ORIGINAL RESEARCH

Molecular Iodine Mouthrinse Antimicrobial Activity Against Periodontopathic Bacteria

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Abstract

Aim: This study compared two molecular iodine mouthrinses for their *in vitro* bactericidal effects against subgingival biofilm bacteria from severe periodontitis patients.

Materials and methods: In a subgingival biofilm eradication assay, dilution aliquots of subgingival microbial specimens from 32 adults with severe periodontitis were mixed *in vitro* with either a mouthrinse containing 100 parts per million (ppm) molecular iodine (Iorinse®) or one containing 150 ppm molecular iodine (iClean®), followed by mouthrinse neutralization after 60 seconds with 3% sodium thiosulfate. The mixtures, along with unexposed subgingival biofilm aliquots, were inoculated onto enriched Brucella blood agar and incubated anaerobically for 7 days to quantitate total viable bacterial counts and selected red/orange complex periodontal pathogens (*Porphyromonas gingivalis, Tannerella forsythia, Prevotella intermedia/nigrescens, Parvimonas micra, Campylobacter rectus,* and *Fusobacterium nucleatum*).

Results: Both molecular iodine mouthrinses significantly reduced total viable bacterial counts in the subgingival biofilm samples, with iClean® providing significantly greater *in vitro* suppression than Iorinse®. Both molecular iodine mouthrinses also significantly reduced total red/orange complex periodontal pathogens, with significantly greater suppression also exhibited by iClean®.

Conclusion: The molecular iodine mouthrinses exerted marked bactericidal activity *in vitro* against human subgingival biofilm microbial species, including red/orange complex periodontal pathogens associated with severe periodontitis, with iClean® providing significantly better antimicrobial activity than Iorinse®.

Clinical significance: These findings suggest potential value of molecular iodine mouthrinses in the treatment and prevention of periodontal diseases.

Keywords: *In vitro*, Laboratory research, Molecular iodine, Mouthrinse, Periodontal pathogens, Periodontitis.

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Introduction

Control of gingival dental plaque growth is integral to maintenance of periodontal health.[1] Poor oral hygiene is closely linked to the onset and perpetuation of gingival inflammation associated with gingivitis and periodontitis.[2] In a study of adults initially examined at 35 years of age, 65% of those with poor oral hygiene at that age had periodontitis when re-examined 15 years later at age 50, in contrast to only 13% of those with good oral hygiene at age 35.[3]

Mechanical tooth brushing and interproximal dental flossing comprise the foundation of daily oral hygiene regimens.[1] However, their frequency of use varies considerably among patients, with better oral hygiene behaviors found in females and people with higher educational attainment, certain oral health beliefs, greater household income, and having a usual source of dental care.[4] In addition, most individuals incompletely remove supragingival plaque with conventional oral hygiene methods. Despite reporting daily oral hygiene activities, 72% of dentate adults in a nationwide study in the United Kingdom had supragingival plaque biofilms visibly present on about one-third of their teeth.[5] Since effective tooth brushing is uncommon and largely ineffective against interproximal plaque biofilms on premolars and molars,[1] antimicrobial mouthrinses are frequently employed to improve clinical outcomes.[6–8] An ideal antimicrobial mouthrinse should be effective, safe, affordable, accessible, and convenient to use.[9] However, classic mouthrinse formulations vary widely in their clinical efficacy against gingival inflammation,[8] *in vitro* antimicrobial activity against intraoral bacteria,[10] and spectrum of potential adverse side effects, such as tooth and soft tissue staining, taste disturbances, and mucosal desquamation.[11,12] Efforts to overcome these shortcomings, as well as increasing consumer demand, continue to spur development of new types of mouthrinse products.[13]

Relative to this, molecular iodine is known to rapidly inactivate a wide range of bacteria, viruses, and fungi,[14] including several putative periodontopathic bacterial species.[15] In recent years, two new commercial mouthrinses are available in the United

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Presentation at a meeting: Research abstract presented in November, 2021 at Research Forum, American Academy of Periodontology 107th Annual Meeting, Miami, entitled "Bactericidal activity of two molecular iodine mouthrinses against selected human red and orange complex periodontal pathogens", co-authored by Shin SS, Rams TE and Sautter J.

