TITLE: Removal of Porphyromonas gingivalis and Escherichia coli from sandblasted acid-etched (SAE) titanium dental implants using chemical agents.

AUTHORS
Cimara Fortes Ferreira, DDS, MSc, PhD
Jegdish Babu, MS, MSc, PhD
David Tipton, DDS, PhD
Timothy Lee Hottel, DDS, MS, MBA

1 Assistant Professor, Department of Periodontology, College of Dentistry University of Tennessee Health Science Center, Memphis, TN.
2 Associate Professor, Dental Research Center, College of Dentistry University of Tennessee Health Science Center, Memphis, TN.
3 Professor, Department of Bioscience Research, College of Dentistry University of Tennessee Health Science Center, Memphis, TN.
4 Professor and Dean, College of Dentistry University of Tennessee Health Science Center, Memphis, TN.

CORRESPONDING AUTHOR
Cimara Fortes Ferreira, DDS, MSc, PhD
Assistant Professor
Director of Implant Dentistry
Department of Periodontology
University of Tennessee College of Dentistry
Dunn Dental Building
875 Union Avenue
Memphis, TN 38163
Phone: (901) 448-4494
Fax: (901) 448-6751
Email: cimarafortes@hotmail.com
ABSTRACT

Introduction: The aim of this study was to compare chemical decontamination techniques used to remove *E. coli* or *Porphyromonas gingivalis* (*P. gingivalis*) from SAE dental implants *in vitro*. Method: SAE dental implants were contaminated with *E. coli* or *P. gingivalis*, and cultured in aerobic and anaerobic conditions for 24 and 48 hr, respectively. Next, the implants were treated with 10 different decontamination techniques: Calcium hydroxide \([\text{Ca(OH)}_2]\) paste for 1 min + saline irrigation for 1 min; \(\text{Ca(OH)}_2\) paste for 1 min + 0.2% chlorhexidine digluconate (CHXD) irrigation for 1 min; 0.2% CHXD for 1 min; DAKIN solution for 1 min; Tetracycline-HCl (T-HCl) \(1\text{g}/20\text{ml}\) solution for 1, 2 and 3 minutes; and, T-HCl paste for 1, 2 and 3 minutes. After the decontamination procedures, all the implants were irrigated with 1 ml of saline solution and incubated in aerobic and anaerobic conditions for 24 hr or 48 hr for *E. coli* and *P. gingivalis*-treated implants, respectively. The control group was submitted to all the procedures except for the chemical treatments. Aliquots were removed and turbidity was measured spectrophotometrically at 600 nm. Statistical analysis was conducted using kruskal-Wallis and Man Whitney for inter- and intra-group statistical significance analysis. Results: Spectrophotometric analysis showed statistically significantly higher decontamination percentages (DC%) for both bacteria using each chemical treatment compared to the control. \(\text{Ca(OH)}_2\) paste alone showed the lowest DC% of 39.3 and 48.7 for *E. coli* and *P. gingivalis*, respectively, and these values were lower than in all other test groups. There was no statistically significant difference in DC% between other groups, which ranged from 95.9-100 and from 92.2-100 for the *E. coli* and *P gingivalis* groups, respectively. The T-HCl paste and solution groups resulted in the highest DC% when compared to all the other treatment groups. Conclusion: The DC% of SAE dental implants contaminated with *E. coli* or *P. gingivalis* by means of chemicals commonly used in dentistry is high, with the exception of \(\text{Ca(OH)}_2\) paste.

Key-words: dental implants, sandblasted acid-etched, tetracycline-HCl, decontamination, bacteria, clinical protocol, tetracycline-HCl, calcium hydroxide, Dakin solution.
INTRODUCTION

Dental implants are successfully used in tooth replacement therapy. Currently, dental implants with different surface characteristics have been developed in the attempt to decrease the time necessary for osseointegration. However, if contaminated, up to 14% of implants placed may result in peri-implantitis, which is defined as an inflammatory lesion of the tissues surrounding the implant subjected to functional loading, with a loss of supporting bone. If peri-implantitis is not adequately addressed, dental implant failure may occur. Other studies have shown that peri-implantitis occurs in 28–56% of patients and in 12–43% of implant sites.

