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By

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I certify that I am the sole author of this thesis, and that any assistance I received in its preparation has been fully acknowledged and disclosed in the thesis. I have cited any sources from which I used ideas, data, or words, and labeled as quotations any directly quoted phrases or passages, as well as providing proper documentation and citations. This thesis was prepared by me, specifically for the M.S. degree and for this assignment.

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Flavia M. Goncalves, D.D.S. May 18, 2015

Synopsis

My research investigated the disinfection of *P. gingivalis* from Biomet 3iT3™ and Osseotite dental implant disks with or without a NanoTite or DCD coating, using three disinfection solutions, and three mechanical methods of applying the disinfection solutions. Then afterwards, I measured the survival and attachment of osteoblasts to the dental implant disks according to the absence or presence of a NanoTite or DCD coating, disinfection solution, mechanical methods of applying the disinfection solutions, and the additional coating of the dental implant disks with growth factors. My research had ten phases: i). Measure the growth and disinfection of *P. gingivalis* in the presence of Biomet 3iT3™ dental implant disks with and without a DCD coating. ii). Measure the effectiveness of using Sodium BiCarbonate, 3% Hydrogen Peroxide, or a 0.12% CHX solution to disinfect P. gingivalis from Biomet 3iT3™ dental implant disks. iii). Compare the effectiveness of using Cavitron ProphyJet spraying, ultrasonic activation or brushing to disinfect *P. gingivalis* from the surfaces of Biomet 3iT3™ dental implant disks. iv). Investigate osteoblast survival in cell culture with the disinfected Biomet 3iT3™ dental implant disks. v). Count the numbers of osteoblasts attached to the disinfected Biomet 3iT3™ implant disk surfaces, vi) Investigate the effectiveness of using growth factors: Emdogain and Growth-factor Enhanced Matrix (GEM 21S) to enhance osteoblast attachment to the Biomet 3iT3™ implant disk surface. vii) Investigate the growth and disinfection of *P. gingivalis* in the presence of Biomet 3i Osseotite dental implant disks with and without a NanoTite coating. viii) Compare the ability of Citric acid and Chlorhexidine to disinfect *P. gingivalis* from Osseotite dental implant disks. ix) Investigate osteoblast vitality in cell culture with the disinfected Osseotite dental implant disks, and x), Compare the numbers of osteoblasts attached to the disinfected Biomet

Osseotite implant disk surfaces to assess the effects of the disinfection treatments on osteoblast attachment.

I used a total of two hundred and seven (n=207) Biomet 3iT3™ dental implant disks without a coating (n=90) or with a NanoTite® surface coating (n=90), in addition to Osseotite implant disks without a coating (n=22) or with a NanoTite surface coating (n=5) which were cultured with *P. gingivalis*. The dental implant disks were divided into twenty treatment groups (n=20), each containing between three to eight dental implant disks. An additional five groups (n=5), each contained three or four dental implant disks as negative controls and received no bacteria or no osteoblasts. The dental implant disks were randomly assigned into the twenty five treatment groups to investigate and compare the effectiveness of three disinfection solutions; Sodium BiCarbonate, 3% Hydrogen Peroxide, or a 0.12% CHX disinfecting solution. The disinfection solutions were applied by Cavitron ProphyJet spraying, ultrasonic activation or brushing the implant surface with a disinfecting solution. After the treatments the implants were maintained in broth for 72 hours to grow any residual *P. gingivalis*. The disinfection of *P. gingivalis* from the dental implant disks was measured by spectrophotometry absorbance at a wavelength of 600nm. Afterwards, I placed the dental implant disks in cell cultures of osteoblasts for 72 hours. I collected the cell culture media and analyzed the amount of osteoblast viability using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. I then counted the attachment of osteoblasts to the dental implant disks from scanning electron microscopy (SEM) micrographs. I analyzed the results of the MTT assay and SEM analysis statistically using a two-way ANOVA test followed by a Tukey post-hoc analysis, at a significance of $P<0.05$.

Several interesting results were observed for the first time in this study. This is the first study to measure the survival and attachment of osteoblasts to 3iT3 and osseotite dental implant disks according to the absence or presence of a DCD or NanoTite coating, disinfection solution, mechanical methods of applying the disinfection solutions, and the additional coating of the dental implant disks with growth factors. This is also the first study to investigate the disinfection of *P. gingivalis* from dental implants by comparing the effectiveness of using three disinfection solutions, and three mechanical methods of applying the disinfection solutions. My conclusions for the ten phases in this research study were:

First, I measured the growth and disinfection of *P. gingivalis* in the presence of Biomet 3iT3™ dental implant disks with and without a DCD coating. The results from this part of the study found that the DCD coating made little difference to the growth or ability of the disinfection chemicals or physical activation to disinfect the *P. gingivalis* from the implant disks.

Second, I measured the effectiveness of using Sodium BiCarbonate, 3% Hydrogen Peroxide, or a 0.12% CHX solution to disinfect *P. gingivalis* from Biomet 3iT3™ dental implant disks. These chemicals all had a similar effectiveness to disinfect *P. gingivalis* from the implant disks.

Third, I compared the effectiveness of using Cavitron ProphyJet spraying, ultrasonic activation or brushing to disinfect *P. gingivalis* from the surfaces of Biomet 3iT3™ dental implant disks. These physical activation methods for the chemicals used to disinfect *P. gingivalis* from the implant disks, all had a similar effectiveness

Forth, I investigated osteoblast vitality in cell culture with the disinfected Biomet 3iT3™ dental implant disks. The 3iT3 dental implant disks without the DCD coating had 13% more osteoblast vitality compared to the 3iT3 dental implant disks with the DCD coating. The results showed that the DCD coating was less favorable for osteoblast vitality. The lack of physical activation of the disinfection chemicals gave the highest osteoblast vitality compared to activation of the chemicals using brushing, ultrasonics or spraying with a Prophy Jet. The vitality of the osteoblasts was least (68%) following the disinfection of the 3iT3 dental implant disks with Chlorhexidine gluconate, suggesting it is more toxic to osteoblasts compared to Hydrogen peroxide and Sodium bicarbonate. The coating of the disinfected 3iT3 dental implant surfaces with GEM21S or Emdogain had little effect on the vitality of osteoblasts suggesting that adding growth factors to the surface of disinfected dental implants is not beneficial for enhancing osteoblast vitality.

Fifth, I counted the numbers of osteoblasts attached to the disinfected Biomet 3iT3™ implant disk surfaces. There were a similar number of osteoblasts attached to the 3iT3 dental implants with or without a DCD coating, suggesting the DCD coating does not promote osteoblast attachment. The lack of physical activation of disinfection chemicals greatly enhanced the attachment of osteoblasts to the 3iT3 dental implant disk surfaces, suggesting that the use of a Prophy Jet, ultrasonics, alter the implant surface making them less optimal for osteoblast attachment. The highest numbers of attached osteoblasts were seen on dental implant surfaces that were disinfected with Hydrogen peroxide, suggesting it is less toxic compared to Chlorhexidine gluconate or Sodium bicarbonate, and that it provided a more biocompatible environment for osteoblast attachment.

Sixth, I investigated the effectiveness of using growth factors: Emdogain and Growth-factor Enhanced Matrix (GEM 21S) to enhance osteoblast attachment to the Biomet 3iT3™ implant disk surface. Surprisingly, the coating of the implant surfaces with Emdogain appeared to promote the migration of osteoblasts way from the implant surfaces, because very few osteoblasts were attached. Many more osteoblasts were attached to the implant surfaces that had been coated with GEM21S, but because of the high variability of osteoblast attachment the effectiveness of GEM21S was similar to the implants without any growth factors.

Seventh, I investigated the growth and disinfection of *P. gingivalis* in the presence of Biomet 3i Osseotite dental implant disks with and without a NanoTite coating. The NanoTite coating made little difference to the disinfection of *P. gingivalis* from the Osseotite dental implant disks.

Eighth, I compared the ability of Citric acid and Chlorhexidine to disinfect *P. gingivalis* from Osseotite dental implant disks. The most effective disinfectants were Chlorhexidine gluconate with ultrasonic activation or with brushing using a titanium brush, the least effective disinfection treatment was Citric acid with ultrasonic activation. These results suggest that Citric acid is the worst choice of treatment for the disinfection of Osseotite dental implants, and that Chlorhexidine should be used to ensure that the implant has been adequately disinfected.

Ninth, I investigated osteoblast vitality in cell culture with the disinfected Osseotite dental implant disks. The vitality of osteoblasts was similar following disinfection with Citric acid and Chlorhexidine gluconate, and the NanoTite coating on some of the Osseotite disks

did not enhance osteoblast attachment, compared to the control osteoblast attachment was 18% less in the disks with a NanoTite surface coating.

Tenth, I counted the numbers of osteoblasts attached to the disinfected Biomet Osseotite implant disk surfaces. There were a similar number of osteoblasts attached to the Osseotite dental implants with or without a NanoTite coating, suggesting the NanoTite coating did not promote osteoblast attachment. There was very little difference in the numbers of osteoblasts attached to the surfaces of the Osseotite dental implant disks following disinfection with Citric acid or Chlorhexidine gluconate. These results suggest these disinfection treatments had a similar effect on osteoblast attachment, however because Citric acid was less effective for disinfecting *P. gingivalis* from the implants it cannot be recommended for disinfecting dental implants.

The results of this study has provided several avenues for further investigation which includes analyzing the effect of the physical activation of disinfection chemicals on the surface roughness properties of dental implants. The surface roughness of dental implants is a key factor in promoting osseointegration hence any treatment which influences roughness could impact the clinical performance of the implants. Another key variable that was identified for further investigation is the toxicity and biocompatibility of the disinfection chemicals to osteoblasts, clearly the use of more toxic chemicals could reduce the ability of implants to heal and cause complications. For this reason it is recommended that all chemicals used to disinfect dental implants should be rinsed away from the dental implant to avoid toxic reactions. The standard concentration of Emdogain used in this study appeared to promote osteoblast migration away from the dental implant surface which is a deleterious reaction. This suggests there is a need to investigate the optimal doses of Emdogain and

GEM21S that will enhance osteoblast attachment and osseointegration of the disinfected dental implants.

My hope is that dentists and patients will benefit from my study data. Dentists can use my data to help guide them to select the most effective disinfectants and disinfection methods to remove bacteria from dental implants and obtain maximal osteoblast attachment. Dental patients will benefit from more successful procedures to disinfect dental implants and have implants that are more resistant to peri-implantitis.

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Abbreviations

ANOVA	Analysis of Variance
BMP	Bone Morphogenic Protein
BSA	Bovine Serum Albumin
CHX	Chlorhexidine gluconate
DCD	Discrete Crystalline Deposition
DMEM	Dulbecco's Modified Eagle Medium
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
FDA	Food and Drug Administration
FGF	Fibroblast Growth Factor
GAG	Glycosaminoglycans
H_2O_2	Hydrogen peroxide
IRB	Institutional Review Board
ISO	International Organization for Standardization
mg	Milligram[10^{-3}]
ml	Microliter [10^{-6}]
ml	Milliliter [10^{-3}]
mm	Millimeter
$NaHCO_3$	Sodium bicarbonate
^oC	Degree Centigrade
PBS	Phosphate Buffered Saline
PRP	Protein Rich Plasma
rhBMP	Recombinant human Bone Morphogenic Protein
SEM	Scanning Electron Microscope
TGF	Transforming Growth Factor
USA	United States of America
wk	Week

Statistical terms

ANOVA	Analysis of variance, a statistical test that provides the probability of whether or not there are any differences among two or more means of several groups.
Scheffe Test	This test is a multiple comparison post hoc ANOVA test to test the probability that there are differences among the means of several groups.
Chi Square	This test is nonparametric statistical test used to determine if a distribution of observed frequencies differs from the theoretical expected frequencies.
P value	The probability of obtaining a result as extreme as the one that was actually observed from chance alone. The P value significance level used in this research was $P<0.05$.

Products and materials in this research

Name	Supplier	City, State
3iT3 dental implant disks	Biomet 3i	Palm Beach Gardens, FL
Amphotericin B	Lonza	Walkersville, MD
Cavitron Prophy Jet	Dentsply	York, PA
Chlorhexidine gluconate	Biomeda	Singapore, Singapore
Citric acid	Vista Dental Products	Racine, WI
Dulbecco's Modification of Eagle's Medium	Mediatech	Herndon, VA
Emdogain	Straumann	Andover, MA
GEM21S	Osteohealth	Shirely, NY
Gentamycin Sulfate	Teknova	CA
Hydrogen peroxide	VWR	Suwanee, GA
Osseotite dental implant disks	Biomet 3i	Palm Beach Gardens, FL
Osteoblasts	ATCC	Manassas, VA
Penicillin – Streptomycin Solution	Mediatech	Herndon, VA
Sodium bicarbonate	Dentsply	York, PA
Standard Fetal Bovine Serum	HyClone	Logan, UT
T-75 culture flasks	BD Biosciences	Bedford, MA
TBS Shur/Mount	Triangle Biomed	Durham, NC
Ultrasonic unit	Dentsply	York, PA

1. Introduction

1.1. History of Dental Implants

Dental metal implants and realistic fake teeth have been discovered in the tomb of an Iron Age woman who died more than 2,300 years ago (1). Some archeological remains in China dated 4,000 years ago, revealed that humans had been using carved bamboo pegs taped into bone to replace their missing teeth (2-4). In ancient Egypt, some 2,000 year-old mummies have been discovered with transplanted human teeth or fake teeth carved from ivory (2-4). The success of ancient dental implants is unclear, but the discoveries of fake teeth in tombs and the fossil record is evidence of the practical and social need for humans to replace their missing teeth. The research of Professor Per Ingvar Brånemark discovered the osseointegration of titanium into rabbit and dog bone (5). Professor Brånemark is credited with developing the modern concept of making dental implants from titanium. According to a newspaper article (Dental Tribune, 2010), the most widely considered "*father of implantology*" is Dr. Leonard Linkow (Unpublished). Dr. Linkow first pioneered the concept of using dental implants to avoid removable prostheses in the 1960s but did not publish his work. Dr. Linkow also started inserting titanium and other metal implants to hold prosthetic teeth in the 1950s, but did not publish his work. The first clinical trials of titanium dental implants began in 1965 (6).

A dental implant, sometimes known as an endosseous dental implant (7) or a dental implant fixture (8) is a surgical component that interfaces with the bone of the jaw or skull to support a dental prosthesis such as a crown, bridge, denture, facial prosthesis or to act as an orthodontic anchor (9). The success of dental implants is dependent on a biologic process called osseointegration where materials, such as

titanium, form an intimate bond to bone (10). Sometimes, pre-implant surgery is needed prior to placing a dental implant in order to create supporting bone with an optimal biomechanical function and aesthetics, the augmentation of atrophied bone can be critical to the longevity of the implant (11). Through attention to the clinical practice and scientific evidence-based procedures for accomplishing the osteointegration, the risks of failing dental implants can be minimized (12). The time needed for dental implant osseointegration before the dental prosthetic tooth, bridge or denture is attached can vary between three to six months before the implant or an abutment is placed which will hold a dental prosthetic device.

1.2. Dental implant survival

The success or failure of dental implants depends on the health of the person receiving it, and habits such as bruxism or smoking can often have a higher rate of failing implants (7), and some medications, such as Bisphosphonate (BP) drugs can reduce the rate of osseointegration, cause osteonecrosis, and have a negative effect on the health of dental tissues (13). After osseointegration of a dental implant has been accomplished, a common cause of implant failure is the physical force of an excessive occlusal load (14). Provided that implants have adequate supporting bone structure, they are positioned correctly and a sufficient number are placed, a normal masticatory stress on dental implants fixed to a prosthesis, should not cause the implants to start failing (15). The prerequisites for the long-term success of osseointegrated dental implants are healthy bone and gingiva (16). A key factor in the survival of implants is to consider these factors when deciding on the numbers and positions of dental implants. Treatment planning to place dental implants must be determined by the position and

angle of adjacent teeth (17). To help accomplish proper implant placement, lab simulations, surgical guides called stents or using computer-aided tomography are useful (18). The prerequisites to long-term success of osseointegrated dental implants are healthy bone and gingiva (19). Since both can atrophy after tooth extraction pre-prosthetic procedures, such as sinus lifts [sinus floor elevation surgery] (20) or gingival grafts, are sometimes required to recreate ideal bone and gingiva (21).

The final prosthetic device can be either fixed, where a person cannot remove the denture or teeth from their mouth or removable, where they can remove the prosthetic (22). In each case an abutment is attached to the implant fixture (23). Where the prosthetic is fixed, the crown, bridge or denture is fixed to the abutment with either lag-screws or cement (24). Where the prosthetic is removable, a corresponding adapter is placed in the prosthetic so that the two pieces can be secured together (25).

In patients with healthy dental tissues, an osseointegrated dental implant not subjected to biomechanical overloads can have long term survival rate over a decade or longer of 77.7% to 100% (16,26-29). Although, the survival rate of dental implants is excellent, improvements to implantology treatments are needed which can more effectively disinfect dental implants and stimulate osteoblast survival and attachment to the dental implant surface, which can promote osseointegration.

The risks, hazards, and complications related to implant treatment could be divided into complications that occur immediately during surgery; such as excessive bleeding or nerve injury (30). Delayed complications that occur in the first six months; such as infection and failure to osseointegrate (31) and complications that occur over months and years, such as peri-implantitis (32) and mechanical overload failures (14). The occurrences of these complications are rare and are mostly treatable. Three million

Americans have dental implants to replace five million missing teeth each year (33). The reason for the high demand for dental implants is their ability to improve smile esthetics (34), masticatory function (35), facial appearance and the quality of life of patients (36,37).

1.3. Dental implant surface modifications

The designs of dental implants, abutments and attachments have evolved to a stage of maturity where there appears to be little remaining scope for innovation. This contrasts with the most rapidly changing aspect of dental implants, which has been to modify the surface characteristics of dental implants (38). Most dental implants are made from commercially pure titanium [Ti], which has various degrees of purity [graded from worst to best; 1 to 4]. The purity and mechanical properties of Ti is altered by oxygen, carbon and iron content (39). Most dental implants are either made from grade 4 commercially pure Ti as it is stronger than other grades, or they are made from Ti alloys such as Ti6AL-4V signifying that the alloy contains 90% Ti, 6% aluminum, and 4% vanadium (40). The Ti is biocompatible with human tissues and does not induce inflammatory responses (41). Over several months the Ti can osseointegrate with bone by creating a Ti-oxide surface layer (42). The commercially pure Ti dental implants have an excellent long-term survival up to 22 years (43). The hydrophilicity of the implant surface is affected by the chemical composition of titanium implants (44). Highly hydrophilic surfaces seem more desirable than hydrophobic ones in view of their interactions with biological fluids, cells and tissues (45). Contact angle measurements give values ranging from 0° (hydrophilic) to 140° (hydrophobic) for titanium implant surfaces (46).

