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 >10^9 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.

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The oral cavity contains a remarkably diverse microbiome, with >700 species or phylotypes reported.[1] Among oral diseases with a bacterial etiology, chronic periodontitis (CP) is the most common, affecting nearly 50% of the United States population.[2] *Porphyromonas gingivalis* is one of several etiologic agents in CP and peri-implantitis.[3,4] This species is a Gram-negative anaerobe identified in the bloodstream and atherosclerotic plaques, most recently, inside migratory antigen-presenting cells.[5] *Enterococcus faecalis* is a Gram-positive facultative anaerobe and one of several species found in periradicular infections of teeth and in infectious endocarditis.[6,7] Bacterial colonization of suture materials is a significant risk factor for wound infections, bacteremia,[8] and endocarditis[9] after dentoalveolar surgeries. Sutures, especially in the oral cavity, can facilitate bacterial adhesion and subsequent wound contamination.[10,11] 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.[12] Recovery of sutures from infected sites in humans revealed colonization by a variety of different microbes, including *Enterococcus* spp.[13] Biofilm formation on these sutures hinders effective wound decontamination.[14] Hence, the development of antibacterial sutures could have a significant effect on risk of wound infections and delayed wound healing.[15,16] 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.[17] 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.[18] 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-octadecanamium, *N*,*N*′-[[3,3-bis[[[3-(dimethyloctadecylammonio)propyl]dihyroxysilyl]oxy]-1,1,5,5-tetrahydroxy-1,5-trisiloxanediyl]di-3,1-propanediyl]bis[*N*,*N*-dimethyl-chloride (1:4).[19]

Preparation of K21-Coated Sutures and Dental Floss Samples

Chromic 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[20,21] 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.[22-24] 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.[‡‡‡]

¶ Ethicon, Johnson & Johnson, Bridgewater, NJ.
# Ethicon, Johnson & Johnson.
** Johnson & Johnson, Skillman, NJ.
†† Coy Laboratory Products, Grass Lake, MI.
‡‡ Acumedia, Neogen, Lansing, MI.
§§ BD Biosciences, Sparks, MD.
‖‖ Eddy Jet 2 Spiral Plating System, Neu-Tec Group, Farmingdale, NY.
¶¶ BD Biosciences.
UVP, Upland, CA.
*** E Test strips, bioMérieux, Marcy l'Etoile, France.
††† Vision Works LS, UVP, Upland, CA.
‡‡‡ Prism 6, GraphPad Software, Jolla, CA.