Dental implant surface characteristics range from relatively smooth machined surfaces to more roughened surfaces. The roughness can be created by: coatings, blasting with various substances, acid etching, or a combination of these. Studies characterizing these implants and surfaces include in vitro experimentation, animal studies, and human clinical trials. Cochran conducted histomorphometric and biomechanical testing assays to evaluate and compare different dental implant surfaces. The data demonstrated that rough implant surfaces increase the bone-to-implant contact requiring greater forces to break that interface compared to smoother surfaces. The documented advantage of implants with a roughened surface in animal and in vitro experiments has been demonstrated in specific clinical cases, indicating a significant advantage for patient care.

A pronounced progression of peri-implantitis around SAE dental implants has been shown in vivo when compared to polished surfaces. Various treatments have been proposed for peri-implantitis, including chemical treatment, air abrasion, antimicrobial use, guided bone regeneration, and sandblasting. Use of air-spray instrumentation units has been associated with increased risks of developing emphysema.
Tetracycline-HCl has been successful as a detoxifying agent in dentistry\textsuperscript{20}, but its pH of 1.8 may decalcify peri-implant bone and delay the healing process. However, tetracycline-based antibiotics have been widely used in regeneration procedures\textsuperscript{21}, and in the treatment of peri-implantitis.\textsuperscript{22-24} Tetracycline has antibacterial effect\textsuperscript{25} and also inhibits collagenase activity.\textsuperscript{26}

On the other hand, calcium hydroxide [Ca(OH)\textsubscript{2}] has a pH above 12 that does not decalcify bone or alter its healing. Apparently, Ca(OH)\textsubscript{2} is effective at higher concentrations against many pathogens, dependent upon the surface onto which the pathogen has adhered. Although not yet determined, a titanium surface may make the bacteria more susceptible than a dentin surface. Ca(OH)\textsubscript{2} paste and subsequent irrigation with 0.2\% CHXD was used as surface detoxifying agents for infected dental implants \textit{in vivo}. Infected implant surfaces and the surrounding bone were successfully treated with Ca(OH)\textsubscript{2} when left on the implant surface and bone defect for less than 30 seconds. It has been suggested that the high pH of Ca(OH)\textsubscript{2} endodontic paste may be better tolerated by osseous tissue than a low decalcifying pH agent such as tetracycline or citric acid. Ca(OH)\textsubscript{2} is bactericidal on contact and must be in direct contact with the bacteria to be lethal.\textsuperscript{27}

Ca(OH)\textsubscript{2} also is a source of calcium ions, which could benefit osteogenesis if the material were left in the site. However, the high pH of this material may produce soft tissue reaction. It is recommended that the site should be irrigated and all visible Ca(OH)\textsubscript{2} removed.\textsuperscript{27}

Another material, sodium hypochlorite, has been investigated for antibacterial activity and tissue toxicity at varying time intervals. Heggers et al. attempted to find the efficacious therapeutic concentration that was both microbicidal and nontoxic. Bactericidal effects were observed for concentrations as low as 0.025\%. Tissue toxicity, both \textit{in vitro} and \textit{in vivo}, was observed at concentrations of 0.25\% but not at a concentration of 0.025\% (Dakin's solution).\textsuperscript{28}
There is a need to establish an effective chemical treatment to reduce and/or eliminate bacterial toxins that can be present on rough dental implant surfaces. Decontamination of dental implant surfaces may promote bone regeneration around previously infected dental implant sites. The aim of this study is to evaluate the capability of decontaminating SAE titanium dental implants using chemicals currently used in dentistry.

MATERIALS AND METHODS

Bacteria

E. coli (25841, ATCC, Rockville, MD) was grown for 24 hours in Todd-Hewitt Broth (T1438, Sigma-Aldrich) at 37ºC under 5% CO₂ overnight. P. gingivalis (25260, ATCC, Rockville, MD) was grown for 48 hours in Schaedler’s broth (, S6056, Sigma-Aldrich) at 37°C in anaerobic conditions. P gingivalis was left for 48 hours due to duplication rate being slower when compared to E coli.

SAE dental implants

Thirty commercially available Screw-Line (K-Series) Promote® Surface (Camlog Biotechnologies AG, Basel, Switzerland) dental implants were used in this study. All the dental implants were the same type with the same diameter (4 mm).

