has been reported as a significant risk factor for early septicemia (debut before 72 h of age) in extremely newborn infants [21]. The impact on the incidence of late onset septicemia (after 72 h of life) is not known. Prior to the study, we had found that standard NPs used on neonates < 24 h were colonized with a multi-flora of bacteria (data not shown). This included several pathogenic species, such as *Klebsiella pneumonia, Enterococcus faecalis, Staphylococcus aureus*, *Staphylococcus epidermides* (CoNS), *Streptococcus agalactiae*

Table 3
Descriptive statistics for results of the study. Actual and ^{10}log values for cfu count at 4 h and 18 h.

Variable	Characteristics	CytaCoat NP	Reference NP
Mean value cfu/mL - 18 h[a]	n	24	24
	Mean	2.04×10^7	1.36×10^{10}
	Std	9.79×10^7	3.05×10^{10}
	Min value	4	1.20×10^8
	q1	152	6.40×10^8
	Median	2935	2.23×10^9
	Q3	5.70×10^4	9.50×10^9
	Max value	4.80×10^8	1.42×10^{11}
^{10}Log mean value cfu/mL - 18 h[a]	n	24	24
	Mean	3.60	9.42
	Std	1.91	0.84
	Min value	0.60	8.08
	q1	2.18	8.80
	Median	3.43	9.34
	Q3	4.75	9.97
	Max value	8.68	11.15
Mean value cfu/mL - 4 h[a]	n	24	24
	Mean	770.83	1.25×10^6
	Std	1826.20	3.34×10^6
	Min value	3	265
	q1	28	2400
	Median	78	3.23×10^4
	Q3	545	2.27×10^5
	Max value	7200	1.28×10^7
^{10}Log mean value cfu/mL - 4 h[a]	n	24	24
	Mean	2.06	4.54
	Std	0.86	1.36
	Min value	0.48	2.42
	q1	1.45	3.38
	Median	1.89	4.48
	Q3	2.72	5.35
	Max value	3.86	7.11

[a] There is no direct correspondence between the statistical data for cfu/mL and ^{10}log(cfu/mL) since they are calculated separately for the 24 coated and 24 uncoated NPs, respectively. The high mean value for the coated NPs at 18 h (2.04×10^7) is due to a max value (4.80×10^8), which is a single value far above the other cfu/mL data (not shown).

Table 4
Results of the study using mixed model. ^{10}Log values of cfu at 4 and 18 h.

Variable	*p*-Value	Treatment difference
Hours with device	0.92	N/A
Log10 mean value cfu/mL - 18 h	< 0.0001	5.82
Log10 mean value cfu/mL - 4 h	< 0.0001	2.48

Table 5
Results of study using non-parametric test (Wilcoxon). Actual and ^{10}log values of cfu at 4 and 18 h.

Variable	*p*-Value
Mean value cfu/mL - 18 h	< 0.0001
Log10 mean value cfu/mL - 18 h	< 0.0001
Mean value cfu/mL - 4 h	< 0.0001
Log10 mean value cfu/mL - 4 h	< 0.0001

(GBS), *Staphylococcus haemolyticus*, species that often are isolated in blood cultures of neonates with sepsis, occurring secondary to ventilator support Since we could demonstrate a strong inhibition of colonization of the coated NPs after 18 h of use, the CytaCoat surface coating on NPs could potentially reduce the frequency of nosocomial infection in the preterm population receiving respiratory support. The study population of healthy subjects reported no severe adverse events, and for the 43 minor and 4 moderate adverse events reported, no clear differences attributed to either type of prong could be found (Supplementary Table S2).

The need for active measures to prevent medical device associated HAIs is recognised by major international health organisations, such as WHO [22], Centre for Disease Control and Prevention, US [23], and European Centre for Disease Prevention and Control [24]. Antibacterial coating of such medical devices is an attractive strategy to reduce HAIs, however current technologies have shown limited effects [25]. The CytaCoat^R technology can be applied on surfaces of different polymeric substrates. In addition to silicone, the substrate of the device in the present investigation, we have demonstrated an antibacterial effect of the coating when applied on polyethylene, polyurethane and polycaprolactone (data not shown). Thus, several other types of medical devices associated with Hospital aquired infections (HAI) are possible to coat with the CytaCoat^R technology. In contrast to existing antibacterial coatings for medical devices such as silver, our novel technology is based on non-releasing active compounds. Furthermore, the CytaCoat^R antibacterial surface is non-toxic and with no predicted negative environmental impact. The antibacterial effects of antibiotic or silver containing coatings involve release of the active substance to the surrounding, with a gradual depletion of the antibacterial silver coating and effect over the number of days used [26]. In addition, a systemic uptake of silver in the body is also of concern. Although claimed not to be clinically signficant, in a study on endotracheal tubes (ETTs) for adults an increase in silver concentrations were detected in plasma of exposed patients [27].

