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Protein antimicrobial barriers to bacterial adhesion: in vitro and in vivo evaluation of nisin-treated implantable materials

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Abstract

A novel approach to controlling unwanted microbial adhesion in clinical environments is to inhibit the initial attachment of bacteria, rather than trying to remove them once they have adhered. Previous investigations have established that antimicrobial peptides such as nisin can adsorb to surfaces and still retain sufficient activity to inhibit pathogenic bacteria. We examined techniques of application of nisin in vitro to elucidate those most effective and practical for use on biomedical implants in vivo. Nisin adsorbed quickly on Polyvinyl chloride (PVC) suction catheter tubing, with only a slight gain in nisin activity as contact times increased from 10 s to 8 h. The activity of nisin adsorbed on PVC suction catheter tubing increased as solution concentrations increased from 0.01 to 2.0 mg/ml, and it decreased with aging in protein-free phosphate buffer for 48 h and with drying for up to 2 months. When exposed to three species of Gram-positive bacteria, nisin-treated PVC tubing demonstrated an ability to inhibit bacterial growth, while bacteria grew unchecked on catheter material that was untreated. We then examined the ability of nisin to retain its activity in vivo when placed on implants in blood vessels or the upper airway and whether nisin causes tissue reactions greater than untreated implants placed in sheep and ponies. Freshly prepared nisin was applied to Teflon® FEP intravenous catheters and to PVC tracheotomy tubes at the time of placement, using a concentration of 1.0 mg/ml and 10-s contact time. Tissue reactions in response to nisin adsorbed on intravenous catheters or tracheotomy tubes did not occur in sheep or ponies, respectively. Nisin activity was retained for more than 5 h but less than 1 week on intravenous catheters placed in the jugular veins of sheep, and the veins with short-term catheters showed fewer and less severe histologic abnormalities compared with controls, indicating a possible protective effect on vascular endothelium. Nisin activity was retained on PVC tracheotomy tubes maintained for 1–2 h in ponies, but not on tubes in place for 24 h. As the first preclinical trial of nisin-treated implantable materials, this study represents an important first step for developing the potentially broad use of protein antimicrobial films on implantable medical devices.

Keywords: Nisin; Antimicrobial proteins

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1. Introduction

Implantable medical devices are commonly used in medical practice. However, infection of implants is a serious complication, and often is due to *Staphylococcus aureus*, coagulase-negative *Staphylococcus epidermidis*, and other Gram-positive bacteria [1–3]. Once bacteria have adhered to an implant, they are difficult to remove. The use of protein antimicrobials adsorbed onto implant surfaces is one possible approach to preventing infection, which would theoretically be easier and more cost-effective than destroying the microorganisms after they have become part of a biofilm. One such protein antimicrobial is nisin.

Nisin is a small (3353 Da) peptide produced by a common milk bacterium, *Lactococcus lactis* [4]. Its molecular structure includes unusual amino acids and thioether rings [5], which are presumed to be important in its activity, although the actual mechanism of bactericidal action has yet to be clearly determined. Nisin has demonstrated activity against Gram-positive bacteria, especially sporeformers. Although, nisin is generally not active against Gram-negative bacteria and fungi, it can be an effective inhibitor of certain Gram-negative bacteria when used in combination with other compounds such as chelating agents [6]. Bacteria susceptible to nisin are killed through a multi-step process that results in pore formation followed by cell lysis [7]. Nisin can retain activity during thermal processing [8], high-pressure treatments [9], and exposure to acidic environments [10]. Additionally, nisin has been found to be non-toxic in foods and has been approved for use as a food preservative in over 50 countries [11]. These characteristics, which have made nisin valuable as a direct food additive to inhibit bacteria and spores, may also prove useful in medical applications, although important questions need to be answered before it can be applied successfully in medical applications.