Contaminating SAE dental implants

A receptacle with Todd-Hewitt Broth (THB) was seeded with E coli and incubated at 37ºC overnight in order to allow for sufficient time to reveal growth of the bacteria after decontamination procedure was conducted. The apices of 11 dental implants were seeded with approximately 2 µl of a suspension of E. coli (1 x 10⁸ cells), Implants were positioned so that only the most apical five threads were immersed in growth broth and incubated at 37ºC overnight (Fig. 1).
A receptacle with THB was seeded with *P. gingivalis* and incubated at 37°C for 48 hr inside anaerobic chamber containing AnaeroGen™ (Oxoid, Fisher Scientific, Waltham, MA, USA) for generation of anaerobic conditions. The apices of 11 dental implants were seeded with approximately 5 µl of a suspension of *P. gingivalis*. This increase in the seeded solution was due to the pilot studies showing insufficient growth of the *P gingivalis* when only 2 µL were seeded. Implants were positioned so that only the most apical five threads were immersed in growth broth and were incubated at 37°C in an anaerobic chamber for 48 hrs. The diameter of the Eppendorf used for these experiments maintained the position of the implant without allowing its surfaces to contact the tube. The objective was to use a tube that would reduce the possibility of contamination beyond the 5 apical threads of the dental implant.

**Chemical treatments**

After 24-hour and 48-hour incubation periods for *E. coli* and *P. gingivalis*, respectively, the contaminated implants were removed from the Eppendorf tubes and dipped 3 x in saline solution. Afterwards, a cotton pellet was saturated with saline solution and burnished on the implant surface for 1 minute. Each implant was then submitted to the following chemical treatments:

**Ca(OH)\textsubscript{2} paste**

Ca(OH)\textsubscript{2} paste (Vista Cal™, Inter-Med, Inc, Racine, WI, USA) has a pH of 12.5 at a concentration of 35% that does not decalcify bone or alter its healing process. Ca(OH)\textsubscript{2} paste alone and as an adjunct to irrigation with 0.2% CHXD have been used as surface detoxifying agents.\textsuperscript{27} Ca(OH)\textsubscript{2} paste was used in two groups, as follows: Ca(OH)\textsubscript{2} paste on a cotton pellet rubbed on the surface of the implant for 1 minute followed by irrigation with saline solution for 1 minute; Ca(OH)\textsubscript{2} paste on a cotton pellet rubbed on the surface of the implant for 1 minute followed by irrigation with 0.2% of CHXD for 1 minute. Afterwards, the implant was irrigated with saline solution for 1 minute.
0.2 % Chlorhexidine digluconate (CHXD) solution
A cotton pellet was saturated with 0.2% of CHXD (Sigma-Aldrich, St. Louis, MO, USA) solution and then rubbed on each implant surface for 1, 2 and minute. Afterwards, the implant was irrigated with saline solution for 1 minutes. Afterwards, the implant was irrigated with saline solution for 1 minute.

Dakin solution
A Clorox™ commercial solution (1.84%) was diluted with saline solution in order to prepare a 0.025% of sodium hypochlorite (Dakin solution).28 A cotton pellet was saturated with this solution and then rubbed on the surface of the implant for 1 minute. Afterwards, the implant was irrigated with saline solution for 1 minute.

Tetracycline-HCl paste
Tetracycline-HCl (T-HCl) powder (Sigma-Aldrich) was solubilized with saline solution to a concentration of 1g/20ml, which is a solution of 5% T-HCl in concentration. A cotton pellet was saturated with this solution and rubbed on the surface of each implant for 1, 2 or 3 minutes. T-HCl powder was also mixed with saline solution to form a paste. The T-HCl paste was placed on a cotton pellet and rubbed on the surface of the implant for 1, 2 or 3 minutes. After both types of treatments, saline solution was used to remove all visible T-HCl.

After each chemical treatment, the implants were placed in fresh culture media and incubated in aerobic conditions for 24 hours at 37°C for E. coli and for 48 hr in anaerobic conditions at 37°C for P. gingivalis.

Controls
The control implants were removed from the Eppendorf tubes and dipped 3 x in saline solution. Afterwards, a cotton pellet was saturated with saline solution and burnished on the implant surface for 1 minute. Next, 1 ml of saline solution
was used to irrigate the implant and it was placed in culture media and incubated for 24 and 48 hr for *E coli* and *P gingivalis*, respectfully.