In conclusion, we have successfully demonstrated an antibacterial effect of the CytaCoat surface coating on a non-invasive medical device in a first-in-human trial. Our encouraging results suggest that the CytaCoat^R technology can reduce HAI associated with the use of other non-invasive devices e.g. endotracheal tubes and urinary catheters. Furthermore, the CytaCoat^R technology may have potential application also on invasive devices such as central venous catheters, which is a type of device associated with increased frequency of HAIs.

Acknowledgements

MSci Marianne Enstsson and MSci Mikael Sundin at the Technical Research Institute of Sweden are gratefully acknowledged for the XPS measurements. Inspiration Healthcare Ltd. is acknowledged for providing uncoated nasal prongs. The study was funded by grants from the Swedish Agency for Innovation (VINNOVA) (grants numbers ´Forska och Väx´, DNR 2012-00321 and 'Innovationsprojekt i företag', DNR 2015-02069).

Declaration of conflict of interest

JO, AW, GHG, and BA are active founders and stockowners of CytaCoat AB, Karolinska Institutet Science Park, Fogdevreten 2, 171 65 Solna, Sweden. The phase one trial was conducted as a sponsored study at the Neonatal Unit, Karolinska University Hospital, with Dr. Baldvin Jonsson as Principal Investigator and study responsible. The responsible CRO, Devicia AB, was contracted by CytaCoat AB to independently organise and audit the study. There has been no financial support for this work that could have influenced the outcome of the study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.msec.2018.08.040.

References

[1] (ECDC) ECfDPaC, Surveillance of Healthcare-Associated Infections and Prevention Indicators in European Intensive Care Units: HAI-Net ICU Protocol, Version 2.2, European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden, 2017.
[2] A.K. Thabit, J.L. Crandon, D.P. Nicolau, Antimicrobial resistance: impact on clinical and economic outcomes and the need for new antimicrobials, Expert. Opin.