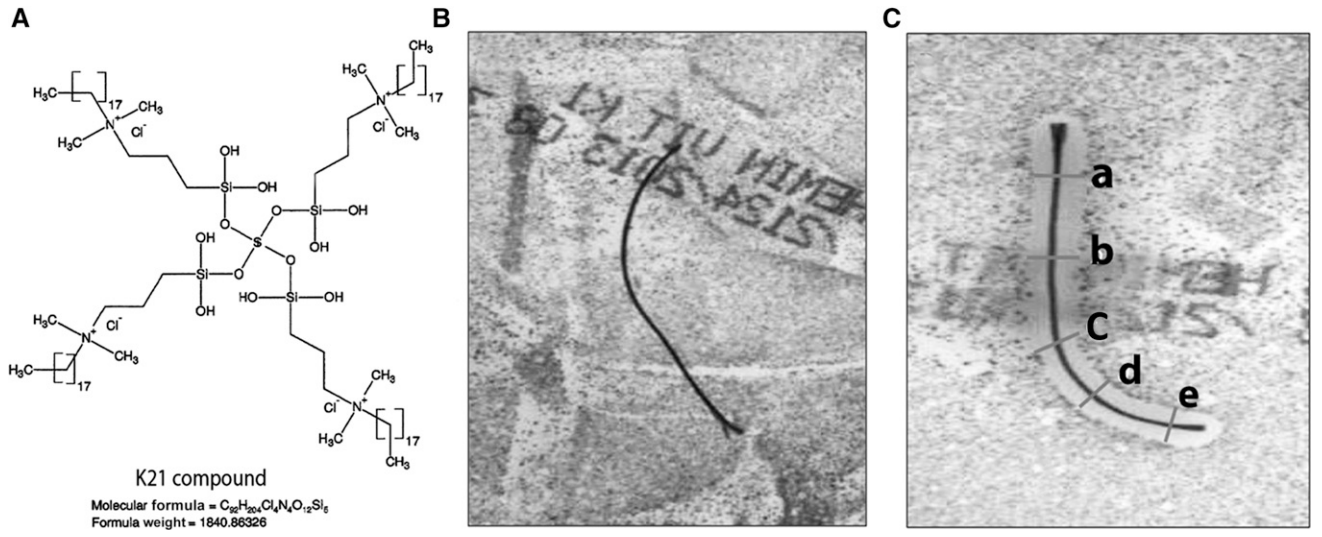

Figure 1.
The K21 compound and representative negative and positive zones of inhibition. ***A)*** *Molecular structure of K21.* ***B)*** *Untreated suture (control) on enriched blood agar showing lawn of* P. gingivalis *with no zone of inhibition.* ***C)*** *Representative silk suture treated with K21 on enriched blood agar showing K21 antimicrobial activity against* P. gingivalis *measured at five different sites (a, b, c, d, e). Mean = ([a + b + c + d + e]/5) ± SE.*

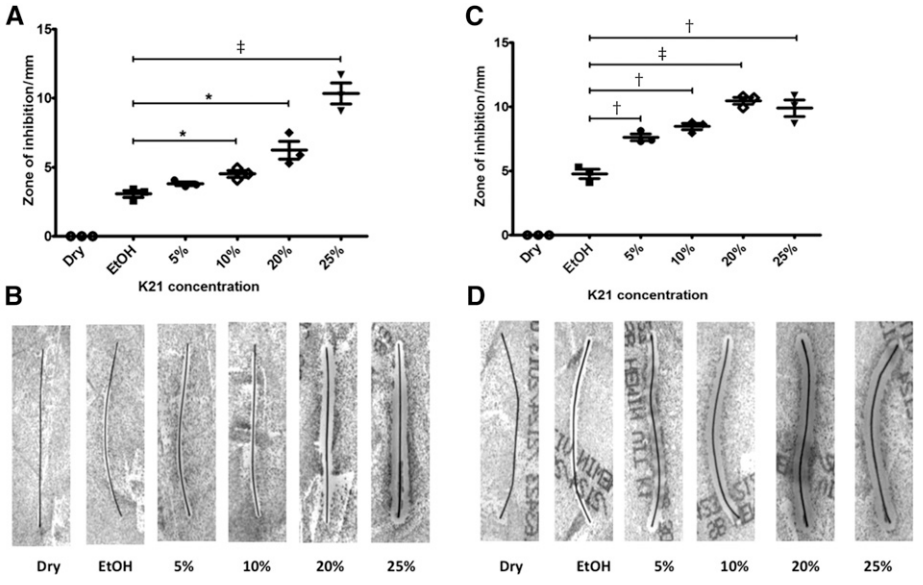

Figure 2.
Dose-dependent inhibition of P. gingivalis *381 growth on K21-treated nylon suture and polyester suture.* ***A)*** *Nylon suture.* ***C)*** *Polyester suture. Error bars represent standard error (n = 3). Untreated samples were considered dry.* ***B*** *and* ***D)*** *Images of samples of nylon and polyester sutures, respectively. Dry (untreated), treated with ethanol (EtOH) and treated with 5%, 10%, 20%, and 25% K21.* *P <0.05, †P <0.01, ‡P <0.001.