**Spectrophotometry**

After the incubation periods, the implants were removed from the Eppendorf tubes and aliquots of 0.1 ml of the culture media were removed. Absorbance of the aliquots was measured at 600 nm using a spectrophotometer (SpectroStar Nano, BMG Lab, Ortenberg/Germany). The absorbance values were compared with the control and the decontamination percentage was calculated for each group.

**Data Analysis**

Each experiment was repeated at least 3 times. Mean values ± SEM from triplicate samples were calculated and the significance of differences was obtained by means of SPSS 10.0 statistics program for Windows. Statistical significance between groups and in each group were assessed with Kruskall-Wallis and Mann Whitney U tests applied (p < 0.05 was considered to indicate statistical significance, respectfully.

**RESULTS**

**Spectrophotometric Analysis**

Spectrophotometric analysis and calculation of the decontamination percentage of SAE dental implants after chemical treatment showed that for both *E. coli* and *P. gingivalis*, all treatments resulted in statistically significantly higher DC% than control groups. In addition, for both bacteria types, there were significant differences in the DC% resulting from the different chemical treatments. For *E. coli*, the DC% caused by Ca(HO)₂ alone was significantly lower than the other treatment groups (which were not significantly different from one another). For *P. gingivalis*, there were statistically significant differences between the Ca(HO)₂ paste group irrigated with saline solution and all of the other groups except for
Dakin and T-HCl paste used for 1 minute). There were no significant differences among the other treatments in their ability to remove *P. gingivalis* from the implant surfaces. When comparing the two bacteria, significantly different DC% were caused only by treatment with 0.2% CHXD solution (*P. gingivalis* > *E. coli*) (Tables 1 and 2).

Ca(HO)$_2$ paste group irrigated with saline solution showed the lowest DC% for both *E. coli* (32.45) and *P. gingivalis* (48.74), respectively. For the *E coli* group, the chemical treatments that showed lowest DC% were: Ca(HO)$_2$ paste group irrigated with saline solution; and Dakin solution (Figure 2). For the *P. gingivalis* group, all the chemical treatments showed high decontamination rates (90-100%), except for Ca(HO)$_2$ paste group irrigated with saline solution (48.74) (Figure 3).

**DISCUSSION**

Threads and rough surface implants improve osseointegration. However, these features render mechanical management of peri-implant infections impractical. Once exposed to bacterial colonization, the coarse texture facilitates bacterial retention. The ease of removal of differently sized and shaped bacteria from different types of surface topographies (smooth or rough), chemistry (titanium oxide), and wettability, was investigated. Atomic force microscopy (AFM) showed that the shape of the bacteria with respect to the shape of the surface influenced the ease of removal of the bacteria from the surface. On smooth surfaces the cocci had a smaller cell:surface contact area (CSCA), whereas the rods had a larger CSCA area. Using engineered surfaces with defined properties, it has been shown that manipulation of surface roughness had an effect on the strength of microbial retention. The present study used *P gingivalis*, which is a non-motile, gram-negative, anaerobic, rod-shaped pathogen, which according to this study, may show stronger retention to surfaces due to its morphology. However, the present study disagrees with the ability to decontaminate this type of bacteria from the rough dental implant surface, due to the higher
decontamination of *P gingivalis* which is a rod shaped bacteria, when compared to the *E coli cocci*. This difference may be attributed to the fast doubling time of *E coli* in comparison to *P gingivalis*.

Changes in surface roughness and topography on the macroscopic scale are known to affect bacterial attachment and retention. An *in vitro* study showed that when surface roughness of 0.04µm (average peak to valley distance, AFM measurements) for polished stainless steel was increased to a roughness of 0.30 µm for abraded surface, bacterial adhesion strength increased. The roughness is indicated as having higher bacterial retention property. The samples selected for the present study were sandblasted acid-etched (SAE) titanium dental implants, but the exact surface topography was not measured in this study. However, this type of surface is most frequently present in commercially available dental implants, with slight variations. Knowledge of the ability of chemicals that are commonly used in dentistry to decontaminate this type of implant surface can be of great value as an adjunct to per-implantitis or peri-mucositis treatment.