Pharmacother. 16 (2015) 159–177.
[3] H.M. Zowawi, P.N. Harris, M.J. Roberts, P.A. Tambyah, M.A. Schembri, M.D. Pezzani, et al., The emerging threat of multidrug-resistant Gram-negative bacteria in urology, Nat. Rev. Urol. 12 (2015) 570–584.
[4] A. Tammelin, I. Qvarfordt, Point-prevalence surveillance of healthcare-associated infections in Swedish hospitals, 2008-2014. Description of the method and reliability of results, J. Hosp. Infect. 91 (2015) 220–224.
[5] E. Bouza, A. Perez, P. Munoz, M. Jesus Perez, C. Rincon, C. Sanchez, et al., Ventilator-associated pneumonia after heart surgery: a prospective analysis and the value of surveillance, Crit. Care Med. 31 (2003) 1964–1970.
[6] M. Cernada, M. Brugada, S. Golombek, M. Vento, Ventilator-associated pneumonia in neonatal patients: an update, Neonatology 105 (2014) 98–107.
[7] Neonatalvårdens omfattning och resultat år 2015, http://www.medscinet.com/pnq/Uploads/SNQ_%C3%85rsrapport 2015.pdf, (2016).
[8] T.A. Gaonkar, L.A. Sampath, S.M. Modak, Evaluation of the antimicrobial efficacy of urinary catheters impregnated with antiseptics in an in vitro urinary tract model, Infect. Control Hosp. Epidemiol. 24 (2003) 506–513.
[9] C. Brun-Buisson, F. Doyon, J.P. Sollet, J.F. Cochard, Y. Cohen, G. Nitenberg, Prevention of intravascular catheter-related infection with newer chlorhexidine-silver sulfadiazine-coated catheters: a randomized controlled trial, Intensive Care Med. 30 (2004) 837–843.
[10] P. Kurtz, P. Rosa, G. Penna, F. Braga, J. Kezen, L.E. Drumond, et al., Antibiotic coated catheter to decrease infection: pilot study, Rev. Bras. Ter. Intensiva. 20 (2008) 160–164.
[11] J.C. Tiller, Antimicrobial surfaces, Adv. Polym. Sci. 240 (2011) 193–217.
[12] J. Hasan, R.J. Crawford, E.P. Ivanova, Antibacterial surfaces: the quest for a new generation of biomaterials, Trends Biotechnol. 31 (2013) 295–304.
[13] J.C. Tiller, S.B. Lee, K. Lewis, A.M. Klibanov, Polymer surfaces derivatized with poly (vinyl-*N*-hexylpyridinium) kill airborne and waterborne bacteria, Biotechnol. Bioeng. 79 (2002) 465–471.
[14] N.M. Milovic, J. Wang, K. Lewis, A.M. Klibanov, Immobilized N-alkylated polyethylenimine avidly kills bacteria by rupturing cell membranes with no resistance developed, Biotechnol. Bioeng. 90 (2005) 715–722.
[15] H. Murata, R.R. Koepsel, K. Matyjaszewski, A.J. Russell, Permanent, non-leaching antibacterial surface-2: how high density cationic surfaces kill bacterial cells, Biomaterials 28 (2007) 4870–4879.
[16] I. Hidalgo Fabrellas, M. Rebollo Pavón, M. Planas Canals, M. Barbero Cabezas, Incidencia de la infección urinaria en pacientes postoperados de cirugía cardiaca: estudio comparativo según el dispositivo de sondaje, Enferm. Intensiva 26 (2015) 54–62.
[17] K. Stenzelius, L. Laszlo, M. Madeja, H. Pessah-Rasmusson, M. Grabe, Catheter-associated urinary tract infections and other infections in patients hospitalized for acute stroke: a prospective cohort study of two different silicone catheters, Scand. J. Urol. 50 (2016) 483–488.
[18] J.T. Li, J. Carlsson, J.N. Lin, K.D. Caldwell, Chemical modification of surface active poly(ethylene oxide)-poly (propylene oxide) triblock copolymers, Bioconjug. Chem. 7 (1996) 592–599.
[19] S. Löfås, B. Johnsson, Å. Edström, A. Hansson, G. Lindquist, R.-M.M. Hillgren, et al., Methods for site controlled coupling to carboxymethyldextran surfaces in surface plasmon resonance sensors, Biosens. Bioelectron. 10 (1995) 813–822.
[20] Medical devices: guidance document, in: European Commission (Ed.), DG Health and Consumer DB, Unit B2 “Cosmetics and Medical Devices”, 2010.
[21] A. Ronnestad, T.G. Abrahamsen, S. Medbo, H. Reigstad, K. Lossius, P.I. Kaaresen, et al., Septicemia in the first week of life in a Norwegian national cohort of extremely premature infants, Pediatrics 115 (2005) e262–e268.
[22] WHO, Infection prevention and control, http://www.who.int/infection-prevention/en/, (2017) (Geneva, Switzerland: WHO).
[23] Prevention CfDCa, https://www.cdc.gov/nhsn/index.html (Atlanta, CDC2017).
[24] Control ECfDPa, http://www.ecdc.europa.eu/en/Pages/home.aspx (Stockholm, Sweden2017).
[25] M.P. Muller, C. MacDougall, M. Lim, Ontario Agency for Health P, Promotion Public Health O, Provincial Infectious Diseases Advisory Committee on Infection P, et al., Antimicrobial surfaces to prevent healthcare-associated infections: a systematic review, J. Hosp. Infect. 92 (2016) 7–13.
[26] J. Rello, B. Afessa, A. Anzueto, A.C. Arroliga, M.E. Olson, M.I. Restrepo, et al., Activity of a silver-coated endotracheal tube in preclinical models of ventilator-associated pneumonia and a study after extubation, Crit. Care Med. 38 (2010) 1135–1140.
[27] J. Rello, M. Kollef, E. Diaz, A. Sandiumenge, Y. del Castillo, X. Corbella, et al., Reduced burden of bacterial airway colonization with a novel silver-coated endotracheal tube in a randomized multiple-center feasibility study, Crit. Care Med. 34 (2006) 2766–2772.