Nisin is a member of a group of ribosomally produced peptides called 'lantibiotics.' The unique physical structure of lantibiotics, (e.g. double bonds, thioether rings, and unusual amino acid residues), makes these antimicrobial peptides highly reactive, and thus different in mode-of-action from traditional antibiotics. Several lantibiotics have demonstrated antimicrobial activity in vitro against pathogenic organisms [12,13]. In addition to reducing the incidence of implant-associated infection, lantibiotic barriers may also offer an alternative method of dealing with multiple-drug-resistant microorganisms, because of the dissimilar mechanism of lantibiotic action as compared with that of conventional antibiotics.

It has been previously demonstrated that lantibiotics such as nisin can adsorb to surfaces, maintain activity, and kill microorganisms that have adhered in vitro [14–17]. The thought that use of nisin-treated devices, such as intravascular catheters, may inhibit implant-associated infection is based solely on such in vitro data. Thus far, the results of in vitro studies of adsorption and adhesion have not been extended to preclinical investigations.

The in vitro studies reported here were designed to determine the techniques of application of nisin that would be most effective and practical for use in vivo. Specifically, the contact time needed for maximal nisin adsorption to implantable materials, the concentration associated with the greatest activity, the effects of aging and of drying on nisin films, and the ability of nisin to inhibit growth of medically significant bacteria were studied. The in vivo studies were designed to obtain preliminary data on the ability of nisin to retain its activity when placed in blood or the upper airway, and to determine if nisin causes tissue reactions such as endothelial and subendothelial damage, inflammation, and thrombosis.

2. Materials and methods

2.1. Nisin preparation

Nisin solutions were aseptically prepared for in vitro studies by solubilizing purified nisin in sodium phosphate buffer (0.01 M; pH 7). For in vivo studies, nisin was solubilized in sterile isotonic saline, and then filter sterilized. Sterility was confirmed by plating the nisin solution onto a Brain Heart Infusion (BHI), (Sigma, St. Louis, MO), agar plate and verifying that there was no

bacterial growth (37 °C; 48 h). All solutions were refrigerated at 4 °C until use.

2.2. Bioassay for nisin activity

Samples were tested for nisin activity using an agar well bioassay procedure [14] that was modified to accommodate solid samples [15]. Bioassay plates were prepared by seeding MRS media (Sigma, St. Louis, MO) with a nisin-sensitive strain of *Pediococcus pentosaceus* FBB 61-2. Known concentrations of nisin were included as controls and were placed onto the bioassay plate with the samples for incubation at 37 °C for 24 h. Zones of inhibition for each sample were measured, and the residual nisin activity was quantified based on the equation derived by plotting the square of each nisin control's zone width against its $\log_{10}$ concentration (U/ml).

2.3. In vitro studies

2.3.1. Implantable materials

Polyvinyl chloride (PVC) suction catheters were used for in vitro studies (Mallinckrodt Inc, St. Louis, MO).

2.3.2. Contact time

Sections of catheter tubing were each placed into a solution of nisin (1.0 mg/ml; pH 7; 25 °C) for 8, 2, 1 h, 30, 10 min, or 10 s. All samples were run in duplicate. Each catheter section was introduced into the nisin solution at an appropriate interval such that all samples would complete their required contact at the same time. Samples were then removed, rinsed in sodium phosphate buffer (0.01 M; pH 7), wicked dry by carefully touching one edge of the catheter to an absorptive material, and then tested for nisin activity using an agar plate bioassay.

2.3.3. Solution concentration

Six different concentrations of nisin (0.01, 0.1, 0.5, 1.0, 1.5, and 2 mg/ml) were prepared in sodium phosphate buffer (0.01 M; pH 7) to determine an optimum nisin concentration for pretreatment of catheters. Sections of catheter tubing were placed into the nisin solutions and incubated at 25 °C. All samples were run in duplicate. After 1 h, the samples were rinsed in sodium phosphate buffer (0.01 M; pH 7), carefully wicked dry, and then tested for nisin activity using an agar plate bioassay.