*P gingivalis* is a gram-negative bacterium and therefore has endotoxin in its cell wall. Endotoxin contains lipid A, which is a region responsible for the toxic effects. Endotoxins do not cause direct cell or tissue pathosis, but instead stimulate macrophages to release mediators that lead to inflammation. Ca(OH)$_2$ has been shown to hydrolyze the highly toxic lipid A molecule that is responsible for the damaging effects of endotoxins, and can also transform lipid A into fatty acids and amino sugars, which are nontoxic. A study that tested serial dilution of Ca(OH)$_2$ against 6 endodontic pathogens found Ca(OH)$_2$ to be effective only at concentrations of approximately 50%. In addition, Ca(OH)$_2$ induced root extension only if it was placed short of the working length of the root. If placed more apically it may interfere with the re-vascularization process of the pulp. In the present study, we did not leave the Ca(OH)$_2$ on site in order to take into account its deleterious effects on re-vascularization. After Ca(OH)$_2$ paste was rubbed on the implant surface for 1 minute, it was thoroughly removed by rubbing saline or 0.2% CHXD irrigation solutions on the
implant surface. The authors suggest that the low DC% of Ca(OH)$_2$ paste alone could be attributed to the difficulty in obtaining direct contact of the paste with the bacteria, which is essential for its decontamination efficiency.$^{17}$ Another explanation of the reduced DC% of Ca(OH)$_2$ paste used alone may be that the concentration indicated to be effective for implant decontamination is for a concentration of 50% and the concentration used in the present study was 35%.$^{35}$

Empirically, the high pH of Ca(OH)$_2$ endodontic paste may be better tolerated by osseous tissue than a low pH decalcifying agent such as T-HCl or citric acid. However, both of these low pH agents have had successful treatment results reported. In the present study, Ca(HO)$_2$ paste group irrigated with saline solution showed the lowest decontamination rate for both *E coli* and *P gingivalis*. However, when Ca(OH)$_2$ paste was combined with a 1 minute exposure to 0.2% CHXD, there was a significant increase in the decontamination rate for *E coli* and *P gingivalis*. This result is in agreement with the earlier study that indicated this combination to have an efficient antimicrobial activity as intracanal medicament when compared to Ca(HO)$_2$ alone.$^{37}$

An *in vitro* study conducted by Dennison and co-workers (1994) compared different types of dental implant surfaces contaminated and treated with different decontamination protocols.$^{38}$ In this study, the implants used were titanium cylindrical press-fit with machined surface, plasma sprayed surface; and hydroxyapatite-coated surface. The implants were contaminated with *P gingivalis* radioactive endotoxin and then treated by burnishing cotton pellet soaked in water, or with citric acid solution, or with 0.12% CHXD, or treated with air powder abrasive. Residual radioactivity on the implants was measured after two treatments. The machined implant surface was decontaminated more effectively than the other surfaces by all treatments except for citric acid treatment, which was equally effective for both machined or hydroxyapatite surfaces. The present study used only rough surface implants because this is most difficult surface to decontaminate.$^{38}$ Dennison’s and co-workers (1994) indicated that LPS decontamination with water or 0.12% CHXD was similar. The present study does not concur with the DC% of CHXD being similar to water or
saline. There was significant mean DC% of 100 and 96.6 for *P. gingivalis* and *E. coli*, respectively, when using 0.2% CHXD burnished for 1 minute on the implant surface. We attribute the success of the decontamination method in our study to the type of contaminant and to the concentration of chlorhexidine used, 0.2% instead of 0.12%. In Dennison and co-workers’ (1994) study, the contaminant was the bacteria and not the LPS. The authors suggest that the bacteria may be more resistant to mechanical debridement when compared to the LPS.

Chlorhexidine is considered a good conditioning agent, but tends to bind to endotoxins and form a complex that may hinder reosseointegration. It has been shown to be effective in endodontics when used with 1% hypochlorite solution. Studies conducted *in vitro*, on infected dentinal tubules, showed that the use of 2% CHXD as an intra-canal irrigant for 10 minutes prior to obturation was more effective as an antimicrobial than placement of Ca(OH)$_2$. This is consistent with the results of the present study, which found that 0.2% CHXD resulted in a high DC% for both bacterial types.