The problem with pure titanium implant surfaces is the amount of time needed for its osseointegration with bone, prior to loading the implants with a stable prosthesis can often take several months up to eighteen months (47). The duration of time required for osseointegration is important because, the survival of the implant depends on rapid healing (48). Osseointegration, defined as a direct structural and functional connection between ordered, living bone and the surface of a load-carrying implant, is critical for implant stability, and is considered a prerequisite for implant loading and long-term clinical success of dental implants (49). The surface characteristics of an implant, which can increase the speed of osseointegration is that, they are; hydrophilic, rough, have an electrical charge, and form a Ti-oxide interface with bone (50). The other surface characteristics that also could influence osseointegration are; chemistry, topography, crystallinity, and resistance to infection (51,52). Osseointegration can also be influenced by the implant loading conditions, implant material and design, surface conditions, quality and thickness of bone, and surgical technique (49). Building on this knowledge, Ti implant surfaces have been modified to accelerate osseointegration, which can benefit patients through reducing the amount of time they are edentulous.

The most common methods used to modify the surface of the Ti implants to accelerate osseointegration are sand blasting, acid etching, anodic oxidation, fluoride treatment, hydroxyapatite and calcium phosphate coating (38). However, the ability of these surface treatments to accelerate osseointegration have often proved to be controversial, several studies have found that the modified surfaces of the implants have had little effect on their survival (53,54). What this demonstrates is not that modifying the surfaces of implants can have no effect, what it demonstrates is that the variables which can accelerate osseointegration are multifactorial.

The most important dental implant surface modifications to accelerate osseointegration are the ones that increase the surface roughness, because osteoblasts prefer to attach to rough surfaces (55) and it increases their rate of osteogenesis (55). Sandblasting and acid etching are common methods for increasing the roughness of Ti implant surfaces (56). The surfaces of the Biomet 3iT3™ implant disks (Biomet 3i, Palm Beach Gardens, FL) used in this study were sand-blasted (57) and processed with nitric, hydrochloric, and sulfuric acids to increase its surface roughness (58). Some of the implants were also coated with a Discrete Crystalline Deposition (DCD) of calcium phosphate. The other types of implants used in this study were Osseotite implant disks (Biomet 3i, Palm Beach Gardens, FL), which are similarly surface modified without or with a NanoTite surface coating of calcium phosphate to accelerate osseointegration (59). A summary of the common procedures to modify the surfaces of dental implants is shown in Table 1.

Table 1. Summary of modifications to the surfaces of dental implants

Type of surface treatment	**Description**
Mechanical	Blasting, grinding, machining, and polishing
Chemical	Acid or alkali etching, hydrogen peroxide treatment, sol-gel, chemical vapor deposition, Fluoride treatment and anodization.
Physical	Plasma spraying, sandblasting, grit-blasted, sputtering, plasma spray coating, laser deposition, and ion deposition.
Surface coating	Calcium-phosphate, hydroxyapatite
Biologically active	Bisphosphonates, simvastatin, antibiotic coating with tetracycline, growth factors

1.4. Dental implants and growth factors

Bone formation and osseointegration is necessary for the clinical success of disinfected dental implants (60). The presence of cytokines and localized growth factors around injured bone can recruit osteoprogenitor cells and modulate inflammatory cells to regenerate bone (61). The adhesion of plasma proteins on the surface of titanium implants has been reported to play an essential role in the process of osseointegration (62). An increase in the proliferation and differentiation of undifferentiated mesenchymal cells, osteoprogenitor cells, and preosteoblasts into osteoblasts may improve bone response and subsequently osseointegration of Ti implants (63). In order to enhance the natural responses of osteoprogenitor cells to remodel bone and to accelerate osseointegration, some studies have coated the surfaces of implants with growth factors such as Emdogain (64) or Growth-factor Enhanced Matrix [GEM21S] (65). However, it remains unclear if growth factors are needed, or which of the growth factors is the most effective to promote osteoblast attachment to the disinfected implant surfaces.

1.5. Reasons for failing dental implants

The failure rate of dental implants is estimated to be between 2% to 14% over five years (66). A major reason why implants can fail is because of a lack of osseointegration (67). Improvements to implantology treatments are needed which can more effectively disinfect dental implants and stimulate osteoblast survival and attachment to the dental implant surface, which can promote osseointegration.

The most common reason for dental implants to fail is because of a disease called Periimplantitis (68,69). Periimplantitis is a localized bacterial infection, which

causes inflammation in adjacent soft tissues, increased pocket depth, and bone loss around an osseointegrated implant in function, which causes a lack of implant stability (68). The etiology of the periimplantitis is conditioned by the status of the tissue surrounding the implant; implant design, degree of roughness, external morphology, and excessive mechanical load (69). Periimplantis is a multifactorial disorder (70). It can also be caused by an inadequate distribution of the mastication pressure on the tissues surrounding the implant, thus leading to loosening of the implant (69), which creates a space for the leakage of bacteria to infect the implant and the adjacent tissues. Periimplantitis is treated by disinfecting the implant socket and the implant surface to remove the bacterial infection, thereby allowing the regeneration of the alveolar bone for the osseointegration of the dental implant.

1.6. *P. ginigivalis* and infection of dental implants

The average mouth contains almost 700 different species of bacteria (71). The presence of several bacterial species has been identified from dental implants with periimplantitis (72-74). This indicates that most perimplantitis infections contain multiple species of bacteria. The leading cause of periimplantitis and the most serious threat to the success of a dental implant is an infection with an oral pathogen called *Porphyromona gingivalis* (*P. gingivalis*) (75,76).

1.7. Chemical disinfection of dental implants

Dental implants diagnosed with periimplantitis must be decontaminated with disinfectant solutions in order to prevent them from failing (75,76). A problem with selecting a dental implant decontamination procedure is the lack of consensus about

which procedure or disinfectant is the most beneficial to remove the *P. gingivalis* and to maintain the vitality of osteoblasts. The common disinfection powder is Sodium BiCarbonate [$NaHCO_3$], and the disinfection solutions include: Hydrogen peroxide [3% H_2O_2], Chlorhexidine gel [2% CHX], Ethylenediaminetetraacetic acid [EDTA], and Tetracycline (75-78). The survival of more epithelial cells on implant discs following disinfection with hydrogen peroxide when compared to chlorhexidine gel (77), suggests that the selection of solutions to disinfect implants can have differences in their toxic effects on cells.

A recent study by a former NSU resident: Dr. Judith Lubin, found that Osseotite implant disks were easier to disinfect compared to the Nanotite implant disks (79). This suggests that modifications to the implant surfaces to accomplish faster osseointegration can also affect the ability of chemicals to disinfect the implant. The reasons may be due to rougher surfaces being more difficult to disinfect, and electrical charges having a neutralizing effect on disinfectants. The same study also found that citric acid and tetracycline were the most effective solutions for the disinfection of *P. gingivalis* from the Osseotite implant disks (79). Dr. Lubins results suggest that different chemicals used for disinfection have vary degrees of effectiveness (24), and so there is a need to continue this line of research to identify the most effective chemicals to disinfect *P. gingivalis* from the surfaces of implants.

1.8. Physical and mechanical disinfection of dental implants

The effectiveness of chemicals to disinfect the surfaces of dental implants diagnosed with periimplantitis could potentially be enhanced by physical and mechanical methods, but a there have been no previous research which have

compared the physical and mechanical activation of disinfectants with the non-activation of disinfectants: A PubMed search of the terms: ‘dental implant’ ‘disinfection’ ‘ultrasonic’ ‘non-ultrasonic’ or ‘physical’ and ‘mechanical disinfection’ did not identify any previous studies, suggesting there has been a lack of research in using or comparing physical and mechanical disinfection methods for disinfecting contaminated dental implants. New technologies for the physical and mechanical disinfection of dental implants include laser disinfection (79), electric current disinfection (80), spraying powder (81), among other methods.

A potential physical or mechanical method to disinfect the surfaces of dental implants is the use of ProphyJet spraying (81,82). Prophyjet is an air polishing prophylaxis system, which uses air, water, and either sodium bicarbonate [NaHCO3], (ProphyJet®) or non-sodium (JET Fresh®) powder. A literature search using PubMed for the terms ‘prophyjet’ ‘dental implant’ ‘disinfection’ identified no previous studies of using a ProphyJet to disinfect dental implants. This suggests that most practitioners are not considering using the ProphyJet to disinfect dental implants, and there is a knowledge gap in the literature about the effectiveness of the ProphyJet to disinfect dental implants.

Most Swiss practitioners use Chlorhexidine gluconate [CHX] as their first choice of disinfectant for cleaning dental implants diagnosed with periimplantitis (83), the other common disinfectants are Citric acid, and Hydrogen peroxide [H_2O_2] (79), these can easily be activated using an ultrasonic tip or a laser to add energy in the solutions and cause fluid movement (79). The increased fluid movement of the disinfectants may be expected to increase their effectiveness to disinfect the surfaces of dental implants

diagnosed with periimplantitis, but there have been no previous investigations as the previous searches of PubMed have indicated.

A titanium brush is sometimes recommended to clean the surface of dental implants by the manufacturer (84). The effectiveness of the brush to remove a biofilm can be influenced by the roughness of the implant surface (84). Thus, further research is needed to determine how the effectiveness the chemicals used to disinfect dental implants are with and without brushing.

It is not known if ProphyJet spraying, ultrasonic activation or brushing the implant surface with a disinfecting solution can have an effect on osteoblast attachment to the disinfected implant surfaces.

1.9. Objectives of this research

The objectives of this research were to evaluate the effectiveness of twenty-five procedures to disinfect implant surfaces intentionally inoculated with *P. gingivalis* and afterwards to evaluate osteoblast attachment to the disinfected implant surfaces. The purpose of this research was to identify the most effective procedures for implant disinfection and osseointegration.

The newest coating applied to Biomet Osseotite dental implants is NanoTite (79), and the newest coating applied to Biomet 3iT3™ dental implants is a Discrete Crystalline Deposition (DCD) of calcium-phosphate. DCD that is intended to promote healing and bone integration (85). However, it is not clear what effect the DCD and NanoTite coatings have on *P. gingivalis* disinfection with Sodium BiCarbonate [NaHCO3], Hydrogen Peroxide [H_2O_2], or a Chlorhexidine gluconate [CHX] disinfecting solution. It is not clear if the NanoTite or DCD coatings can promote osteoblast survival

and attachment, or which disinfection procedure is optimal for these types of implant coatings (86). There is a lack of consensus among periodontists and dentists who place dental implants about which disinfection procedures are the most optimal to remove *P. gingivalis* from the surface of implants (87). Disinfection procedures using $NaHCO_3$, H_2O_2], or CHX have been suggested (88-91), but these have not been investigated using osteoblasts. The results of this research may be helpful to provide guidance on the selection of implants and disinfection procedures to maximize implant disinfection and osseointegration.

2. MATERIALS AND METHODS

2.1. Regulatory approvals

The Chair of NSU IRB: Dr. David Thomas has previously ruled that cell cultures do not meet the federal or institutional standards to be regarded as a human individual: This study does not require an IRB or IACUC review and approval, because it does not involve animals or human subjects.

2.2. Calculation of Sample Sizes

Prior to the commencement of this research the sample effect size and Alpha were calculated as follows:

Effect size will use Cohen's d at 6.78 and power = 0.80 (Two-Tailed Test). The effect size in this study is achieved towards a large magnitude, which implies higher power value, and the probability of detecting a real effect will be achieved.

Alpha which is related to the P values is set at the $P < 0.05$ significance level. A type I error where the null hypothesis tests no difference should not be encountered, in

order to reject the null hypothesis. The results should delineate which of the disinfection solutions and physical methods of applying the disinfection solutions is the more effective for the disinfection of dental implant disks contaminated with *P gingivalis*. Alternatively, if results find no differences and since there should be differences, this implies a type II error P = *Beta*; therefore, null hypothesis will not be rejected.

The treatment groups in this study use a sample size of six to eight replicates that has previously been adequate to detect statistically significant differences in a research study (92). Therefore, an average sample size of ten (n = 8) samples per group was used in this study, as it is cost effective and has sufficient power to detect significant differences.

2.3. Dental implant disks

The dental implants disks used in this study were 3iT3™, 3iT3 with a Discrete Crystalline Deposition (DCD), Osseotite, and Osseotite with a NanoTite coating. All the implant disks were supplied by Biomet 3i, Palm Beach Gardens, FL. The disks were prepared from the same titanium and surface coating materials as commercially available dental implants that are used for testing implant properties. The Biomet 3iT3™ disks and Osseotite disks had dimensions of 10 mm x 1.5 mm. All the dental implant disks were sterilized prior to testing by autoclaving them in sealed sterilization pouches placed inside a steam autoclave 250^{o}C for 15 minutes.

2.4. Osteoblast cells

This study used a human osteoblast cell line (CRL-1427) supplied from the American Tissue and Cell Culture Collection (ATCC, Manassas, VA). The osteoblasts were used for the cell survival and cell attachment experiments. The osteoblasts have

been used in previous studies (93,94). The osteoblasts were cultured in Dulbeccos Modified Eagles Medium (GIBCO, Grand Island, NY) supplemented with 10% fetal calf serum, 1% penicillin/streptomycin antibiotics and fungizone maintained at 37ºC in a humidified atmosphere of 5% CO_2 with the culture media being replenished every second day (95). The confluent osteoblast cultures were collected by trypsinization (0.2% trypsin/EDTA) and subcultured in T-75 culture flasks (BD Biosciences, Franklin Lakes, NJ).

2.5. Porphyromonas gingivalis

Porphyromonas gingivalis (*P. gingivalis)* is an oral pathogen associated with peridontitis and gingivitis (96), and also periimplantitis or failing implants (75,76). *P. gingivalis* was obtained from the American Type Culture Collection (ATCC 53977) supplied as a stock solution (Kwik-Stik Microbiologics, St. Cloud, MN). The *P. gingivalis* was grown in Trypticase soy broth supplemented with 10% vitamin K/Hemin (BD BBL, Franklin Lakes, NJ). The *P. gingivalis* was maintained in an anaerobic jar containing an anaerobic gas-producing pouch (AnaeroPack Kenki A-03, Mitsubishi Gas Chemical Company, Inc., Tokyo, Japan) inside a 37^{o}C incubator on a shaking platform.

2.6. Dental implant disk contamination with *P. gingivalis*

An aseptic technique with sterile gloves and forceps was used to handle the implant disks inside a sterile laminar flow hood to prevent accidental infections. The dental implant disks were infected with *P. gingivalis*. Each disks was submerged in 15ml test tubes containing 10mls Trypticase soy broth supplemented with 10% vitamin K/Hemin (BD BBL, Franklin Lakes, NJ). To contaminate the disks I will add 0.5mls of

viable *P. gingivalis* containing 10^6 cells to each test tube. The samples were maintained inside an anaerobic jar containing an anaerobic gas-producing pouch (AnaeroPack Kenki A-03, Mitsubishi Gas Chemical Company, Inc., Tokyo, Japan). The samples were then placed inside a 37^{o}C incubator on a shaking platform to keep the samples in motion.

2.7. P. gingivalis growth measurement with dental implant disks

The 207 samples (shown in Tables 2 and 3) were placed in Trypticase soy broth, and were maintain in anaerobic conditions for 72 hours. Afterwards, the samples were removed using an aseptic handling technique inside the sterile environment of a laminar flow hood. The Trypticase soy broth was collected from each of the specimens to be analyzed using a spectrophotometer at an absorbance of 600nm (97). The negative controls; where no pathogen was added to the Trypticase soy broth with disks (Table 2) was used to calibrate the spectrophotometer (Genesys 20, Thermo Spectron Corp., Madison, Wisconsin, USA).

2.8. P. gingivalis disinfection from dental implant disks

Groups 1-3, 9-11, and 17-20: The 3iT3 and 3iT3-DCD dental implant disks were disinfected using a Cavitron Prohy Jet (Dentsply, York, PA) with Sodium BiCarbonate ($NaHCO_3$) powder (Dentsply, York, PA) for 90 seconds. There is no published standardized time to use the Cavitron Prophy Jet with $NaHCO_3$ to disinfect dental implants: 90 seconds was used because this is the amount of time has been used previously to investigate the effects of Cavitron Prohy Jet with $NaHCO_3$ on the surface roughness of dental implants, (98). **Groups 4 and 12:** The 3iT3 and 3iT3-DCD

dental implant disks will be disinfected by submerging them in hydrogen peroxide (3% H_2O_2, VWR, Suwanee, GA) at room temperature for 5 minutes with ultrasonic activation with a size 2 retrotip (Obtura-Spartan, Fenton, MI, USA) attached to a Spartan ultrasonic unit (Obtura-Spartan) using the highest ultrasonic setting. There is no published standardized time to use the H_2O_2 with ultrasonics to disinfect dental implants. We selected 5 minutes because a previous study used 5 minutes for implant disinfection with an experimental disinfectant solution (99). **Groups 5 and 13:** Similar to groups 4 and 12, except that the implant disks will be submerged in Chlorhexidine gluconate (0.12% CHX, Singapore) with ultrasonic activation. **Groups 6 and 14:** The 3iT3 and 3iT3-DCD dental implant disks were disinfected by submerging them in hydrogen peroxide (3% H_2O_2, VWR, Suwanee, GA) at room temperature for 5 minutes while being brushed with a titanium wire brush. There is no published standardized time to use the H_2O_2 with a wire brush to disinfect dental implants: 5 minutes was used to be consistent with the ultrasonics groups in this study. **Groups 7 and 15:** Similar to groups 6 and 14, except that the implant disks were be submerged in Chlorhexidine gluconate (0.12% CHX, Biomeda, Singapore) during brushing with a titanium brush. **Groups 8 and 16:** The 3iT3 and 3iT3-DCD dental implant disks were disinfected by submerging them in Chlorhexidine gluconate (0.12% CHX, Biomeda) at room temperature for 5 minutes. The disks were rinsed with sterile saline three times to remove remnants of the disinfectants. **Groups 17 and 19**: Are control groups where no osteoblasts were added to the 3iT3-DCD dental implant disks. **Groups 18 and 20**: Are control groups where no *P. gingivalis* was added to the added to the 3iT3-DCD dental implant disks. The treatments are summarized in Table 2.

