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Abstract

Title of Thesis: The Effects of Air Polishing Abrasives on the TiUnite® Implant Surface at Standardized Distance, Exposure Time, and Pressure Level: An *in vitro* Scanning Electron Microscopic Evaluation

Joshua Adam Metzger, Master of Science, 2014

Thesis Directed by Dr. Mary Elizabeth Aichelmann-Reidy, Department Chair, Post Graduate Periodontics

Background: Air polishing abrasives are becoming increasingly popular as a part of the treatment for decontaminating implants with identified peri-implantitis. This study sought to evaluate via scanning electron microscopy (SEM) the effect of three commercially available air abrasive powders on the TiUnite® implant surface, at pre-determined standardized settings.

Methods: 20 NobelReplace® Select implants were mounted on a custom jig which travelled uni-directionally at a constant speed. The implants passed across a fixed air polisher unit with the tip set 5mm from the implant surface. Each implant provided evaluation of one test abrasive (or control) which included three grooves of the TiUnite® surface, and was aligned with an orientation notch scored on the implant collar. The formulations of abrasives tested included sodium bicarbonate (EMS®, 60-70μm), glycine (EMS®, 60-70μm), and calcium carbonate (KaVo®, 65μm). For each test implant at 1000x magnification, an SEM image was captured at the center of each of the first three grooves of the TiUnite® surface for visual and statistical analyses. The characteristic micro-porosities of this particular roughened surface were identified and recorded within a standardized 100μm^2 area, then subjected to statistical analysis (ANOVA) to determine significance between the abrasives on the surface.

Results: It was found that both sodium bicarbonate (mean 145.1, SD 4.4) and calcium carbonate formulations (mean 20.0, SD 11.0) demonstrated significant reductions of identifiable micro-porosities and alteration of the anodized surface, when compared to the control group (mean 205.5, SD 2.2). The glycine abrasive powder, however, did not demonstrate a statistically significant difference (mean 199.3, SD 7.7) nor was there any notable alteration at magnifications up to 5000x.

Conclusions: Sodium bicarbonate and calcium carbonate air abrasives formulations induce extensive irreversible surface effects when compared to glycine, which did not demonstrate a significant loss of the porous topography. Whether this alteration in surface topography is beneficial or facilitates equivalent biofilm removal that is compatible with re-osseointegration remains to be evaluated.

The Effects of Air Polishing Abrasives on the TiUnite® Surface at Standardized Distance, Exposure Time, and Pressure Level: An *in vitro* Scanning Electron Microscopic Evaluation

Joshua Adam Metzger

Thesis submitted to the faculty of the Graduate School of the University of Maryland, Baltimore in partial fulfillment of the requirements for the degree of Master of Science
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Introduction:

Tooth loss has been explored from many perspectives into the 21st century, and is well established to be considered an event with multiple and compound etiologies. These can include most notably caries, periodontal disease, trauma or resorption, and the loss of even a single tooth can lead to reduced function and drifting of adjacent teeth [1]. Additional and continued remodeling of residual hard and soft tissues can occur, as well as possible esthetic, psychological, and social concerns among others can develop as a result of tooth loss [2, 3]. Tooth replacement options that are commonly offered in modern practice include conventional removable dentures, fixed bridges where applicable, and dental implant therapy to support either fixed or removable prostheses. Dental implants have received much focus as a predictable means of tooth replacement in the appropriate patient and are accepted to be successful in the range of over 90-95% [3]. Despite this, trends in research and development particularly for the last 10-15 years had gone through a philosophical shift in surface design, from that of a machined smooth surface to a significantly rougher surface produced by one of a variety of treatment methods unique to the manufacturer. These newer implant designs have not yet had the opportunity for comparable longitudinal research largely because of the rapid nature of advancing technologies. Additionally, it is clear they are not free of their own subset of disease; now an established disease category properly termed peri-implantitis. This process involves the inflammation and loss of supporting tissues, which can lead to reduced support, pain, and possible failure [4]. With more clinicians including both specialists and general dentists placing growing numbers of implants into the general population, this disease category is anticipated to present in clinical practice at an exponential rate. As a result, further research and understanding are required not only in terms of the accepted etiologies but of the effects of currently available peri-implantitis treatment modalities on implant retention.

Prevalence and Etiologies of Tooth Loss

The prevalence of partial and complete tooth loss in adults has generally decreased over the last several decades and through the latest NHANES data surveyed from 1999-2004. In spite of this marked improvement, adults between the ages of 20 to 64 have an average of less than 25 (24.92) remaining teeth, and 3.75% of adults 20 to 64 are fully edentulous. Additionally, adults 65 years or greater, current smokers, and those with lower education levels and socioeconomic status have positive correlations with fewer remaining teeth [5]. Furthermore, a look at the NHANES data by Albandar showed that there has been a gross underestimation of periodontal disease, and estimated that 50% of Americans over the age of 30 have periodontitis after adjustment for biases caused by the study protocols and data collection methods [6].

As demonstrated by authors of population-based tooth loss studies, the distribution of tooth lost was significantly skewed with a minority of subjects losing the majority of teeth [7]. Age, number of carious teeth, loss of attachment of 7mm or greater, mobility and sub-gingival calculus are significant predictors for tooth loss. At the subject level, both caries and periodontal disease were equally important in influencing tooth loss, but at the tooth level, caries was the main etiology of tooth loss in all age groups [7]. To elaborate, a study of 509 Greek subjects between ages 18-44, found a total number of

1,231 teeth extracted due to periodontitis (34.4%), caries (32.2%) and other reasons (33.4%) [8]. Additionally in a United States population of 736 male adults, caries was found to be the cause of tooth loss in one third of the extracted teeth, while extraction for prosthetic reasons accounted for 31.3%, and for periodontal disease 18.7% of teeth lost [9]. The first and second molars in both maxillary and mandibular arches, were found to be the most commonly extracted teeth due to periodontal disease [9]. Although tooth loss is dependent on patient's age and socioeconomic status, both caries and periodontal disease processes are impacted by genetic pre-disposition and individual disease susceptibility [10]. Additionally, as primary etiologies for tooth loss, attrition, orthodontic therapy, supra-eruption, neoplasm, supernumerary teeth, cystic lesions and hypoplasia all contribute to tooth extraction and loss of teeth [11].

