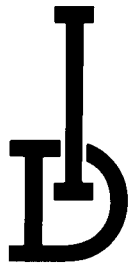
DETOXIFICATION OF ENDOTOXIN-CONTAMINATED TITANIUM AND HYDROXYAPATITE-COATED SURFACES UTILIZING VARIOUS CHEMOTHERAPEUTIC AND MECHANICAL MODALITIES



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The surgical repair of the ailing implant may be complicated by the surface effects of pathogenic bacteria and their products. This study evaluated the ability of various chemotherapeutic modalities to detoxify endotoxin-contaminated titanium alloy and hydroxyapatite-coated test strips. Grit-blasted titanium alloy and hydroxyapatite-coated test strips were contaminated with purified outer membranes of Escherichia coli labeled with radioactive ^{14}C. The titanium alloy strips were treated with citric acid, stannous fluoride, tetracycline HCl, chlorhexidine gluconate, hydrogen peroxide, chloramine T, sterile water, a plastic sonic scaler tip, and an air-powder abrasive unit. Hydroxyapatite-coated strips were treated with chloramine T, citric acid, or burnished with sterile water on cotton pellets. Residual lipopolysaccharide levels were measured by liquid scintillation spectrometry. The air-powder abrasive unit removed significantly greater amounts of lipopolysaccharide than all other treatment modalities on titanium samples ($P < 0.05$). A 60-second burnish with sterile water was able to remove significant amounts of lipopolysaccharide when compared with untreated controls ($P < 0.05$). Citric acid was superior in the removal of lipopolysaccharide from hydroxyapatite-coated surfaces when compared with the controls or chloramine T ($P < 0.01$). Detoxification of an implant infected surface may be beneficial when surgical repair of the ailing implant is indicated. (Implant Dent 1992;1:154–158)

Implant dentistry has made great strides in the past decade. Long-term studies confirm the predictable rehabilitation of partially and completely edentulous patients utilizing both titanium and hydroxyapatite-coated implant systems.[1,2] In spite of these advances, complications can and do occur in a small percentage of cases. Peri-implantitis can be the result of biomechanical and occlusal overload as well as have a microbiologic etiology.[3,4] The dental implant may be more susceptible to dental plaque than the natural tooth, as the predictability of a stable soft tissue attachment complex has not yet been confirmed.[5] A circular fiber arrangement[6] may be responsible for the perimucosal seal around the implant abutment.[7,8] This hypothesis has led to the thought that a plaque-induced gingivitis may progress to a peri-implantitis much more readily.[3,9]

The ingress of plaque with its periodontopathic organisms and by-products cause progressive pocketing and peri-implant bone loss. When attempting regenerative procedures, the question of how to address the potentially pathologic implant surface must be considered. A number of investigators have demonstrated that conventional instrumentation will alter titanium or the hydroxyapatite (HA) surface coating.[10–14] It has been hypothesized that using a dissimilar metal to clean titanium may leave residues and lead to galvanic corrosion.[15] Short applications of citric acid (pH 1) have been found to detoxify the HA-coated implant surface without significantly altering the residual coating[16] (Zablotsky et al, unpublished data, 1991).

Lozada et al[17] described a protocol for detoxification of the implant surface utilizing both an air-powder abrasive unit (Prophy-Jet; Dentsply International Inc., York, PA) and a 1 percent application of chloramine T (OraChlor; OraTec Corporation, Herndon, VA).

The purpose of this study was to determine whether various chemotherapeutic and mechanical modalities can detoxify contaminated grit-blasted titanium alloy. In addition, chloramine T was compared with citric acid in the detoxification of endotoxin contaminated HA-coated surfaces.

MATERIALS AND METHODS

Contaminated Grit-Blasted Titanium Alloy—Untreated

Endotoxin was applied to nine titanium alloy test strips (10 mm × 10 mm × 1.537 mm) from the same

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production batch. Each strip contained the same grit-blasted titanium alloy surface onto which HA is plasma sprayed for the commercially marketed Integral implant (Calcitek, Inc., Carlsbad, CA).

The endotoxin, also known as lipopolysaccharide (LPS), applied to the titanium alloy surfaces is present in its natural state as partially purified outer membranes of *Escherichia coli* CS1919. This strain of *E. coli* has been described in detail by Schnaitman and Austin.[18] It is a rough K-12 strain carrying a mutation in *galE* and permits specific radioactive labeling of the Ra chemotype endotoxin with [1-^{14}C]galactose. The bacteria were grown in radioactive media according to Diedrich et al[19] and the outer membranes prepared by breakage in a French pressure cell followed by differential ultracentrifugation according to the method of Schnaitman and McDonald.[20] The outer membranes were treated with lysozyme, which was removed by ultracentrifugation and two washes in 10 mM Hepes buffer at pH 7.4 and 4°C.[19] The LPS content of the outer membranes was determined from analysis of their 2-keto-2-deoxyoctonic acid content.

