The effectiveness of a pre-procedural mouthrinse in reducing bacteria on radiographic phosphor plates

Allison Hunter[1,*], Sajitha Kalathingal[1], Michael Shrout[2], Kevin Plummer[2], Stephen Looney[1,3]

[1]Radiology Oral Health and Diagnostic Sciences, Georgia Regents University, College of Dental Medicine, Augusta, GA, USA
[2]Department of Oral Rehabilitation, Georgia Regents University, College of Dental Medicine, Augusta, GA, USA
[3]Department of Biostatistics and Epidemiology Georgia Regents University Medical College of Georgia, Augusta, GA, USA

ABSTRACT

Purpose: This study assessed the effectiveness of three antimicrobial mouthrinses in reducing microbial growth on photostimulable phosphor (PSP) plates.

Materials and Methods: Prior to performing a full-mouth radiographic survey (FMX), subjects were asked to rinse with one of the three test rinses (Listerine®, Decapinol®, or chlorhexidine oral rinse 0.12%) or to refrain from rinsing. Four PSP plates were sampled from each FMX through collection into sterile containers upon exiting the scanner. Flame-sterilized forceps were used to transfer the PSP plates onto blood agar plates (5% sheep blood agar). The blood agar plates were incubated at 37°C for up to 72 h. An environmental control blood agar plate was incubated with each batch. Additionally, for control, 25 gas-sterilized PSP plates were plated onto blood agar and analyzed.

Results: The mean number of bacterial colonies per plate was the lowest in the chlorhexidine group, followed by the Decapinol, Listerine, and the no rinse negative control groups. Only the chlorhexidine and Listerine groups were significantly different (p=0.005). No growth was observed for the 25 gas-sterilized control plates or the environmental control blood agar plates.

Conclusion: The mean number of bacterial colonies was the lowest in the chlorhexidine group, followed by the Decapinol, Listerine, and the no rinse groups. Nonetheless, a statistically significant difference was found only in the case of Listerine. Additional research is needed to test whether a higher concentration (0.2%) or longer exposure period (two consecutive 30 s rinse periods) would be helpful in reducing PSP plate contamination further with chlorhexidine. (**_Imaging Sci Dent 2014; 44: 149-54_**)

KEY WORDS: Diagnostic Imaging; Listerine; Chlorhexidine; Delmopinol

Introduction

Digital imaging using photostimulable phosphor (PSP) plates has been available in medical radiology since 1981 and in dental radiology since 1994.[1,2] PSP plates are similar to conventional intraoral films in the sense that they are thin and flexible and may be used with the same position-ing devices for image acquisition.[1] In contrast to direct digital sensors, also used for intraoral imaging, the entire surface area of a PSP plate is active. Additionally, PSP plates have been shown to exhibit a wider range of exposures, less patient discomfort, and a decreased requirement for retakes.[3-5] For these reasons, PSP plates may be favored in a teaching environment such as dental school clinics. However, because of the number of personnel involved in the process and the inexperience of the operators, keeping PSP plates disinfected in this type of environment can be challenging. Previously, two studies were conducted by the authors to determine whether, despite precautions, PSP plates can become contaminated over time.[6,7] In the first

study conducted by Kalathingal et al in 2009, approximately 57.8% of the PSP plates demonstrated bacterial growth and a microscopic analysis indicated oral flora as the source of the Gram-positive rods sampled from the contaminated plates.[6] In the second study conducted by Kalathingal et al in 2010, Mitis-Salivarius agar was used to confirm oral streptococci as a source of contamination.[7]

Chemotherapeutic mouthrinses have been used in dentistry for many years to aid in the reduction and removal of plaque. Several different products have been used and evaluated for numerous applications. Chlorhexidine is one of the most widely used and most effective mouthrinses and is therefore considered the gold standard.[8-11] Chlorhexidine has both bactericidal and bacteriostatic activity and has been shown to be the most effective antiplaque agent for both short- and long-term use.[10,11] Listerine® is an over-the-counter product approved for the control of supragingival plaque.[9] The bactericidal effect of Listerine® is accomplished through the disruption of the cell wall and the inhibition of enzyme activity.[9] Listerine® has been shown to significantly reduce gingivitis and plaque without extrinsic staining like that reported with chlorhexidine.[9] Decapinol® contains delmopinol hydrochloride, which is bactericidal and reduces the adherence of plaque-forming bacteria.[8,12,13] Delmopinol has also been shown to dissolve existing plaque.[14] Similar to Listerine®, delmopinol does not share the tendency toward tooth staining with chlorhexidine.[8]