Dental Implant History and Materials

The earliest evidence of the first dental implant to replace a tooth dates beyond 5,000 years ago in Egypt. Archeological evidence has shown a shell carving resembling a maxillary incisor based on its crown and root forms[12]. Dental implants were accidentally discovered in the 1950's by Dr. Per-Ingvar Brånemark, an orthopedic surgeon. During his experimentation on rabbit subjects implanted with titanium-based devices into their tibias, he noted that they were inseparable and incorporated within the bone tissue when he attempted their removal [13]. He stated that "the osseointegrated implant is directly connected to living remodeling bone without any intermediate soft tissue component" [14]. The landmark event sparked the interdisciplinary collaboration, which then led to the development of titanium-based implants for dental use.

Commercially pure titanium is classified according to its oxygen content and can range from grade 1 which has the lowest amount of oxygen at 0.18%, through grade 4 containing the greatest amount at 0.4% [15]. The different grades have mechanical properties that are a result of the various trace elements present. Prominent trace elements present in titanium alloys include Vanadium (V), Aluminum (Al), Iron (Fe), Carbon (C), and Nitrogen (N). It is the condition and purity of the oxide layer formed, which is based upon the alloy composition, that affects the biologic outcome of implant osseointegration [16]. The stability of the oxide layer protects the titanium from corrosive breakdown and foreign body reaction [17]. Titanium forms an oxide layer within immediate contact with air, which thickens to anywhere between 2-10nm within a second, and it is this layer that is the main active interface with the body and its fluids [18,19].

Implant Surface Designs

Titanium surfaces can be broadly classified based on the average surface roughness, and into having either smooth or rough surfaces [20]. The leading trends in implant dentistry relating to surface design characteristics have shifted significantly since their initial acceptance and entry into common practice. The more recent philosophy is that a highly roughened surface, as opposed to a machined polished design, can have a profound effect on its osteoconductive ability and the differentiation of osteogenic cells on the surface of the implant.

Implant surfaces have been roughened by a variety of innovative and patented processing methods that vary between each manufacturing company. Rough surface implants demonstrate higher bone-to-implant contact, higher removal torque values and improved long-term survival compared with that of machined or smooth surfaced implants [21-23]. The surface preparation of the modern dental implant is created by a variety of processing technologies include blasting with aluminum or titanium oxide, plasma coating with titanium or ceramics, acid-etching and porous sintered surfaces, as well as combinations thereof [24].

The TiUnite® surface of NobelBiocare® Replace implants is the primary implant surface evaluated in this study, and is commercially pure grade 4 titanium that has a roughened oxide layer characterized by a high crystalline and phosphorous content. In the treatment process, implants are anodized in a galvanic cell with a phosphoric acid electrolyte, such that once the current is run through the galvanic cell, an oxide layer forms on the surface of 5nm to nearly 10,000nm thickness [24]. Ivanoff and colleagues reported that the TiUnite® surfaces histomorphometrically had a higher bone-to-implant contact compared to machine-turned surfaces [25]. Rough-surface implants have increased available surface area for fibrin attachment [26], allowing development of earlier bone contact [16], and higher torque removal values at six weeks [27]. There is a higher incidence of fibrous encasement with smooth implant surfaces relative to the greater direct bone contact which was evident with the roughened implants [28].

Microscopic Examination of Osseointegration

Osseointegration has been defined as the direct contact between bone and a dental implant surface, and absence of movement between the implant and the bone to which it's embedded. In essence, it is a functional ankylosis of the implant into its surrounding bone tissue [29]. Microscopic studies show a close topographical relation between bone and implant, where collagen fibers from the bone are touching the implant surface without connective tissue or encapsulating soft tissues found between. Albrektsson and colleagues ultimately listed parameters consistent with successful implant osseointegration and specified recommendations to promote the greatest osseointegration including the use of threaded, unalloyed titanium implants with a defined finish and surface geometry [16].

In a study by Linder et al, under transmission electron microscopy (TEM), it was noted that the titanium was separated from the bone by at least 20nm. The nearest collagen filaments were at least 20nm away and the zone consisted of proteoglycans, predominately hyaluronic acid and chondroitin sulfate. Calcified deposits were noted in this zone, and Linder concluded that no antagonism between titanium implants and biologic tissues exist due to the oxide layer [30].

The TiUnite® surface, a titanium-oxide surface added through spark anodization processing, was examined by Schupbach et al under multiple forms of magnification including the light microscope and SEM to further analyze bone to implant interfaces with this surface [31]. Under light microscopy, intimate contact between the oxidized implant surface layer and the newly formed bone had been demonstrated. This newly formed bone migrated from adjacent bone surfaces toward the implant, and also by bone growth directly onto the implant surface. The SEM analysis showed a micro-porous surface between 1 to 7μm and nano-porosities of less than 1μm in diameter, in which bone matrix and tissues deposited by nearby osteoblasts lead to a strong interlock. It was concluded that osteoblasts were located around the surface pore orifices and bone matrix was deposited into these implant surface pores, leading to a strong interlock between the bone and the titanium oxide layer of the implant [31].

Peri-Implantitis Disease

As described by Meffert, disease around implants, if left untreated, can lead to their loss just as untreated periodontitis can lead to loss of teeth [32]. Based on the collaboration at the European Workshop on Periodontology, the definition of peri-implantitis was established as an inflammatory process affecting tissues around an osseointegrated implant in function, thereby resulting in pocket formation and the loss of supporting bone. Peri-implant mucositis, to be discriminated as a separate entity, was defined as a reversible inflammatory process present within the soft tissues surrounding a functional implant without associated bone loss [33]. A detailed classification system was subsequently developed in 2012 by Froum and Rosen, defining categories of peri-implantitis as early, moderate, and advanced. They are all characterized with bleeding and/or suppuration combined with deepened probing depths. Early peri-implantitis is described as implants with probing depths ≥4mm and bone loss <25%, moderate peri-implantitis as ≥6mm and bone loss <50%, and advanced peri-implantitis as probing depths ≥8mm and bone loss >50% [34].

The signs and symptoms of peri-implant disease are similar but may be more difficult to detect than analogous disease around a natural tooth, with a functional and responsive associated periodontal ligament. Tooth mobility becomes clinically detectable with less cumulative bone loss when compared to an osseointegrated implant, and there is less pain around implants. In a long-term follow-up study of 1,057 Brånemark implants, the overall survival rate was over 95%, with a significant relationship noted between implant loss and periodontal bone loss of remaining teeth at time of implant placement. This indicates that a history of periodontitis conveys susceptibility to the development of peri-implantitis and possible implant loss [35].

Inflammation around implants undoubtedly causes bleeding and/or suppuration with light probing forces (approximately 0.25N) and subsequent detectable radiographic evidence of bone loss, which tends to be crater-like or run circumferentially around the implant with sharp demarcation [36].