Table 2. Summary of 3iTi implant disk decontamination procedures

#	Name of group	Cell type	Contamin--ation	Physical decontam-ination	Decontamin-ation agent	Implant type or coating	Growth factor	Sample numbers
1	Cavitron Prophy Jet	Osteoblasts	*P. gingivalis*	Prophy Jet	$NaHCO_3$	3iT3	None	n =10
2	Cavitron Prophy Jet	Osteoblasts	*P. gingivalis*	Prophy Jet	$NaHCO_3$	3iT3	Emdogain	n =10
3	Cavitron Prophy Jet	Osteoblasts	*P. gingivalis*	Prophy Jet	$NaHCO_3$	3iT3	GEM21S	n =10
4	Ultrasonics	Osteoblasts	*P. gingivalis*	Ultrasonics	3% H_2O_2	3iT3	None	n =10
5	Ultrasonics	Osteoblasts	*P. gingivalis*	Ultrasonics	0.12% CHX	3iT3	None	n =10
6	Titanium brush	Osteoblasts	*P. gingivalis*	Titanium brush	3% H_2O_2	3iT3	None	n =10
7	Titanium brush	Osteoblasts	*P. gingivalis*	Titanium brush	0.12% CHX	3iT3	None	n =10
8	CHX	Osteoblasts	*P. gingivalis*	None	0.12% CHX	3iT3	None	n =10
9	Cavitron Prophy Jet	Osteoblasts	*P. gingivalis*	Prophy Jet	$NaHCO_3$	3iT3 with DCD	None	n =10
10	Cavitron Prophy Jet	Osteoblasts	*P. gingivalis*	Prophy Jet	$NaHCO_3$	3iT3 with DCD	Emdogain	n =10
11	Cavitron Prophy Jet	Osteoblasts	*P. gingivalis*	Prophy Jet	$NaHCO_3$	3iT3 with DCD	GEM21S	n =10
12	Ultrasonics	Osteoblasts	*P. gingivalis*	Ultrasonics	3% H_2O_2	3iT3 with DCD	None	n =10
13	Ultrasonics	Osteoblasts	*P. gingivalis*	Ultrasonics	0.12% CHX	3iT3 with DCD	None	n =10
14	Titanium brush	Osteoblasts	*P. gingivalis*	Titanium brush	3% H_2O_2	3iT3 with DCD	None	n =10
15	Titanium brush	Osteoblasts	*P. gingivalis*	Titanium brush	0.12% CHX	3iT3 with DCD	None	n =10
16	CHX	Osteoblasts	*P. gingivalis*	None	0.12% CHX	3iT3 with DCD	None	n =10
17	Control	None	*P. gingivalis*	Prophy Jet	$NaHCO_3$	3iT3 with DCD	None	n = 5
18	Control	Osteoblasts	None	Prophy Jet	$NaHCO_3$	3iT3 with DCD	None	n = 5
19	Control	None	*P. gingivalis*	Prophy Jet	$NaHCO_3$	3iT3 with DCD	None	n = 5
20	Control	Osteoblasts	None	Prophy Jet	$NaHCO_3$	3iT3 with DCD	None	n = 5
					Total			n = 180

After the Osseotite dental implant disks were contaminated with *P. gingivalis* they were removed from the test tubes using an aseptic handling technique inside a laminar flow hood. The dental implant disks were randomly assigned to receive one of the

treatments shown in Table 3. **Group 21:** The osseotite dental implant disks were disinfected using 0.12% CHX with ultrasonic activation for 5 minutes. **Group 22:** The osseotite-NanoTite dental implant disks were disinfected using 0.12% CHX with ultrasonic activation for 5 minutes. **Group 23:** The osseotite dental implant disks were disinfected using Citric acid (Vista Dental Products, Racine, WI) with ultrasonic activation for 5 minutes. **Group 24:** The osseotite dental implant disks were disinfected using 0.12% CHX with a titanium brush to apply physical forces on the implant surface for 5 minutes. **Group 25:** As a control, the osseotite dental implant disks were not disinfected. The treatments are summarized in Table 3.

Table 3. Summary of Osseotite implant disk decontamination procedures

#	Name of group	Cell type	Contamina-tion	Physical decontamin-ation	Decontamin-ation agent	Implant type or coating	Growth factor	Sample numbers
21	Ultrasonics	Osteoblasts	*P. gingivalis*	Ultrasonics	0.12% CHX	Osseotite	None	n = 6
22	Ultrasonics	Osteoblasts	*P. gingivalis*	Ultrasonics	0.12% CHX	Osseotite with NanoTite	None	n =6
23	Ultrasonics	Osteoblasts	*P. gingivalis*	Ultrasonics	Citric acid	Osseotite	None	n =6
24	Titanium brush	Osteoblasts	*P. gingivalis*	Titanium brush	0.12% CHX	Osseotite	None	n =6
25	Control	Osteoblasts	*P. gingivalis*	None	None	Osseotite	None	n = 3
					Total			n = 27

2.9. Percentage of *P. gingivalis* disinfection from dental implant disks

An aseptic handling technique inside a laminar flow hood was used to submerge the specimens in 15ml test tubes containing 10mls Brain Heart Infusion broth supplemented with 10% vitamin K/Hemin (BD BBL, Franklin Lakes, NJ). The specimens were maintained in anaerobic conditions inside an anaerobic jar containing an anaerobic gas-producing pouch (AnaeroPack Kenki A-03, Mitsubishi Gas Chemical Company, Inc., Tokyo, Japan), which were placed inside a 37^{o}C incubator on a shaking

platform to keep the samples in motion. After 72 hours the specimens were removed from the anaerobic conditions, using an aseptic handling technique. As a control, some test tubes had no implant disks, to ensure that there are no accidental infections of the broth occurring. Some test disks had no *P. gingivalis* added to check that there were no accidental disinfections of the disks occurring. The effectiveness of the *P. gingivalis* disinfection procedures was measured by collecting the broth and analyzing its absorbance at 600nm (97) using a spectrophotometer (Genesys 20, Thermo Fisher Scientific, Waltham, MA). The absorbance of the broth infected with *P. gingivalis* without any disks was used as the 100% growth measurement, and all the raw data was converted from each of the specimens to a percentage of the normal growth rate.

2.10. Osteoblast viability following disinfection of implant disks

An aseptic handling technique inside a laminar flow hood was used to prevent accidental infection of the dental implant disks. The Brain Heart Infusion broth with vitamin K and hemin broth were rinsed from each of the disks three times using a sterile saline solution. The disks were disinfected again using the disinfection procedures described previously in Tables 2 and 3. Each dental implant disk was placed into a 10mm well of a six-well culture plate (BD Biosciences, Franklin Lakes, NJ). Each culture plate contained confluent cultures (>80% cell coverage of plates) of human osteoblast cell line (CRL-1427). The osteoblasts will be cultured in Dulbeccos Modified Eagles Medium (GIBCO, Grand Island, NY) supplemented with 10% fetal calf serum, 1% penicillin/streptomycin antibiotics and fungizone maintained at 37ºC in a humidified atmosphere of 5% CO_2 (95). After 72 hours the culture media was collected and analyzed with the MTT kit (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

(MTT Cell Proliferation Assay Kit, Biotium, Hayward, CA) to measure osteoblast viability. The optical density of the MTT reaction was measured at an absorbance of 562nm absorbance using a spectrophotometer (Genesys 20) as a measure of osteoblast cell viability (100).

2.11. Growth factors

The growth factors used in this study to coat some of the disinfected implant surfaces were Growth-factor Enhanced Matrix (GEM21S, Osteohealth, Shirley, NY) and Emdogain (Straumann, Andover, MA). The GEM21S and Emdogain were added in at full strength concentrations prior to culture with osteoblasts. The growth factors were added to the dental implant disks in groups 2, 3, 10 and 11 show in Table 2.

2.12. Scanning electron microscopy of osteoblast attachment to dental implant disks

The attachment of osteoblasts to the implant disks was assessed using high-power micrograph images of the disk surfaces obtained using a scanning electron microscope (SEM, Joel, Tokyo, Japan). The disks and osteoblasts were prepared for use in the SEM by fixing the samples in 10% neutral-buffered formalin solution at 18^{o}C for 24 hours. The samples were then dehydrated in a graded series of ethanol solutions (20%, 50%, 70%, 90%, for 2 hours each, followed by one day of 100% ethanol). The samples were removed from the solutions and placed in hexamethyldistilazane for 5 minutes to fix the dehydrated specimens. The samples were dried on filter paper for 30 minutes. The dried tooth specimens were mounted onto aluminum stereoscan stubs (Electron Microscopy Sciences, Hatfield, PA) with conductive carbon adhesive tabs

(Electron Microscopy Sciences, Hatfield, PA) (87). The dried mounted specimens were coated with a 20-30nm thin metallic layer of gold/palladium in a Polaron E5000 sputter coater (BioRad, Hercules, CA, USA). SEM micrographs were obtained at x2,000 magnification using digital image analysis software (95). All the specimen surfaces were examined and the micrograph images stored as digital files in an Acer Computer connected to the SEM. The number of osteoblasts attached to the dental implant disks, was counted by two double blind independent reviewers using semi-quantitative criteria (94). The images of cells in the SEM micrographs were colorized using the "magnetic loop" and "color fill" functions of imaging software (Photoshop, Adobe, San Jose, CA). The morphology of the osteoblast cells in the SEM micrographs was assessed using a modified phenotypic criteria (score 0-2) described by Al-Nazhan (101): 0) Round cells. 1) Oblong cells. 2) Flattened cells. 3) Oblong cells, and 4) Star-shaped cells.

2.13. Biohazard procedures and research waste disposal

The bacteria, cells, culture flasks, pipettes, and flammable chemicals were disposed of according to NSU standard OSHA protocols for handling potentially bio-hazardous waste. A sterile handling technique was used with the *P. gingivalis* and osteoblast cell cultures to prevent contamination. At the end of experimentation, the bacteria, cells, culture flasks, and pipettes were placed in biohazard bags and autoclaved prior to removal by NSU clinical waste services. Flammable chemicals (waste alcohols) were stored in a fume cupboard and were collected after use for disposal by NSU waste services.

2.14. Statistical analysis and data interpretation.

The raw data was collected in a de-identified manner to help avoid any experimenter bias. The specimens were de-identified by Dr. Elazizi by assigning them random codes, prior to the collection of data by Dr. Goncalves. Once the data collection was complete; Dr. Elazizi identified the data treatment groups. Then, Dr. Goncalves entered the data into SAS statistical spreadsheets for statistical analysis.

The effectiveness of the disinfection of *P. gingivalis* from the dental implant disks was measured as 600nm absorbance raw data. The raw data was analyzed using a two way analysis of variance (ANOVA) statistical test, followed by Post hoc Tukey tests to compare statistical differences between treatment groups (SAS Inc, Cary, NY) at the $P<0.05$ significance level.

The attachment of osteoblasts to the dental implant disks were counted as continuous real numbers which will also be analyzed using (ANOVA) statistical tests followed by Post hoc Tukey tests between treatment groups (SAS Inc, Cary, NY) at the $P<0.05$ significance level.

The phenotype (shape) of the osteoblasts attached to the 3iT3™ implant disks were assessed using the following criteria: 0) Round cells. 1) Oblong cells. 2) Flattened cells, and 3) Star-shaped described by Al-Nazhan (101). The categorical data was analyzed statistically using Chi-Square tests (SAS Inc, Cary, NY) at the $P<0.05$ significance level.

3. RESULTS

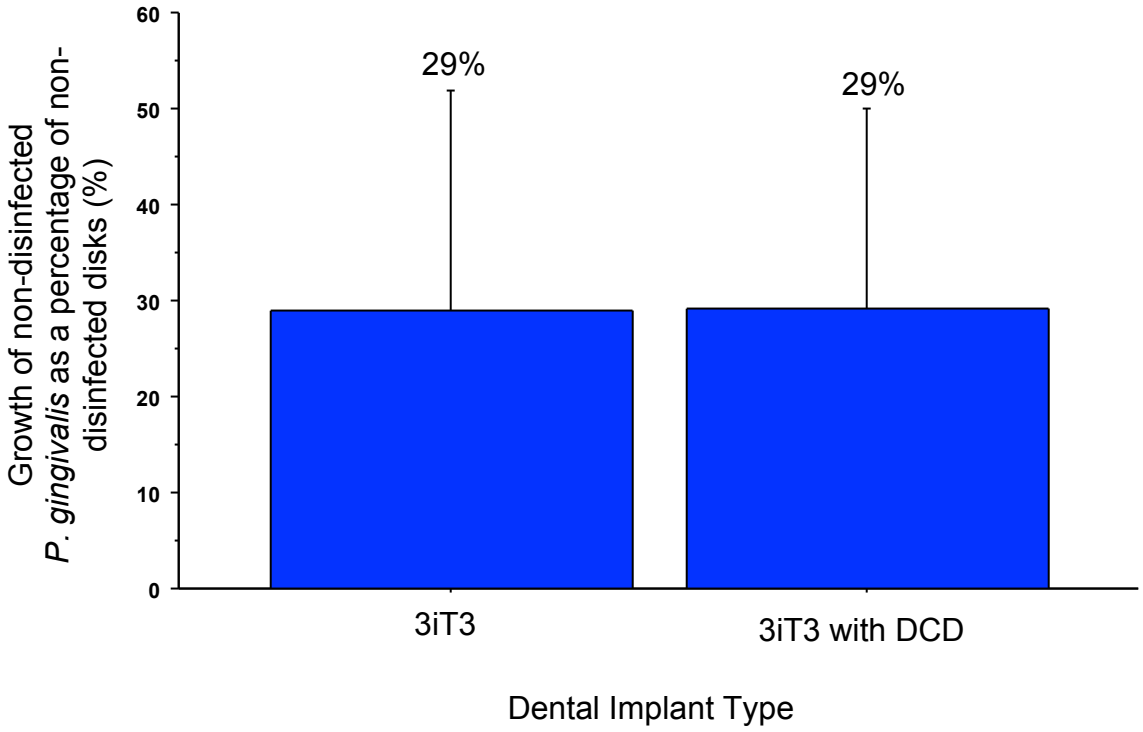
3.1. Disinfection of 3iT3 dental implants

The disinfection of *P. gingivalis* was not very effective following most of the chemical treatments, because an average residual growth of 29% of *P. gingivalis* was observed from all the disinfected 3iT3 and 3iT3 with DCD dental implant disks (ANOVA, P=0.9593, Power 0.05) (Figure 1). Among the chemical treatments, the Hydrogen peroxide (18% and 29%) was only slightly more effective than the Sodium bicarbonate (32%) and Chlorhexidine gluconate effective (23%, 30%, and 37%) (Figure 2). However, there was no significant difference between the three chemical treatments: Sodium bicarbonate, Hydrogen peroxide, or Chlorhexidine gluconate to disinfect the dental implant disks (ANOVA, P=0.1689, Power 0.427). The physical activation of the three chemical disinfectants by Prophy Jet, Ultrasonics, or Brushing, or with no physical activation, was not found to have much effect on their ability to disinfect the dental implant disks (ANOVA, P=0.2043, Power 0.323).

3.2. Disinfection of Osseotite dental implants

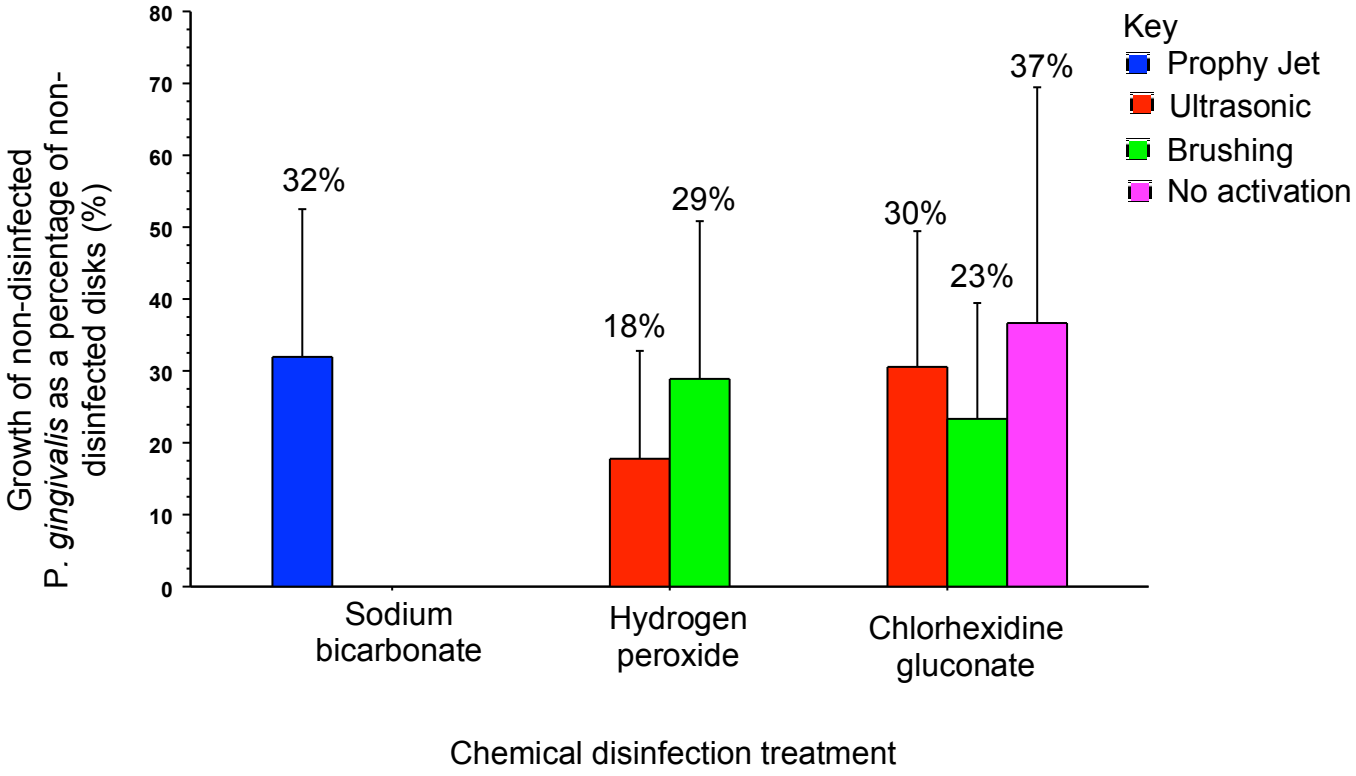
The disinfection of *P. gingivalis* from the Osseotite and Osseotite with NanoTite dental implant disks was most effective using Chlorhexidine gluconate, which had very low residual amounts of *P. gingivalis* equal to a half of one percent (0.52-0.53%) (Figure 3). The least effective disinfection agent was Citric acid (51%) (Figure 3) (ANOVA, P=0.0025, Power 0.937). A comparison of the Chlorhexidine gluconate to disinfect Osseotite and Osseotite with NanoTite dental implant disks, found that the NanoTite coating had very little effect on the disinfection of *P. gingivalis* from the implant disks (0.52% versus 0.53%) (Figure 3) (ANOVA, P=0.3017, Power 0.165).