2.3.4. Film aging

To determine the effect of aging in protein-free phosphate buffer on the activity of adsorbed nisin films, 24 sections of suction catheter tubing were pretreated with nisin solution (1.0 mg/ml; pH 7). Half of the sections were placed in the nisin solution for 10 min, and the other half for 1 h. The tubing was then placed in phosphate buffer (0.01 M; pH 7) to age for up to 48 h. Antimicrobial activity was tested using the bioassay procedure described above.

2.3.5. Film drying

To verify that nisin-treated materials will retain sufficient activity to kill bacteria after prolonged storage, sections of catheter tubing were immersed in a solution of nisin (1.0 mg/ml; 30 min; pH 7), dried (35 °C; 30 min), and then stored at room temperature. Bioassay plates were used to compare residual nisin activities of samples subjected to long-term storage (2 months), short-term storage (30 min), and no storage (fresh samples). All tests were run in duplicate.

2.3.6. Antibacterial effect

Nisin's ability to inhibit the growth of medically important bacteria on catheters was evaluated by immersing catheter tubing in a solution of nisin (1.0 mg/ml; 1 h), and then challenging the samples with *Staphylococcus aureus*, *Staphylococcus epidermidis*, or *Streptococcus faecalis* (@10^8 CFU/ml; 25 °C). After one h, each sample was rinsed with sterile phosphate buffer (0.01 M; pH 7) and aseptically transferred to a disposable cuvette containing a sterile bacterial growth medium (BHI). Growth of pathogens from the catheter samples was followed with a spectrophotometer at 600 nm for 24 h. Catheter tubing was also tested in three control solutions. The first contained only sodium phosphate buffer (0.01 M; pH 7). The second solution consisted of BHI, a complex multi-protein broth capable of encouraging bacte-

rial growth. The third control solution contained heat-inactivated nisin, prepared by autoclaving nisin (1.0 mg/ml) in sodium phosphate buffer (0.01 M; pH 7) for 3 h at 121 psi. Inactivation of nisin was confirmed when no zones of inhibition were detected on an agar bioassay plate. All samples were run in duplicate.

2.4. In vivo studies

In a randomized, double-blind study, commonly used sterile Teflon® FEP (fluorinated ethylene propylene) intravenous catheters (Abbocath, Abbott Park, IL), and PVC tracheotomy tubes (Concord/Protex, Keene, NH) were treated with either nisin, at a concentration of 1.0 mg/ml suspended in sterile saline, or in sterile isotonic saline alone. The catheters and tracheotomy tubes were treated for 10 s immediately before placement. Sterility of the nisin solution was confirmed by plating it onto a BHI agar plate and verifying there was no bacterial growth (37 °C; 48 h). Vials containing the nisin and saline solutions were labeled only as A or B, so people placing catheters and tracheotomy tubes and evaluating jugular veins and tracheal areas were not aware of the contents of each vial.

2.4.1. Intravenous catheters

Intravenous catheters were placed in one jugular vein of each of 12 sheep undergoing surgery as part of a training laboratory for veterinary students. The jugular vein area was clipped and a standard surgical prep was performed on the skin using povidone iodine and alcohol. Catheters were placed using aseptic technique. The sheep were randomly divided into two groups (A and B). Depending on the group assignment, half of the sheep received catheters treated with nisin, and the other half received saline-treated catheters.

The catheters were maintained under sterile bandages for 1 week, and were flushed twice daily with heparinized saline to retain patency. Sheep received daily physical examinations, and the jugular veins were examined for any signs of inflammation or thrombus formation.

These catheters were removed after 1 week and were assigned a number, which was correlated with a particular sheep and solution (A or B), so the personnel performing microbiologic testing would be unaware of the treatment each catheter had received. When each long-term catheter was removed, a new catheter was placed in the opposite jugular vein of the 12 sheep, using the same technique described above. These catheters were removed after 5 h. The same double-blind study design was used for pretreating these with nisin or saline and performing the laboratory evaluations.