Radiographic presentation of bone resorption due to deep placement, proximity of adjacent implants or structures, and abutment design have all been associated with subsequent bone remodeling determined to be unrelated to the pathologic disease process of peri-implantitis. Additionally, not all probing depths is indicative of peri-implantitis as the implant, its abutment, and prosthetic designs can affect the ultimate dimensions of the peri-implant soft tissues [37]. As a result, it is recommended that long-term monitoring of the peri-implant hard and soft tissues should be performed once the peri-implant tissues have become established subsequent to the completion of all prosthetic work.

Peri-Implantitis Microbiology

Microorganisms play a major role in peri-implantitis, as outlined in a review by Mombelli and coworkers [37]. In human subjects, plaque deposition on implants leads to peri-implant mucositis. There is a distinct difference of the microflora when comparing failing implants to those with established health. This was confirmed in animal studies; plaque-retentive ligatures lead to shifts in the microflora and eventual peri-implantitis [37]. Additionally, oral hygiene level correlates with long-term implant success, and the clinical status of peri-implantitis patients improves with anti-microbial therapy [38].

Highly roughened implant surface treatments inherently produce vulnerable implant fixtures due to the morphologic irregularities once exposed to contamination. The bacterial colonization sequence of implant surfaces largely parallels that of teeth. In a healthy environment, there is a predominance of gram-positive, facultative rods and cocci, however, in disease the peri-implant pockets contain bacterial profiles similar to that seen in periodontitis [39]. This includes the presence of the red complex (*P. gingivalis, T. forsythia, T. denticola),* as well as *A. actinomycetemcomitans, P. intermedia, P. nigrescens,* and *C. rectus* [40]. Concurrent with an expanding presence of species in diseased sites, it was noted the more beneficial, gram-positive species were reduced compared to healthy implants sites [41].

Peri-Implantitis Treatment Options

Treatment for peri-implant disease has become an emerging field of interest, and similar to periodontal disease can be approached clinically in either a nonsurgical (closed), or surgical (open) fashion. The general principles of peri-implant treatment can be summarized as follows: the mechanical disturbance and removal of the peri-implant bacterial biofilm, chemical decontamination of the implant surface, correction of hard and soft defects non-maintainable by oral hygiene measures, establishment of adequate homecare by the patient, and finally, regeneration and re-osseointegration of deficient peri-implant bone [40]. Both the surgical and non-surgical treatment of peri-implantitis produce similar clinical outcomes based on the clinical parameters outlined in a systematic review by Claffey et al, however radiographic and histologic improvements were superior in the surgical group [42]. The predominance of current evidence suggests that peri-implantitis does not respond to traditional nonsurgical therapy [43]. The surgical treatment of peri-implantitis involves either a resective or regenerative approach. The regenerative approach involves the use of graft and barrier membrane materials, when moderate bone loss and containable intra-bony defects surround the dental implants. Re-osseointegration was found to be more robust on rough surface implants than on smooth surface implants [36]. When advanced bone loss is present, removal of the implant and subsequent regeneration of the deficient ridge site may, in fact, be indicated if the prognosis of the implant is in question [33].

Mechanical decontamination using different instruments and methods has been studied to evaluate their effects on implant surfaces. These include the use of titanium, steel, carbide, and ePTFE curettes, ultrasonic scalers with carbon fiber or plastic tip inserts, rubber polishing cups with pumice pastes, and various air abrasive applications [33]. Chemical decontamination, using antimicrobial agents such as chlorhexidine 0.12%, tetracycline, citric acid, betadine (povidone-iodine solution), and hydrogen peroxide have been evaluated and compared [33, 44-46]. When compared to rough surfaces such as plasma-sprayed or hydroxylapatite-coated, machined surface implants were more effectively decontaminated by chemical means. Also, it was noted that air abrasion was consistently the most effective method for removal of endotoxin [44].

Air Abrasives in Dentistry

Air abrasive devices have a range of applications in dentistry, which includes intra- and extra-oral polishing of teeth and other materials, cavity preparation, and more recently surface decontamination of ailing implants. Systems generally feature the use of an abrasive powder, propelled by a stream of compressed air with water between 65 to 100 pounds per square inch pressure, to assist in removal of biofilm or extrinsic stain [47]. Examples of currently available formulations of air abrasives include aluminum oxide, sodium bicarbonate, calcium carbonate, and glycine. Aluminum oxide has an abrasive index (Mohs scale of hardness) of approximately 9 of 10, which allows for a different set of applications than the abrasives marketed for surface decontamination of implants. The other formulations fall within a lower and closer range to each other: sodium bicarbonate 2.4, calcium carbonate 3.0, and glycine 2.0 [48].

Air Abrasives for Surface Decontamination

Air abrasive techniques have been demonstrated in both *in vitro* and *in vivo* studies to be effective in cleaning previously contaminated implant surfaces [4]. A 2012 review by Tastepe et al included 27 articles reporting the efficacy of this approach in cleaning the implant surface, as well as the clinical response to implants treated using this method. It was concluded that the cleaning efficiency, evaluated by endotoxin removal, ranged from 84% to 98% and the removal of the bacterial biofilm was up to 100% in the *in vitro* studies included [49]. Some currently accepted implant treatment protocols, like that proposed in 2013 by Froum and Rosen et al with 3 to 7.5 years of successful follow up, advocate a multi-step sequence that incorporates the use of a sodium bicarbonate air-abrasive delivery system twice for 60 seconds [50].

It remains controversial whether this treatment modality physically alters the structure of the implant surface thereby altering cell attachment. This may, in part, be due to the recent and rapid evolution of implant surfaces and abrasives such that thorough testing has not been performed. It has been demonstrated by Mengel et al that that the delivery of sodium bicarbonate abrasive on Screw-vent®, titanium plasma coated, and standard Branemark® implant designs does not appear to cause marked implant surface changes based on scanning electron microscopy and optical laser profilometry and thus is permitted for use on implant surfaces for decontamination [51]. Conversely, Chairay and coworkers demonstrated by scanning electron microscopy that a single air abrasive treatment on machined and plasma-sprayed implant surfaces modified portions of their exposed surfaces to some degree [52]. Another aspect important to consider, as highlighted in a study by Mouhyi et al, is although the use of an air abrasive technique may be efficient to remove biofilm, it may also result in contamination with residual particles attached to the implant surface [53].

There are no *in vivo* studies in which complete re-osseointegration has been demonstrated by the use of air powder abrasives alone; however some animal studies have shown bone regeneration when combined with use of grafts and barrier membrane for guided bone regeneration [54-56]. It can only be substantiated that air powder abrasive can contribute to the detoxification of the implant surface and can improve the clinical outcomes when used in combination with surgical regenerative procedures. Sodium bicarbonate based abrasive materials are the most commonly available on the market, however more recently other alternative formulations have been introduced and released such as glycine, calcium-carbonate, and erythritol-based abrasives. Furthermore, there is no available evidence with respect to the effects of these air abrasives on TiUnite® roughened implant surface, developed by NobelBiocare®.