Figure 1. Bar chart of the effectiveness of disinfection of *P. gingivalis* from 3iT3 versus 3iT3 with DCD dental implants.



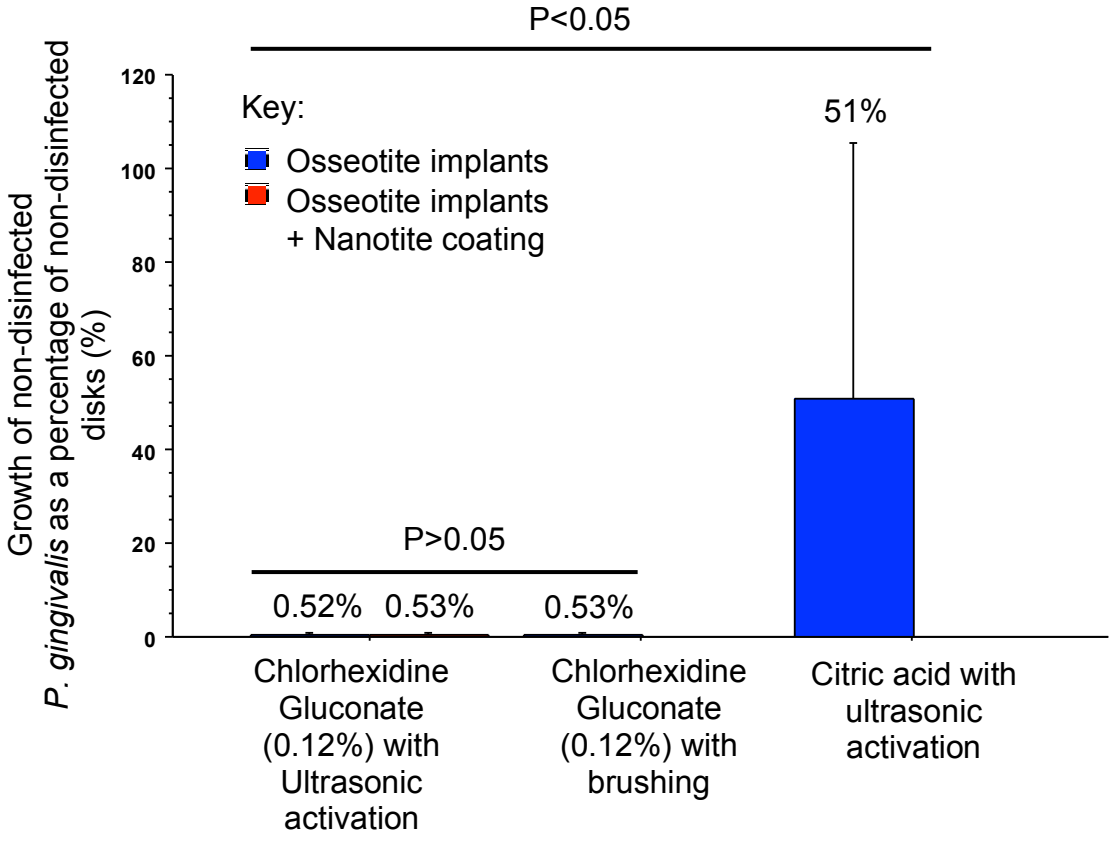
The bar char represents the means of each disinfection treatment group which contained eight specimens. Each mean shows the growth of residual non-disinfected *P. gingivalis* as a percentage of non-disinfected disks (%) following disinfection. A sample absorbance of 0.271 was used as the non-disinfected 100% control. The zero absorbance controls were calculated as 0% when the calibrating of the spectrophotometer. The standard error bars represent the standard deviation of the means. The amount of residual *P. gingivalis* was measured following disinfection using a spectrophotometer at an absorbance at 600nm.

Figure 2. Bar chart of the effectiveness of chemicals and physical disinfection treatments to disinfect *P. gingivalis* from 3iT3 dental implant disks.



The bar char represents the means of each disinfection treatment group which contained eight specimens. Each mean shows the growth of residual non-disinfected *P. gingivalis* as a percentage of non-disinfected disks (%) following disinfection. A sample absorbance of 0.271 was used as the non-disinfected 100% control. The zero absorbance controls were calculated as 0% when the calibrating of the spectrophotometer. The standard error bars represent the standard deviation of the means. The amount of residual *P. gingivalis* was measured following disinfection using a spectrophotometer at an absorbance at 600nm.

Figure 3. Bar chart of the effectiveness of chemicals and physical disinfection treatments to disinfect *P. gingivalis* from Osseotite and Osseotite with NanoTite dental implants.



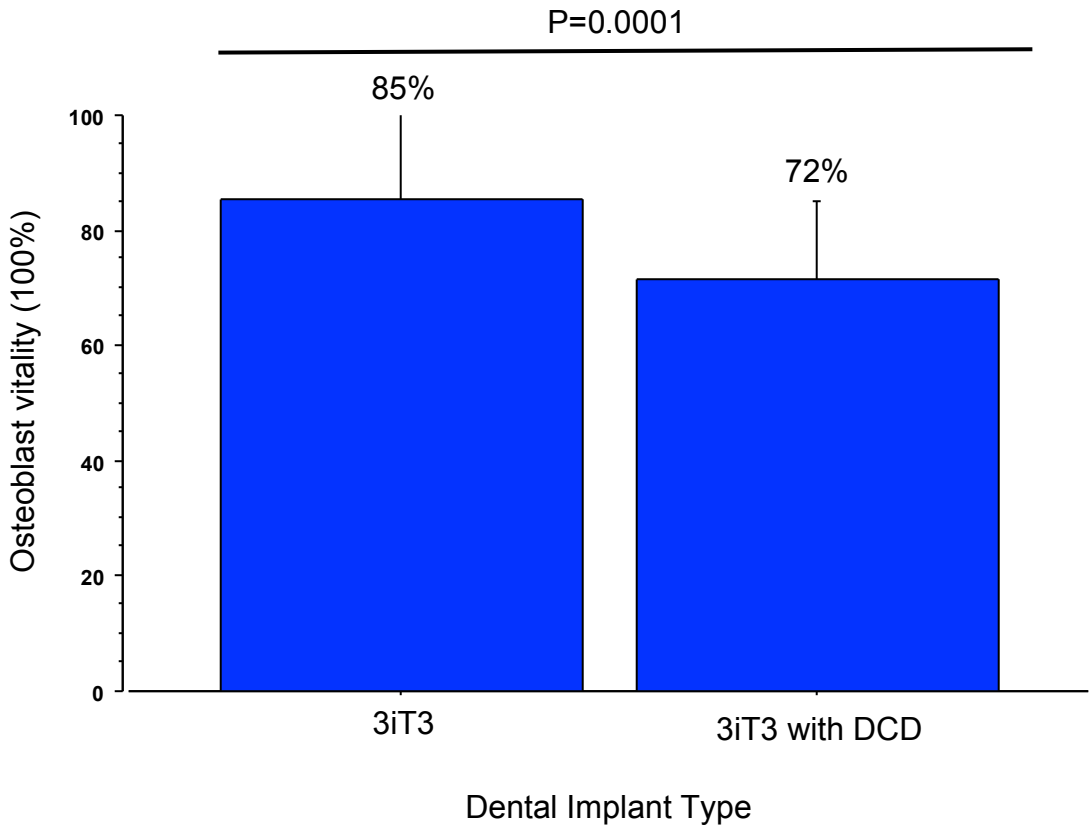
The bar char represents the means of each disinfection treatment group which contained six specimens. Each mean shows the growth of residual non-disinfected *P. gingivalis* as a percentage of non-disinfected disks (%) following disinfection. A sample absorbance of 1.522 was used as the non-disinfected 100% control. The zero absorbance controls were calculated as 0% when the calibrating of the spectrophotometer. The standard error bars represent the standard deviation of the means. The amount of residual *P. gingivalis* was measured following disinfection using a spectrophotometer at an absorbance at 600nm.

3.3. Osteoblast viability to 3iT3 disks following the disinfection of *P. gingivalis*.

The viability of osteoblasts was 13% higher in the presence of 3iT3 dental implant disks (85%) compared to the 3iT3 with DCD dental implant disks (72%) (ANOVA, P=0.0001, Power 1.0) (Figure 4). The use of four physical disinfection treatments: Prophy Jet, Ultrasonic activation, brushing, and no activation, had an effect on the viability of osteoblasts. The lack of physical activation of the chemical disinfectants to remove *P. gingivalis* from the dental implant disk had the lowest amount of osteoblast vitality at 70% of the control (Figure 5). Brushing the implant surfaces with a chemical disinfectant had an osteoblast vitality at 73% of the control (Figure 5). Both the Prophy Jet and ultrasonic activation allowed the highest amount of osteoblast vitality at 81% of the control (Figure 5). These results suggest that the physical decontamination method for disinfecting dental implants can influence osteoblast vitality by up to 11% (ANOVA, P=0.0346, Power 0.689).

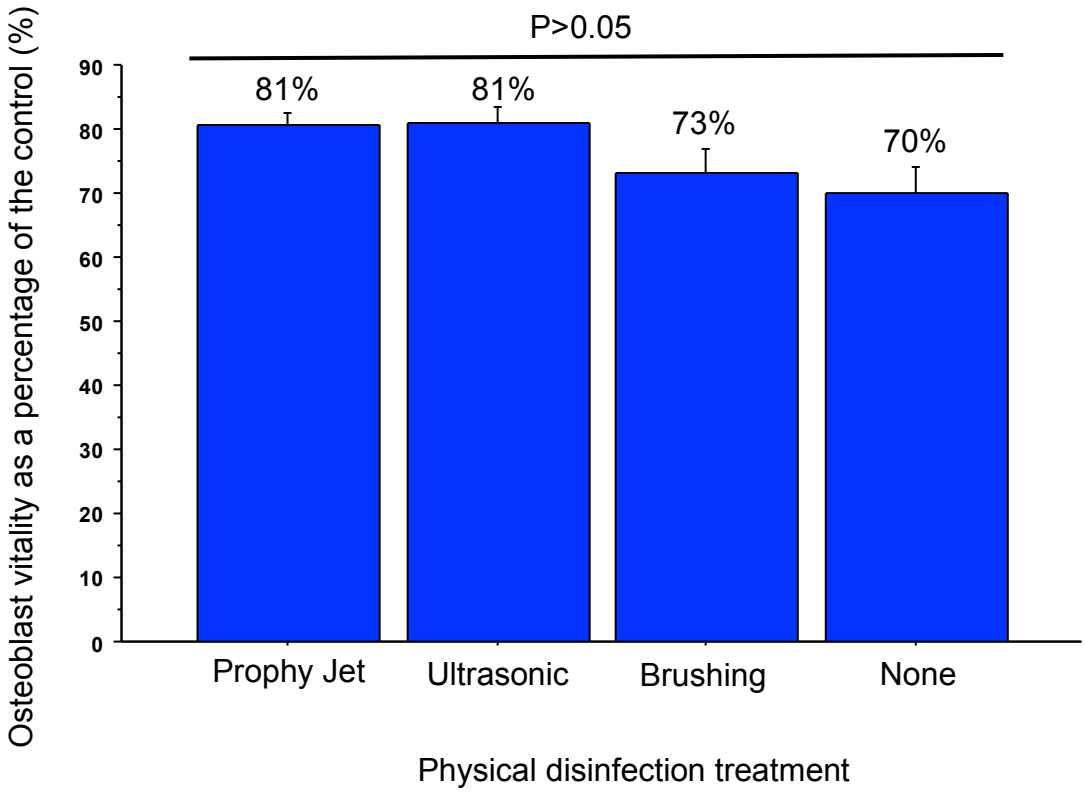
A comparison of the osteoblast vitality on 3iT3 dental implant disks versus the 3iT3 with DCD dental implant disks for each of the four physical disinfection treatments found that osteoblast vitality was higher between 28% and 8% on the 3iT3 dental implant disks (Figure 6). The physical decontamination of the 3iT3 with DCD dental implant disks always gave a lower amount of osteoblast vitality compared to the 3iT3 dental implant disks (ANOVA, P=0.008, Power 0.847). A Scheffe post hoc ANOVA found that osteoblast vitality was higher in each of the four physical decontamination treatments: Prophy Jet (P=0.0001), ultrasonic activation (P=0.0001) and no physical activation (P=0.0001), except brushing (P=0.2883). The combined effects of the implant type and physical activation of chemical disinfectants, found that because osteoblast vitality was always highest on the 3iT3 dental implant disks,

Figure 4. Bar chart of osteoblast viability on 3iT3 disks versus 3iT3 disks with DCD following the physical disinfection of *P. gingivalis*.



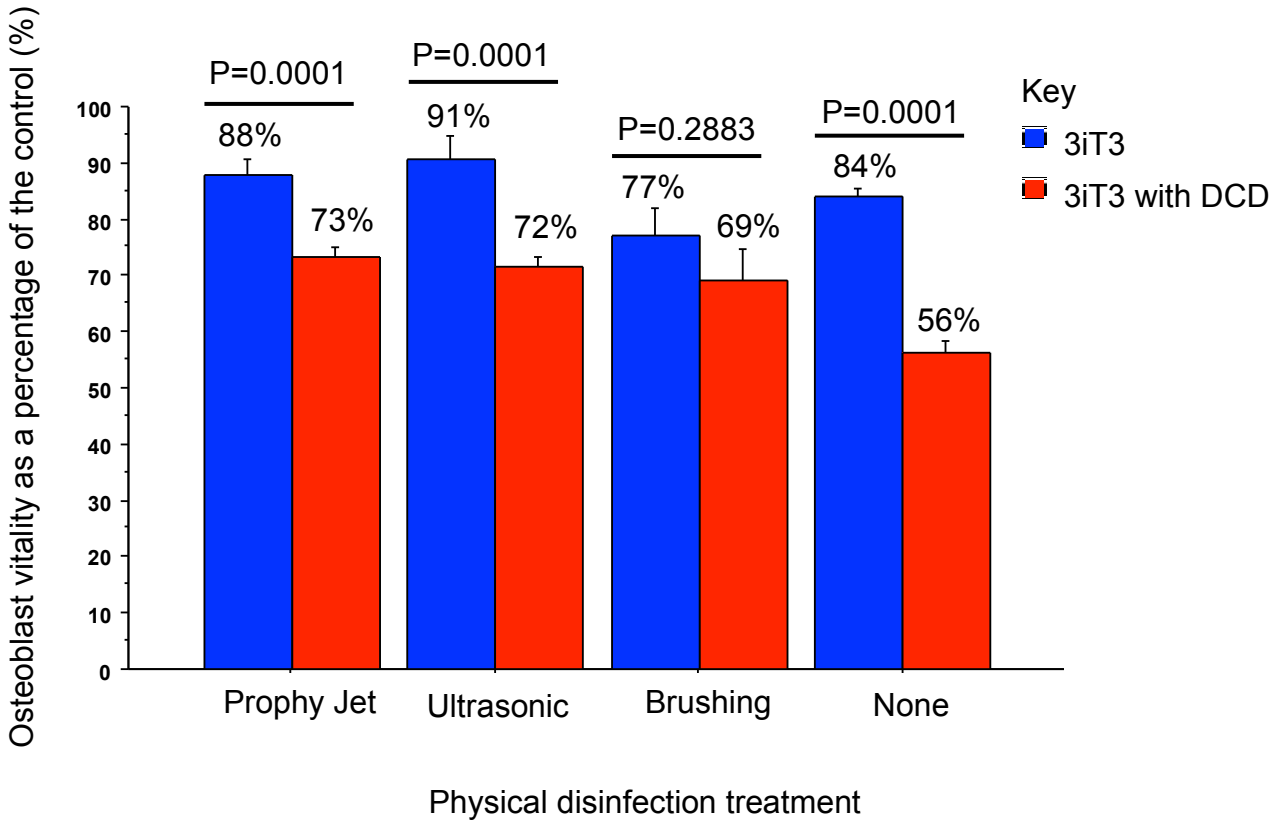
The bar char represents the means of each treatment group which contained fifty-nine specimens. The standard error bars represent the standard deviation of the means. The osteoblast vitality is represented as a percentage of the mean osteoblast vitality of the untreated dental implant groups. The vitality of the osteoblasts for each of the specimens was measured using the MTT assay at an absorbance at 600nm.

Figure 5. Bar chart of osteoblast viability on 3iT3 disks following the physical disinfection of *P. gingivalis*.



The bar char represents the means of each treatment group which contained 16 to 48 specimens. The standard error bars represent the standard deviation of the means. The osteoblast vitality is represented as a percentage of the mean osteoblast vitality of the untreated dental implant groups. The vitality of the osteoblasts for each of the specimens was measured using the MTT assay at an absorbance at 600nm.

Figure 6. Bar chart of osteoblast viability on 3iT3 disks versus 3iT3 with DCD disks following the physical disinfection of *P. gingivalis*.



The bar char represents the means of each treatment group which contained 16 to 48 specimens. The standard error bars represent the standard deviation of the means. The osteoblast vitality is represented as a percentage of the mean osteoblast vitality of the untreated dental implant groups. The vitality of the osteoblasts for each of the specimens was measured using the MTT assay at an absorbance at 600nm.

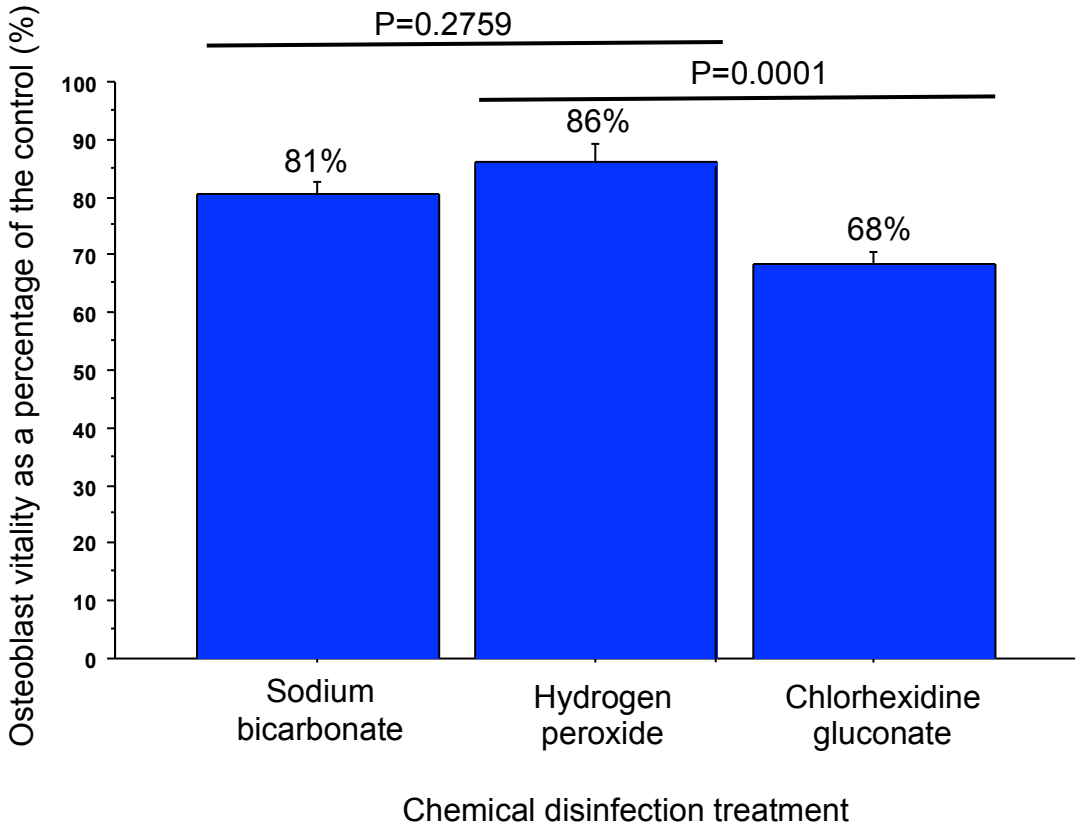
that overall effect of the physical decontamination procedures on osteoblast vitality was relatively much weaker (ANOVA, P=0.1085, Power 0.508).