Each surface received an amount of outer membrane which possessed 66 μg of LPS based on 2-keto-2-deoxyoctonic acid analysis (3-deoxy-D-manno-octulosonic acid).[21] This was allowed to almost dry on the surface in a stream of air in a laminar flow hood. A Teflon instrument was used periodically to spread the drying liquid evenly on the coated surface so that the concentration of membrane per unit of surface area would be consistent. When the surface appeared to be only damp the coated surfaces were rinsed by dipping them into Hepes buffer followed by storage at 4°C submerged in 1.0 ml of Hepes buffer in 12-well tissue culture plates. The test strips were allowed to soak in this preparation for 3 days.

Five of the strips were rocked for 4 hours on a rocker table. The remaining four strips were left untreated for scintillation counting. Each strip was placed in a glass scintillation vial containing 10 ml of CytoScint (ICN Biomedicals, Irvine, CA) counting fluid and the radioactivity counted in a liquid scintillation spectrometer. The amount of radioactivity washed from each test strip was expressed in total radioactive counts. The radioactivity in the fluid in which the strips were washed/rocked was counted in a similar manner. The surface area of each strip was then measured and divided into the total radioactive LPS count which remained on that strip. The units of measurement evaluated were designated as LPS counts per minute per square millimeter. The washed (rocked) and unwashed control strips were then compared to determine to what extent LPS remained on the titanium alloy surfaces.

Contaminated Grit-Blasted Titanium Alloy—Treated

Thirty additional titanium alloy test strips were prepared in an identical manner with the labeled LPS. Three test strips were exposed to each of the 10 treatment regimens listed in Table 1. After the appropriate treatment was performed, each test strip was placed in a glass liquid scintillation vial and analyzed as before in a liquid scintillation spectrometer.

HA-Coated Strips—Treated

Ten HA-coated test strips with the identical coating used for Integral implants (Calcitek, Inc.) were treated with labeled LPS. The endotoxin-bound HA-coated surfaces were then treated with either citric acid, chloramine T, or sterile water (Table 2). After the appropriate application, the strips were placed in glass scintillation vials and analyzed as before.

Data was analyzed using a one-way analysis of variance with follow-up Duncan's multiple range tests for comparisons of groups.

RESULTS

Contaminated Grit-Blasted Titanium Alloy—Untreated

The control strips (not rocked) exhibited an average of 80,847 counts/strip or 808 counts/min/mm^2. Strips which had been rocked displayed significantly lower amounts of LPS on their surfaces ($P < 0.05$). Forty-seven percent of the applied LPS remained on the rocked surfaces (Table 3).

Table 1. Treatment Modalities Evaluated ("Contaminated Grit-Blasted Titanium Alloy—Treated")

1. Stannous fluoride 1.64 percent—burnished with cotton pellets for 1 minute.
2. Chlorhexidine gluconate 0.12 percent—burnished with cotton pellets for 1 minute.
3. Tetracycline HCl (50 mg/ml)—burnished with cotton pellets for 1 minute.
4. Hydrogen peroxide 3 percent—burnished with cotton pellets for 1 minute.
5. Chloramine T 1 percent—burnished with cotton pellets for 1 minute.
6. Citric acid pH 1—burnished with cotton pellets for 1 minute.
7. Plastic sonic scaler—utilized with light pressure for 1 minute.
8. Air-powder abrasive—applied 2–3 mm from surface for 30 seconds.
9. Untreated control.
10. Burnished control—burnished with cotton pellets with saline for 1 minute.

Table 2. Treatment Modalities Evaluated ("HA-Coated Strips—Treated")

1. Chloramine T 1 percent—burnished with cotton pellets for 3 minutes.
2. Citric acid pH 1—burnished with cotton pellets for 1 minute.
3. Burnished control—burnished with cotton pellets with sterile water for 3 minutes.