The sheep were euthanized, and both jugular veins were removed and fixed in 10% neutral-buffered formalin. Veins were labeled only by sheep number and left or right vein; therefore, histologic evaluation was also performed in a blinded fashion. Veins were divided into proximal, middle, and distal thirds, with reference to the site of catheter placement (middle). Proximal segments were considered control regions. Segments were embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E) and Masson's trichrome. Sections were assessed for endothelial and subendothelial (intimal) proliferation or damage as well as the presence of fibrin thrombi.

In five sheep, intravenous jugular catheters were placed using the same aseptic technique. Three catheters were dipped in 1 mg/ml nisin solution, and two in saline solution before placement using a single- blind protocol in which the catheter treatment was not known at the time of testing for nisin activity. Catheters were removed after 5 h and flushed inside and out with sterile saline. Evaluation of nisin activity was performed using the *Pediococcus* bioassay described above.

2.4.2. Tracheotomy tubes in ponies

Using the same randomized, double-blind study design, tracheotomy tubes were treated with nisin or saline immediately before being placed in the mid-cervical region of the trachea in 12 ponies. Tracheotomy was performed using standard surgical technique during a surgery-training laboratory for veterinary students. Tubes were placed through an incision made in the ventral surface of the middle of the neck, with an incision between

tracheal rings. Six ponies received a nisin-treated tube and six received a saline-treated one. In half the ponies, tracheotomy tubes were removed 18–24 h after surgery, and in the other half they were removed at the end of the laboratory (1–2 h after placement). Tracheotomy incision sites of ponies were cleaned and evaluated daily for swelling, inflammation, and infection. Abnormalities were graded mild, moderate, or severe.

2.4.3. Laboratory evaluation of catheters and tracheotomy tubes

Direct bacterial count (catheters): Attached material on the surface of each catheter was transferred to a glass slide and Gram stained. Relative quantities of bacteria on each catheter were microscopically evaluated by viewing each slide under a 100 × oil-immersion lens.

Culture of viable bacteria: Each catheter was cut into eight lengths (1-cm each) and each tracheotomy tube was sectioned into eight circular cut outs using a sterile cork borer. Two samples from each catheter and tracheotomy tube were sequentially touched to BHI agar plates (until no more bacteria were likely to detach), and then the plates were cultured at 37 °C for 48 h. The relative number of colonies on each plate was used to determine the effect of nisin or saline treatments on bacterial growth.

Assay of nisin activity: Segments of catheters and tracheotomy tubes were transferred to MRS agar bioassay plates seeded with a nisin-sensitive strain of *Pediococcus pentosaceus* FBB 61-2 and incubated at 37 °C for 24 h. The zone of inhibition around each sample was measured to determine the relative amount of nisin present, as compared with a 1.0 mg/ml nisin control on each plate.

3. Results

3.1. In vitro studies

3.1.1. Contact time

The amount of time catheter materials were immersed in nisin solution had minimal effect on the quantity of nisin retained by each surface. The size of nisin inhibition zones increased only slightly throughout the entire 8 h of testing, suggesting that the vast majority of nisin adsorption to the catheter tubing occurred within seconds of contact. Therefore, 10-s contact time was chosen for the in vivo studies.

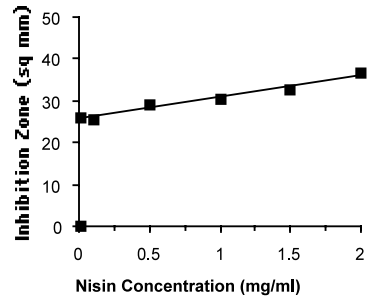

Fig. 1. The effect of nisin concentration on the antimicrobial activity retained by catheter tubing.

3.1.2. Solution concentration

As the concentration of nisin applied to catheter tubing increased (from 0.01 to 2 mg/ml), the zone of inhibition also increased (Fig. 1). The area of each inhibition zone directly corresponds to the quantity of nisin retained by each sample. Based on activity and nisin's maximum solubility at pH 7, a concentration of 1 mg/ml was selected for the in vivo studies.