RESULTS

Inhibition of P. gingivalis *and* E. faecalis *Growth by K21-Impregnated Suture*

The results indicate that K21-impregnated surgical sutures, placed on blood agar, plated previously with *P. 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. faecalis* was also susceptible to K21 on silk, chromic gut, and polyester sutures and unwaxed dental

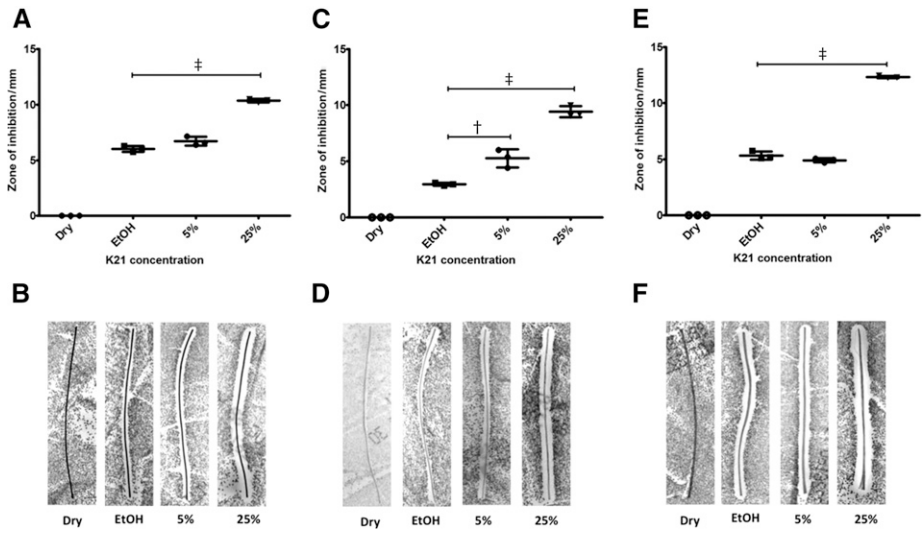

Figure 3.
Dose-dependent inhibition of P. gingivalis *381 growth on K21-treated silk* ***(A)****, chromic gut* ***(C)****, and unwaxed dental floss* ***(E*** *and* ***F)*** *Controls were dry or treated with ethanol (EtOH) alone while experimental groups were treated with or 5% or 25% K21 (n = 3). Sample pictures of silk* ***(B)****, chromic gut* ***(D)****, and unwaxed dental floss. †P <0.01, ‡P <0.001.*

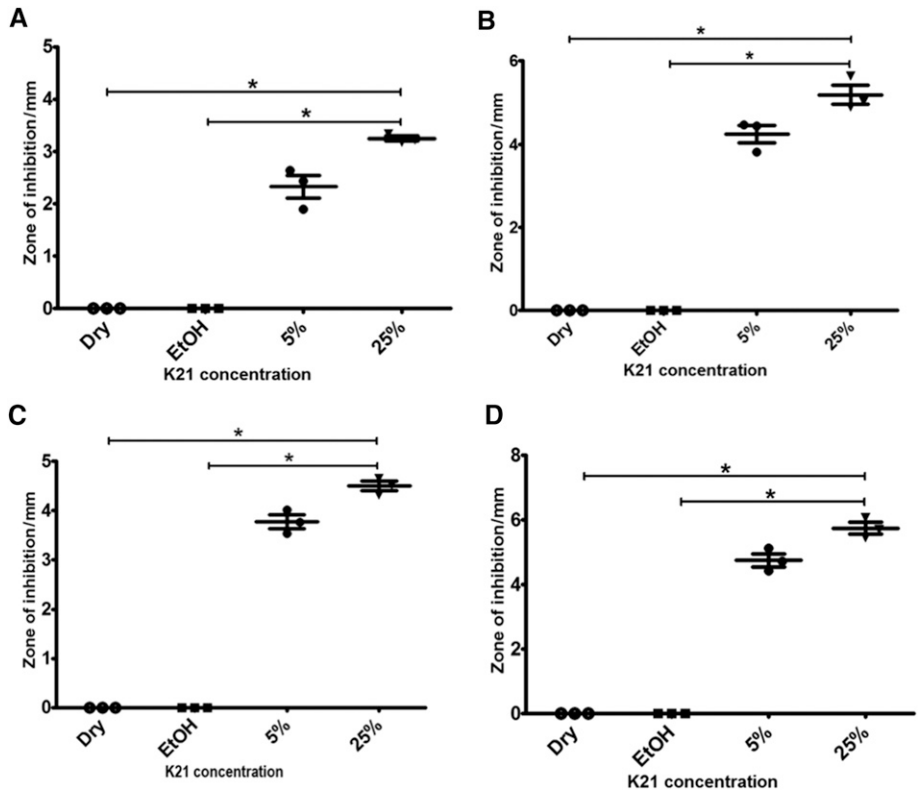

Figure 4.
Analysis of antimicrobial activity of K21 compound against E. faecalis. E. faecalis *growth zones of inhibition with treatment of silk* ***(A)****, chromic gut* ***(B)****, polyester suture* ***(C)****, and unwaxed dental floss* ***(D)*** *with ethanol (EtOH) and 5% and 25% K21 (n = 3).* *******P <0.05.*

floss at 25% (P <0.05), although the zones were lower than those of *P. gingivalis* (Fig. 4).