Table 3. Mean Residual LPS Counts/min/mm^2 ("Contaminated Grit-Blasted Titanium Alloy—Untreated")

Treatment	LPS counts (washed off)	LPS counts (remaining)	Total
Unrocked controls		808	808
Rocked (washed)	429 (53 percent)	385 (47 percent)	814

Contaminated Grit-Blasted Titanium Alloy—Treated

Table 4 shows the mean LPS counts per minute per square millimeter. A 30-second application of the Plaque Sweep® (Dentsply International Inc.) was the only treatment able to remove significantly greater amounts of LPS from surfaces when compared with burnishing for 1 minute with a sterile saline pellet ($P < 0.05$). Although a 60-second application of the plastic sonic scaler tip or citric acid was superior to burnishing with saline, the differences were not statistically significant. Burnishing with saline, hydrogen peroxide, chloramine T, citric acid, or applying the plastic sonic scaler tip significantly reduced residual LPS levels when compared with the untreated control strips ($P < 0.05$). Stannous fluoride applications left significantly greater levels of LPS on surfaces when compared with untreated controls ($P < 0.05$).

HA-Coated Strips—Treated

Table 5 gives the mean LPS counts per minute per square millimeter. Overall significant differences were seen at a level of $P < 0.0002$. Citric acid was significantly better than the other treatments for the removal of bacterial endotoxin ($P < 0.01$).

DISCUSSION

The rationale for utilizing outer membranes from a member of the Enterobacteriaceae is three-fold:

1. The outer membranes contain LPS in its natural configuration (associated with phospholipids and membrane proteins in a membrane matrix). The binding of outer membranes to a titanium alloy surface simulates the natural interaction of both intact bacteria and surface components (vesicles) shed from gram-negative bacteria with an implant/abutment surface. Extracted LPS is less desirable for studies such as this because the extraction procedure can chemically alter the LPS and generate aggregates in a variety of physical forms.[22]
2. The LPS and outer membrane of *E. coli* are well characterized genetically and biochemically, making them well suited to experimental manipulation.
3. The toxic lipid A portion of LPS has the same general structure in almost all the Eubacterium. This conservation of structure is manifested by the cross-reactivity of lipid A-specific monoclonal antibodies with LPS from several genera.[23] Although preparations of LPS can exhibit a spectrum of biologic activities based on their origins and methods of extraction, LPS is amphiphilic and its physical interactions with a surface such as titanium alloy will be similar. That is, *E. coli* LPS should bind to the titanium alloy implant/abutment in the same manner and to the same degree as LPS from Bacteroides, Treponema, and other genera.

Table 4. Mean Residual LPS Counts

Treatment	No.	LSP counts/ min/mm^2
Stannous fluoride (1.64 percent)	3	302*
Untreated control	3	197
Chlorhexidine gluconate (0.12 percent)	3	170
Tetracycline HCl (50 mg/ml)	3	141
Hydrogen peroxide (3 percent)	3	108**
Burnished control	3	98**
Chloramine T	3	86**
Citric acid (pH 1)	3	68**
Plastic sonic scaler tip	3	63**
Air-powder abrasive	3	12***

* Significantly greater amounts of LPS than untreated control ($P < 0.05$).
** Significantly less amounts of LPS than untreated control ($P < 0.05$).
*** Significantly less amounts of LPS than burnished control ($P < 0.05$).

Table 5. Mean Residual LPS Counts

Treatment	No.	LPS counts/ min/mm^2
Chloramine T 1 percent (3 minutes)	4	103
Burnished control (3 minutes)	3	116
Citric acid pH 1 (60 seconds)	3	5*

* Significantly less amounts of LPS than burnished control ($P < 0.01$).

The treatment modalities in this investigation addressed the physical properties of the LPS binding and not the biologic properties. The LPS preparation utilized has been found to inhibit the early growth and spreading of human gingival fibroblasts on HA-coated implant surfaces (Zablotsky et al, unpublished data, 1991). This model was utilized to eliminate other potential variables, such as bacterial by-products and enzymes. The degree of adherence to the HA-coated surface by these by-products is not clear at this time.

When comparing the binding of LPS to titanium alloy with that of HA-coated surfaces,[16] it appears that there is a much greater affinity of LPS for the hydroxyapatite surface. Charge interactions must play a role in this phenomenon as the grit-blasted titanium surface and the plasma-sprayed HA surface both have significant surface irregularities. It can be concluded that LPS would have a reduced affinity for polished abutments when compared with the grit-blasted titanium alloy surface. It is interesting to note that burnishing the contaminated grit-blasted titanium surface with a cotton pellet dipped in sterile saline for 1 minute was effective in reducing LPS levels significantly below untreated controls. Treatments with chlorhexidine gluconate, tetracycline HCl, hydrogen peroxide, and chloramine T were all unable to reduce LPS levels significantly below those from burnishing alone.