The vitality of osteoblasts on 3iT3 dental implant disks was measured following the use of the chemicals; Sodium bicarbonate, Hydrogen peroxide, and Chlorhexidine gluconate to disinfect *P. gingivalis*. The type of chemical used to disinfect *P. gingivalis* from the 3iT3 implants had an effect on the vitality of osteoblasts (ANOVA, P=0.0001, Power 1.0). The use of hydrogen peroxide had the highest osteoblast vitality at 86%, sodium bicarbonate had an osteoblast vitality of 81%, and the lowest osteoblast vitality of 68% was found following the use of chlorhexidine gluconate (Figure 7). A scheffe post hoc ANOVA found that the vitality of osteoblasts was similar following the use of Sodium bicarbonate and Hydrogen peroxide to disinfect the implants (P=0.2759), while the implant disinfected with Chlorhexidine gluconate always had a lower osteoblast vitality (P<0.05).

The vitality of osteoblasts on 3iT3 dental implant disks was 17% to 15% higher on the 3iT3 disks compared to the 3iT3 disks with a DCD coating following the use of chemical treatments to disinfect *P. gingivalis* (ANOVA, P=0.0001, Power 1.0) (Figure 8). A scheffe post hoc ANOVA found that the vitality of osteoblasts was always higher on the 3iT3 dental implant disks, compared to the 3iT3 dental implant discs with a DCD coating (P<0.0001) (Figure 8).

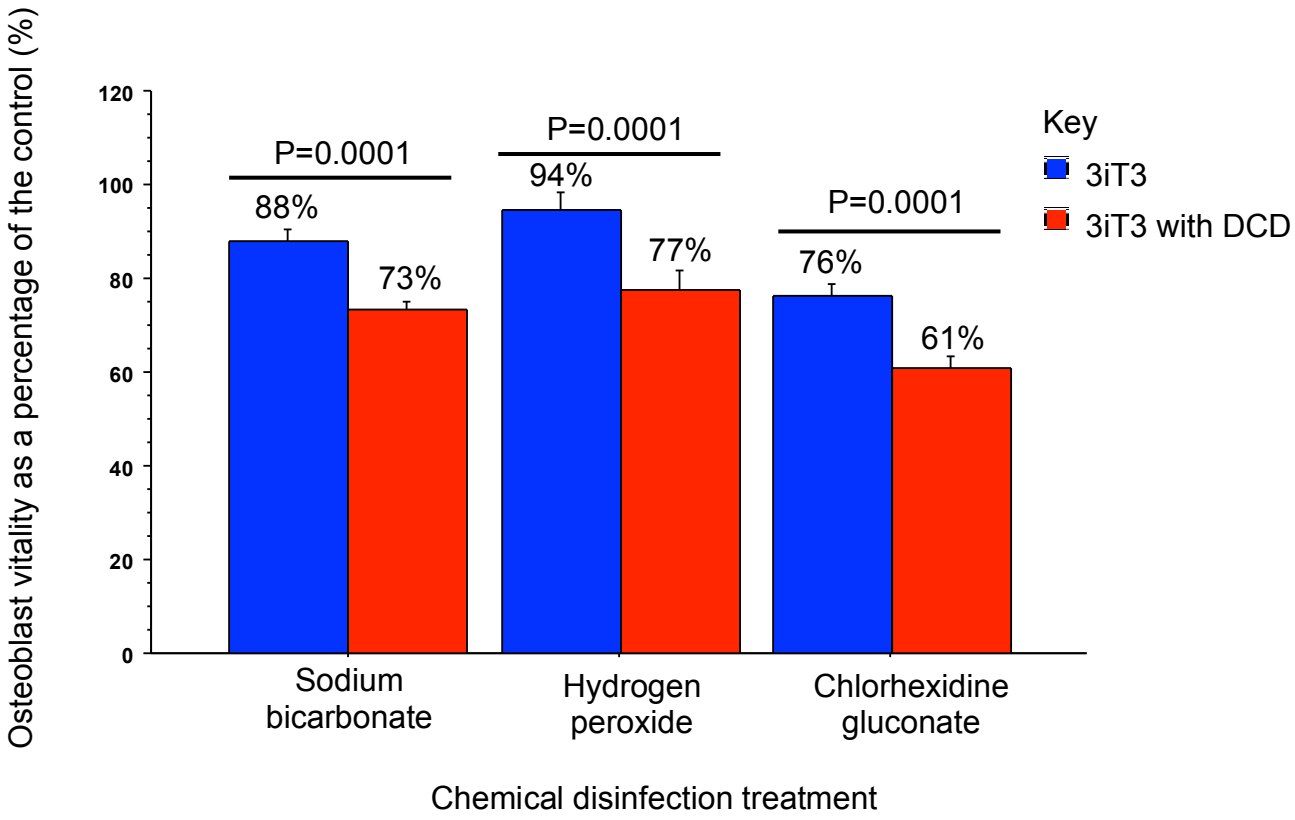
The vitality of osteoblasts on 3iT3 dental implant disks and 3iT3 dental implant disks with a DCD coating were highest after the disks had been decontaminated with Hydrogen peroxide which had been brushed (90%) and ultrasonically activated (82%) (Figure 9). The vitality of osteoblasts on the dental implant disks was 81% following Prophy Jet disinfection with Sodium bicarbonate (Figure 9).

Figure 7. Bar chart of osteoblast viability on 3iT3 disks following the chemical disinfection of *P. gingivalis*.



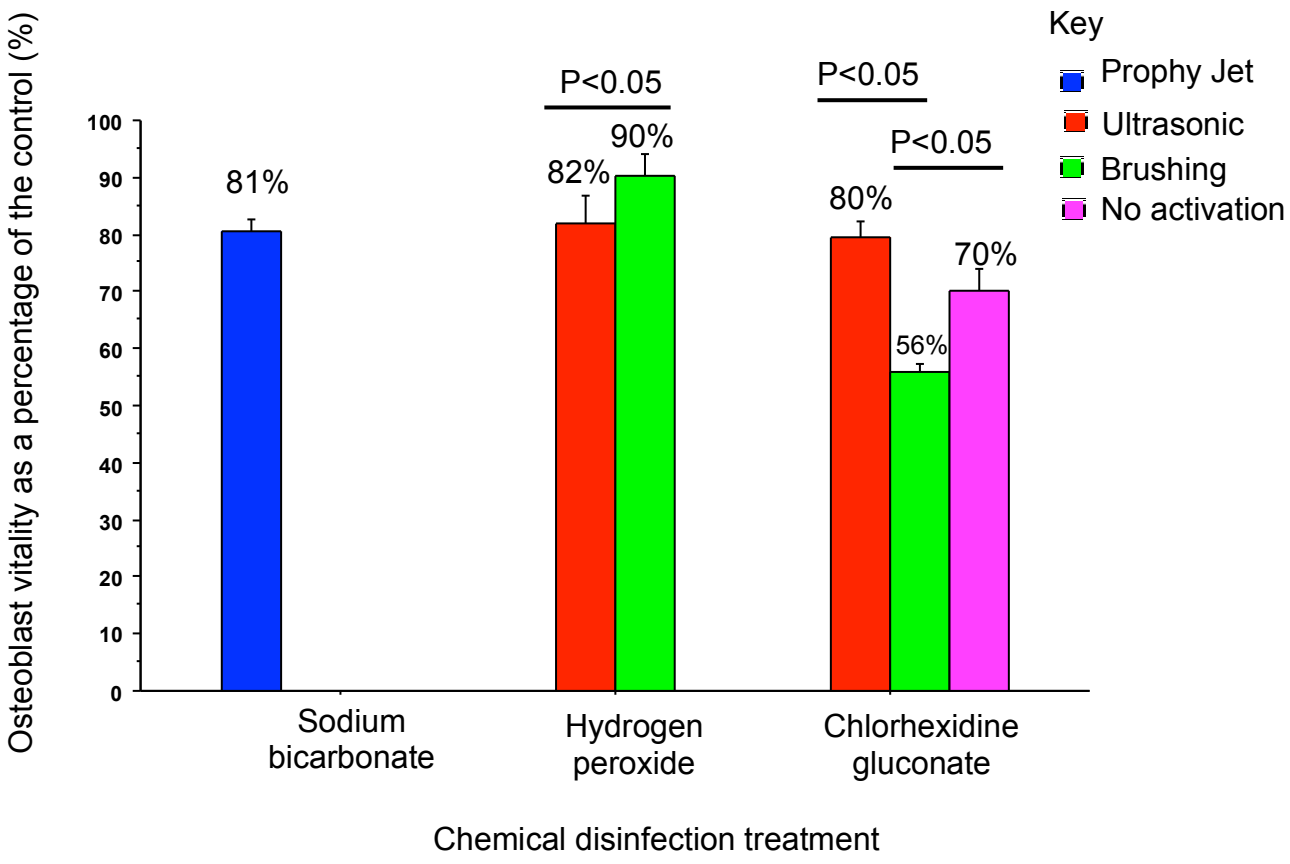
The bar char represents the means of each treatment group which contained 16 to 48 specimens. The standard error bars represent the standard deviation of the means. The osteoblast vitality is represented as a percentage of the mean osteoblast vitality of the untreated dental implant groups. The vitality of the osteoblasts for each of the specimens was measured using the MTT assay at an absorbance at 600nm.

Figure 8. Bar chart of osteoblast viability on 3iT3 disks versus 3iT3 disks with DCD following the chemical disinfection of *P. gingivalis.*



The bar char represents the means of each treatment group which contained 16 to 48 specimens. The standard error bars represent the standard deviation of the means. The osteoblast vitality is represented as a percentage of the mean osteoblast vitality of the untreated dental implant groups. The vitality of the osteoblasts for each of the specimens was measured using the MTT assay at an absorbance at 600nm.

Figure 9. Bar chart of osteoblast viability on 3iT3 disks following the chemical and physical disinfection of *P. gingivalis*.



The bar char represents the means of each treatment group which contained 16 to 48 specimens. The standard error bars represent the standard deviation of the means. The osteoblast vitality is represented as a percentage of the mean osteoblast vitality of the untreated dental implant groups. The vitality of the osteoblasts for each of the specimens was measured using the MTT assay at an absorbance at 600nm.

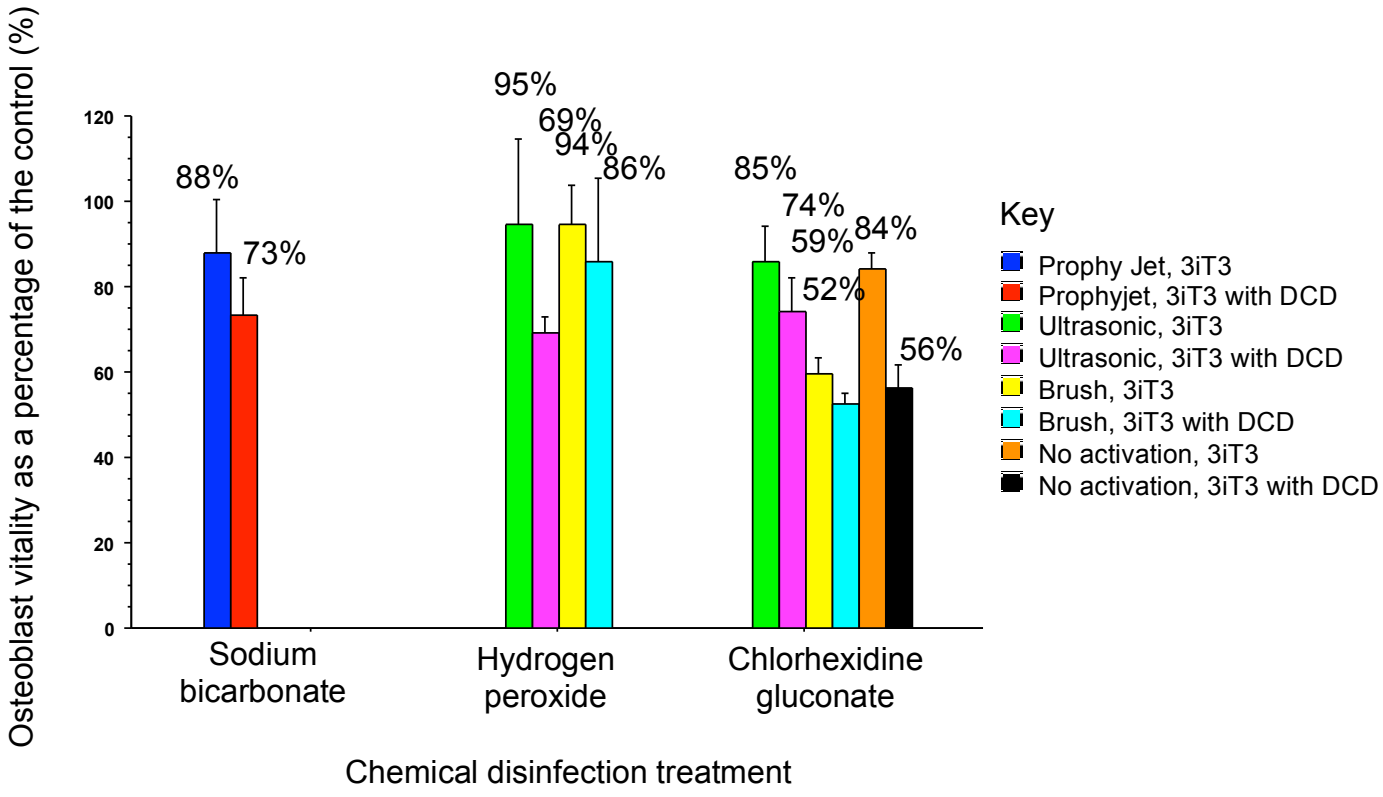
The vitality of osteoblasts in the presence of dental implant disks disinfected with Chlorhexidine gluoconate was highest following ultrasonic activation (80%), less without any activation (70%) and worst when used with brushing (56%) (Figure 9). A scheffe post hoc ANOVA found that the vitality of osteoblasts was mostly different within the chemical treatments depending on the type of physical activation that was used ($P<0.05$) (Figure 9).

A comparison of the vitality of osteoblasts on 3iT3 dental implant disks versus the 3iT3 dental implant disks with a DCD coating found that after the chemical and physical disinfection of *P. gingivalis* that osteoblast vitality was always highest on the 3iT3 dental implant disks (Figure 10). The interaction between the implant surface types, physical and chemical disinfection methods all had a combined effect on the vitality of osteoblasts (ANOVA, $P=0.0213$, Power 0.816). A scheffe post hoc ANOVA found that the vitality of osteoblasts was mostly different within the chemical treatments depending on the type of physical activation that was used ($P<0.05$) (Figure 10).

The coating of the disinfected 3iT3 dental implant disks with growth factors had little effect on the vitality of osteoblasts (ANOVA, $P=0.1172$, Power 0.422). The coating of the dental implant disks with GEM21S increased the vitality of osteoblasts by 6% compared to the control (absence of growth factors), and the addition of Emdogain reduced the vitality of the osteoblasts by 4% compared to the control (Figure 11).

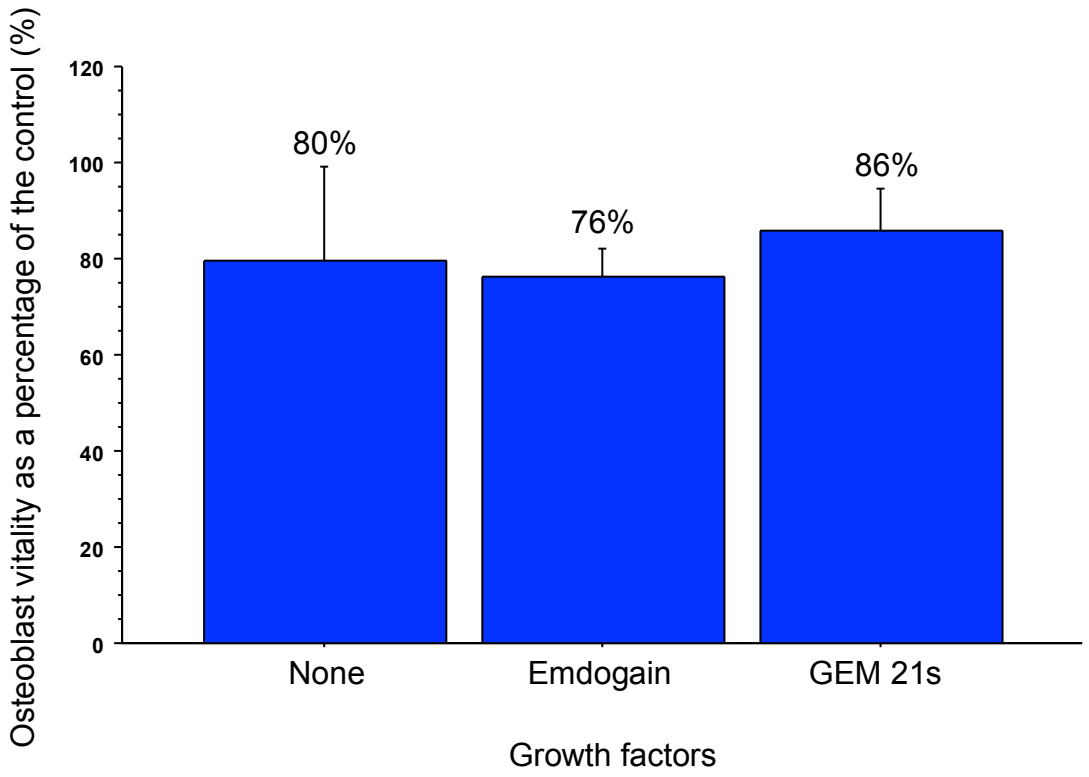
The combined effects of different dental implant surfaces and the addition of Emdogain and GEM21S growth factors were able to effect osteoblast vitality (ANOVA, $P=0.0001$, Power 1.0). The vitality of the osteoblasts was 34% higher in the presence of 3iT3 dental implant disks compared to the 3iT3 disks with a DCD coating without growth factors (Figure 12).