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States of America, following marketing clearance by the United States Food and Drug Administration, which claim to possess high levels of free molecular iodine. One of the mouthrinses, Iorinse® RTU (Iotech International, Boca Raton, FL, USA), claims a free molecular iodine level of 100 parts per million (ppm), while the other, iClean® (iClean Company, San Antonio, TX, USA), claims a 150 ppm concentration of free molecular iodine. These free molecular iodine levels are markedly greater than those obtained from over-the-counter povidone-iodine products, where only 2 ppm of free molecular iodine is eluted from undiluted 10% solutions, and up to only 25 ppm after a fresh 1/100 dilution of a 10% solution to better release iodine complexed to the polyvinylpyrrolidone carrier.[16]

It is not known whether Iorinse® and/or iClean® molecular iodine mouthrinses are active against major periodontal bacterial pathogens associated with severe forms of periodontitis, such as red/orange complex bacterial species[17] that may populate supragingival biofilm communities as well as periodontal pockets in periodontitis patients.[18] If molecular iodine mouthrinses possess antimicrobial activity against microorganisms associated with human periodontal disease, they may serve as a new therapeutic and preventive tool for clinical use in dental practices.

As a result, the purpose of this study was to compare the *in vitro* bactericidal effects of Iorinse® and iClean® molecular iodine-based mouthrinses against subgingival biofilm bacteria isolated from adults with severe periodontitis.

Materials and Methods

This study was carried out in the Oral Microbiology Testing Service (OMTS) Laboratory at Temple University School of Dentistry in Philadelphia, PA, USA, from February to March 2021. The OMTS Laboratory is licensed by the Pennsylvania Department of Health and Clinical Laboratory Improvement Amendments (CLIA) certified by the United States Centers for Medicare and Medicaid Services for high complexity bacteriological analysis.

Subgingival Biofilm Specimens

Subgingival biofilm specimens from 32 adults with severe periodontitis were tested with the two mouthrinses as approved by the Temple University Human Subjects Protections Institutional Review Board and in accordance with the Declaration of Helsinki. The 32 subgingival biofilm sample size provided an acceptable *post hoc* statistical power level of ≥83.5%. The subgingival biofilm specimens were collected and submitted to the OMTS Laboratory for microbiological analysis by each patient's periodontist, following standardized procedures, by removing supragingival plaque from 3 to 5 periodontal sites in each patient which exhibited moderate (5–6 mm) to deep periodontal probing depths (≥7 mm) and gingival bleeding on probing, and isolating them with cotton rolls and air drying to avoid saliva contamination during microbiological sampling. Subgingival biofilm samples were then collected by placement of one to two sterile paper points into each isolated periodontal site for approximately 10 seconds. All paper points from each patient were pooled together into a single glass vial containing 2.0 mL of pre-reduced and anaerobically sterilized Möller's VMGA III transport media.[19]

After receipt at the microbiology laboratory within 24 hours, the VMGA III specimens were warmed to 37°C for 10 minutes to liquefy gelatin in the transport medium, vortexed at a maximal instrument setting for 45 seconds, and then subjected to serial 10-fold dilutions using Möller's VMG I anaerobic dispersion solution, which was comprised of pre-reduced and anaerobically sterilized 0.25% tryptose-0.25% thiotone E peptone-0.5% NaCl.[20]

Subgingival Biofilm Eradication Assay

A subgingival biofilm eradication assay evaluated the *in vitro* bactericidal effects of a 60-second exposure of subgingival biofilms to each of two molecular iodine mouthrinses, similar to *in vitro* methods described by Pozhitkov et al.[21] for testing the antimicrobial activity of dilute sodium hypochlorite on human subgingival bacteria, and by Rams et al.[22] for assessing the effects of silver diamine fluoride on human subgingival biofilms.

