Novel Coating of Surgical Suture Confers Antimicrobial Activity Against *Porphyromonas gingivalis* and *Enterococcus faecalis*

Mohamed M. Meghil,* Frederick Rueggeberg,† Ahmed El-Awady,‡ Brodie Miles,§ Franklin Tay,‖ David Pashley,* and Christopher W. Cutler‡

**Background:** The oral cavity is colonized by >10⁹ bacteria, many of which can increase heart disease risk when seeded into the bloodstream. Most dentoalveolar surgeries require the use of surgical sutures. Suture placement and removal can increase the risk of postoperative infection and bacteremia. The aim of this study is to evaluate the antimicrobial activity of a novel quaternary ammonium compound, K21, when coated on different suture materials.

**Methods:** The periodontal pathogen *Porphyromonas gingivalis* and the endodontic species *Enterococcus faecalis* were grown to early log phase and inoculated on enriched Brucella blood agar, on which were placed identical lengths of surgical suture (chromic gut, polyester suture, silk, and nylon suture) and control unwaxed dental floss impregnated with K21 at 5%, 10%, 20%, and 25% volume/volume in ethanol vehicle. Controls included the following: 1) sutures treated with vehicle; 2) untreated sutures; and 3) unwaxed floss. Zones of inhibition in millimeters were measured at five randomized sites per suture/floss for each concentration and material used. Mean ± SD of zones of inhibition were calculated, and analysis of variance (P < 0.05) was used to determine whether differences were statistically significant.

**Results:** The results indicate that K21-coated suture at concentrations ranging from 5% to 25%, depending on the type of suture, have antimicrobial activity for *P. gingivalis* and *E. faecalis*. Nylon suture coated with K21 at 5%, 10%, 20%, and 25% resulted in zones ranging from 3 to 11 mm. Polyester suture was more effective at lower K21 concentrations with 5% (P = 0.0031), 10% (P = 0.0011), and 20% (P = 0.0002), yielding 7.5, 8.3, and 10.5 mm zones of inhibition. K21-coated silk suture yielded significant zones of inhibition at 25% (P < 0.0001), whereas chromic gut was effective at K21 concentrations of 5% (P = 0.0081) and 25% (P < 0.0001).

**Conclusion:** It is concluded that K21-coated surgical sutures have antimicrobial activity for bacterial species of direct relevance to postoperative infection and bacteremia. *J Periodontol* 2015;86:788-794.

**KEY WORDS**

Bacteremia; *Enterococcus faecalis; Porphyromonas gingivalis*; quaternary ammonium compounds; wound infection.

---

* Department of Oral Biology, Georgia Regents University, Augusta, GA.
† Division of Infectious Diseases, Anschutz Medical Campus, University of Colorado Denver, Aurora, CO.
‡ Department of Oral Rehabilitation, Georgia Regents University.
§ Department of Periodontics, Georgia Regents University.
‖ Department of Endodontics, Georgia Regents University.

doi: 10.1902/jop.2015.140528
The oral cavity contains a remarkably diverse microbiome, with >700 species or phylotypes reported. Among oral diseases with a bacterial etiology, chronic periodontitis (CP) is the most common, affecting nearly 50% of the United States population. Porphyromonas gingivalis is one of several etiologic agents in CP and peri-implantitis. This species is a Gram-negative anaerobe identified in the bloodstream and atherosclerotic plaques, most recently, inside migratory antigen-presenting cells. Enterococcus faecalis is a Gram-positive facultative anaerobe and one of several species found in periradicular infections of teeth and in infectious endocarditis. Bacterial colonization of suture materials is a significant risk factor for wound infections, bacteremia, and endocarditis after dentoalveolar surgeries. Sutures, especially in the oral cavity, can facilitate bacterial adhesion and subsequent wound contamination. Clinical studies have shown that P. gingivalis and other oral species attach to barrier membranes, including collagen, expanded polytetrafluoroethylene, and polylactic acid, that are closed with sutures. Recovery of sutures from infected sites in humans revealed colonization by a variety of different microbes, including Enterococcus spp. Biofilm formation on these sutures hinders effective wound decontamination. Hence, the development of antibacterial sutures could have a significant effect on risk of wound infections and delayed wound healing. Quaternary ammonium compounds (QACs) have been reported to be potent antimicrobial agents with wide applications in the dental and medical fields. The bactericidal action of QACs is mainly by disruption of the electrostatic interactions of the cytoplasm and bacterial cell wall. In dental bonding agents, QACs showed lasting antibacterial activity after photo-cure. Using the QACs in polymers provided durable antibacterial capabilities in these dental materials. In the present study, K21, a novel QAC, is tested for its antimicrobial ability when coated on surgical sutures. It was shown that K21-coated sutures and dental floss have dose-dependent antimicrobial activity for P. gingivalis and E. faecalis in vitro.