3.1.3. Film aging

When adsorbed nisin films on catheter tubing were placed in phosphate buffer to age, the antibacterial activity of the films decreased substantially over time (Table 1), with catheter tubing contacted with nisin for 10 min retaining slightly more activity than samples contacted for a longer

Table 1
The percentage of antimicrobial activity retained by nisin films on catheter tubing

Aging (h)	10-min Nisin contact (%)	1-h Nisin contact (%)
0	100	100
2	77	68
48	49	37

Table 2
Percentage of antimicrobial activity retained by dried nisin films on catheter tubing

Storage time of dried films	Catheter tubing (%)
0 min	100
30 min	93
60 days	50

interval. Because of the effect of film aging, freshly prepared nisin was applied to catheters and tracheotomy tubes immediately before placement.

3.1.4. Film drying

The antibacterial activity of nisin films adsorbed onto catheter tubing decreased when dried and stored at room temperature for 60 days (Table 2). A small loss of activity was immediately apparent in the 30-min samples. The catheter tubing tested 60 days later retained about half of its original nisin activity.

3.1.5. Antibacterial effect

As shown in Fig. 2, nisin-treated catheters displayed the lowest levels of bacterial growth for all three bacteria tested, (*Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus faecalis*). Bacteria grew unchecked on catheter material that was untreated, exposed to protein-rich BHI solution, or exposed to inactive nisin.

3.2. In vivo studies

3.2.1. Clinical findings

Sheep: On daily physical examination, none of the sheep had clinical abnormalities associated with the intravenous catheters. All jugular veins and catheters remained patent for the duration of the study.

Ponies: There was no clinically apparent difference in the degree of swelling, inflammation, or discharge during healing of tracheotomy sites in ponies in the nisin or control groups. During the first week after the tracheotomies were performed, all ponies had mild to moderate swelling of the skin and subcutaneous tissue around the incision.

3.2.2. Laboratory evaluations

Sheep: No bacteria were directly observed on Gram stains or later grew in culture on any of the catheters tested regardless of the experimental group (nisin or saline) or the time the catheter was in place (1 week or 5 h). Cellular debris and red blood cells were visible on most samples, except for the five catheters flushed with saline after removal.