For each of the 32 subgingival biofilm samples, 0.1 mL aliquots of 10^{-6} specimen dilutions were mixed with either (1) 0.05 mL of Iorinse® mouthrinse, (2) 0.05 mL of iClean® mouthrinse, or (3) no mouthrinse to serve as a control for comparison with mouthrinse-exposed samples. After a 60-second contact time, the molecular iodine mouthrinse in each exposed mixture was neutralized with 0.05 mL of 3% sodium thiosulfate,[23] which by itself has no effect on bacterial viability (unpublished data).

After mouthrinse neutralization, the exposed and nonexposed subgingival biofilm specimens were then plated with a sterile glass rod onto pre-reduced and enriched Brucella blood agar (EBBA), comprised of 4.3% Brucella agar (BBL Microbiology Systems, Cockeysville, MD, USA) supplemented with 0.3% bacto-agar, 5% defibrinated sheep blood, 0.2% hemolyzed sheep red blood cells, 0.0005% hemin, and 0.00005% menadione. The inoculated EBBA culture plates were incubated at 37°C for 7 days in an upright heated incubator (Caron, Marietta, OH, USA) in jars containing an 85% N_2-10% H_2-5% CO_2 anaerobic atmosphere introduced by an Anoxomat™ Mark II automatic jar evacuation-replacement system (Advanced Instruments, Inc., Norwood, MA, USA). Bacterial species growing on EBBA plates after *in vitro* exposure to a molecular iodine mouthrinse were considered resistant to the molecular iodine mouthrinse. Flowchart 1 summarizes how the subgingival biofilm species eradication assay was carried out.