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 NR_4^+, 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 periimplantitis 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

with bacteremias.[32] *E. faecalis* undergoes molecular changes that enable it to adapt and grow when it gains access to the bloodstream.[33] The present data indicate that growth of *P. gingivalis* and *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 $C_{92}H_{204}Cl_4N_4O_{12}Si_5$ to $C_{44}H_{90}ClNO_{18}Si_5$. The resultant compound lost its activity for *E. faecalis* but retained its activity for *P. gingivalis* (data not shown).

Regarding surgical suture, the present study has therapeutic implications for the prevention of postoperative 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.[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.[40] Previous studies reported marked differences in antibiotic susceptibility among periodontal pathogens, whereas others indicated geographic variations of antimicrobial resistance of periodontal pathogens.[41,42] Furthermore, *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.[43,44] In addition, *P. gingivalis* fimbriae have also been shown to interact with $\alpha5\beta1$ integrin and, subsequently, enhance bacterial adhesion to epithelial cells.[45] A recent study tested the influence of *P. gingivalis* on normal reepithelialization by incubating *P. gingivalis* with oral keratinocyte cultures.[46] *P. gingivalis* was shown to interfere with normal wound healing by causing keratinocyte apoptosis and interfering with keratinocyte proliferation. Thus, both *P. gingivalis* and *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.

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.

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REFERENCES

1. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. *J Clin Microbiol* 2005;43:5721-5732.
2. Eke PI, Dye BA, Wei L, Thornton-Evans GO, Genco RJ; CDC Periodontal Disease Surveillance workgroup: James Beck (University of North Carolina, Chapel Hill, USA), Gordon Douglass (Past President, American Academy of Periodontology), Roy Page (University of Washington). Prevalence of periodontitis in adults in the United States: 2009 and 2010. *J Dent Res* 2012;91:914-920.
3. Colombo AP, Boches SK, Cotton SL, et al. Comparisons of subgingival microbial profiles of refractory periodontitis, severe periodontitis, and periodontal health using the human oral microbe identification microarray. *J Periodontol* 2009;80:1421-1432.
4. Botero JE, González AM, Mercado RA, Olave G, Contreras A. Subgingival microbiota in peri-implant mucosa lesions and adjacent teeth in partially edentulous patients. *J Periodontol* 2005;76:1490-1495.
5. Carrion J, Scisci E, Miles B, et al. Microbial carriage state of peripheral blood dendritic cells (DCs) in chronic periodontitis influences DC differentiation, atherogenic potential. *J Immunol* 2012;189:3178-3187.
6. Subramanian K, Mickel AK. Molecular analysis of persistent periradicular lesions and root ends reveals a diverse microbial profile. *J Endod* 2009;35:950-957.
7. Chuang-Smith ON, Wells CL, Henry-Stanley MJ, Dunny GM. Acceleration of *Enterococcus faecalis* biofilm formation by aggregation substance expression in an ex vivo model of cardiac valve colonization. *PLoS ONE* 2010;5:e15798.
8. Otten JE, Wiedmann-Al-Ahmad M, Jahnke H, Pelz K. Bacterial colonization on different suture materials — A potential risk for intraoral dentoalveolar surgery. *J Biomed Mater Res B Appl Biomater* 2005;74:627-635.
9. Giglio JA, Rowland RW, Dalton HP, Laskin DM. Suture removal-induced bacteremia: A possible endocarditis risk. *J Am Dent Assoc* 1992;123:65-66, 69-70.
10. Grigg TR, Liewehr FR, Patton WR, Buxton TB, McPherson JC. Effect of the wicking behavior of multifilament sutures. *J Endod* 2004;30:649-652.
11. Banche G, Roana J, Mandras N, et al. Microbial adherence on various intraoral suture materials in patients undergoing dental surgery. *J Oral Maxillofac Surg* 2007;65:1503-1507.

