A Pilot Study on the Efficacy of a Treatment Algorithm to Detoxify Dental Implant Surfaces Affected by Peri-implantitis

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This in vitro pilot study was performed to determine whether a treatment algorithm including mechanical debridement, followed by air powder abrasion with glycine, followed by citric acid conditioning with vigorous flushing of the surface with sterile water after each step, is capable of decontaminating an infected implant surface. A total of 14 dental implants that were deemed hopeless due to advanced peri-implantitis were extracted. Of these, 6 implants served as tests and had their exposed surfaces treated with the decontamination protocol, 6 served as untreated controls, and 2 were mechanically treated only, followed by rubbing the surface with sterile saline. All implants were placed in culture with human osteoprogenitor cells for 72 hours, and evaluation was performed using scanning electron microscopy. The 6 test implants all demonstrated attachment and proliferation of the normal human osteoprogenitor cells on their prior exposed and decontaminated surfaces. All of the untreated control and the mechanically debrided, sterile water–treated implants failed to demonstrate this same success. The results suggest that this protocol can decontaminate an implant surface affected by peri-implantitis. Further studies are warranted to determine if this technique would demonstrate similar success over a greater number of implants and whether this outcome may occur in humans. Int J Periodontics Restorative Dent 2018;38:261–267. doi: 10.11607/prd.3203

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include surface decontamination, these efforts may be related to an inability to decontaminate the dental implant’s surface.

Several recent reports have demonstrated the success of a regenerative treatment algorithm to provide a steady state of health as evidenced by stable or improved bone levels and soft tissue gains. For this to occur, the affected surface must be disinfected such that hard and soft tissue regeneration are possible. While Lindhe and Møller commented that no one method of decontamination is adequate to render the surface healthy, the current treatment involves a combination of air powder abrasion with glycine powder followed by citric acid treatment with vigorous flushing of the surface for approximately 1 minute with sterile water spray after each step. Both air powder abrasion and citric acid have animal and in vitro evidence to support their use. With this in mind, the present pilot study was undertaken to determine whether this combined approach for surface decontamination could allow reattachment of osteoprogenitor cells in situ.

Materials and Methods

A total of 14 implants were used in this experiment, which had IRB approval (H-33173). The implants that were the subject of this investigation had an anodized surface and were obtained from patients at the Department of Periodontics at the Henry M. Goldman School of Dental Medicine at Boston University in Boston, Massachusetts, USA. All implants had been diagnosed with severe peri-implantitis and were determined by faculty members to require removal as part of their overall care (Fig 1a). The implants were explanted by reverse torque, trephine, or piezoelectric unit, with care given to avoid damaging their surface. They were assigned numbers to allow for anonymity and blinded randomization for treatment and evaluation. Assignment to the test, control, or mechanical-sterile saline group was done by selection from sealed unmarked envelopes, and this information was masked until the conclusion of the analysis was performed. Treatment was performed by a single clinician (P.S.R.) familiar with the protocol for surface decontamination (SD), which has met with clinical success as in a regenerative technique in the treatment of peri-implantitis in humans. The test group began with mechanical removal of large debris such as calculus and residual bone with ultrasonic and hand scaler instrumentation, and care was taken to avoid as much as possible deforming the implant surface. This was followed by SD using air powder abrasion with glycine powder (Air-n-Go, Acteon) for 1 minute followed by rinsing the implant with sterile water. The surface was then treated with citric acid (pH = 1) in a 50% saturated solution that was burnished onto the surface with cotton pellets for approximately 30 seconds to 1 minute followed by vigorously rinsing with sterile water for 1 minute (Fig 1b). Control implants remained untreated, with no effort made to remove any of the surface contaminants. The mechanically treated arm underwent the same mechanical debridement as the test group, followed by rubbing the surface with sterile water on cotton pledgets for approximately 2 minutes in an attempt to remove any residual contaminants. All implants were then individually placed into sterile containers, labeled, and transferred to the tissue culture lab.

Isolation and preparation of osteoprogenitor cells used in this study were derived from human osseous tissues and was performed in vitro in the laboratories of Dental Biomaterial Sciences at Boston University. The detailed protocol used was previously published. All lab work was performed by a single

Fig 1 (a) Pretreatment image of one of the test implants prior to the treatment protocol. (b) The same implant after mechanical removal of the calculus and biofilm with scalers, followed by air powder abrasion, citric acid application, and rinsing with sterile water.
individual (M.Q.) familiar with the protocol. Implants were individually placed into sterile cell-culture tubes that contained $3 \times 10^6$ osteoprogenitor cells for 1 hour to allow for seeding to occur, followed by placement into a 24-well plate. Culture plates were stored in an incubator for 72 hours to allow for cellular proliferation to occur. The growth media was changed daily to ensure optimal cellular survival.