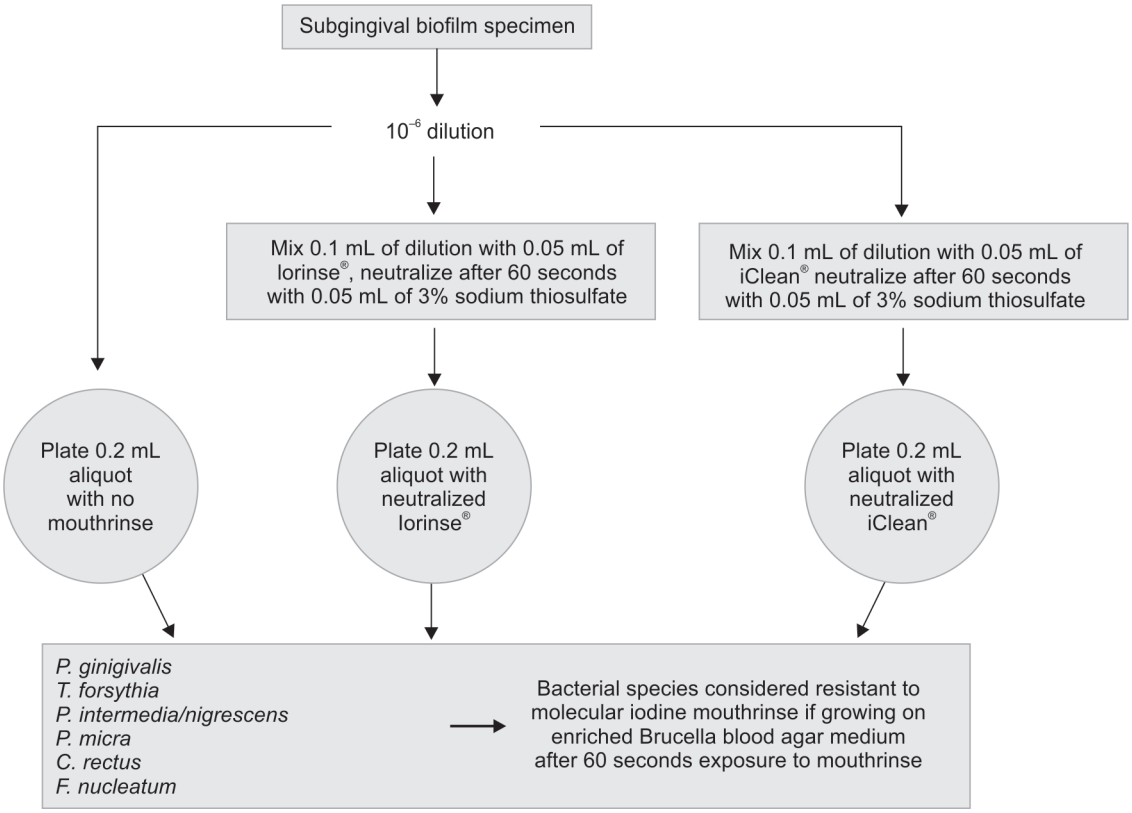
In Vitro Antibiotic Resistance Testing

Additional 0.1 mL aliquots of subgingival biofilm specimen dilutions were inoculated onto EBBA plates supplemented with either metronidazole at 16 mg/L, doxycycline at 4 mg/L, amoxicillin at 8 mg/L, or clindamycin at 4 mg/L (all antibiotics obtained as pure powder from Sigma–Aldrich, St Louis, MO, USA), and incubated anaerobically for 7 days. These antimicrobial concentrations represent non-susceptible/resistant breakpoint concentrations against anaerobic bacteria for amoxicillin, clindamycin, and metronidazole as recommended by the Clinical and Laboratory Standards Institute,[24] and for doxycycline as recommended by the French Society for Microbiology.[25] *In vitro* resistance to an antibiotic breakpoint concentration was recorded when test species growth was detected on an antibiotic-supplemented EBBA plate.[26] *Bacteroides thetaiotaomicron* ATCC 29741, *Clostridium perfringens* ATCC 13124, and a multi-antibiotic-resistant clinical periodontal isolate of *Fusobacterium nucleatum* were used as positive and negative controls for antibiotic resistance testing on antibiotic-supplemented EBBA plates.[26]

Microbiological Identification and Quantitation

For all EBBA plates, total viable bacterial counts were quantitated using an automated colony counter system (AccuCount™ 1000 Macroscopic Automated Colony Counter, BioLogics, Inc., Manassas, VA, USA), validated to reliably detect bacterial surface

Flowchart 1: Flowchart for a subgingival biofilm species eradication assay for two molecular iodine mouthrinses



colonies ≥0.3 mm in diameter,[27] to determine the total number of microbial colony-forming units (CFUs) per culture plate. Established phenotypic criteria were used to presumptively identify and enumerate the following selected red/orange complex periodontal pathogens: *Porphyromonas gingivalis, Tannerella forsythia, Prevotella intermedia/nigrescens, Parvimonas micra, Campylobacter rectus,* and *F. nucleatum*.[26,28]

Proportional recovery of the test bacterial species was calculated as the percent recovery of each test species CFU among total viable bacterial counts as determined on EBBA plates where the inoculated subgingival specimen was not exposed *in vitro* to either of the molecular iodine mouthrinses.

Data Analysis

Statistical analysis compared total viable bacterial counts and total counts of red/orange complex periodontal pathogens among subgingival biofilm samples exposed *in vitro* to (1) Iorinse®, (2) iClean®, or (3) no mouthrinse.

For subgingival biofilm species eradication assay testing outcomes, mean total viable bacterial counts were calculated and transformed to $\log_{10}$ values for each patient subgingival sample exposed and not exposed *in vitro* to the molecular iodine mouthrinses. For each of the selected red/orange complex bacterial species evaluated in this study, the number and proportion of species-positive patient samples were determined, along with the mean proportional recovery and standard deviation (SD) of the bacterial species.