As proven antiplaque agents, mouthrinses may also be effective in reducing the contamination of PSP with oral streptococci. In an effort to evaluate whether a mouthrinse protocol might be efficacious, three antimicrobial rinses were chosen for this study: chlorhexidine oral rinse 0.12% (Peridex 3M ESPE, St. Paul, USA,), Listerine® (Johnson & Johnson, Skillman, USA), and Decapinol® (Sinclair Pharmaceuticals, Milan, Italy). Chlorhexidine was chosen to serve as a positive control for comparing the efficacy of chemotherapeutic agents.[9-11] Listerine® was chosen as a representative of an effective over-the-counter antiplaque agent. Decapinol® was chosen due to its ability to dissolve plaque and its decreased association with extrinsic staining.[8,14] Because of their antimicrobial activity, it was proposed that the use of a pre-procedural mouthrinse may assist in reducing microbial growth on PSP plates and aid in reducing cross-contamination in a dental school's clinical environment.

The purpose of this study was to evaluate the effectiveness of using a mouthrinse prior to acquiring a full-mouth radiographic survey in reducing the contamination of PSP plates in a dental school clinical environment. The three

mouthrinses tested included: chlorhexidine oral rinse 0.12%, Listerine®, and Decapinol®.

Materials and Methods

A total of 130 subjects were recruited from the screening population of the Georgia Regents University College of Dental Medicine (GRU CDM) to be included in the four treatment groups. Subjects with mandibular premolars and molars that were deemed suitable for a full-mouth radiographic survey upon review of their clinical needs were included in the study. Subjects with a reported history of allergy to chlorhexidine, Listerine®, or Decapinol® were excluded from the study. There were four test groups: one each for the three oral rinses and one no rinse group. This study was approved by the Human Assurance Committee of the GRU CDM (HAC file number: 10-10-082). Informed consent was obtained from all the subjects prior to their inclusion in the study.