After 72 hours, the implants were removed from the tubes and cellular fixation was done using glutaraldehyde, osmium tetroxide, alcohol, and hexamethyldisilazane (HMDS). Samples were then mounted on scanning electron microscope (SEM) discs and sputter coated with palladium. A single individual performed the imaging of each implant. Analysis was made of the top 5 mm of the implant from the flange, as this area consistently demonstrated bone loss in all cases. Analysis determined whether there was any proliferation of the osteoprogenitor cells in this region.

**Results**

There was no loss of any test or control implants due to contamination during the incubation period, allowing for SEM examination of all specimens. The untreated control group displayed the characteristic pattern associated with the anodized implant surface preparation (Figs 2a and 2b) with some foreign material/smear layer still remaining adherent (Fig 2c). No osteoprogenitor cells were seen attaching to or proliferating on the surfaces exposed to the oral environment throughout the entire 24- to 72-hour time frame of the study. The mechanically debrided, sterile saline–treated implants demonstrated the characteristic pattern of the anodized implant’s surface. In lower magnification, some osteoprogenitor cells appear to be attaching...
to the surface (Fig 3a). However, their attachments are few and far between and in higher magnification appear unhealthy (Figs 3b and 3c). The test surfaces that received the air powder/citric acid decontamination were considerably different. While all these implants demonstrated the classic anodized surface morphology, all were absent of any smear layer as was seen in the controls. Scattered osteoprogenitor cells (Fig 4a) could be seen as early as 24 hours and increased in number throughout the 72-hour time frame. The attached osteoprogenitor cells demonstrated an elongated morphology that in some cases bridged the titanium threads (Figs 4b and 4c). This cellular morphology with their extended projections suggested healthy attachment and proliferation. The treated surfaces also demonstrated some rough micronodules adherent to the surface that may have been associated with the surface adherence of the osteoprogenitor cells.

Discussion

The goals of any regenerative therapy that attempts to successfully treat a bacterially contaminated dental implant are to eliminate not only the microorganisms but their cytotoxic byproducts and elements as well, allowing a blood clot to stabilize and osteoblasts to attach and proliferate. This would provide the potential for osseointegration to occur. A number of articles with either individual or case series reports have suggested that regeneration is attainable.\textsuperscript{12-14,19-21} All these reports use some form of clinical measure, such as attachment level gains, radiographic bone fill, or re-entry where bone is present in the prior defect, to attest to the end points being reached. The clinician is left to wonder whether the exposed surface actually had contact with bone, or the bone suggested to have formed had fibrous connective tissue interposed between it and the dental implant surface, or the implant surface was truly decontaminated of bacteria and their cytotoxic byproducts, or the bone had simply been lost secondary to an exuberant inflammatory reaction without direct bacterial contact. In the current study, all microscopic analysis was performed in the top 5 mm from the implant platform, since bone loss could be corroborated by a radiograph taken prior to implant removal and by what appeared to be calculus attaching above, at, or in many cases apical to this level on the dental implant surface.

The current study does not quantify the number of osteoprogenitor cells present for a given area, which could be considered a shortcoming. However, the purpose of the study was to provide proof of principle determination as to whether the contaminated or treated implant surface could support the reattachment and proliferation of the osteoprogenitor cells. Furthermore, the study...
involved normal human osteoprogenitor cells and not those derived from osteosarcoma lineage. The reaction and bone formation associated with osteosarcoma cells may not be consistent with healing seen in a healthy wound in the human body, calling into question whether the surface decontamination might work with healthy bone cells.

Historically, decontamination of a bacterially contaminated dental implant surface by any one procedure has not been considered feasible on a consistent basis.¹ The regenerative protocol used in the current study was originally devised and used to successfully treat contamination from several different directions. First, mechanical removal of gross deposits is accomplished mechanically with scalers and ultrasonics. While some may opt for rotary instrumentation (ie, implantoplasty of the implant’s surface) based on prior evidence,¹⁹,²² this may not be a prudent idea. First, the titanium particles released are contaminated and may not be readily removed from the surrounding environment. Second, with narrower diameter implants, thinning the implant body could lead to fracture of the fixture over the long term, or heat might be generated in the implantoplasty procedure that could adversely affect the existing osseointegration. Following this, air powder abrasion with glycine was performed to further remove any residual plaque, calculus, and biofilm from the macro- and microstructure of the implant. Parlar et al²³ have demonstrated the benefits of air powder abrasion in vivo and in vitro to detoxify the implant surface.