The number and proportion of patient samples with red/orange complex periodontal pathogens that were resistant to either of the molecular iodine mouthrinses were also determined. After $\log_{10}$-transformation, total counts of the evaluated red/orange complex periodontal pathogens were determined by summing together individual species data for each patient and then calculating total mean values across all patients.[22]

As recommended by Socransky et al.[29] for oral biofilm count data, nonparametric statistical analysis, using the Wilcoxon matched pairs signed-rank test, compared mean total viable bacterial counts, and mean total counts of red/orange complex periodontal pathogens for subgingival biofilm samples exposed and not exposed to either of the two molecular iodine mouthrinses, with $p \leq 0.05$ required for statistical significance. The PC-based STATA/SE 16.1 for Windows (StataCorp PL, College Station, TX, USA) 64-bit statistical software package was used for data analysis.

Results

Table 1 lists subgingival red/orange complex biofilm species recovered by culture from 32 adult patients with severe periodontitis. All of the subgingival specimens were positive with one or more of the evaluated red/orange complex periodontal pathogens. Orange complex periodontal pathogens were more frequently found in subgingival biofilm specimens than red complex bacterial species in the study patients. *P. micra* and *F. nucleatum* were the most often recovered, with 100 and 93.8% of patient subgingival samples positive for the species, respectively. Mean $\log_{10}$ total red/orange complex periodontal pathogen counts per patient were 1.4031 ± 0.4880 (SD) $\times 10^6$ CFU, with $\log_{10}$ total viable subgingival counts averaging 2.1781 ± 0.4837 (SD) $\times 10^6$ CFU per study patient specimen.

Table 2 provides the prevalence of *in vitro* antibiotic resistance for red/orange complex periodontal pathogens recovered from

Table 1: Recovery of red/orange complex periodontal pathogens in subgingival biofilm samples from 32 severe periodontitis patients

Species	*No. (%) of positive patients*	*% recovery in species-positive patients ± SD*	*Range %*
Red complex species:			
P. gingivalis	1 (3.1)	8.2 ± 0.0	8.2
T. forsythia	1 (3.1)	0.7 ± 0.0	0.7
Orange complex species:			
P. intermedia/nigrescens	25 (78.1)	4.9 ± 5.6	0.1–23.7
P. micra	32 (100)	6.9 ± 5.2	0.8–25.0
C. rectus	9 (28.1)	0.1 ± 0.0	*0.1*
F. nucleatum	30 (93.8)	9.1 ± 7.2	1.4–30.0

Table 2: *In vitro* antibiotic resistance of red/orange complex periodontal pathogens

Species (no. of positive patients)	MET (16 mg/L[a])	AMOX (8 mg/L)	CLIN (4 mg/L)	DOX (4 mg/L)
Red complex species:				
P. gingivalis (1)	0[b]	0	0	0
T. forsythia (1)	0	0	1 (100)	0
Orange complex species:				
P. intermedia/nigrescens (25)	0[b]	12 (48.0)	17 (68.0)	9 (36.0)
P. micra (32)	0	3 (9.4)	15 (46.9)	14 (43.8)
C. rectus (9)	0	0	0	0
F. nucleatum (18)	0	0	0	0

[a]Non-susceptible breakpoint concentration of antibiotic used *in vitro*; [b]no. (%) of organism-positive patient samples demonstrating *in vitro* resistance of organism to non-susceptible breakpoint concentration of antibiotic; AMOX, amoxicillin; CLIN, clindamycin; DOX, doxycycline; MET, metronidazole

subgingival biofilms. No metronidazole *in vitro* resistance was found among red/orange complex species recovered in the 32 patient samples. Over two-thirds (68%) of *P. intermedia/nigrescens* clinical isolates were resistant to clindamycin, with 48% resistant to amoxicillin, and 36% resistant to doxycycline. Among *P. micra* strains, 46.9% were resistant to clindamycin, 43.8% resistant to doxycycline, and 9.4% resistant to amoxicillin. All *C. rectus* and *F. nucleatum* clinical isolates were inhibited *in vitro* by threshold breakpoint concentrations of each of the four tested antibiotics.