Citric acid has been shown to inhibit the growth of bacteria on periodontally diseased root surfaces.[24] Tanaka et al[25] found citric acid (pH 1) removed virtually all debris from partially scaled periodontally diseased surfaces. Decalcification of superficial residual calculus was demonstrated after 3 minutes of burnishing.[25] Zablotsky et al[16] studied the LPS-infected HA-coated implant surface and found that a short (30 to 60 seconds) application of citric acid removed LPS without significantly altering the residual coating. This treatment was also successful in returning the contaminated HA coating to a biologically acceptable surface on which human gingival fibroblasts could proliferate (Zablotsky et al, unpublished data, 1991).

The mechanism of action of citric acid on the HA surface is thought to be a demineralization phenomenon of the most superficial layer of HA. This obviously does not occur with the toxic titanium or titanium alloy surface. Although a 1-minute application of citric acid was superior to saline burnishing for the same time interval, residual levels were not significantly less. It is not known, but it is unlikely, that a longer application period would enhance the results seen with the 1-minute application.

The use of plastic instrumentation on commercially pure titanium or titanium alloy surfaces is the standard of care at this time due to the detrimental effects of metal mechanical cleaning devices.[26] Nishimine and O'Leary[27] and Smart et al[28] measured LPS on treated and untreated diseased root surfaces and found ultrasonics were capable of removing bacterial endotoxin. A prototype plastic Cavitron (Dentsply International Inc.) and plastic sonic scaler tips have been shown to be clinically effective in removing plaque and calculus without significantly altering the titanium surface.[14, 29]

The modified plastic Cavitron tip has been shown to remove endotoxin from the HA surface.[16] The Protip (Dynadent Inc., Santa Ana, CA) was utilized in this study. Although this instrument was able to remove greater amounts of LPS than burnished controls, these reduced LPS levels were not significant. Ultrastructural plastic residues have been seen when utilizing the plastic Cavitron tip on titanium and HA.[29] The effects of these residues are not presently known.

In recent years the air-powder abrasive unit has been used to remove stains on teeth.[30] Some clinicians have utilized this modality in the surgical treatment of pathologically altered dental implant surfaces.[17] The Plaque Sweep is a portable hand-held unit similar to the Prophy-Jet. Parham et al[31] evaluated the effects of air-powder abrasive on plasma-sprayed titanium implant surfaces and concluded that the abrasive did not significantly alter the implant surface, but was able to remove all bacterial colonization.

Lozada et al[17] investigated the use of the Plaque Sweep along with the application of 1 percent chloramine T in treating the infected implant surface. A 30-second application of the Plaque Sweep was the only modality able to detoxify the titanium alloy surface when compared with burnishing with saline. It may be concluded that the positive results reported by Lozada et al[17] were the result of the air-powder abrasive system and not the application of chloramine T.

Extreme care should be exercised when utilizing an air-powder abrasive in a surgical setting. Protection of the mucoperiosteal flaps, marrow spaces, and fascial spaces are necessary as postoperative sequelae, such as air emboli and emphysema, have been reported from the use of air-driven handpieces in implant dentistry.[32]

The authors are unaware of any reports of subcutaneous emphysema in over 30 years of clinical use of high-speed handpieces for osseous reduction in periodontal surgery. The use of air-powder abrasive has been shown to be efficacious when used in periodontal surgery.[33] Crooks[34] was unable to induce emphysema when an air-powder abrasive spray was intentionally directed into the reflected gingival tissues of dogs.

An air-powder abrasive should be used with caution when treating the medically compromised patient, as the effect of abrasive polishing on systemic absorption of electrolytes is not fully known at this time.[35]

The application of stannous fluoride to contaminated titanium alloy appears to bind greater levels of LPS to the surface when compared with untreated controls. This agrees with findings on infected HA coatings.[16] The mechanism for this is unknown, but may be due to changes in charge interactions among stannous fluoride, LPS, and HA and/or to the binding of the glycerol carrier in the stannous fluoride solution.

When evaluating a 3-minute application of 1 percent chloramine T and a 1-minute application of citric acid (pH 1) to LPS-contaminated HA-coated surfaces, it appears that chloramine T does not offer any benefit when compared with burnished controls. Results from this study confirm the ability of citric acid to detoxify endotoxin-contaminated HA surfaces.

CONCLUSIONS

Titanium alloy surfaces are at a lower risk to the binding of endotoxin than HA-coated surfaces. If fixture modification is performed as part of a pocket elimination procedure, the use of chemotherapeutic agents or air-powder abrasives are probably not indicated. If regenerative techniques, such as osseous grafting and guided tissue regeneration, are contemplated, chemotherapeutic agents or air-powder abrasive techniques appear to have merit in the treatment of the toxic surface. *In vivo* studies are warranted to follow-up this *in vitro* investigation.

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