MATERIALS AND METHODS

Structure of K21 Compound

The chemical formula for K21 is shown in Figure 1A. The chemical name is 1-octadecanaminium, \( N,N' - [3,3\text{-bis}([3\text{-dimethyloctadecylammonio}]\text{propyl}])\text{di-hyroxysilyl]oxy} - 1,1,5,5\text{-tetrahydroxy-1,5-trisiloxanediyl]}\text{di-3,1-propanediyl]bis[N,N-dimethyl-chloride (1:4)]}.\)

Preparation of K21-Coated Sutures and Dental Floss Samples

Chronic gut, polyester suture, silk, and nylon suture and control unwaxed dental floss were cut into 40-mm strips and soaked in the following concentrations of K21 dissolved in vehicle ethanol: 5%, 10%, 20%, and 25% for 30 minutes. Specimens were then placed on glass slides to allow for solvent evaporation. Control groups included the following: 1) untreated sutures and dental floss (dry); and 2) sutures and dental floss treated with ethanol.

Bacterial Growth Conditions and Antimicrobial Susceptibility Testing

Wild-type P. gingivalis 381 was grown to late log phase under anaerobic conditions (10% H\(_2\), 10% CO\(_2\), and 80% N\(_2\)) in a type C vinyl anaerobic system glove box at 37°C in Wilkins-Chalgren anaerobe broth. E. faecalis ATCC 29212 strain was grown overnight at 37°C in brain–heart infusion broth. Bacteria were harvested by centrifugation, washed, and resuspended in phosphate-buffered saline to an optical density reading of 0.11 at 660 nm. P. gingivalis was plated using sterile glass rods, and E. faecalis was plated using a plating system on enriched Brucella blood agar with 5% sheep blood, hemin, and vitamin K. For P. gingivalis, suture and dental floss samples were placed on the blood agar plates and then incubated anaerobically for 12 days to allow for the formation of a lawn of black-pigmented colonies. For E. faecalis, plates were incubated overnight after suture material and dental floss placement. Purity of the cultures was confirmed by Gram staining. Images for the plates were then taken using an imaging system. Azithromycin, amoxicillin, and metronidazole test strips were used to confirm patterns of susceptibility of P. gingivalis to standard antimicrobials. Five sites per strip were randomly selected for measurement, which was performed using image analysis software. Shown in Figure 1B is control untreated silk suture with no zone of inhibition and K21-treated silk suture (Fig. 1C) with zones of bacterial growth inhibition. Analysis was performed in triplicate, and results expressed as mean ± SE zones of inhibition.

Data Analyses

Data analyses were conducted using the individual sample as the unit of analysis. Analysis of variance served to examine the effect of K21 treatment on P. gingivalis and E. faecalis growth using statistical software.
RESULTS

Inhibition of \(P. \text{gingivalis}\) and \(E. \text{faecalis}\) Growth by K21-Impregnated Suture

The results indicate that K21-impregnated surgical sutures, placed on blood agar, plated previously with \(P. \text{gingivalis}\) and incubated for 12 days, led to significant inhibition of colony growth. Zones of inhibition (in millimeters) were measured at five randomly selected sites per sample. These zones of inhibition were dose dependent and influenced by the suture material. For example, nylon suture, coated with K21 at 5%, 10%, 20%, and 25% resulted in zones ranging from 3 to 11 mm (Figs. 2A and 2B). These zones were significantly greater, relative to ethanol control (vehicle), for 10% \((P = 0.0135)\), 20% \((P = 0.0107)\), and 25% \((P = 0.0008)\) K21. Polyester-coated suture was more effective at lower K21 concentrations with 5% \((P = 0.0031)\), 10% \((P = 0.0011)\), and 20% \((P = 0.0002)\), yielding 7.5-, 8.3-, and 10.5-mm zones of inhibition, respectively, whereas 25% yielded a drop off in inhibition (Figs. 2C and 2D). K21-coated silk suture yielded significant zones of inhibition at 25% \((P < 0.0001)\) (Figs. 3A and 3B), whereas chromic gut at K21 concentrations of 5% \((P = 0.0081)\) and 25% \((P < 0.0001)\) was effective relative to control vehicle (Figs. 3C and 3D). Unwaxed dental floss was highly effective at the highest concentration of 25% \((P < 0.0001)\), with zones of inhibition >12 mm (Figs. 3E and 3F). \(E. \text{faecalis}\) was also susceptible to K21 on silk, chromic gut, and polyester sutures and unwaxed dental floss.
DISCUSSION