Figure 1 reveals the effects of the molecular iodine mouthrinses on total viable bacterial counts in the subgingival biofilm samples. Log_{10} total viable bacterial counts in subgingival specimens exposed *in vitro* to Iorinse® averaged 1.5902 ± 0.8165 (SD) × 10^6 CFU per patient, while those exposed to iClean® were a mean 0.7885 ± 0.8233 (SD) × 10^6 CFU per patient. Subgingival biofilm specimens exposed to either of the molecular iodine mouthrinses yielded significantly lower average log_{10} total subgingival viable bacterial counts (ranging from 27.0 to 63.8% less) than nonexposed control specimens (both $p < 0.0001$). The iClean® mouthrinse produced significantly lower mean log_{10} total subgingival viable counts than Iorinse® ($p < 0.0002$).

Figure 2 shows the effects of the molecular iodine mouthrinses on red/orange complex periodontal pathogens. Mean log_{10} red/orange complex periodontal pathogen counts in subgingival specimens exposed *in vitro* to Iorinse® were 0.3589 ± 0.5853 (SD) × 10^6 CFU/patient, while those exposed to iClean® were 0.0368 ± 0.2079 (SD) × 10^6 CFU/patient, which were both significantly lower than in nonexposed control specimens (both $p < 0.0001$), inducing 74.4 and 97.4% greater reductions, respectively. In addition, iClean® significantly lowered red/orange complex periodontal pathogen counts more than Iorinse® ($p < 0.0044$).

Among Iorinse® mouthrinse-exposed subgingival specimens, all evaluated red/orange complex periodontal pathogens were suppressed below detection in 17 (53.1%) of the subgingival biofilm samples. Subgingival species resistant *in vitro* to Iorinse® were *P. intermedia/nigrescens* (8 of 25 patient strains), *P. micra* (7 of 32 patient strains), and *F. nucleatum* (6 of 30 patient strains).

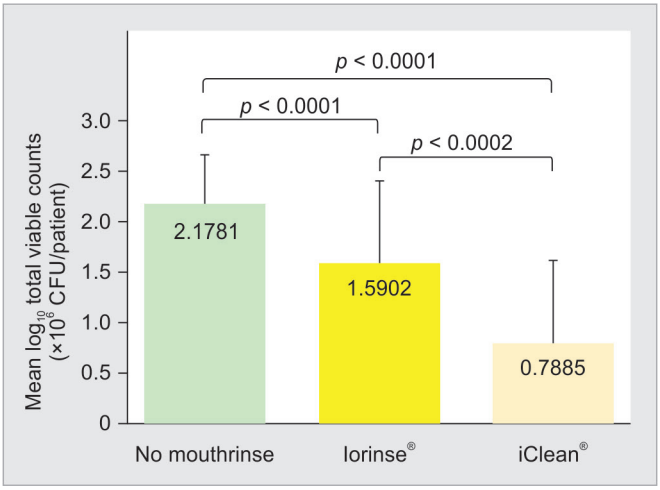


Fig. 1: Average log_{10} total viable bacterial counts in subgingival biofilms exposed and not exposed *in vitro* to Iorinse® or iClean® mouthrinses

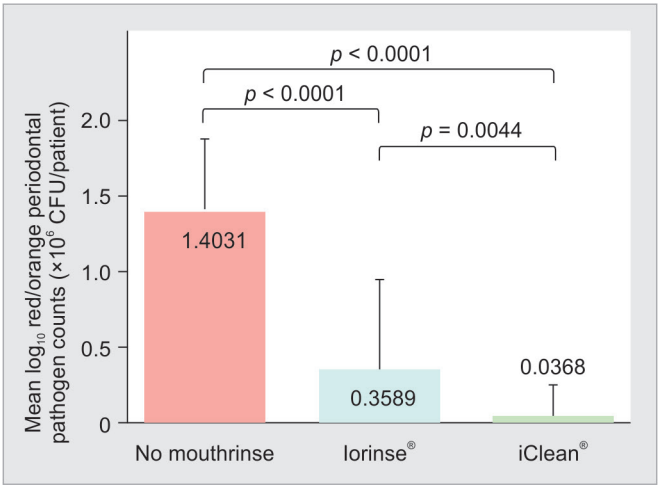


Fig. 2: Average $\log_{10}$ red/orange complex periodontal pathogen counts in subgingival biofilms exposed and not exposed to Iorinse® or iClean® mouthrinses