Purpose:

The aim of this study is to evaluate *in vitro* the effect of a single pass of three different air abrasives on the TiUnite® surface of NobelBiocare® Replace implants under scanning electron microscopy (SEM). The objective is to quantify and describe changes to the surface topography and porosity after treatment with three commercially available materials with equivalent particle size formulations (sodium bicarbonate, glycine, and calcium carbonate) at a set distance, exposure time, and pressure level relative to the implant surface.

Materials and Methods:

Air Abrasive Polisher and Powder Systems

For these experiments, an EMS® Air-Flow® Handy 2+ unit was utilized, and attached to a conventional dental chair unit. Implants were exposed to three different air abrasive powder materials commonly available and marketed for use with the selected air polisher unit. The unit was held static at a fixed position of 5mm, and pressure of 95%, as the air-water-abrasive stream was activated and exposed to the entire length of the implant surface. The three formulations of air abrasives tested include sodium bicarbonate (EMS®, average particle size 60-70μm), glycine (EMS®, average particle size 60-70μm), and calcium carbonate (KaVo®, average particle size 65μm) formulations, all of which are compatible with the selected unit. These settings and selections were chosen in effort to closely simulate a clinical scenario in a reproducible, standardized manner.

Titanium Implants and Procedure

A custom implant jig was constructed using a rail mounted to a leveled wooden block and pulled uni-directionally by an implant motor (see Figures 3 and 5). The implant motor was set to 15rpm pulling each implant on the jig at a constant speed of 1.4mm/s. The air abrasive unit tip was set at a constant distance of 5mm to the implant surface, using an assembled transparent plexi-glass enclosure and verified with a periodontal probe each trial (see Figure 5). The enclosure was stabilized and prepared with a 3mm hole at the height of the implant mount center for consistent alignment as well as containment of the air-water-abrasive mix. A custom gutter or spillway was fixed to the wooden block to prevent any migration of abrasive material into the rail-track assembly and clean the apparatus between trials. The implants were exposed to the air abrasive material released by the polisher unit, the tip of which was set at a 90° angle to the lateral implant surface when the distance was held constant at 5mm. The air abrasive unit was activated from approximately 10mm prior to and held on through 10mm beyond completed exposure of the implant surface from platform end to apex.   Each implant was rinsed with sterile water for 30 seconds and then allowed to air dry fully following exposure with compressed air from the dental unit.

20 NobelReplace® Tapered implants (NobelBiocare®) with a TiUnite® surface and a machined polished collar were mounted onto the jig and pulled with the implant motor for each air abrasive tested at the same repeated settings. The implants tested all had a 3.5mm diameter and a length of TiUnite® surface between 10mm and 16mm, of which the machined polished collar consisted of the first two millimeters from the platform side. All implants were handled under sterile conditions during handling and air abrasive application therapy.

Each implant was treated with one test abrasive or control of the TiUnite® surface, and the first three grooves below the polished collar were designated to be imaged for surface analysis. The abrasive was applied based on an orientation notch that had been marked with a 1/4 round bur (see Figures 1 and 2). The total experimental group consisted of 20 implants, with five implants exposed to each of the three tested abrasives, and five control implants exposed identically but without abrasive (water-air). The data was then averaged to produce a mean value for each implant tested, giving a total of n=5 per treatment group.

Figure 1: Orientation notch view from implant platform

Figure 2: Orientation notch view from lateral side

Figure 3: Implant mounted on onto jig with notch facing out

Figure 4: View of SEM machine used

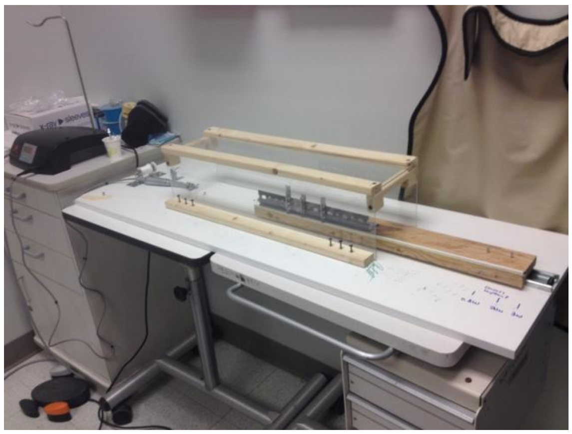


Figure 5: Complete view of implant mount, track, plexi-glass enclosure, and implant motor

Scanning Electron Microscopy

After treatment with the selected air abrasives, the test and control implants were each placed on a custom mount and aligned according to the reference notch for imaging by scanning electron microscopy (see Figure 4 - Quanta™ 200, FEI systems. Hillsboro, Oregon) at the following magnifications: 50x, 500x, 1000x, 5000x. The lower 50x and 500x magnifications were used for initial reference alignment and to center the area of the first three grooves. The 1000x magnification level was used for detailed visualization of the micro-porosities. An additional 5000x magnification image was taken for a more qualitative and descriptive detail of the second groove of each implant analyzed.

Statistical Analysis

The primary parameter identified and measured is defined as the total number of visible pores present in the standardized surface area ($100\mu m^2$) within the SEM images captured of each groove, at 1000x. TiUnite® is a titanium oxide rendered into an osteoconductive biomaterial though spark anodization such that the high volume and presence of micro-porosities are the hallmark of the surface. The maintenance or loss of this characteristic surface feature was assessed by counting individually and marking each visible pore, with computer aid, within the standardized surface area of each groove included in the samples. One-way analysis of variance was then performed, along with Tukey HSD post hoc test, to determine statistical significance between each of the tested air abrasives on the selected surface. These analyses were performed using JMP (JMP Software, Statistical Discovery™, Cary, NC, USA).

Results:

The data obtained from the visual and computer-aided numerical analysis are listed in Tables 1, 2 and in Figure 6. The control surface group demonstrated a mean of 205.5 pores (SD 2.2, range 198-217) per standardized surface area of $100um^2$. The sodium bicarbonate group demonstrated an average of 145.1 pores (SD 4.4, range 132-161), the glycine group had an average of 199.3 pores (SD 7.7, range 189-215), and the calcium carbonate had an average 20.0 pores remaining (SD 11.0, range 9-37).