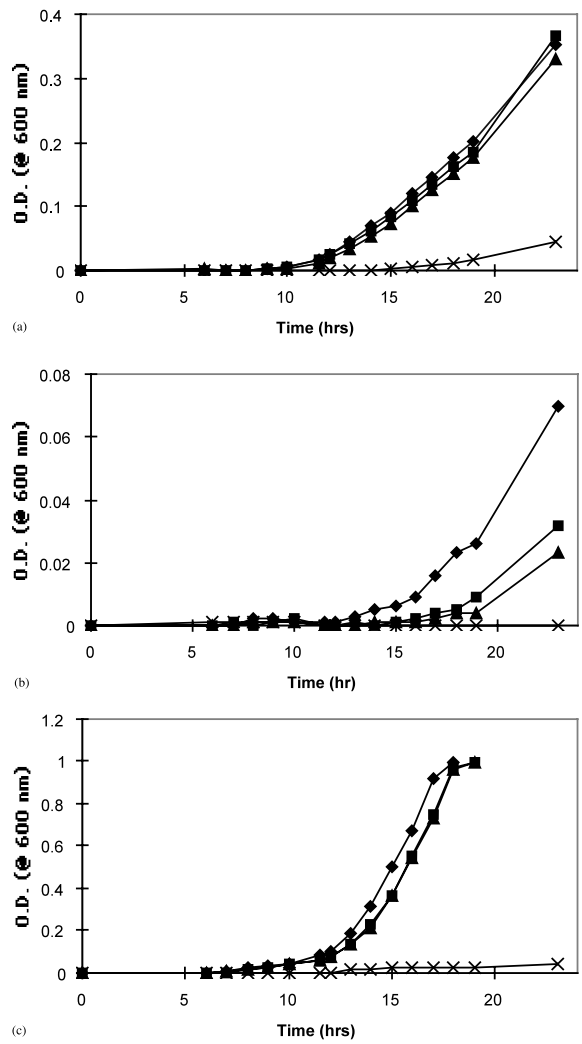

Fig. 2. Growth curves for catheters exposed to bacteria: (a) *Staphylococcus aureus*; (b) *Staphylococcus epidermidis*, and (c) *Streptococcus faecalis*. Overlapping curves represent catheter tubing that was either untreated (◆), exposed to a protein-rich BHI solution (▲), or exposed to inactive nisin (■). Growth curves for nisin-treated samples (X) are clearly visible having lower levels of bacterial colonization.

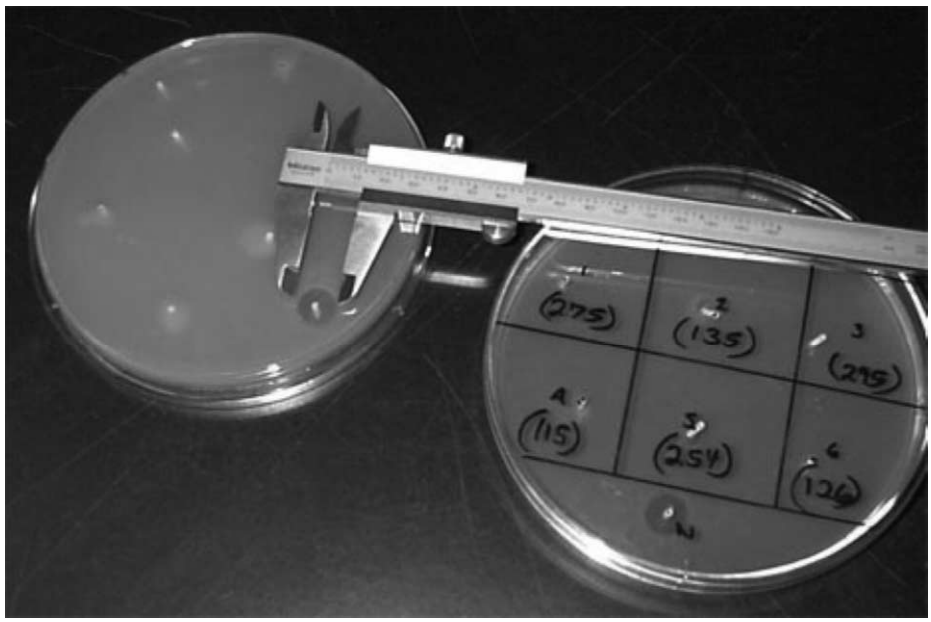

Fig. 3. Bioassay plates for detecting nisin activity. Intravascular catheters from sheep were tested against a nisin-sensitive strain of *Pediococcus* to detect and quantify active nisin retained on the catheter. Large inhibition zones are visible around nisin controls. The presence of blood around the sample catheters may be obscuring small nisin zones.

No zones of activity were present for any of the 1-week catheter samples, regardless of catheter treatment (Fig. 3). On the first group of catheters in place for 5 h, zones of activity were present surrounding three catheters in the nisin group and three catheters in the saline group. Neither saline nor blood normally produces zones of activity on bioassay plates seeded with *Pediococcu*s, therefore, the zones of activity on saline-coated catheters were not indicative of nisin activity. These zones were found to be associated with barbiturate solution, used for euthanasia, remaining in the catheter lumen. On the second group of five catheters in place for 5 h, inhibition zones ranging from 7 to 12% of the original activity were present surrounding only the nisin-treated catheters. There were no zones of activity surrounding the 2 saline-coated catheters.

Ponies: Short-term airway studies (1–2 h) produced large zones of activity (up to 25% the size of the nisin-treated control) for all nisin-treated tracheotomy tubes, and no zones of activity for saline-treated samples. When tracheotomy tubes were left in place for 24 h, no zones of activity were present on either nisin- or saline-treated samples. However, bacteria grew on all BHI plates (48 h at 37 °C), regardless of the pretreatment (nisin or saline), or the time the tracheotomy tubes were in place.