All *P. intermedia/nigrescens* and six *P. micra* strains resistant to Iorinse® were also resistant *in vitro* to one or more of the test antibiotics.

All evaluated red/orange complex periodontal pathogens were below detection in 29 (90.6%) of iClean® mouthrinse-exposed subgingival specimens. Subgingival species resistant *in vitro* to iClean® were *P. intermedia/nigrescens* (1 of 25 patient strains), *P. micra* (2 of 32 patient strains), and *F. nucleatum* (2 of 30 patient strains). The single *P. intermedia/nigrescens* and two *P. micra* strains resistant to iClean® were also resistant *in vitro* to one or more of the test antibiotics.

Both Iorinse® and iClean® were bactericidal to 9 of the 17 *P. intermedia/nigrescens* and 9 of the 15 *P. micra* strains exhibiting *in vitro* resistance to one or more of the test antibiotics.

Microbial species present among the low number of organisms growing after exposure to the molecular iodine mouthrinses were predominately viridans streptococci and *Actinomyces*-like species, as recognized by their typical colony morphology and phenotypic appearance. No yeasts or Gram-negative enteric rods/pseudomonads were noted on any of the molecular iodine mouthrinse-exposed EBBA culture plates.

Discussion

This study assessed the *in vitro* bactericidal effects of two molecular iodine mouthrinses against total viable bacterial counts and selected red/orange complex bacterial pathogens in subgingival biofilms from adults with severe periodontitis. Even though these microbial specimens originated from periodontal pockets, many of the bacterial species are also found in supragingival plaque biofilms in periodontitis patients,[18] and thus may be impacted by mouthrinses, although generally for only several minutes of *in vivo* exposure time.[11] In regard to this, several major periodontal pathogens, such as *P. gingivalis*, *P. intermedia/nigrescens*, *F. nucleatum*, and *Aggregatibacter actinomycetemcomitans*, require chlorhexidine concentrations of 0.5–2% to attain bactericidal effects within a 5-minute *in vitro* contact time, and remain viable when exposed for 5 minutes to 0.12% and 0.2% chlorhexidine concentrations found in commercial mouthrinse products.[15] The present study employed a subgingival biofilm eradication assay[21,22] with a shorter *in vitro* exposure time, attained by neutralizing the molecular iodine mouthrinses with sodium thiosulfate[23] 60 seconds after being mixed with the microbial specimens, to mimic the contact time mouthrinses most likely have with coronal tooth surfaces during *in vivo* oral rinsing,[11] or within inflamed periodontal pockets when used as a subgingival irrigant prior to their rapid clearance by the flow of gingival crevicular fluid.[30]

The major finding from the present study is that both molecular iodine mouthrinses exhibited rapid bactericidal activity within 60 seconds against total subgingival viable bacterial counts and red/orange complex periodontal pathogens from severe periodontitis patients. However, the iClean® mouthrinse reduced total viable bacterial counts and red/orange complex periodontal pathogens significantly more than Iorinse®. Among the 32 subgingival biofilm samples tested, 90.6% were culture-negative for all evaluated red/orange complex periodontal pathogens after a 60-second exposure to iClean®, but only 53.1% of the specimens were similarly affected after the same exposure time to Iorinse®. Thus, iClean® significantly outperformed Iorinse®, at least under the laboratory-based testing conditions used in the present study. No prior studies have been published on the antimicrobial potential of the two molecular iodine mouthrinses on freshly cultivated subgingival biofilms from severe periodontitis patients.