Cassettes of PSP plates (Soredex/Orion Corp., Helsinki, Finland) were checked out from the junior clinic dispensary. The infection control policy of our institution involves sterilizing PSP plates at the end of the work week with ethylene oxide gas.[7] Therefore, in order to ensure the results represented an equal distribution of dispensed PSP plates, the study was conducted towards the beginning and the end of the work week. Prior to performing a full-mouth radiographic survey, the subjects were asked to either rinse with one of the three test rinses or to refrain from rinsing. The no rinse group served as the negative control group for the study.[15] Pre-procedural rinsing with the three test rinses was performed according to the manufacturer's instructions. Chlorhexidine: 15 mL for 30 s; Listerine®: 20 mL for 30 s; Decapinol®: 10 mL for 30 s. The radiographic survey was conducted according to the normal school infection control protocol described previously.[7] Four PSP plates were sampled from each full-mouth survey. To select a PSP plate with the highest probability of salivary contamination, the plates used to acquire the mandibular premolar and molar periapical views were collected. After processing the images, each of the four PSP plates was captured into a separate sterile container upon exiting from the scanner (Digora Optime, Soredex, Helsinki, Finland). The plates were not allowed to be collected in the plate receptacle attached to the scanning unit by the manufacturer. The PSP plates were removed from their sterile container with flame-sterilized forceps and plated onto separate blood agar plates (5% sheep blood agar, Lampire Biological Laboratories, Pipersville, USA).[6,7] The blood
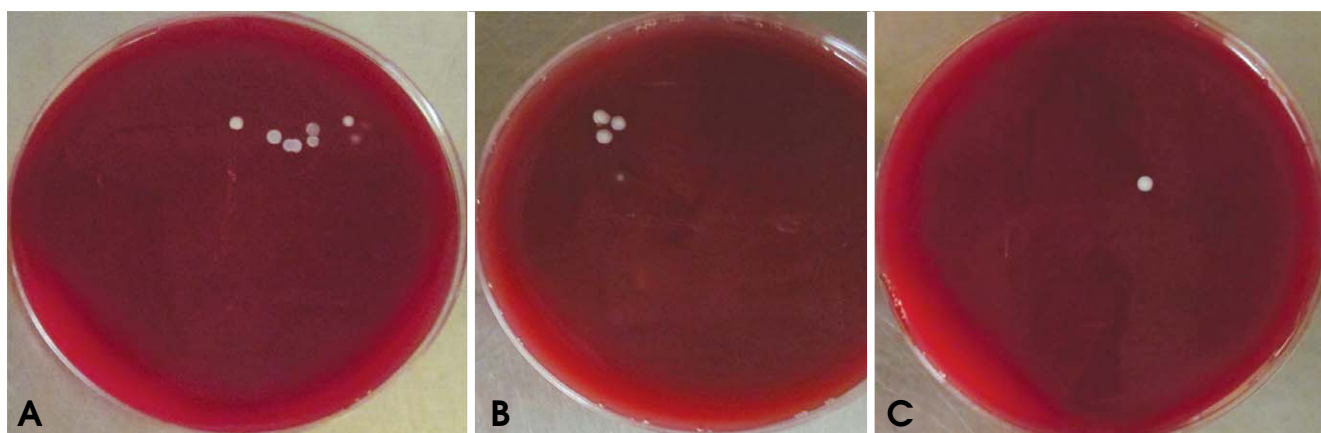
Fig. 1. Bacterial growth is seen on blood agar plates. A. Listerine test group, B. Decapinol test group, C. Chlorhexidine test group.

agar plates were labeled with the date, batch number, and test group. Each set of four blood agar plates were placed in plastic wrap and incubated at 37°C for up to 72 h. After incubation, the blood agar plates were evaluated for the presence or absence of microbial growth. When microbial growth was detected, the number of colonies was recorded (Fig. 1). For control, 25 gas-sterilized PSP plates were plated onto blood agar (5% sheep blood agar, Lampire Biological Laboratories, Pipersville, USA) and analyzed using the same protocol.[7] Additionally, a control blood agar plate exposed to the environment during the plating of the PSP plate was incubated with each sample batch. The inclusion of the control agar plates exposed to the environment allowed for the detection of any cross contamination occurring during the plating of the PSP plate on the blood agar. A similar baseline technique was used by Logothetis and Martinez-Welles in 1995 and Feres et al in 2010 for the collection of bacteria in aerosols on blood agar plates.[15,16]

The negative control group (no rinse group) and the three mouthrinse groups were compared in terms of the mean number of colonies per plate by using statistical methods for comparing two or more groups in the presence of clustered data.[17] Statistical methods for clustered data were used since four phosphor plates (the mandibular premolar and molar periapicals) were examined for each subject in each treatment group. Thus, each patient was treated as a cluster, and the intra-cluster correlation (ICC) was taken into account when comparing the four treatment groups by using cluster-based analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons. This cluster-based analysis was carried out using mixed-effects regression models, as implemented in the MIXED procedure in SAS 9.3 (SAS Institute, Inc., Cary, NC, 2009). Mixed-effects

regression models were required for the analysis of the study data in order to account for the clustered nature of the data (PSP plates were clustered within the patients), as well as the fact that not all patients had data for all four plates. The MIXED procedure in SAS is particularly well-suited for dealing with both of these data situations. The Shapiro-Wilk test was used to assess the normality of the data in each treatment group, and if violations of normality were found, rank-based statistical methods were used. A significance level of 0.05 was used for all statistical tests. There were no preliminary data or data from previously published studies that could be used to estimate the anticipated effect size for the comparison of the four treatment groups. A sample size of n=30 patients in each group was chosen because this would yield 80% power for detecting a medium-to-large effect size of 0.32 in the ANOVA comparison of the four groups by using a significance level of 0.05.[18]