3.2.3. Tissue evaluations

On postmortem evaluation, no vessels were occluded with thrombi, nor did the veins or perivascular tissues show signs of infection. Abnormalities on histologic evaluation of the jugular veins were confined to the middle and distal segments, except for one vein with a long-term control catheter in which a non-occlusive intraluminal fibrin thrombus was present in the proximal segment. Endothelial and subendothelial proliferation and damage were comparable for the veins with long-term catheters regardless of the pretreatment. However, after short-term catheterization, five of six veins with control catheters compared with one of six veins with nisin-treated catheters had evidence of endothelial and subendothelial proliferation in middle or distal segments, and three of six control veins had

non-occlusive intraluminal fibrin thrombi present compared with one of six in the veins with nisin-treated catheters.

4. Discussion

Surfaces placed into protein-containing solutions tend to become quickly covered by protein 'films' that can profoundly affect the properties of the material and may also serve to harbor bacteria [18]. Adsorption of biologically active proteins, such as nisin, may impart antimicrobial activity to a synthetic material surface [14]. We found that nisin adsorbs within seconds at a concentration that remains in solution at a physiologic pH, so that long contact times are not needed for large quantities of nisin to adsorb. Therefore, nisin was applied to catheters and tracheotomy tubes at the time of placement in sheep jugular veins. Because of the rapid adsorption, it is possible that nisin could be reapplied intermittently, for example by injecting a small amount through the catheter. Further studies are warranted to determine if this is feasible and safe.

Proteins adsorbing to a surface typically undergo conformational changes to optimize their surface interactions [19]. The degree of structural rearrangement can be influenced by the available space on the surface, with fewer proteins allowing more space per protein and therefore more molecular unfolding. For biologically active proteins, even slight conformational changes can affect the activity [20]. Conversely, adsorption from a more concentrated protein solution generally produces a more crowded layer of molecules on the surface where the shortage of space inhibits extensive unfolding. Since conformational changes can alter the active site of proteins, it was important to evaluate the effect on activity of adsorption from solutions of high and low concentrations of nisin. In this case, as nisin concentration increased so did the activity. Since nisin is less soluble at higher pH's [21], a concentration of 1 mg/ml, near the maximum solubility of nisin at pH 7, was chosen for the in vivo studies.

Adsorbed proteins can lose activity over time as the degree of unfolding increases. Previous studies have been designed to characterize conformational change in terms of size, shape, charge, and tendency of the protein to unfold, as well as the hydrophobicity of both the protein and the contact surface [19,22]. However, there is currently no means of reliably predicting the degree of unfolding and subsequent loss of activity for any specific protein. Actual measurement of the change in activity of an adsorbed protein over time is required, and this has not been published previously for any lantibiotic peptide adsorbed onto synthetic materials designated for in vivo use. Our in vitro 'aging' measurements, where adsorbed protein films were allowed to stand in a buffer solution for up to 2 days, show that considerable antimicrobial activity remains. However, to maximize the efficacy of nisin in clinical applications, freshly prepared nisin would be most advantageous.

In vitro studies of the effect of drying on nisin were performed to determine if nisin could be applied to catheters and stored for future use, which would make clinical application more convenient. Substantial nisin activity was retained for 2 months after drying, although the fresh films had the most activity. Consequently, although storage is possible, nisin that has not been subjected to drying is likely to have the strongest antimicrobial activity in clinical applications. However, this requires further evaluation, since susceptibility to nisin's antimicrobial activity is likely to vary among different strains and species of Gram-positive bacteria [23].

Our in vitro evaluation of nisin's ability to inhibit Gram-positive bacterial growth indicates that few bacteria attached to the surfaces, or those that did attach were subsequently killed. This has considerable clinical relevance, since a variety of Gram-positive bacteria are responsible for many serious infections in hospitalized patients. Whether nisin can inhibit bacterial growth on implants placed in vivo remains to be determined.