The enhanced antimicrobial impact of iClean® is likely due to its higher concentration of free molecular iodine (150 ppm), as compared to Iorinse® (100 ppm), since higher levels of free molecular iodine are associated with greater antimicrobial activity.[16] The mechanism of antimicrobial action of molecular iodine is thought to involve multiple bacterial cellular targets, including creation of pores in negatively charged cell walls of microorganisms, leading to leakage of intercellular contents and cell death, as well as electrophilic-mediated disruption of microbial metabolic pathways, leading to denaturation of microbial enzymes.[31,32]

Importantly, no opportunistic overgrowth of fungi or Gram-negative enteric rods/pseudomonads was noted after exposure of subgingival biofilms to the molecular iodine mouthrinses. This is in contrast to chlorhexidine-based mouthrinses, where many pathogenic Gram-negative enteric rod/pseudomonad species, such as *Enterobacter cloacae, Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Pseudomonas cepacia*, and *Serratia marcescens*, often survive and grow in the presence of 70 mg/L of chlorhexidine,[33] potentially leading to opportunistic overgrowth of the species in the oral cavity after prolonged chlorhexidine mouthrinse use, as has been found in animal studies.[34]

Only some clinical isolates of *P. intermedia/nigrescens*, *P. micra*, and *F. nucleatum* exhibited *in vitro* resistance to the molecular iodine mouthrinses in the present study. Instead, viridans streptococci and *Actinomyces*-like species were prominent among the low number of CFU growing after *in vitro* exposure to the molecular iodine mouthrinses. Thus, the molecular iodine mouthrinses, particularly iClean®, were more active against putative pathogenic microorganisms in subgingival biofilms, and less active against bacterial species considered to have a low or negligible periodontopathic potential. This effect of molecular iodine mouthrinses, if clinically replicated *in vivo*, may be useful as an adjunct to mechanical debridement of dental plaque biofilms in promoting a desirable shift in the composition of the oral microbiome in periodontitis from a pathogenic dysbiotic state associated with progressive periodontal breakdown to one found with periodontal health and clinical periodontal stability.[35]

Interestingly, all eight *P. intermedia/nigrescens* strains and six of the seven *P. micra* strains exhibiting *in vitro* resistance to either Iorinse® or iClean® were also resistant *in vitro* to one or more of the evaluated antibiotics. Molecular mechanisms underlying these concurrent antimicrobial resistance patterns were not studied. In contrast, the majority (9 of 17 *P. intermedia/nigrescens* and 9 of 15 *P. micra* strains) of test species strains exhibiting resistance *in vitro* to one or more of the test antibiotics were susceptible to the molecular iodine mouthrinses. As a result, molecular iodine mouthrinses may help mitigate against the oral cavity microbiota as a potential source for antibiotic resistance genes in the human microbiome.[36] An added benefit of molecular iodine mouthrinses, not studied in the present investigation, is their potential for rapid inactivation of viruses, particularly SARS-CoV-2.[37,38]

The present study is limited by its laboratory-based design without any *in vivo* clinical assessments. Only selected cultivable red/orange complex periodontal pathogens were identified in the subgingival biofilm specimens, and not a wider range of microorganisms detectable with molecular methods.[39] It is also not clear if the antimicrobial activity of molecular iodine mouthrinses is adversely affected by blood or protein, as occurs with chlorhexidine.[40] Patient-based studies are needed to better delineate the potential clinical value of molecular iodine mouthrinses in comparison to other types of mouthrinses.

Conclusion

Two commercial molecular iodine mouthrinses exerted marked bactericidal activity *in vitro* against human subgingival biofilm microbial species, including red/orange complex periodontal pathogens associated with severe periodontitis, with iClean® providing significantly better antimicrobial activity than Iorinse®.

Clinical Significance

Two commercially available molecular iodine mouthrinses may aid in the treatment and prevention of periodontal diseases via their bactericidal effects against periodontal bacterial pathogens in subgingival biofilms.

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