Sodium hypochlorite has broad antimicrobial activity, rapid bactericidal action, and relative non-toxicity at concentrations commonly used in dentistry. Subgingival irrigation with 0.5% sodium hypochlorite causes a significantly higher and longer lasting reduction in plaque and gingivitis than irrigation with water. The present study used Dakin solution (0.025% sodium hypochlorite), which resulted in greater %DC when compared to the control (saline solution irrigation). However, the DC% caused by treatment with Dakin solution was approximately 2 to 3 fold lower for *E coli* and *P gingivalis*, respectively, than DC% values obtained with other treatment groups. The lower concentration of sodium hypochlorite used in the present study would avoid tissue toxicity if used *in vivo*. Its use is considered at a higher concentration for decontamination protocols when the oral environment is intact. When the indicated procedure involves flap elevation, e.g. treatment of peri-implantitis for exposure of the dental implant threads, 0.5% of sodium hypochlorite wouldn’t be the chemical option to use.
Tetracycline-HCl (100mg/ml; pH 1.8) has been used to decontaminate root surfaces and is comparable to lower pH citric in demineralizing dentin.\textsuperscript{43} Tetracycline has been used as part of a decontamination protocol for dental implants \textit{in vivo}\textsuperscript{21-24} and is regarded as the most promising agent for implant decontamination because it has several unique functional properties that would promote tissue repair and reosseointegration.\textsuperscript{44} The present study showed the decontamination percentage of tetracycline-HCl (1g/20ml) \textit{in vitro} in paste form which is commonly used clinically for decontamination purposes, even though the literature does not indicate the exact manner in which the T-HCl paste is prepared. In the present study, the paste was prepared as is in clinical settings, by adding saline solution to T-HCL powder until it had a consistency that allowed it to be delivered to the site with a cotton pellet.

To our knowledge, so far, no quantitative \textit{in vitro} study exists showing the detoxification capacity of currently used dental chemicals for treatment of contaminated commercially available dental implants with rough surfaces. Further studies are suggested to verify cytotoxicity of the proposed chemicals \textit{in vivo}.

**CONCLUSION**

The proposed treatments for dental implant decontamination showed significant decontamination percentages of \textit{E coli} and \textit{P gingivalis} from SAE type dental implants, except when compared to the use of Ca(OH)\textsubscript{2} paste alone. Therefore, the authors suggest that these treatments may improve peri-implantitis and peri-mucositis therapy for patients presenting SAE type dental implants.

**ACKNOWLEDGEMENTS**

The authors would like to thank the University of Tennessee College of Dentistry Alumni Endowment Fund for its support of this project.
FIGURES

**Figure 1**) Position of the dental implant inside an Eppendorf tube after treatment. Note that the dental implant mount is secured to the top of the tubes impeding the implant from contacting the sides of the tube.

![Image of dental implant in Eppendorf tube](image.png)

**Figure 2**) Decontamination percentage and standard error (STDE), values from spectrophotometric assay (600 nm absorbance) measurements for each chemical treatment used to remove *E. coli* from SAE implants. All treatments were statistically significant.

![Spectrophotometric Assay E coli](image.png)

**Figure 3**) Decontamination percentage and standard error (STDE), values from spectrophotometric assay (600 nm absorbance) measurements for each chemical treatment used to remove *E. coli* from SAE implants. All treatments were statistically significant.
chemical treatment used to remove \textit{P. gingivalis} from SAE implants. All treatments were statistically significant.

\begin{center}
\textbf{Spectrophotometric Assay \textit{P gingivalis}}
\end{center}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure.png}
\caption{Decontamination percentage for various chemicals used to remove \textit{P. gingivalis} from SAE implants.}
\end{figure}

\section*{Tables}

\textbf{Table 1} Sum (\(\Sigma\)), average (MV), standard deviation (STDV), standard error (STDE), p value, and decontamination (DC) percentage values from spectrophotometric assay (600 nm absorbance) measurements for each chemical treatment used to remove \textit{E. coli} from SAE implants. All treatments were statistically significant.