Figure 10. Bar chart of osteoblast viability on 3iT3 disks versus 3iT3 disks with DCD following the chemical and physical disinfection of *P. gingivalis*.



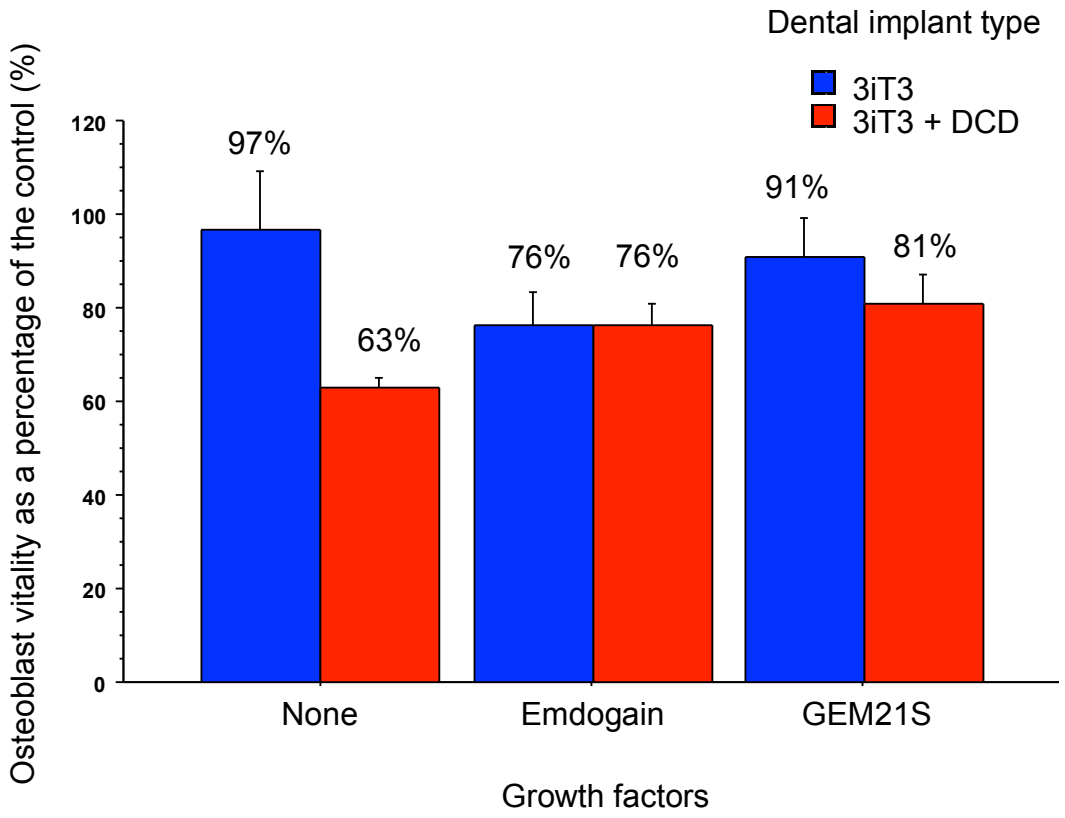
The bar char represents the means of each treatment group which contained 16 to 48 specimens. The standard error bars represent the standard deviation of the means. The osteoblast vitality is represented as a percentage of the mean osteoblast vitality of the untreated dental implant groups. The vitality of the osteoblasts for each of the specimens was measured using the MTT assay at an absorbance at 600nm.

Figure 11. Bar chart of osteoblast viability on 3iT3 disks treated with growth factors following the chemical and physical disinfection of *P. gingivalis*.



The bar char represents the means of each treatment group which contained 16 to 48 specimens. The standard error bars represent the standard deviation of the means. The osteoblast vitality is represented as a percentage of the mean osteoblast vitality of the untreated dental implant groups. The vitality of the osteoblasts for each of the specimens was measured using the MTT assay at an absorbance at 600nm.

Figure 12. Bar chart of osteoblast viability on 3iT3 disks versus 3iT3 disks with DCD treated with growth factors following the chemical and physical disinfection of *P. gingivalis.*



The bar char represents the means of each treatment group which contained 16 to 48 specimens. The standard error bars represent the standard deviation of the means. The osteoblast vitality is represented as a percentage of the mean osteoblast vitality of the untreated dental implant groups. The vitality of the osteoblasts for each of the specimens was measured using the MTT assay at an absorbance at 600nm.

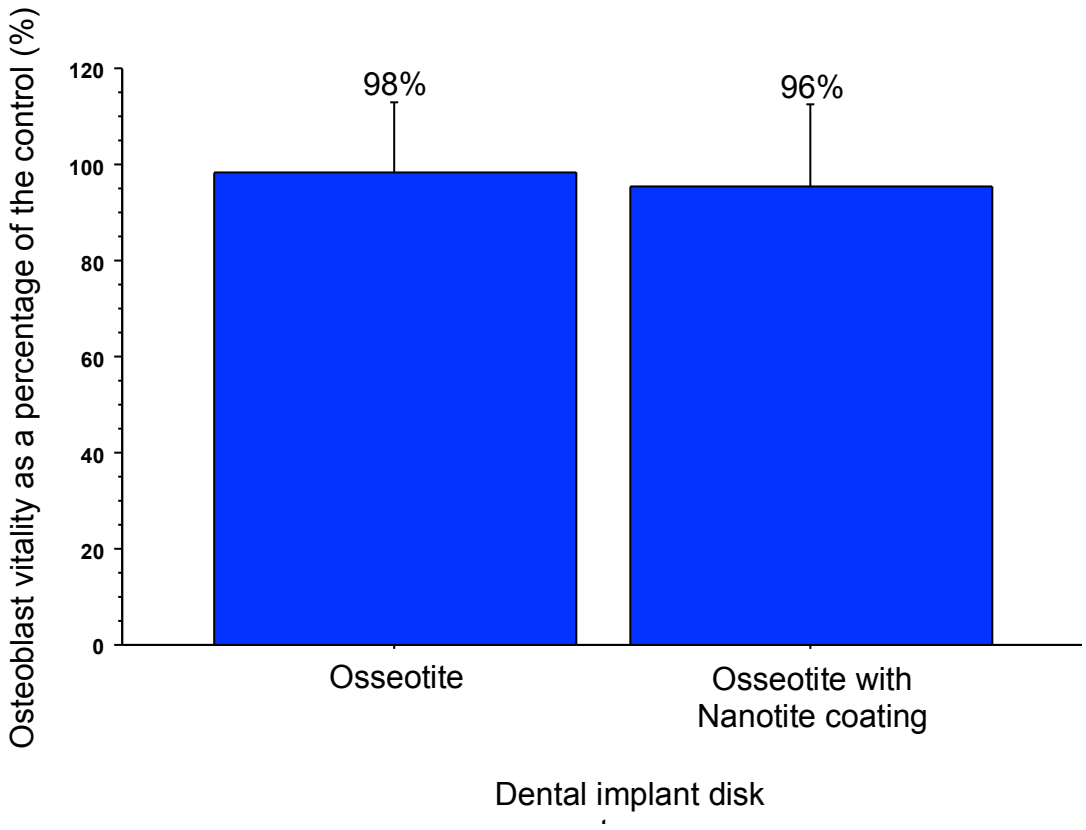
The coating of 3iT3 disks with Emodgain reduced osteoblast vitality by 21%, but increased the vitality of 3iT3 disks with a DCD surface coating by 13% (Figure 12). The effect of coating both types of the 3iT3 implant disks with Emdogain is that it equalized the vitality of osteoblasts at 76%. The 3iT3 disks with DCD surface coating and also coated with GEM21s, had an 18% increase in osteoblast vitality (Figure 12). The 3iT3 disks coated with GEM21s, had a 6% reduction in osteoblast vitality (Figure 12).

3.4. Osteoblast viability to Osseotite disks following the disinfection of *P. gingivalis*

The vitality of osteoblasts in the presence of Osseotite dental implant disks and Osseotite dental implant disks with a NanoTite coating were similar at 98% and 96% respectively (ANOVA, P=0.6453, Power 0.073) (Figure 13).

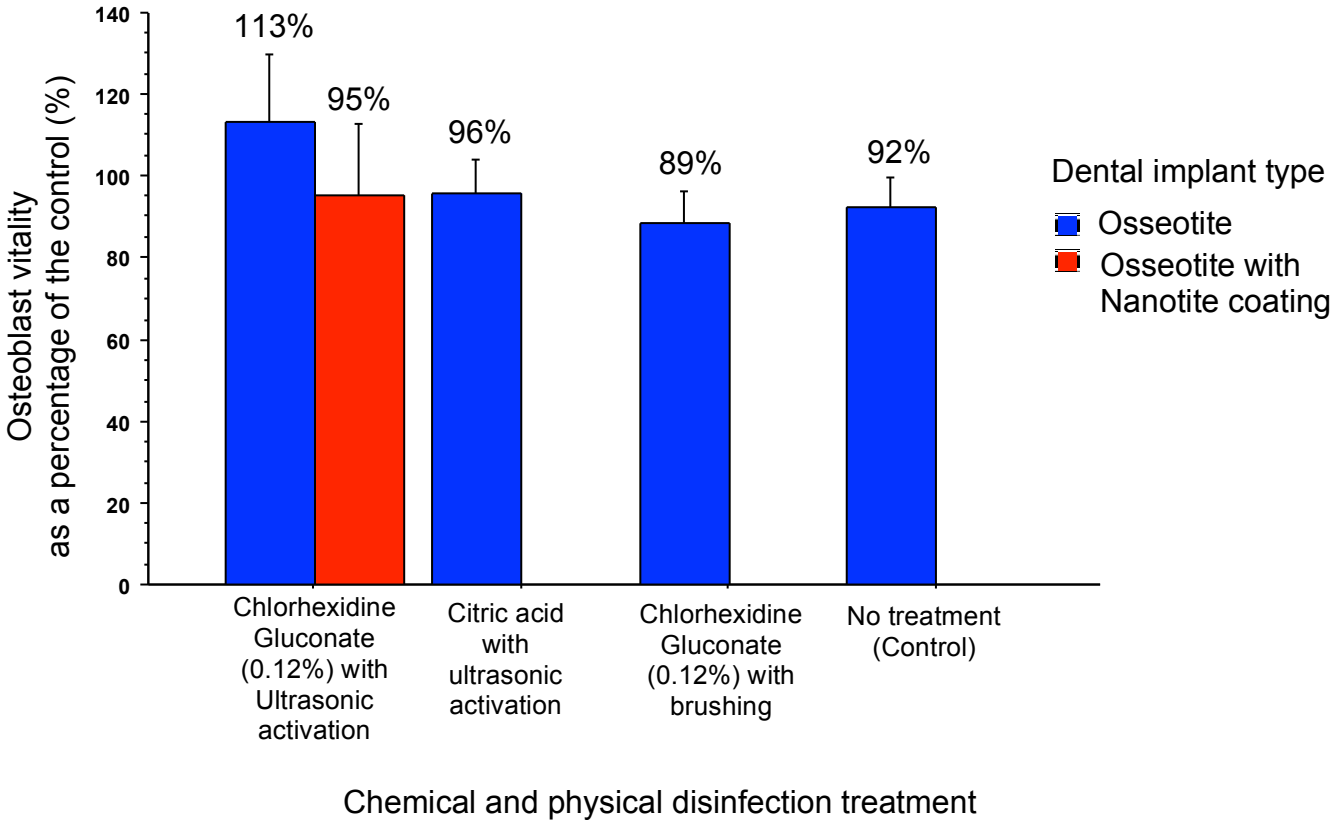
A comparison of the effect of chemical and physical disinfection of Osseotite disks found that osteoblast vitality was similar with all the disinfection treatments (ANOVA, P=0.0676, Power 0.581) (Figure 14). The low power of the P value suggests that if the sample numbers had been increased that a significant difference may be found. The vitality of osteoblasts was highest at 113% following the disinfection of Osseotite implant disks with Chlorhexidine gluconate with ultrasonic activation (Figure 14). The vitality of the osteoblasts in the presence of the Osseotite dental implant disks for all the dental implant types and disinfection treatments were similar. The brushing of Osseotite dental implant disks with Chlorhexidine gluconate gave the lowest osteoblast vitality at 89% (Figure 14).

Figure 13. Bar chart of osteoblast vitality on Osseotite versus Osseotite with Nanotite disks.



The bar char represents the means of each treatment group which contained 8 to 28 specimens. The standard error bars represent the standard deviation of the means. The osteoblast vitality is represented as a percentage of the mean osteoblast vitality of the untreated dental implant groups (0.034). The vitality of the osteoblasts for each of the specimens was measured using the MTT assay at an absorbance at 600nm.

Figure 14. Bar chart of osteoblast vitality on Osseotite disks following disinfection.

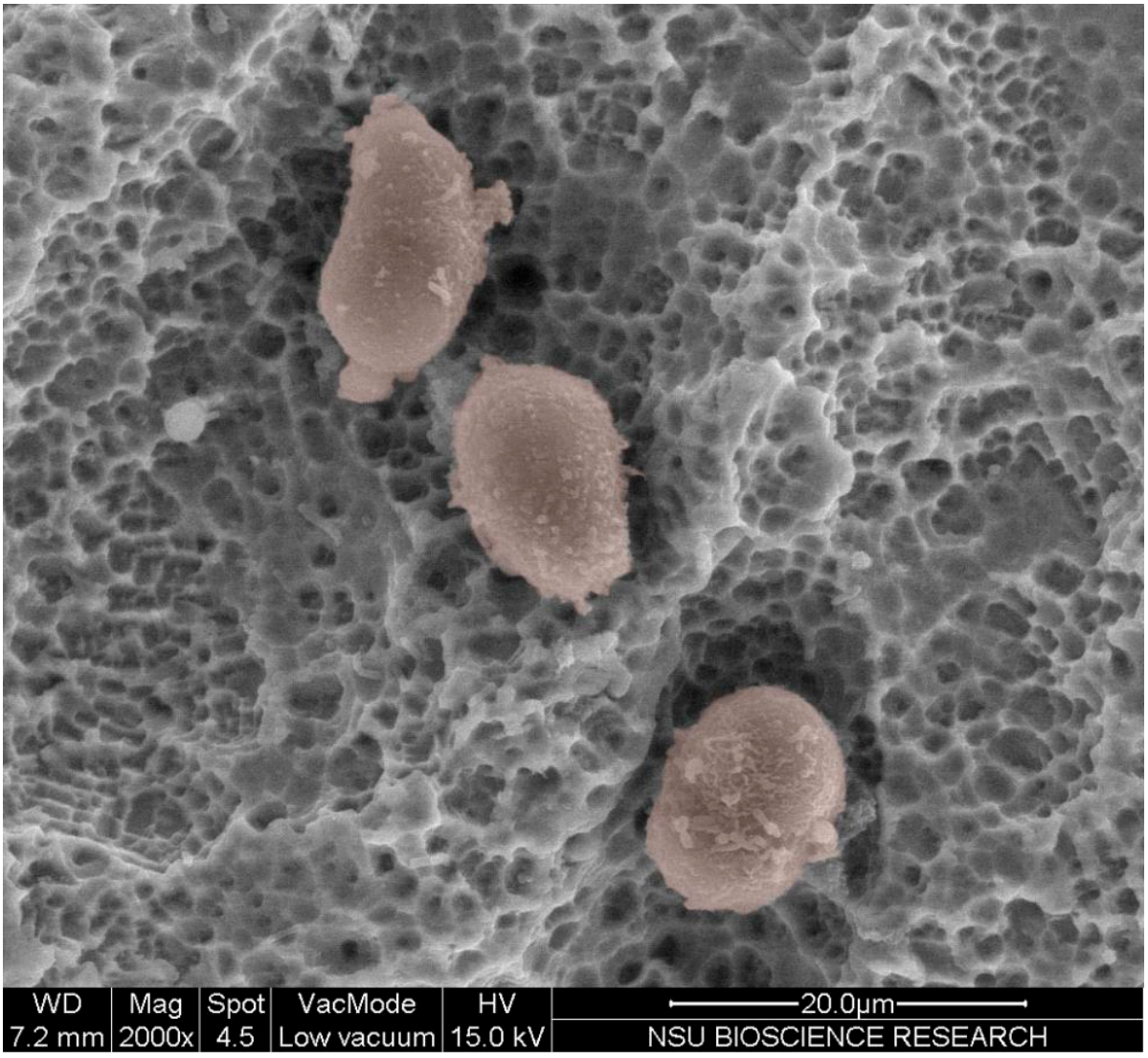


The bar char represents the means of each treatment group which contained 6 to 4 specimens. The standard error bars represent the standard deviation of the means. The osteoblast vitality is represented as a percentage of the mean osteoblast vitality of the untreated dental implant groups (0.034). The vitality of the osteoblasts for each of the specimens was measured using the MTT assay at an absorbance at 600nm.