This study was performed to establish whether the K21 compound would have antimicrobial effects against oral microbes when used to impregnate surgical suture and a control fiber (unwaxed dental floss). K21 consists of positively charged or cationic polyatomic ions of the structure NR₄⁺, with R being alkyl or aryl groups. Figure 1A illustrates the structure and formula weight of this complex and stable QAC. Because bacteria have a net negative charge, compounds such as K21 will attach to bacteria and cause the cytoplasmic membrane to leak, killing the bacteria.²⁵ Anaerobic Gram-negative species P. gingivalis has been implicated as a major etiologic agent in periodontitis. This pathogen has been isolated from individuals with peri-implantitis and from periodontally diseased natural teeth.²⁶,²⁷ Specific virulence factors of species such as P. gingivalis contribute to the inflammatory process occurring around natural teeth and implants.²⁸ Although the present study uses a laboratory strain, it is important to note that P. gingivalis can develop resistance to many antibiotics used in adjunctive therapy for periodontitis.²⁹ Moreover, this pathogen has the ability to transfer chromosomal and plasmid DNA that would provide an important route for transfer of resistance genes, as well.³⁰ E. faecalis behaves as both a commensal and opportunistic pathogen depending on the host environment. Enterococci are associated with almost 15% of cases of infective endocarditis.³¹ Additionally, E. faecalis is the most frequent causative agent of surgical wound and nosocomial infections, and it is also the most common pathogen associated floss at 25% (P < 0.05), although the zones were lower than those of P. gingivalis (Fig. 4).
with bacteremias.\textsuperscript{32} \textit{E. faecalis} undergoes molecular changes that enable it to adapt and grow when it gains access to the bloodstream.\textsuperscript{33} The present data indicate that growth of \textit{P. gingivalis} and \textit{E. faecalis} is inhibited by K21 at concentrations ranging from 5\% to 25\% when applied to different suture materials and unwaxed dental floss. The versatility of these classes of compounds with regard to antimicrobial spectrum was shown when the formula was changed from C\textsubscript{6}H\textsubscript{2}O\textsubscript{4}Cl\textsubscript{4}N\textsubscript{4}O\textsubscript{12}Si\textsubscript{5} to C\textsubscript{44}H\textsubscript{90}ClNO\textsubscript{18}Si\textsubscript{5}. The resultant compound lost its activity for \textit{E. faecalis} but retained its activity for \textit{P. gingivalis} (data not shown).

Regarding surgical suture, the present study has therapeutic implications for the prevention of post-operative wound infections. Bacterial colonization of suture leading to bacteremia is reported to contribute to post-surgical complications of dentoalveolar surgery, head and neck cancer surgery, eye prosthetic implants, colorectal surgery, and hepatobiliary surgery.\textsuperscript{8,34-39} Sutures serve as a wick to draw microbes into the underlying wound. Antibiotics have the disadvantage of high cost, insufficient antibacterial spectrum, and the risk of more antibiotic-resistant strains from overuse.\textsuperscript{40} Previous studies reported marked differences in antibiotic susceptibility among periodontal pathogens, whereas others indicated geographic variations of antimicrobial resistance of periodontal pathogens.\textsuperscript{41,42} Furthermore, \textit{P. gingivalis} has been shown to interfere with the normal process of wound healing. It causes degradation of the integrin-related signaling proteins paxillin and focal adhesion kinase.\textsuperscript{43,44} In addition, \textit{P. gingivalis} fimbrae have also been shown to interact with \(\alpha\)5\(\beta\)1 integrin and, subsequently, enhance bacterial adhesion to epithelial cells.\textsuperscript{45} A recent study tested the influence of \textit{P. gingivalis} on normal reepithelialization by incubating \textit{P. gingivalis} with oral keratinocyte cultures.\textsuperscript{46} \textit{P. gingivalis} was shown to interfere with normal wound healing by causing keratinocyte apoptosis and interfering with keratinocyte proliferation. Thus, both \textit{P. gingivalis} and \textit{E. faecalis} are virulent pathogens that influence wound healing locally or inflammation systemically; therefore, preventing submucosal infection with these species should be a high priority.

\textbf{CONCLUSIONS}

This preliminary in vitro study demonstrates that suture materials and dental floss can be rendered antimicrobial when coated with the novel QAC K21. These included such commonly used suture materials as nylon suture, polyester suture, silk, and chromic gut, although polyester suture seemed to be more effective at lower K21 concentrations, perhaps because of increased absorbance of the K21. Additional testing will be needed to confirm long-term efficacy, whether other suture types, such as polyglactin 910, can be rendered similarly antimicrobial, and whether the coating will affect the flexibility and handling characteristics of the sutures.

\textbf{ACKNOWLEDGMENTS}

The spiral plater, K21, and K18 were donated by fiteBac, Atlanta, Georgia. The authors report no conflicts of interest related to this study.

\textbf{REFERENCES}


Correspondence: Dr. Christopher Cutler, Department of Periodontics, Georgia Regents University, 1120 15th St., GC1352. Augusta, GA 30912. Fax: 706/723-0215; e-mail: chcutler@gru.edu.

Submitted September 16, 2014; accepted for publication January 8, 2015.