Glycine is used rather than sodium bicarbonate to avoid the potential for a foreign body reaction, should residual particles remain at the end of the procedure. While concern has been voiced for causing an air emphysema, this can be avoided by keeping the spray tip as close to the implant surface as possible and packing the site with lint-free gauze to prevent air from getting under the flap. Another advantage of air powder abrasion is its ability to reach areas that may be obviated by lesion morphology, rendering mechanical efforts futile, such as in extremely narrow moat lesions. This is followed by vigorous rubbing of the exposed implant surface with a saturated solution of citric acid. Introducing the citric acid at this point facilitates the removal of any residual organic debris from the surface, which may include the glycine powder itself. Citric acid has also been shown to successfully decontaminate root surfaces affected by periodontitis²⁴ and to help to stabilize a clot²⁵ to the implant surface. Again, there is concern that the citric acid may be cytotoxic to the bone if there is contact during the decontamination process. However, de Rezende et al²⁶ demonstrated in vitro that bone surfaces demineralized by citric acid for 30 seconds increased the spreading of preosteoblasts and the surface area covered by these cells. Between each step, the implant is flushed with sterile water to help remove any residual material. The advantage of sterile water over sterile saline is that it creates an osmotic gradient that may lyse the bacterial cell wall.

Since all six of the anodized implants treated with the decontamination protocol demonstrated successful attachment of osteoprogenitor cells, this protocol may have a positive consistency. Two prior clinical studies²²,²⁴ suggested this would be the case, as the regenerative approach that incorporated this surface decontamination as a part of its overall treatment algorithm was able to predictably achieve positive clinical outcomes that remained at a steady state for up to 10 years.

While the decontamination protocol appeared to be successful, there are two plausible reasons no osteoprogenitor cells were seen on any of the contaminated or mechanically debrided control specimens. First, beyond the physical presence of viable bacteria, endotoxins in the biofilm and calculus may have been present that were cytotoxic, prohibiting cells from attaching. This cause-and-effect basis has been proven with periodontitis research²⁷,²⁸ and could extend to affected implant surfaces. Second, the failure of attachment to the untreated surfaces could be based on the theory of contact guidance of cells. Normal human cells must attach to solid substance to achieve healthy attachment, proliferation, and differentiation. In the presence of a smear layer, normal human cells will not find such solid anchorage and this will prevent them from attaching. The question might be posed as to why other protocols that employ only mechanical instrumentation and sterile saline wipes have been successful.²⁰ Implants lack the normal circumferential soft
tissue attachment apparatus, as do teeth. If a more superficial irritant were present around the neck of the dental implant, it could set off an exuberant inflammatory reaction that would be destructive to the bone without contaminating the implant more apically. Simply removing the causative bacteria/cement in this acute phase would set the scenario to achieve a strong healing response in kind.

Two other interesting findings resulted from this pilot work. The first was that this particular chemotherapeutic treatment algorithm may have created a change in the implant surface’s chemical composition to make it more favorable for cellular attachment to a commercially pure titanium. The second was that the treated surfaces demonstrated what appeared to be exposed, fresh, rough micronodules of their surfaces, which also may result in a better surface attachment.

While the results appear encouraging, further research is needed to better understand this decontamination algorithm. Does this treatment affect surface the chemistry of the implant, making it more or less hydrophilic? Are biological additives needed after decontamination to enhance clot stability? Can the endpoint of osseointegration, as demonstrated with histology, consistently be reached? Which regenerative materials or protocols are indicated to provide the most consistent and stable outcomes?

Conclusions
This study suggests that a protocol in which mechanical debridement is followed by air powder abrasion with glycine and citric acid conditioning followed by vigorous rinsing with sterile water after each step can lead to sufficient decontamination of an infected dental implant surface, allowing human osteoprogenitor cells to attach and proliferate. Further studies are needed to determine whether this finding could be translated in vivo over a larger number of dental implants.

Acknowledgments
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References