\begin{table}
\centering
\begin{tabular}{|l|c|c|c|c|c|c|}
\hline
R/S & \(\Sigma\) & MV & STDV & STDE & P value & DC\% \\
\hline
\text{Ca(OH)\textsubscript{2} paste + saline} & 129.9 & 48.74 & 13.97 & 7.030 & * & 39.3 \\
\text{Ca(OH)\textsubscript{2} paste + 0.2% CHXD 1'} & 387.7 & 100 & 6.15 & 3.000 & * & 95.9 \\
\text{0.2\% CHXD 1'} & 338 & 100 & 24.78 & 12.39 & * & 96.6 \\
\text{DAKIN solution 1'} & 266.6 & 92.77 & 39.32 & 19.78 & * & 55.5 \\
\text{T-HCl 1g/20ml 1'} & 371.9 & 100 & 4.89 & 2.415 & * & 90.6 \\
\text{T-HCl 1g/20ml 2'} & 390.7 & 100 & 2.77 & 1.315 & * & 96.9 \\
\text{T-HCl 1g/20ml 3'} & 396.2 & 100 & 1.9 & 1.000 & * & 98.7 \\
\text{T-HCl paste 1'} & 390.7 & 96.65 & 2.82 & 1.667 & * & 98.3 \\
\text{T-HCl paste 2'} & 495.1 & 100 & 0 & .000 & * & 100 \\
\text{T-HCl paste 3'} & 300 & 100 & 0 & .000 & * & 100 \\
\hline
\end{tabular}
\end{table}

* Comparison between Ca(OH)\textsubscript{2} paste alone with the other groups showed statistical significance (p < 0.05).
Table 2) Sum (Σ), average (MV), standard deviation (STDV), standard error (STDE), p value, and decontamination (DC) percentage for each chemical treatment used to remove *P. gingivalis* from SAE implants. All treatments were statistically significant, except for comparison between Ca(OH)$_2$ paste + saline and Ca(OH)$_2$ paste + 0.2% CHXD 1' which showed no statistical significance.

<table>
<thead>
<tr>
<th>R/S</th>
<th>Σ</th>
<th>MV</th>
<th>STDV</th>
<th>STDE</th>
<th>p value</th>
<th>DC%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca(OH)$_2$ paste + saline</td>
<td>146.23</td>
<td>48.743</td>
<td>44.3097</td>
<td>25.58</td>
<td>48.74</td>
<td></td>
</tr>
<tr>
<td>Ca(OH)$_2$ paste + 0.2% CHXD 1'</td>
<td>300.00</td>
<td>100.0</td>
<td>.00000</td>
<td>.0000</td>
<td>* *100</td>
<td></td>
</tr>
<tr>
<td>0.2% CHXD 1'</td>
<td>300.00</td>
<td>100.0</td>
<td>.00000</td>
<td>.0000</td>
<td>* *100</td>
<td></td>
</tr>
<tr>
<td>DAKIN solution 1'</td>
<td>278.32</td>
<td>92.773</td>
<td>12.5169</td>
<td>7.226</td>
<td>92.77</td>
<td></td>
</tr>
<tr>
<td>T-HCl 1g/20ml 1'</td>
<td>300.00</td>
<td>100.0</td>
<td>.00000</td>
<td>.0000</td>
<td>* *100</td>
<td></td>
</tr>
<tr>
<td>T-HCl 1g/20ml 2'</td>
<td>300.00</td>
<td>100.0</td>
<td>.00000</td>
<td>.0000</td>
<td>* *100</td>
<td></td>
</tr>
<tr>
<td>T-HCl 1g/20ml 3'</td>
<td>300.00</td>
<td>100.0</td>
<td>.00000</td>
<td>.0000</td>
<td>* *100</td>
<td></td>
</tr>
<tr>
<td>T-HCl paste 1'</td>
<td>289.96</td>
<td>96.653</td>
<td>5.79660</td>
<td>3.346</td>
<td>96.65</td>
<td></td>
</tr>
<tr>
<td>T-HCl paste 2'</td>
<td>300.00</td>
<td>100.0</td>
<td>.00000</td>
<td>.0000</td>
<td>* *100</td>
<td></td>
</tr>
<tr>
<td>T-HCl paste 3'</td>
<td>300.00</td>
<td>100.0</td>
<td>.00000</td>
<td>.0000</td>
<td>* *100</td>
<td></td>
</tr>
</tbody>
</table>

* Comparison between Ca(OH)$_2$ paste alone with other groups showed statistical significance (p < 0.05).

REFERENCES