Many different antibiotic and antiseptic coatings have been applied to catheters in an effort to prevent infections [2]. Because lantibiotics, such as nisin, have a different mechanism of action compared with conventional antibiotics, they may

prove to be a useful alternative in the clinical setting, in which development of bacterial resistance is a serious concern. The need for alternatives to conventional antibiotic therapy to help address the problem of resistant bacteria prompted the current preliminary studies. It is likely that some bacteria will be able to develop resistance to lantibiotics as well. Therefore, as with conventional antibiotics, it would be best to limit any use of nisin, which proves to be clinically effective, to patients in need of that specific therapy or at risk for development of Gram-positive infections of implants.

The in vivo studies showed no clinically evident adverse effects from placement of nisin-treated intravenous catheters and tracheotomy tubes in sheep or ponies during the experimental period. It is possible for compounds introduced in vivo to cause tissue reactions that result in clotting of vessels or increase in infection associated with the implant or vein. In these preliminary studies, none of the catheterized veins developed infection or thrombosis despite half of the catheters being left in place for 1 week, which significantly exceeds the 72 h typical for this type of catheter. More histologic abnormalities involving endothelium and intima (the lining of blood vessels), with microscopically evident fibrin thrombi, were present in the control veins compared with veins with nisin-treated catheters in the short-term catheter study. This may indicate that nisin has properties that diminish the adverse effects commonly associated with intravenous catheters. Because endothelial damage is often the first step in the development of the thrombosis and subsequent embolization associated with intravenous implants, this is an important finding. Endothelial damage and thrombosis also predispose to bacterial colonization by providing a favorable environment for bacterial adhesion and growth. Because nisin activity was not retained for the entire week of catheter placement, this protective effect would also not be likely to persist, explaining the comparable findings in long-term catheters regardless of treatment. Further studies to include repeated application of nisin, by intermittent flushing of the catheter for example, are needed to determine whether this protective effect can be extended over time.

Nisin activity was not retained on the intravenous catheters left in place 1 week, although it was retained for at least 5 h. Most of the loss of activity was likely associated with proteins in blood interacting with nisin adsorbed to the catheter surface, rather than changes in the degree of unfolding of nisin molecules over time. Nisin adsorbs to surfaces in multiple layers, with loosely attached outer layers believed to be more biologically active [17]. However, this also allows for more easy displacement, in this case by blood proteins, and subsequent loss of biological activity. Evaluation of methods to retain or restore nisin activity of implants in situ is in progress.

The absence of both clinical infection and bacteria on the catheters is consistent with catheters placed aseptically and maintained under sterile bandages. Future studies should involve exposure of the implant to bacteria, once the duration of nisin activity or methods to reapply nisin have been established. The tracheotomy tubes retained substantial nisin activity during short-term placement, although nisin activity was not present after 24 h. Intra-tracheal devices are typically changed on at least a daily basis, so the duration of nisin activity may be sufficient to prevent secondary infections associated with contamination of tracheotomy tubes. All tracheotomy tubes had bacterial growth, which indicates that nisin did not inhibit their presence on the tube. However, nisin would only be expected to inhibit Gram-positive bacteria, and the type of bacteria was not determined in this study. Bacterial challenge studies are needed to determine if nisin can prevent growth of pathogenic Gram-positive bacteria on tracheotomy tubes.

In these in vivo studies, the first of their kind, we were able to use nisin-treated implants in a clinical situation, without prolonging procedures, and without causing adverse effects in the animals. Nisin did not cause systemic or local adverse effects when applied to intravenous catheters and tracheotomy tubes in sheep and ponies, respectively. Additionally, nisin appeared to have a protective effect on vascular endothelium. The duration of nisin activity in vivo appears to be short, and reapplication of nisin is likely to be needed to retain activity in implants

left in place. However, as the first preclinical trial of nisin-treated implantable materials, this study represents an important first step for developing the potentially broad use of protein antimicrobial films on implantable medical devices.

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