12. Chen YT, Wang HL, Lopatin DE, O'Neal R, MacNeil RL. Bacterial adherence to guided tissue regeneration barrier membranes exposed to the oral environment. *J Periodontol* 1997;68:172-179.
13. Edmiston CE Jr., Krepel CJ, Marks RM, et al. Microbiology of explanted suture segments from infected and noninfected surgical patients. *J Clin Microbiol* 2013; 51:417-421.
14. Katz S, Izhar M, Mirelman D. Bacterial adherence to surgical sutures. A possible factor in suture induced infection. *Ann Surg* 1981;194:35-41.
15. Harnet JC, Le Guen E, Ball V, et al. Antibacterial protection of suture material by chlorhexidine-functionalized polyelectrolyte multilayer films. *J Mater Sci Mater Med* 2009;20:185-193.
16. Pratten J, Nazhat SN, Blaker JJ, Boccaccini AR. In vitro attachment of *Staphylococcus epidermidis* to surgical sutures with and without Ag-containing bioactive glass coating. *J Biomater Appl* 2004;19: 47-57.
17. Melo MA, Wu J, Weir MD, Xu HH. Novel antibacterial orthodontic cement containing quaternary ammonium monomer dimethylaminododecyl methacrylate. *J Dent* 2014;42:1193-1201.
18. Imazato S, Ehara A, Torii M, Ebisu S. Antibacterial activity of dentine primer containing MDPB after curing. *J Dent* 1998;26:267-271.
19. Justinger C, Moussavian MR, Schlueter C, Kopp B, Kollmar O, Schilling MK. Antibacterial [corrected] coating of abdominal closure sutures and wound infection. *Surgery* 2009;145:330-334.
20. Davey M, Liu X, Ukai T, et al. Bacterial fimbriae stimulate proinflammatory activation in the endothelium through distinct TLRs. *J Immunol* 2008;180: 2187-2195.
21. Takahashi Y, Davey M, Yumoto H, Gibson FC 3rd, Genco CA. Fimbria-dependent activation of proinflammatory molecules in *Porphyromonas gingivalis* infected human aortic endothelial cells. *Cell Microbiol* 2006;8:738-757.
22. Japoni A, Vasin A, Noushadi S, Kiany F, Japoni S, Alborzi A. Antibacterial susceptibility patterns of *Porphyromonas gingivalis* isolated from chronic periodontitis patients. *Med Oral Patol Oral Cir Bucal* 2011;16:e1031-e1035.
23. Pajukanta R. In vitro antimicrobial susceptibility of *Porphyromonas gingivalis* to azithromycin, a novel macrolide. *Oral Microbiol Immunol* 1993;8:325-326.
24. Pinheiro ET, Gomes BP, Drucker DB, Zaia AA, Ferraz CC, Souza-Filho FJ. Antimicrobial susceptibility of *Enterococcus faecalis* isolated from canals of root filled teeth with periapical lesions. *Int Endod J* 2004;37:756-763.
25. Tischer M, Pradel G, Ohlsen K, Holzgrabe U. Quaternary ammonium salts and their antimicrobial potential: Targets or nonspecific interactions? *ChemMedChem* 2012;7:22-31.
26. Mombelli A, Marxer M, Gaberthüel T, Grunder U, Lang NP. The microbiota of osseointegrated implants in patients with a history of periodontal disease. *J Clin Periodontol* 1995;22:124-130.
27. Slots J. Subgingival microflora and periodontal disease. *J Clin Periodontol* 1979;6:351-382.
28. Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. *Periodontol 2000* 1994;5:78-111.
29. van Winkelhoff AJ, Herrera Gonzales D, Winkel EG, Dellemijn-Kippuw N, Vandenbroucke-Grauls CM, Sanz M. Antimicrobial resistance in the subgingival microflora in patients with adult periodontitis. A comparison between The Netherlands and Spain. *J Clin Periodontol* 2000;27:79-86.
30. Tribble GD, Lamont GJ, Progulske-Fox A, Lamont RJ. Conjugal transfer of chromosomal DNA contributes to genetic variation in the oral pathogen *Porphyromonas gingivalis*. *J Bacteriol* 2007;189:6382-6388.