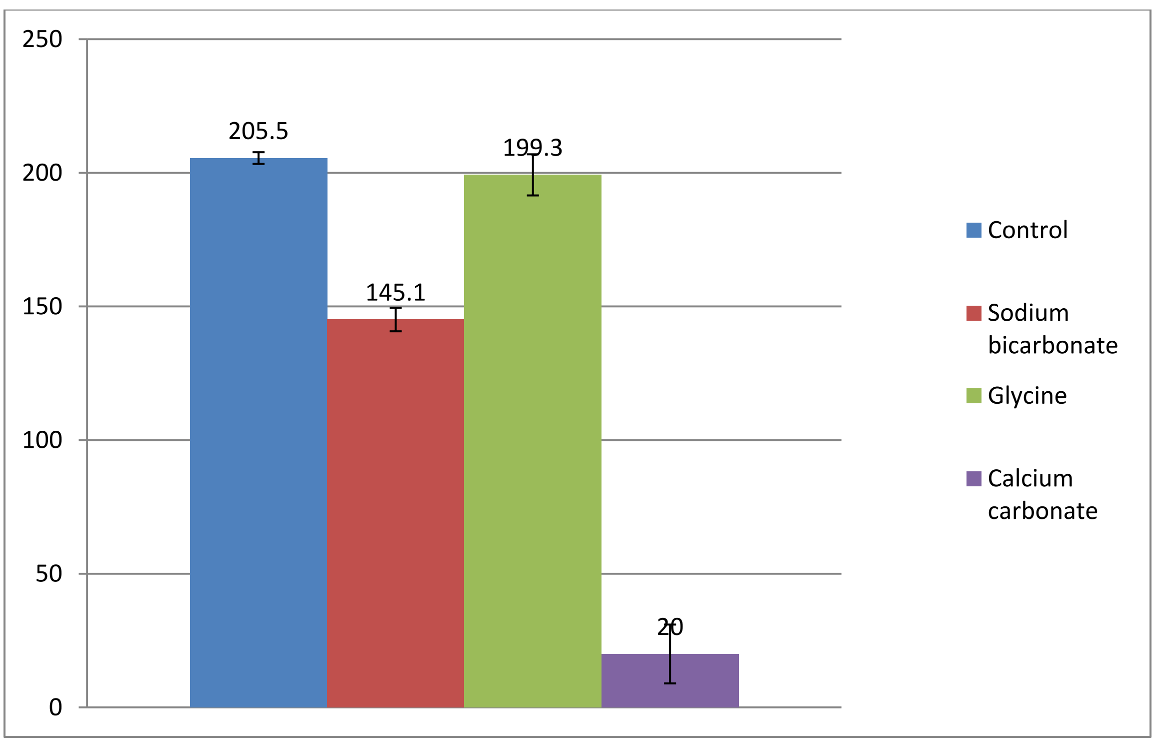
Table 1: Raw data of number of pores identified by treatment group, per $100um^2$

	Control	Sodium bicarbonate (SB)	Glycine (Gly)	Calcium carbonate (CC)
Implant 1, Groove 1	**217** (LOT 381757, REF 29401)	**158** (LOT 381757, REF 29401)	**197** (LOT 394791, REF 29401)	**36** (LOT 394784, REF 29401)
Implant 1, Groove 2	**203** (LOT 381757, REF 29401)	**144** (LOT 381757, REF 29401)	**194** (LOT 394791, REF 29401)	**15** (LOT 394784, REF 29401)
Implant 1, Groove 3	**207** (LOT 381757, REF 29401)	**136** (LOT 381757, REF 29401)	**207** (LOT 394791, REF 29401)	**26** (LOT 394784, REF 29401)
Implant 2, Groove 1	**204** (LOT 396397, REF 29403)	**141** (LOT 394791, REF 29401)	**201** (LOT 381757, REF 29401)	**9** (LOT 381757, REF 29401)
Implant 2, Groove 2	**198** (LOT 396397, REF 29403)	**148** (LOT 394791, REF 29401)	**191** (LOT 381757, REF 29401)	**29** (LOT 381757, REF 29401)
Implant 2, Groove 3	**213** (LOT 396397, REF 29403)	**138** (LOT 394791, REF 29401)	**202** (LOT 381757, REF 29401)	**18** (LOT 381757, REF 29401)
Implant 3, Groove 1	**206** (LOT 394791, REF 29401)	**149** (LOT 393301, REF 29402)	**191** (LOT 381757, REF 29401)	**12** (LOT 394791, REF 29401)
Implant 3, Groove 2	**201** (LOT 394791, REF 29401)	**161** (LOT 393301, REF 29402)	**206** (LOT 381757, REF 29401)	**30** (LOT 394791, REF 29401)
Implant 3, Groove 3	**210** (LOT 394791, REF 29401)	**142** (LOT 393301, REF 29402)	**208** (LOT 381757, REF 29401)	**19** (LOT 394791, REF 29401)
Implant 4, Groove 1	**201** (LOT 394784, REF 29401)	**153** (LOT 381757, REF 29401)	**211** (LOT 393301, REF 29402)	**26** (LOT 381757, REF 29401)
Implant 4, Groove 2	**205** (LOT 394784, REF 29401)	**141** (LOT 381757, REF 29401)	**189** (LOT 393301, REF 29402)	**15** (LOT 381757, REF 29401)
Implant 4, Groove 3	**209** (LOT 394784, REF 29401)	**148** (LOT 381757, REF 29401)	**196** (LOT 393301, REF 29402)	**37** (LOT 381757, REF 29401)
Implant 5, Groove 1	**207** (LOT 394791, REF 29401)	**145** (LOT 394791, REF 29401)	**194** (LOT 394784, REF 29401)	**17** (LOT 381757, REF 29401)
Implant 5, Groove 2	**199** (LOT 394791, REF 29401)	**132** (LOT 394791, REF 29401)	**215** (LOT 394784, REF 29401)	**24** (LOT 381757, REF 29401)
Implant 5, Groove 3	**203** (LOT 394791, REF 29401)	**141** (LOT 394791, REF 29401)	**207** (LOT 394784, REF 29401)	**31** (LOT 381757, REF 29401)

Table 2: Mean number of pores identified per implant, by treatment group, per 100μm^2