3.5. Osteoblast attachment to 3iT3 dental implant disks following the disinfection of *P. gingivalis*

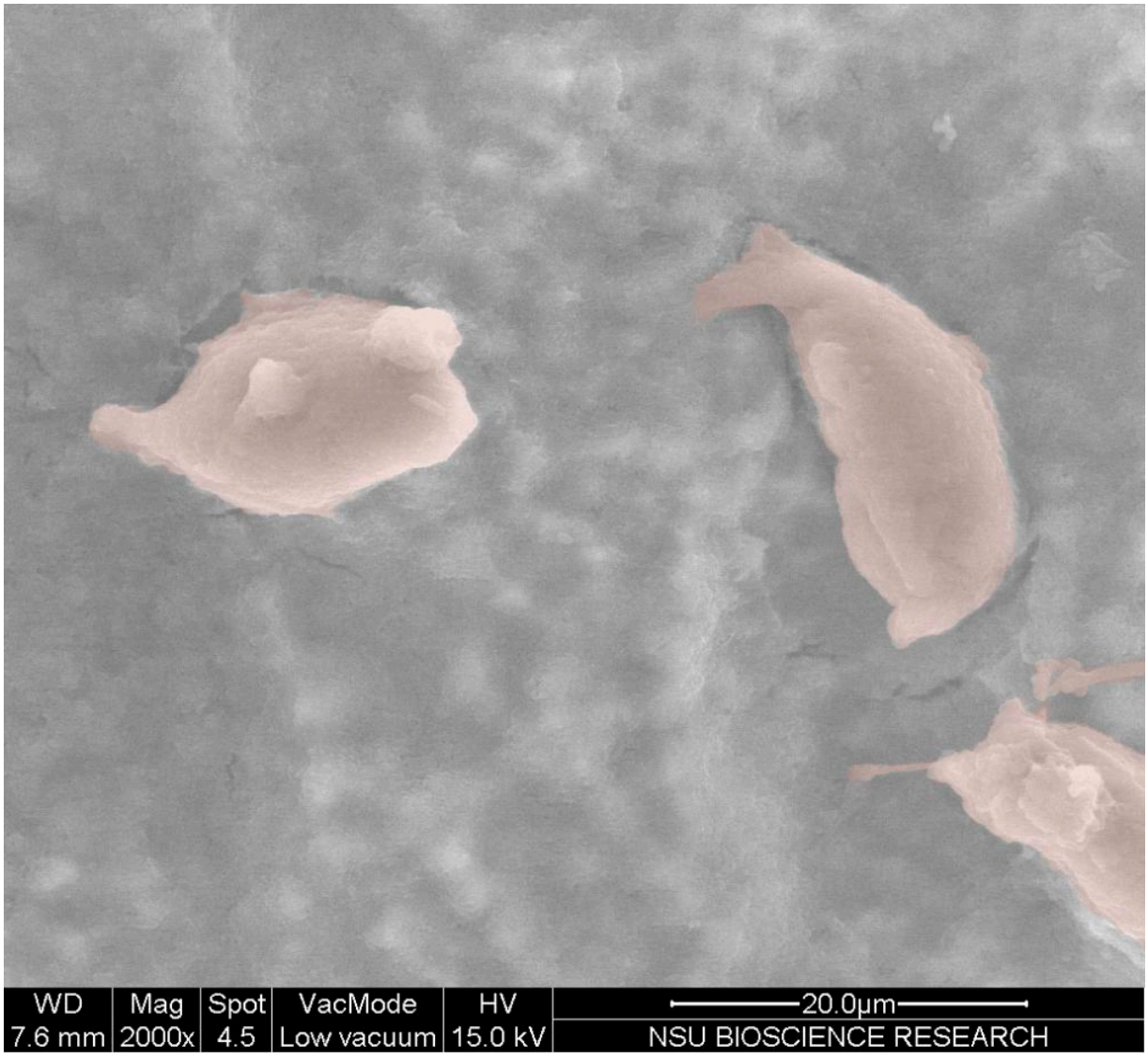
The osteoblasts were observed attached to the surfaces of the 3iT3 dental implant disks following disinfection treatments using scanning electron microscopy at a magnification of x2,000. Some osteoblasts were seen attached to the 3iT3 dental implants following disinfection with a Prophy Jet and Sodium bicarbonate (Figure 15). After adding Emdogain to the 3iT3 dental implants following disinfection with a Prophy Jet and Sodium bicarbonate, some osteoblasts were seen to be attached to the surfaces (Figure 16). Osteoblasts were seen attached to the surface of 3iT3 dental implants that had been coated with GEM21S after disinfection with a Prophy Jet and Sodium bicarbonate (Figure 17). A few osteoblasts were seen attached to the surface of 3iT3 dental implants following disinfection with ultrasonically activated hydrogen peroxide (Figure 18). After disinfecting 3iT3 dental implants with ultrasonically activated chlorhexidine gluconate, some osteoblasts were observed to have attached to the implant surface (Figure 19). Two osteoblasts had attached to the surface of 3iT3 dental implants following brushing with hydrogen peroxide disinfection (Figure 20). After disinfecting 3iT3 dental implants by brushing them with chlorhexidine gluconate, some osteoblasts were seen attached to the implant surface (Figure 21). Some osteoblasts were observed attached to the surface of 3iT3 dental implants following chlorhexidine gluconate disinfection (Figure 22).

Figure 15. Scanning electron micrograph of osteoblasts attached to 3iT3 dental implants following disinfection with a Prophy Jet and $NaHCO_3$



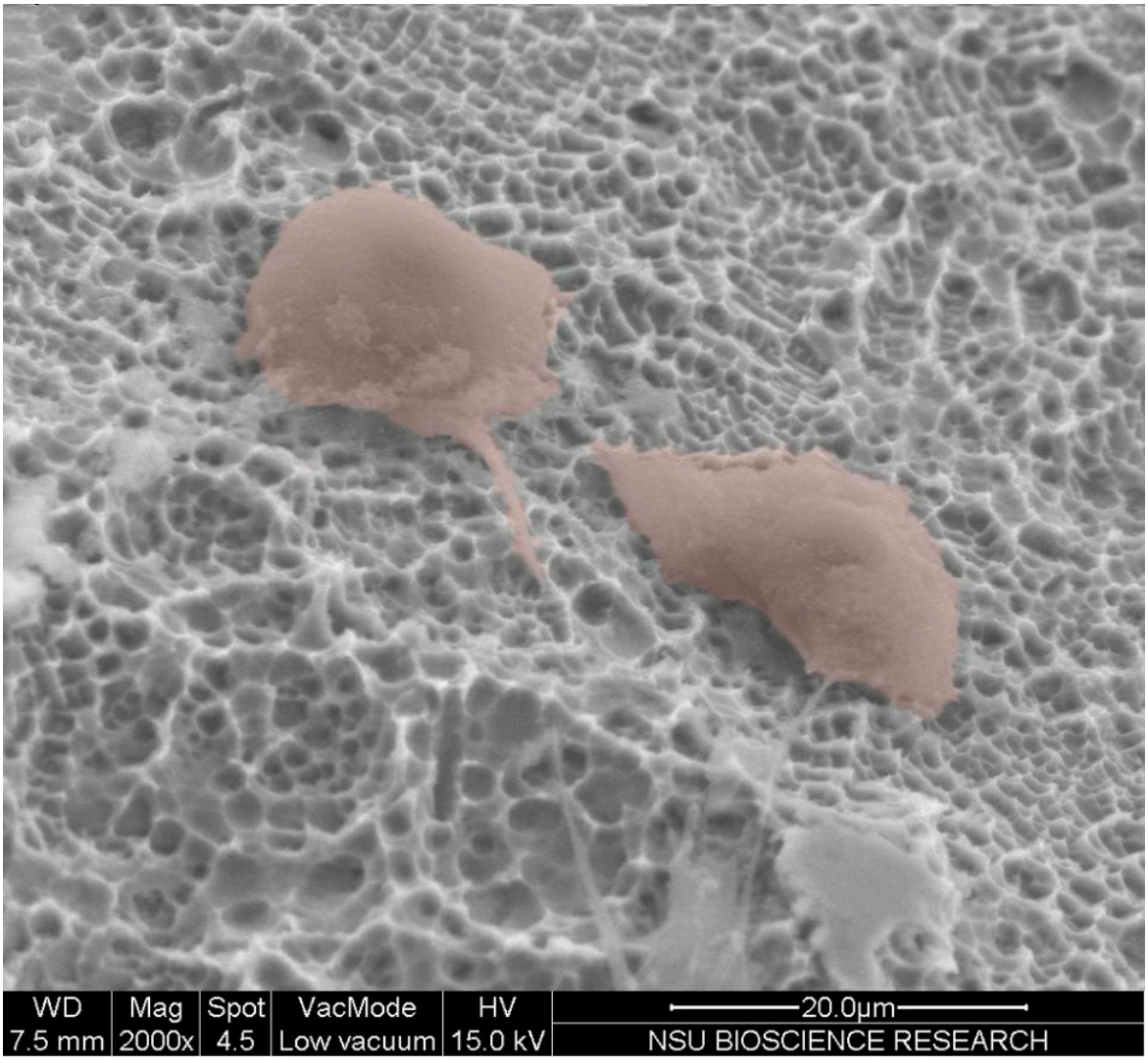
Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

Figure 16. Scanning electron micrograph of osteoblasts attached to 3iT3 dental implants coated with Emdogain following disinfection with a Prophy Jet and $NaHCO_3$.



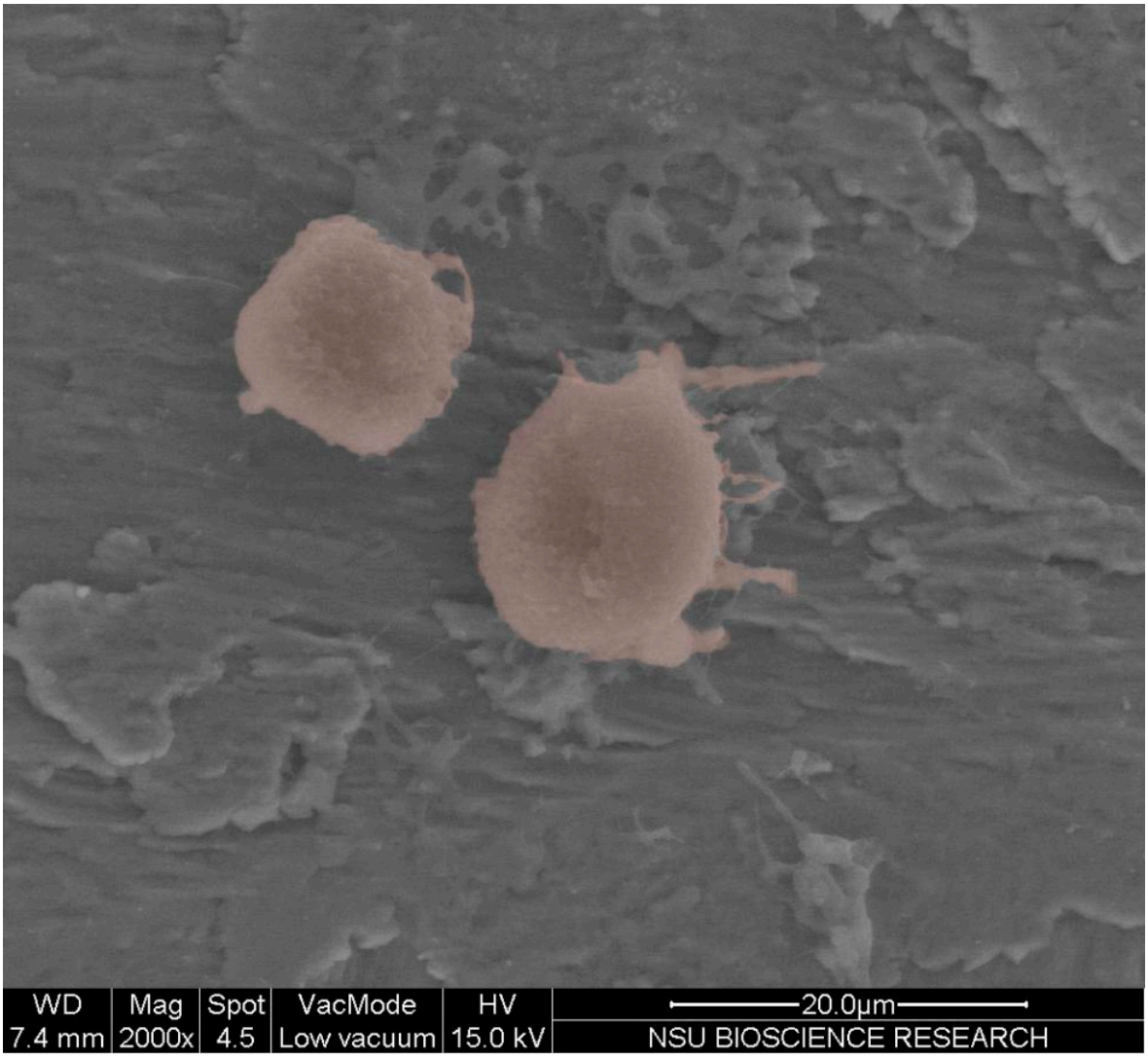
Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

Figure 17. Scanning electron micrograph of osteoblasts attached to disinfected 3iT3 dental implants coated with GEM21s following Prophy Jet disinfection with $NaHCO_3$.



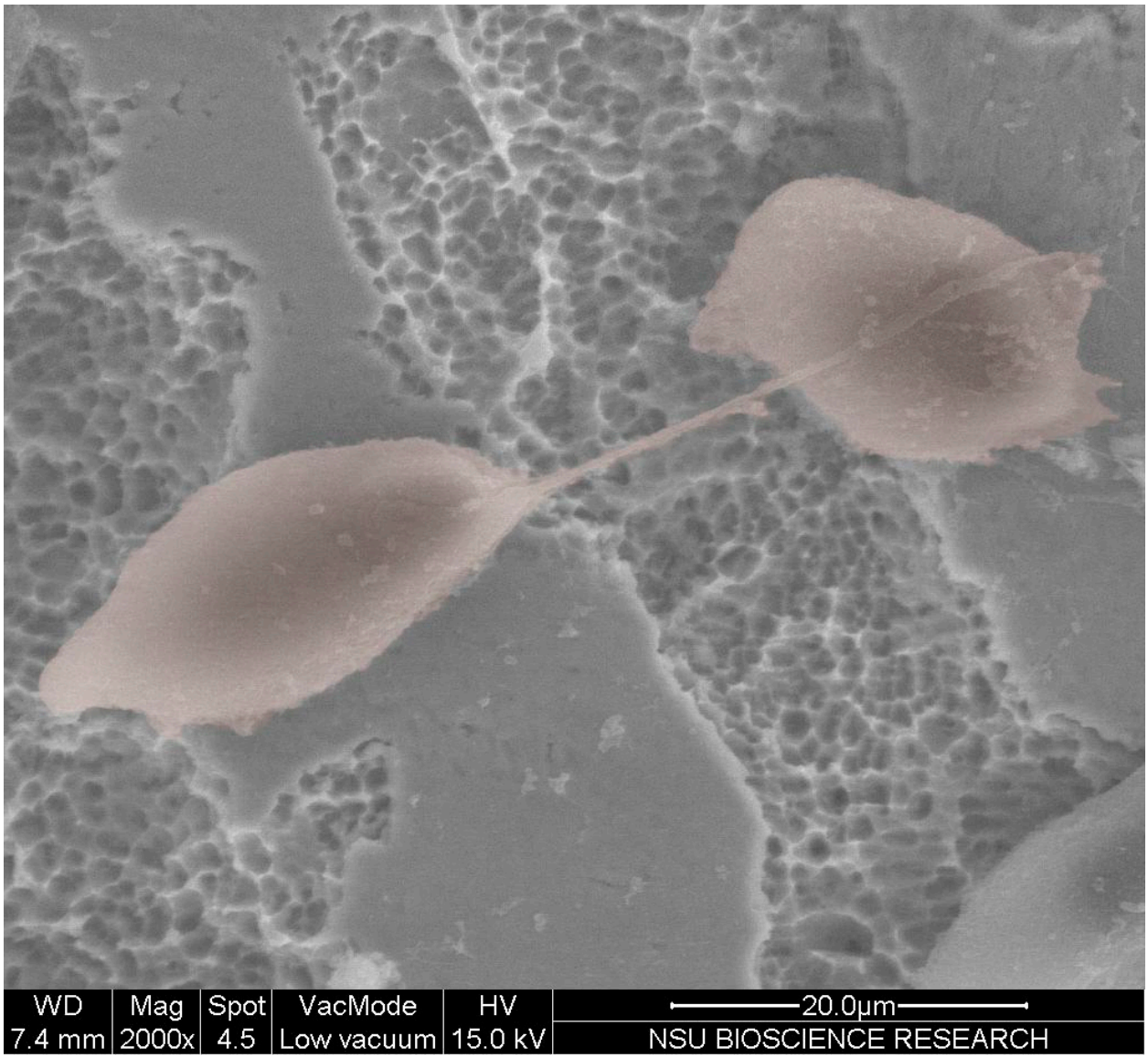
Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

Figure 18. Scanning electron micrograph of osteoblasts attached to 3iT3 dental implants following ultrasonic hydrogen peroxide disinfection.



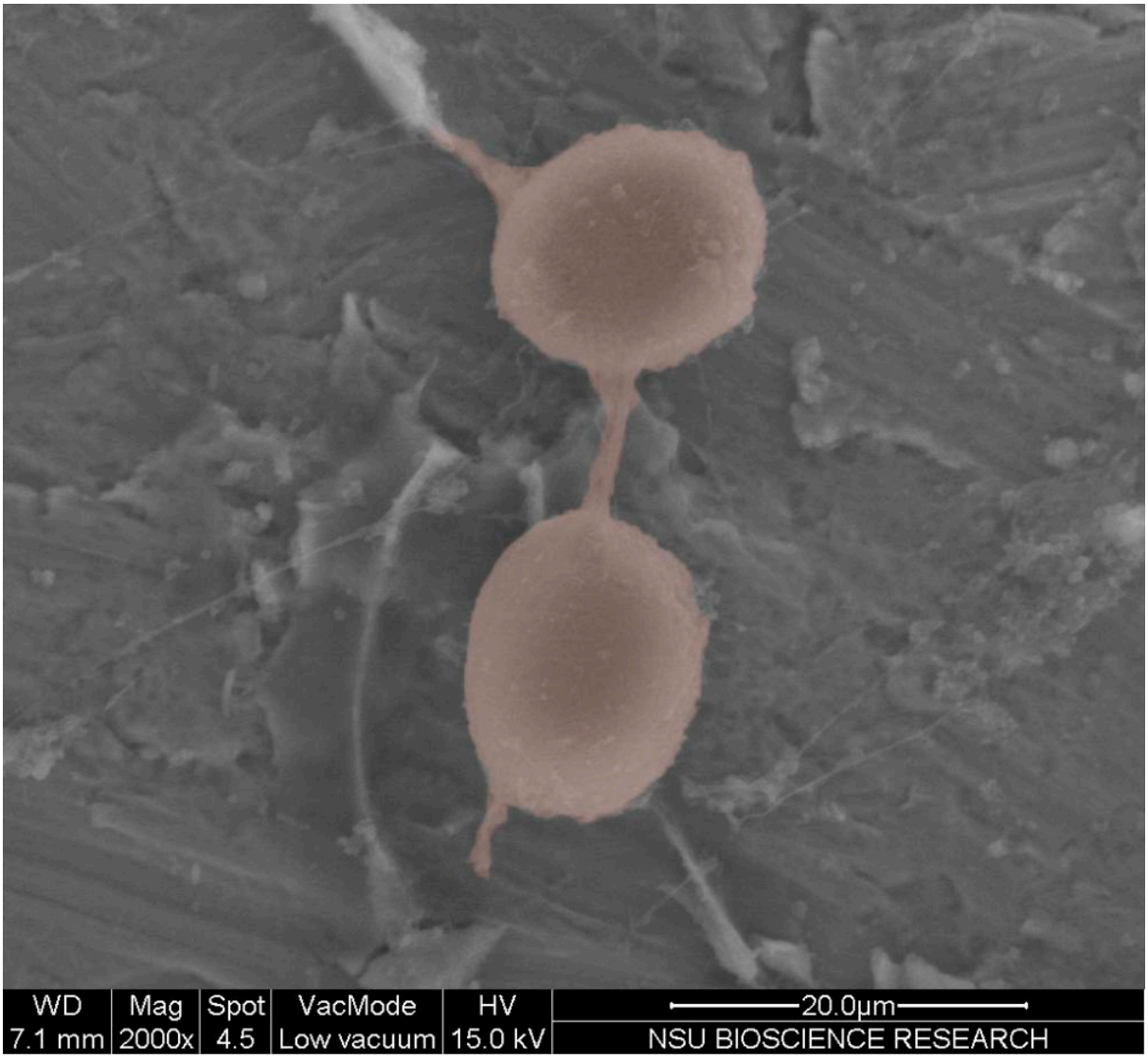
Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

Figure 19. Scanning electron micrograph of osteoblasts attached to 3iT3 dental implants following ultrasonic chlorhexidine gluconate disinfection.