Results

Contamination data were available for a total of 500 PSP plates. Additionally, the 25 control plates sterilized using ethylene oxide gas and the control blood agar plates exposed to the environment during the plating procedure were evaluated. Table 1 contains a summary of the data for the negative control group and the three mouthrinse groups in terms of the mean, standard deviation, median, and range of bacterial colonies per plate. The mean number of colonies per plate was the lowest in the chlorhexidine group, followed by the Decapinol, Listerine, and the no rinse negative control groups. Column 2 in Table 1 represents the number of PSP plates available with contamination data relative to the planned sample size. For example, the plann-

Table 1. Comparison of negative control and mouthrinse groups in terms of mean and median number of bacterial colonies per plate

Treatment group (Sample size)	Number of PSP plates with contamination data	Number of bacterial colonies per plate (Mean ± S.D.)	95% confidence interval for comparison with chlorhexidine	Median (Range)
No rinse (n=40)	151	6.6 ± 16.2	(−1.7, 9.3)	2.0 (0-130)
Chlorhexidine (n=30)*	118	2.8 ± 8.2	–	1.0 (0-66)
Listerine® (n=30)*	115	5.8 ± 10.8	(0.6, 5.4)	2.0 (0-80)
Decapinol® (n=30)	116	3.7 ± 7.3	(−0.7, 2.5)	1.0 (0-57)

PSP: photostimulable phosphor. *: $p < 0.05$ by Tukey-Kramer method. S.D.: standard deviation

ed sample size for the no rinse group was 160 PSP plates; however, data were available for only 151 plates. Loss of PSP plates occurred for reasons such as PSP plate contact with the receptacle on the Digora Optime scanning unit and handling of the PSP plate as it exited the scanner. Since the data for the number of colonies were non-normally distributed in all four groups according to the Shapiro-Wilk test ($p < 0.05$ in each group), rank-based methods were used. Treating each patient as a cluster yielded an intra-cluster correlation in the number of colonies per plate of 0.19. After adjusting for the clustered nature of the data, we found that the overall F-test based on the ranks indicated a significant difference among the groups in terms of the mean number of colonies per plate (F=3.83; d.f.=3, 370; p=0.010). Tukey-Kramer multiple comparisons based on the ranked data indicated a significant difference only between the Listerine and the chlorhexidine groups (t= 3.35; d.f.=370; adjusted p=0.005). No other significant pair-wise differences were found among the treatment groups. No growth was observed for the 25 gas-sterilized control plates or the control blood agar plates exposed to the environment.

Discussion

Feres et al in 2010 demonstrated that 0.12% chlorhexidine was effective in reducing aerosolized bacteria produced during ultrasonic scaling procedures.[15] Logothetis and Martinez-Welles in 1995 showed that both Listerine® and chlorhexidine reduced bacterial contamination in aerosols,[16] and Hase et al in 1998 reported that chlorhexidine and delmopinol significantly reduced *mutans streptococci* when compared to a placebo.[19] Therefore, it is not surprising that the use of these three products reduced the mean number of bacterial colonies isolated from PSP plates.

Chlorhexidine performed the best of the three mouthrinses tested. However, a statistically significant difference was detected only with the Listerine group. Based upon the mean and the median colonies per plate, it would appear that chlorhexidine also performed better than the no rinse group; unfortunately, a statistically significant difference was not detected. The failure to identify a statistically significant difference between the chlorhexidine group and the no rinse group may be attributable to the larger standard deviation (S.D.) and therefore, higher variability in the no rinse group. In fact, the S.D. in the no rinse group was much larger than that in any other group and almost twice the S.D. in the chlorhexidine group (16.2 vs. 8.2). This increased variability would affect any comparison with the no rinse group. Although a statistically significant difference was not detected between the chlorhexidine group and the no rinse group, it is worth noting that both the mean and the median colonies per plate were reduced by one-half with chlorhexidine in comparison to the no rinse group.