	Control	Sodium bicarbonate (SB)	Glycine (Gly)	Calcium carbonate (CC)
Implant 1	**209** (LOT 381757, REF 29401)	**146** (LOT 381757, REF 29401)	**199.3** (LOT 394791, REF 29401)	**36** (LOT 394784, REF 29401)
Implant 2	**205** (LOT 396397, REF 29403)	**142.3** (LOT 394791, REF 29401)	**201** (LOT 381757, REF 29401)	**9** (LOT 381757, REF 29401)
Implant 3	**205.7** (LOT 394791, REF 29401)	**150.7** (LOT 393301, REF 29402)	**191** (LOT 381757, REF 29401)	**12** (LOT 394791, REF 29401)
Implant 4	**205** (LOT 394784, REF 29401)	**147.3** (LOT 381757, REF 29401)	**211** (LOT 393301, REF 29402)	**26** (LOT 381757, REF 29401)
Implant 5	**203** (LOT 394791, REF 29401)	**139.3** (LOT 394791, REF 29401)	**194** (LOT 394784, REF 29401)	**17** (LOT 381757, REF 29401)
MEAN	**205.5**	**145.1**	**199.3**	**20.0**
Std Dev	**2.2**	**4.4**	**7.7**	**11.0**

Figure 6: Effects of Air Abrasive Exposure on TiUnite® Surface



X-axis = treatment groups; Y-axis = number of identified pores, per 100μm^2

Control group:

At 50x (Figure 7), the reference notch at the platform extending down the first six grooves of the TiUnite® surface are captured. At this magnification, only the macroscopic features of the implant were distinguishable, and this was primarily used to ensure the reference notch was aligned properly within the SEM chamber for the subsequent increased magnifications. At 500x (Figure 8), the SEM image captured one complete groove between external threads of the TiUnite® surface, which could be seen at the lateral borders of the image. The central area of each selected groove is where the 1000x and 5000x images were taken for statistical and qualitative analysis. At 1000x (Figure 9), the regular distribution of the micro-porous surface was clearly seen. Additionally, a notably raised perimeter surrounds the pore, and therefore the pore plus surrounding raised perimeter was defined as one micro-porosity in the statistical analysis. It was at this magnification that a $100\mu m^2$ surface area was selected from each of the SEM images and incorporated in the statistical analysis, which included the first three grooves of each of the treatment implants. A mean of 205.5 porosities were measured after the data was combined for the control implants, per standardized $100\mu m^2$ surface area selected. At 5000x (Figure 10), the highly detailed microscopic features were appreciated. Some of the micro-porosities were clearly more irregular in configuration, and in addition the nano-porosities became distinguishable with smaller diameter and lack of well-defined raised border. A generalized uniform albeit random distribution of these porosities was noted.

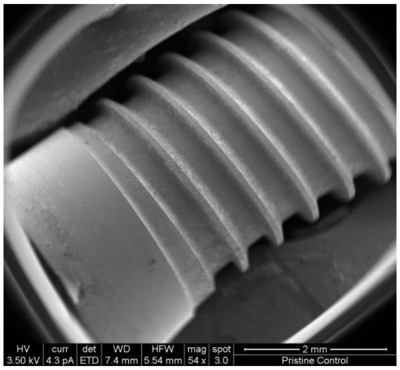


Figure 7: Control implant at 50x. Note reference notch, threads, and grooves present

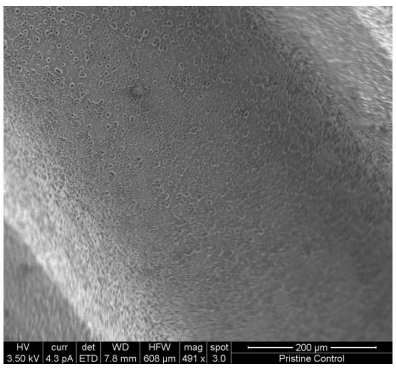


Figure 8: Control at 500x. Note the groove centered within the threads

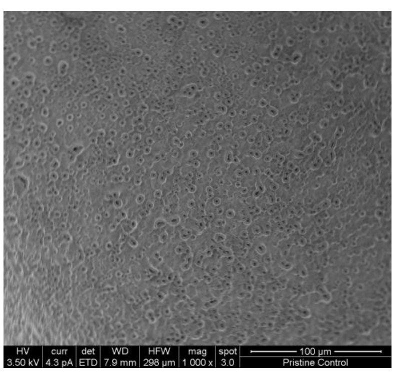


Figure 9: Control at 1000x used for analysis. Micro-porosities clearly visible

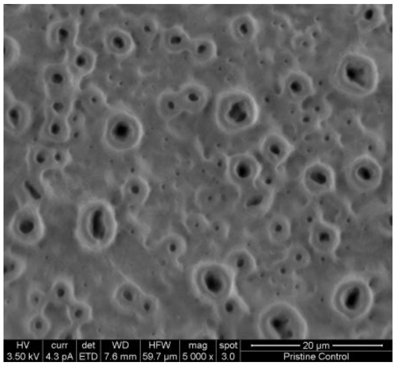


Figure 10: Control at 5000x. Irregular geometry and nano-porosities evident

Sodium bicarbonate:

At 50x (Figure 11), in addition to the reference notch and previous features present in the control images, a clear residual coating of the air abrasive material could be seen. This appears on both the polished collar as well as the TiUnite® surface visually (Figure 12), and despite the standardized rinsing of each implant with sterile water for 30 seconds right after the completed exposure to the abrasive and removal from the mounting jig. At 500x (Figure 13), there was loss of the anodized surface and areas were found to be devoid of the highly porous topography. Upon greater magnification at 1000x (Figure 14), both partially intact porosities as well as obliterated or coated pores were evident, and these were not incorporated into the statistical analysis. Areas of completely flat or smooth appearing surface, as well as partial and occluded porosities were further discernible at 5000x (Figure 15). These alterations would account for the lower mean of 145.1 micro-porosities as compared to 205.5 per $100um^2$ found for the control surface. This difference was determined to be statistically significantly, and examination at 5000x magnification demonstrated the unquestionably altered nature of the TiUnite® surface.

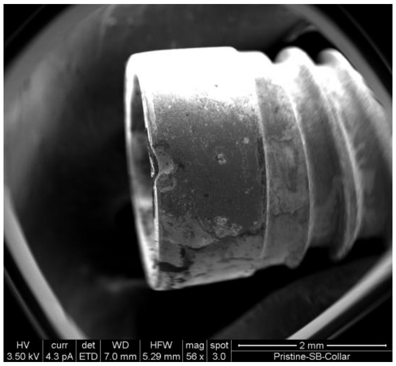


Figure 11: Sodium bicarbonate exposure at 50x. Notch and surface coating noted


Figure 12: Visualization of sodium bicarbonate surface coating

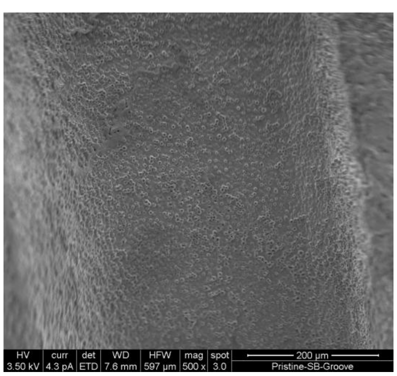


Figure 13: Sodium bicarbonate exposure at 500x. Note the groove centered within threads