31. Hill EE, Herijgers P, Claus P, Vanderschueren S, Herregods MC, Peetermans WE. Infective endocarditis: Changing epidemiology and predictors of 6-month mortality: A prospective cohort study. *Eur Heart J* 2007;28:196-203.
32. Richards MJ, Edwards JR, Culver DH, Gaynes RP. Nosocomial infections in combined medical-surgical intensive care units in the United States. *Infect Control Hosp Epidemiol* 2000;21:510-515.
33. Vebø HC, Snipen L, Nes IF, Brede DA. The transcriptome of the nosocomial pathogen *Enterococcus faecalis* V583 reveals adaptive responses to growth in blood. *PLoS One* 2009;4:e7660.
34. Chen SY, Chen TM, Dai NT, et al. Do antibacterial-coated sutures reduce wound infection in head and neck cancer reconstruction? *Eur J Surg Oncol* 2011; 37:300-304.
35. Maxwell DP Jr., Diamond JG, May DR. Surgical wound defects associated with endophthalmitis. *Ophthalmic Surg* 1994;25:157-161.
36. Hood CT, Lee BJ, Jeng BH. Incidence, occurrence rate, and characteristics of suture-related corneal infections after penetrating keratoplasty. *Cornea* 2011;30: 624-628.
37. Suárez Grau JM, De Toro Crespo M, Docobo Durántez F, Rubio Chaves C, Martín Cartes JA, Docobo Pérez F. Prevention of surgical infection using reabsorbable antibacterial suture (Vicryl Plus) versus reabsorbable conventional suture in hernioplasty. An experimental study in animals (in Spanish). *Cir Esp* 2007;81:324-329.
38. Baracs J, Huszár O, Sajjadi SG, Horváth OP. Surgical site infections after abdominal closure in colorectal surgery using triclosan-coated absorbable suture (PDS Plus) vs. uncoated sutures (PDS II): A randomized multicenter study. *Surg Infect (Larchmt)* 2011; 12:483-489.
39. Justinger C, Schuld J, Sperling J, Kollmar O, Richter S, Schilling MK. Triclosan-coated sutures reduce wound infections after hepatobiliary surgery — A prospective non-randomized clinical pathway driven study. *Langenbecks Arch Surg* 2011;396:845-850.
40. Slots J. Selection of antimicrobial agents in periodontal therapy. *J Periodontal Res* 2002;37:389-398.
41. van Winkelhoff AJ, Herrera D, Oteo A, Sanz M. Antimicrobial profiles of periodontal pathogens isolated from periodontitis patients in The Netherlands and Spain. *J Clin Periodontol* 2005;32:893-898.
42. Ardila CM, Granada MI, Guzmán IC. Antibiotic resistance of subgingival species in chronic periodontitis patients. *J Periodontal Res* 2010;45:557-563.
43. Hintermann E, Haake SK, Christen U, Sharabi A, Quaranta V. Discrete proteolysis of focal contact and adherens junction components in *Porphyromonas gingivalis*-infected oral keratinocytes: A strategy for

cell adhesion and migration disabling. *Infect Immun* 2002;70:5846-5856.
44. Kato T, Kawai S, Nakano K, et al. Virulence of *Porphyromonas gingivalis* is altered by substitution of fimbria gene with different genotype. *Cell Microbiol* 2007;9:753-765.
45. Tsuda K, Furuta N, Inaba H, et al. Functional analysis of alpha5beta1 integrin and lipid rafts in invasion of epithelial cells by *Porphyromonas gingivalis* using fluorescent beads coated with bacterial membrane vesicles. *Cell Struct Funct* 2008;33:123-132.
46. Bhattacharya R, Xu F, Dong G, et al. Effect of bacteria on the wound healing behavior of oral epithelial cells. *PLoS One* 2014;9:e89475.

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.

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