Listerine® is an over-the-counter product proven effective for the control of plaque and gingivitis and has been shown to reduce the microbial content of aerosols during ultrasonic scaling when used as a preprocedural rinse.[20,21] However, when Logothetis and Martinez-Welles compared Listerine® to chlorhexidine as a preprocedural rinse, chlorhexidine performed significantly better.[16] These results agree with the results of our study and the literature supporting chlorhexidine as the gold standard.[11] Our results showed that Listerine® performed similarly to the group that refrained from rinsing and the Decapinol (delmopinol) group. Based on the means, the no rinse group and the Listerine group did not exhibit as much reduction of the contamination of the PSP plates as the Decapinol (delmopinol) group did; nevertheless, a statistically significant difference was not detected. The failure to detect a statistical difference between the Decapinol (delmopinol) group and the no rinse group may be attributable to the large S.D. in the no rinse group, similar to the comparison with the chlorhexidine group. Due to the variability of the data, a larger sample size may be needed in order to detect a statistical difference between Decapinol® (delmopinol) and Listerine®, and between Decapinol (delmopinol) and chlorhe-

xidine.

Kalathingal et al in 2010 demonstrated that the oral cavity serves as a source of PSP contamination.[7] Due to the fact that the oral cavity contributes to the contamination of PSP plates and that chlorhexidine is currently the most effective antimicrobial agent, it seems that the use of chlorhexidine as a preprocedural mouthrinse for radiographic examination would provide the greatest reduction in cross-contamination. However, it is important to note that contamination was still detected even with the use of chlorhexidine. In a meta-analysis, Berchier et al in 2010 found a small but statistically significant difference favoring 0.2% versus 0.12% chlorhexidine for plaque control.[11] In a study by Logothetis and Martinez-Welles in 1995, 0.12% chlorhexidine significantly reduced colony-forming units produced during polishing procedures when used as a preprocedural mouthrinse.[16] However, in this study, two consecutive 30 s rinsing periods were used. Similar to Logothetis and Martinez-Welles, Veksler et al in 1991 demonstrated a statistically significant reduction in the number of colony-forming units when using 0.12% chlorhexidine as a preprocedural mouthrinse.[22] Again, two consecutive 30 s rinsing periods were used. Therefore, perhaps, it is worth testing a concentration of 0.2% chlorhexidine or two consecutive 30 s rinse periods with 0.12% chlorhexidine to evaluate the effect of chlorhexidine in reducing the cross-contamination on PSP plates.

An additional source of contamination that should be considered is the scanning procedure. The Digora Optime unit comes equipped with an internal ultraviolet (UV) disinfection feature. This UV disinfection feature has been shown to eliminate the contamination of the Digora Optime scanning unit when contaminated with *C. albicans* and *S. oralis*.[23] However, this feature was not always included in the construction of the Digora Optime units and was not a feature of the Digora Optime units used in this study. Therefore, the contamination of the scanning unit could have contributed to the bacterial colonies isolated from the PSP plates. The Digora Optime system is also equipped with cardboard sheaths and plastic envelopes that provide a "touch-free" operation of the PSP plate during the scanning process. This "touch-free" system allows for hygienic PSP plate handling and works well with experienced users such as faculty and trained dental personnel. However, this system is less effective for inexperienced users such as dental students, and some cross-contamination may occur during the scanning process. This cross-contamination is most likely to occur along the edges of the PSP, while the operator is preparing to insert it into the scanning unit.[6] There-fore, in addition to the oral flora contamination through the plastic sheath, these two sources of contamination must be considered as well.

In conclusion, the mean number of bacterial colonies detected was the lowest in the chlorhexidine group. Unfortunately, a statistically significant difference was detected only between the chlorhexidine group and the Listerine group. The lack of detecting a statistically significant difference when compared to the other treatment groups may be attributable to the need for a larger sample size due to the variability of the data. There is a possibility that a higher concentration or longer exposure period may be helpful in further reducing contamination with chlorhexidine; however, additional research is needed to test this hypothesis. It is important to note the fact that the bacterial contamination of PSP plates was still present, even with the use of chlorhexidine. This source of contamination may have occurred during the scanning process due to the improper "touch-free" handling of the PSP plate and/or the contamination of the scanning unit. Therefore, strict adherence to an infection control policy during clinic operations must be ensured to minimize cross-contamination.

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