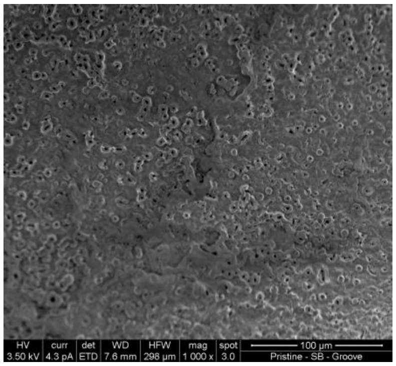


Figure 14: Sodium bicarbonate at 1000x. Devoid and altered areas noted

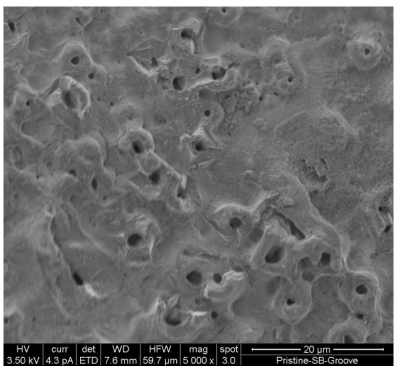


Figure 15: Sodium bicarbonate at 5000x. Partial and full obliteration of some pores

Glycine:

In contrast to the sodium bicarbonate abrasive, at 50x (Figure 16) magnification there was no visible surface film or remnants of glycine abrasive left on the implant surface. In fact, minimal differences were observed in comparison to the control implant images at this power, and the same held true at magnifications of 500x (Figure 17) and 1000x (Figure 18). A trend of slight reduced mean pores was found with 199.3 identified per $100\mu m^2$; however this was not found to be statistically significant. At 5000x (Figure 19) similar to the sodium bicarbonate group, there was evidence of some partial porosities, though the majority identified remained fully intact like that of the control surface.

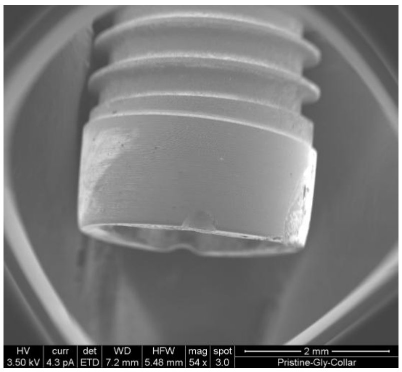


Figure 16: Glycine at 50x magnification showing notch and lack of surface coating

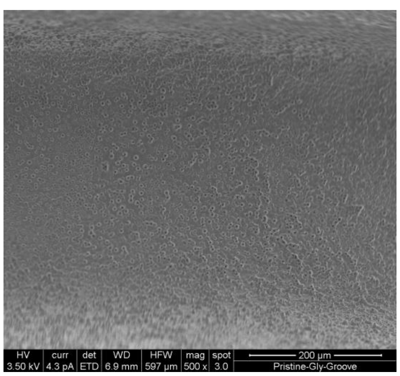


Figure 17: Glycine exposure at 500x. Note the groove is centered within the threads

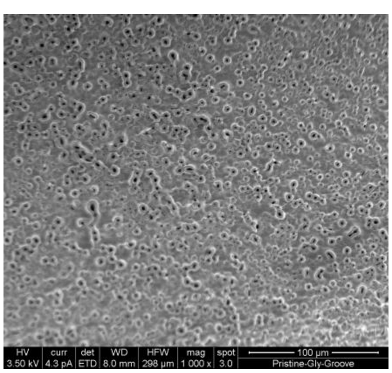


Figure 18: Glycine exposure at 1000x, and minimal changes noted vs. control

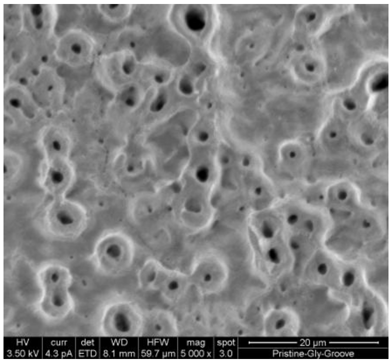


Figure 19: Glycine exposure at 5000x shows similar complete and partial pores with border

Calcium carbonate:

At 50x magnification (Figure 20), several prominent gouges were visualized all in association with the notch and the path of abrasive along the implant. At 500x (Figure 21), the most notable and characteristic difference was the almost complete absence of the porosities within the grooves and lost anodized surface. Additionally a second 500x image (Figure 22) was taken, which focused on the third thread of a test implant (LOT 381757, REF 29401), highlighting one of these prominent gouges in finer detail. It appeared as though the abrasive material caused the jagged dissociation of the peripheral surface in this area of the thread, and this was found to protrude similarly in the other affected areas. At 1000x (Figure 23) magnification, there was a highly statistical significant reduction in remaining porous surface topography, such that there was a mean of only 20.0 pores per standardized 100um^2 area identified. This heavily altered, obliterated surface seen at 5000x (Figure 24) resulted from a single pass of this air abrasive over the TiUnite® surface.

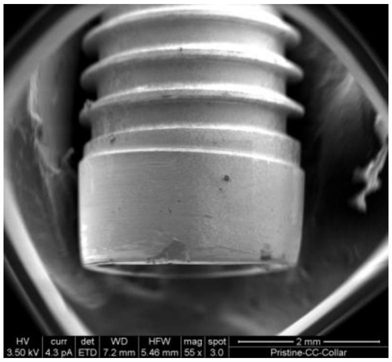


Figure 20: Calcium carbonate at 50x demonstrates several gouges

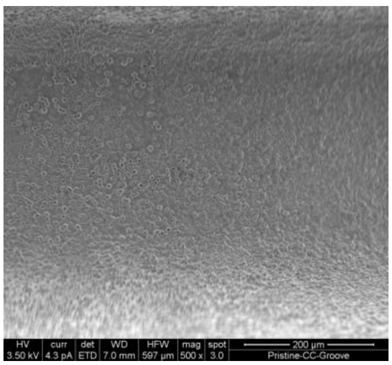


Figure 21: Calcium carbonate at further magnification at 500x shows absence of pores

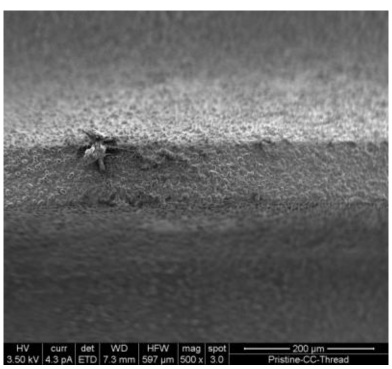


Figure 22: Calcium carbonate induced gouge at 500x. Note the jagged appearance

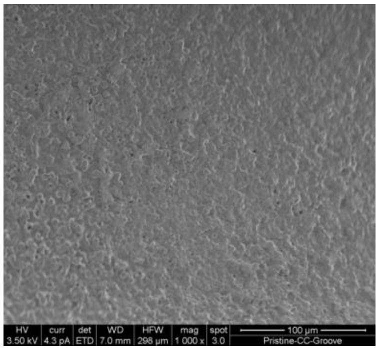


Figure 23: Calcium carbonate at 1000x shows minimal identifiable pores present

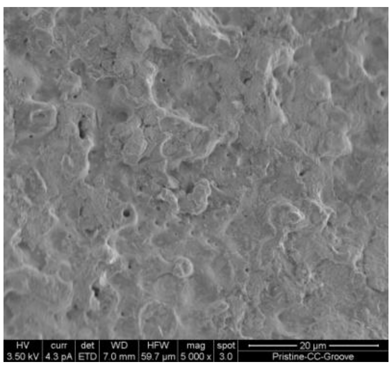


Figure 24: Calcium carbonate causing extensive obliteration of pores at 5000x

Discussion:

With the growing trends in implant therapy and associated rise in peri-implantitis, air abrasive applications have been used for the decontamination process of current rough surface implants [57]. Studies have assessed the use of air abrasive formulations in the treatment of implant surfaces but are limited and difficult to compare, particularly since their conclusions vary largely based on the implant systems and surfaces both popular and available at the time of publication. This study sought to investigate the microscopic effect of surface treatment of TiUnite® implants with three commercially available air abrasive formulations at a pre-selected and standardized exposure time, distance, and pressure level for its application. The results demonstrate a significant disparity and range of effects, which can be produced after these three air abrasive formulations were applied to the TiUnite® implant surface.

Based on the 2009 systematic review by Renvert et al., re-osseointegration was concluded to be possible to obtain on a previously contaminated implant surface and can occur in experimentally induced peri-implantitis defects following therapy. The amount of re-osseointegration varies considerably within and between studies, as well as the particular implant surface characteristics may in fact influence the amount of potential re-osseointegration [57]. Additionally, the most common abrasive for which literature is most abundant and available is sodium bicarbonate, and interestingly the only material found to remain bound to the implant surface despite significant and consistent post-treatment surface rinsing. This finding was consistent and had been mentioned by other studies, particularly one by Mouhyi et al which confirmed residual sodium bicarbonate material coating the implant surface and concluded its use introduced a different form of contamination than what was initially being treated [53].

Now that surface alteration has been established, it is important to determine whether this apparent modification of the surface is, in fact, better or worse for potential re-osseointegration? More qualitative studies are necessary to determine, for example, if the gentler glycine material is as effective in biofilm removal as is either of the more surface altering formulations. There remains the possibility that the loss of porous surface might actually be beneficial by inherently eliminating all possible reservoirs for bacteria and therefore creating a newly biocompatible surface. Therefore, even though the alterations may reduce the potential surface area for re-integration, they may also potentially facilitate a more complete bacterial elimination. As a result of this possibility, the glycine abrasive could actually be the poorest in regards to therapeutic outcome based on this study, which only examines whether the treatment alters the physical surface *in vitro*. Another possibility is that all three agents equally remove biofilm contamination, and any consequent surface alterations are clinically irrelevant in nature if the surface is clean and re-osseointegration takes place. To provide more information it would be helpful to consider the incorporation of a bio-mimetic for biofilm contamination, or perform *in vivo* trials in coordination with these abrasives for a better understanding of therapeutic outcomes.

Albuoy and colleagues in a recent study evaluated several different common implant surfaces after surgical mechanical debridement of experimentally induced peri-implantitis. Among the surfaces investigated was TiUnite® which continued to have bone loss after treatment, whereas other implants surfaces (TiOblast™ and SLA®) had radiographic bone gain after treatment of implants. They attributed these differences in treatment outcomes to implant surface characteristics [56]. Future research should focus on a comparison of these air abrasives on other commercially available implant surfaces and under similar surgical parameters and study protocol.

It is possible that the set distance, pressure, or length of exposure time standardized in this in vitro analysis could have been more influential on the outcome as compared to its clinical use. To elaborate, only one pass was used in this study where clinically a surgeon may choose to go over the affected implant surface with multiple passes and for significantly longer duration than for each of the tested implants exposed in this study. Additionally, a greater variation in relative angle of abrasive stream contacting the implant surface may exist clinically, which in this case was standardized at a right angle in each trial. This may translate to more or less variation in overall surface changes between the three tested abrasives, which otherwise could have been given greater impact due to the single pass design. In this regard, the grain shape of the KaVo® formulation is marketed as having the advantage of being spherical and more homogenous than competitor sodium bicarbonate formulations [58]. It is claimed that better contact transfer exists between the particles and target accounting for the greatest observed effect on the TiUnite® surface.

Within the parameters of this study, a single pass application of three different air abrasive powder formulations on anodized titanium surfaces produced surface alterations that were unique to the specific material selected. It was found that sodium bicarbonate and calcium carbonate formulations induced statistically significant loss of the porous features. Alternatively, the glycine formulation did not produce a statistical significantly change in the surface characteristics when compared to the untreated controls. Further studies are required to determine the possibility of re-osseointegration of TiUnite® implants after decontamination efforts with each of these materials *in vivo*. Additionally, it remains to be investigated whether this surface alteration and loss of the characteristic porosities that was observed in this study benefits potential re-osseointegration after completed treatment of these implant surfaces. Ultimately, in addition to effective mechanical biofilm elimination, possible future ventures may consider development of an air abrasive application that can perform-additional chemotherapeutic effects and thus further aid effective treatment of peri-implantitis disease in this rapidly growing age of implant-related dentistry.

Conclusions:

Sodium bicarbonate and calcium carbonate air abrasives were found to induce significant irreversible surface alterations *in vitro* when compared to glycine formulation. Implant surfaces treated with glycine did not show similar loss of micro-porous structure within the grooves under SEM magnification of 1000x. Whether this alteration is beneficial, necessary, or facilitates equivalent biofilm removal and compatibility for re-osseointegration remains to be evaluated. Additional research including the use of a bio-mimetic agent, or performing *in vivo* trials in animal or human subjects would further this growing area of clinical interest based on the results obtained in this investigation.

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