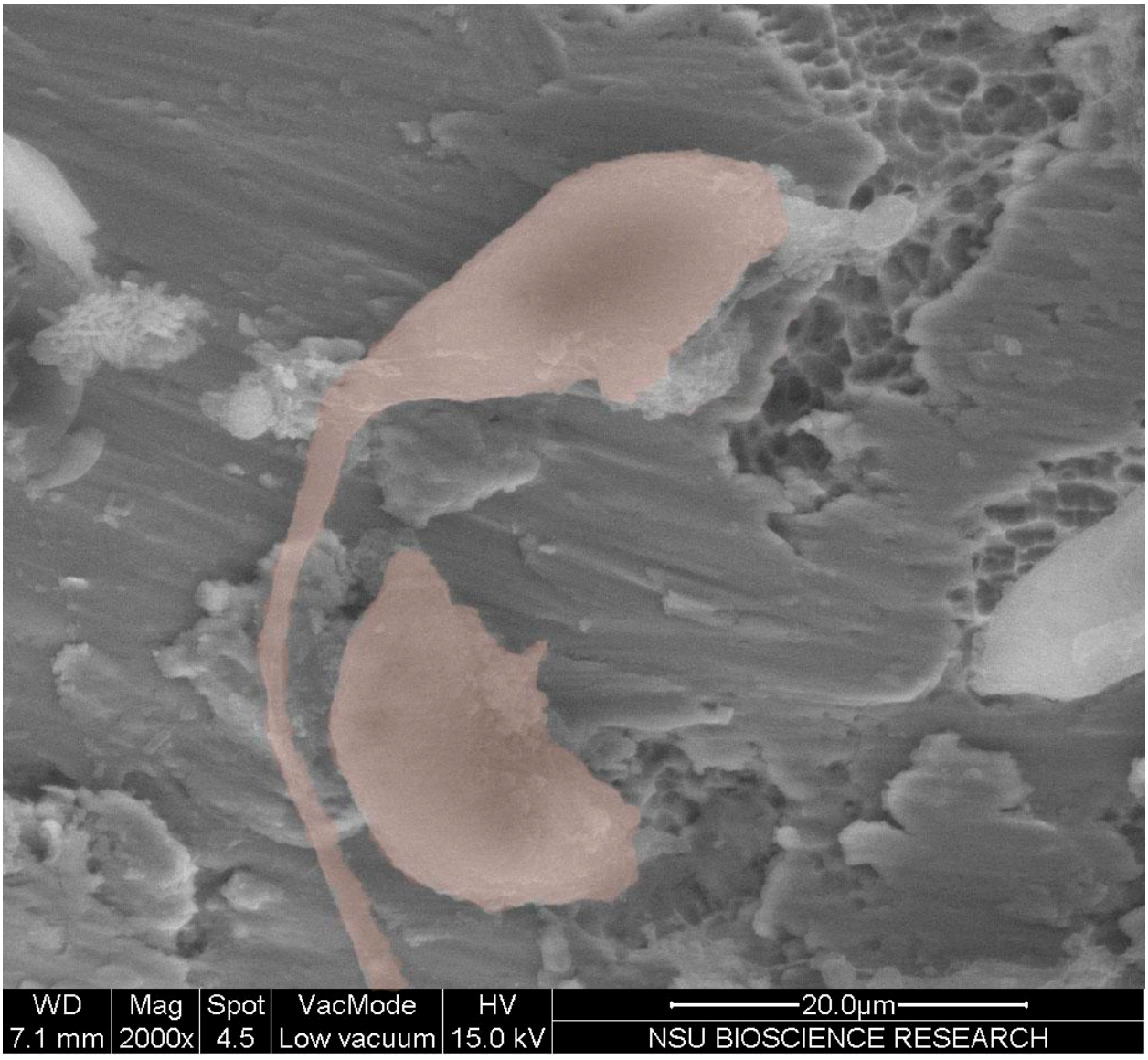
Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

Figure 20. Scanning electron micrograph of osteoblasts attached to 3iT3 dental implants following brushing with hydrogen peroxide disinfection.



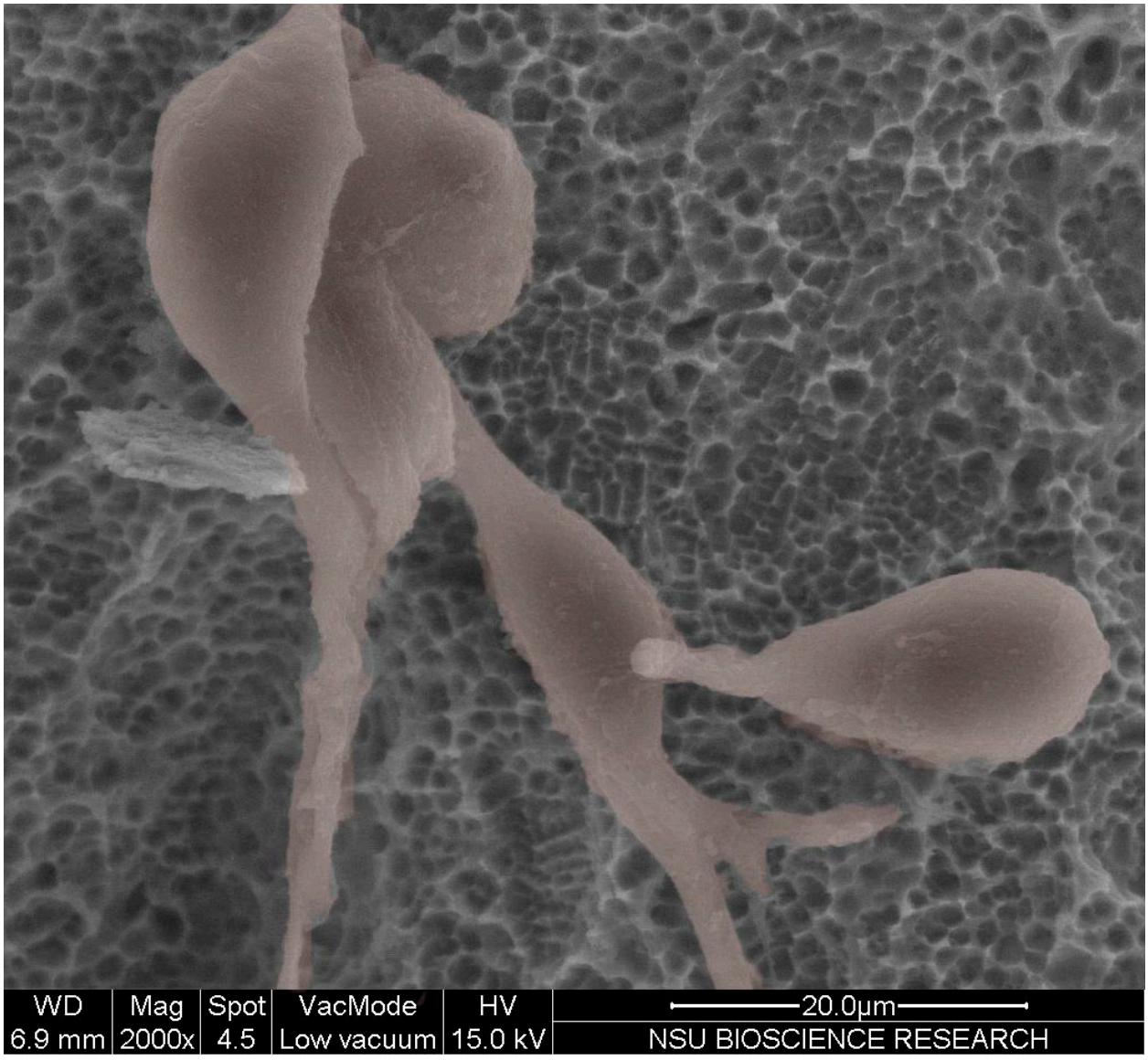
Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

Figure 21. Scanning electron micrograph of osteoblasts attached to 3iT3 dental implants following disinfection by brushing with chlorhexidine gluconate.



Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

Figure 22. Scanning electron micrograph of osteoblasts attached to 3iT3 dental implants following chlorhexidine gluconate disinfection.

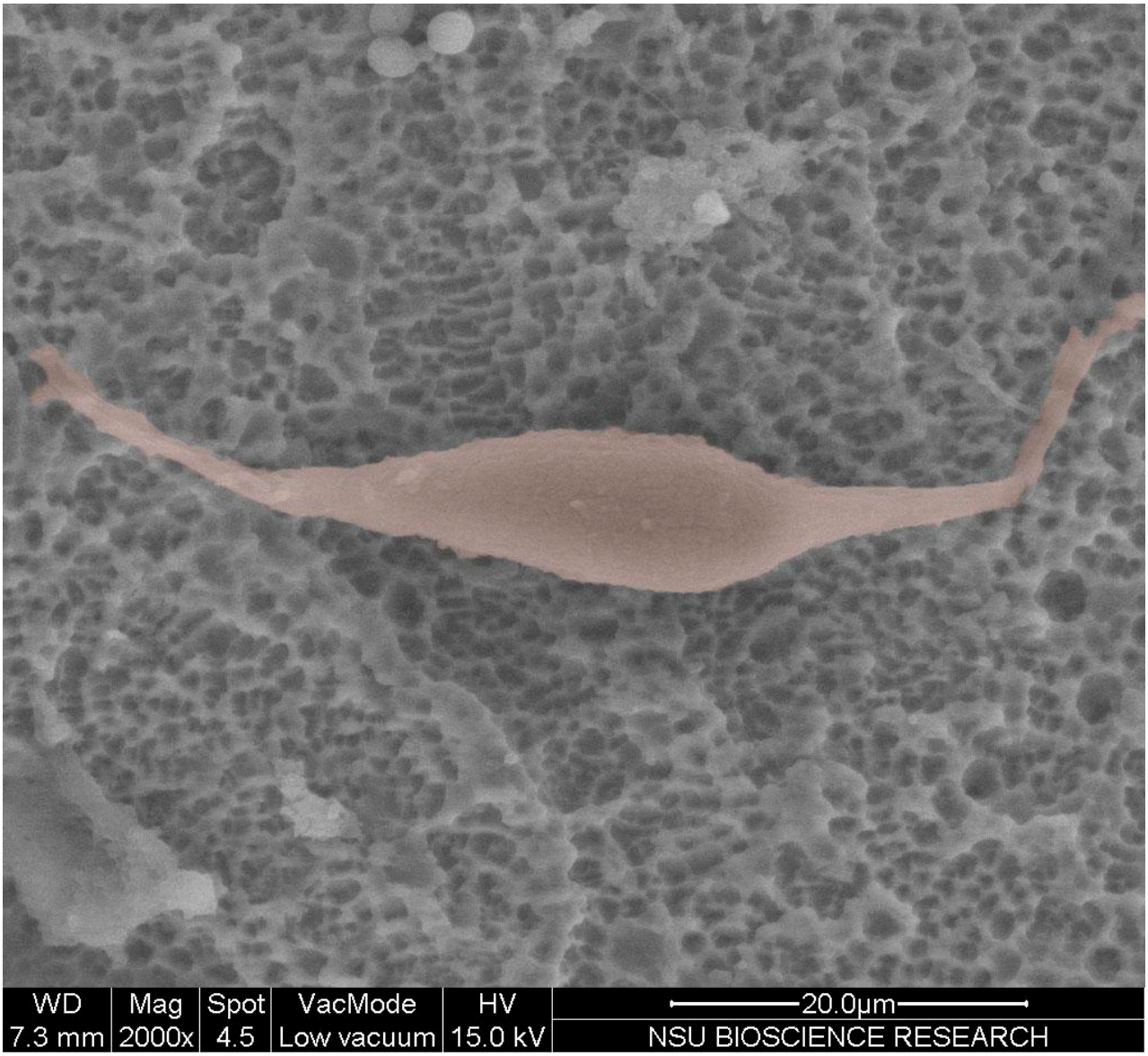


Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

3.6. Osteoblast attachment to 3iT3 dental implant disks with a DCD coating following the disinfection of *P. gingivalis*

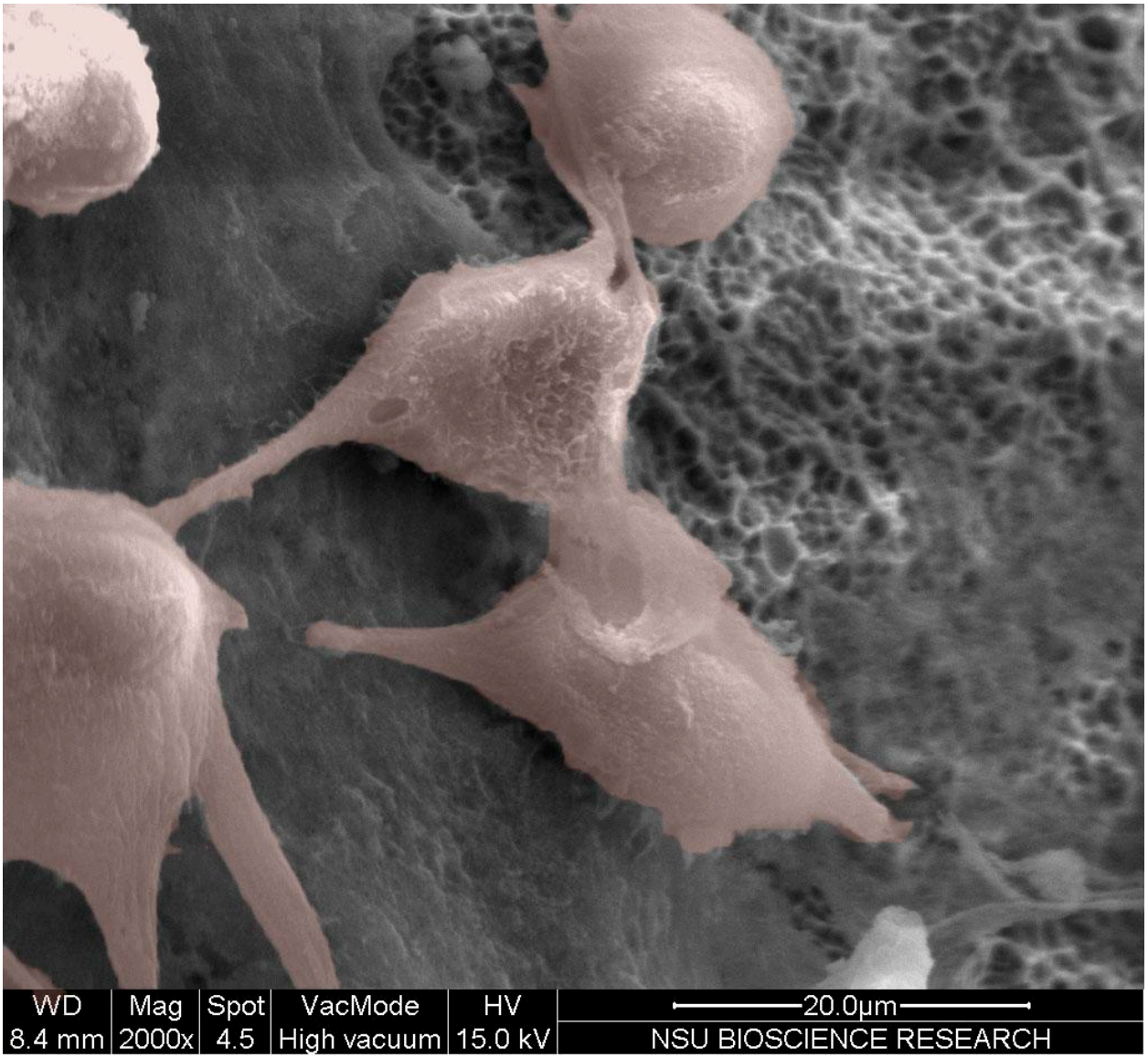
The osteoblasts were observed attached to the surfaces of the 3iT3 dental implant disks with a DCD coating following disinfection treatments using scanning electron microscopy at a magnification of x2,000. Some osteoblasts were seen attached to the 3iT3 dental implants with a DCD coating following disinfection with a Prophy Jet and Sodium bicarbonate (Figure 23). After adding Emdogain to the 3iT3 dental implants with a DCD coating following disinfection with a Prophy Jet and Sodium bicarbonate, some osteoblasts were attached to the surfaces (Figure 24). Osteoblasts were seen attached to the surface of 3iT3 dental implants with a DCD coating that had also been coated with GEM21s after disinfection with a Prophy Jet and Sodium bicarbonate (Figure 25). A few osteoblasts were seen attached to the surface of 3iT3 dental implants with a DCD coating following disinfection with ultrasonically activated hydrogen peroxide (Figure 26). After disinfecting 3iT3 dental implants with a DCD coating using ultrasonically activated chlorhexidine gluconate, some osteoblasts were observed to have attached to the implant surface (Figure 27). Two osteoblasts had attached to the surface of 3iT3 dental implants with a DCD coating following brushing with hydrogen peroxide disinfection (Figure 28). After disinfecting 3iT3 dental implants with a DCD coating by brushing them with chlorhexidine gluconate, some osteoblasts were seen attached to the implant surface (Figure 29). Some osteoblasts were observed attached to the surface of 3iT3 dental implants with a DCD coating following chlorhexidine gluconate disinfection (Figure 30). The attachment of osteoblasts was observed to the surface of a 3iT3 dental implant with a DCD coating following Prophy Jet disinfection with sodium bicarbonate (Figure 31).

Figure 23. Scanning electron micrograph of osteoblasts attached to 3iT3 dental implants with a DCD coating following disinfection with a Prophy Jet and $NaHCO_3$.



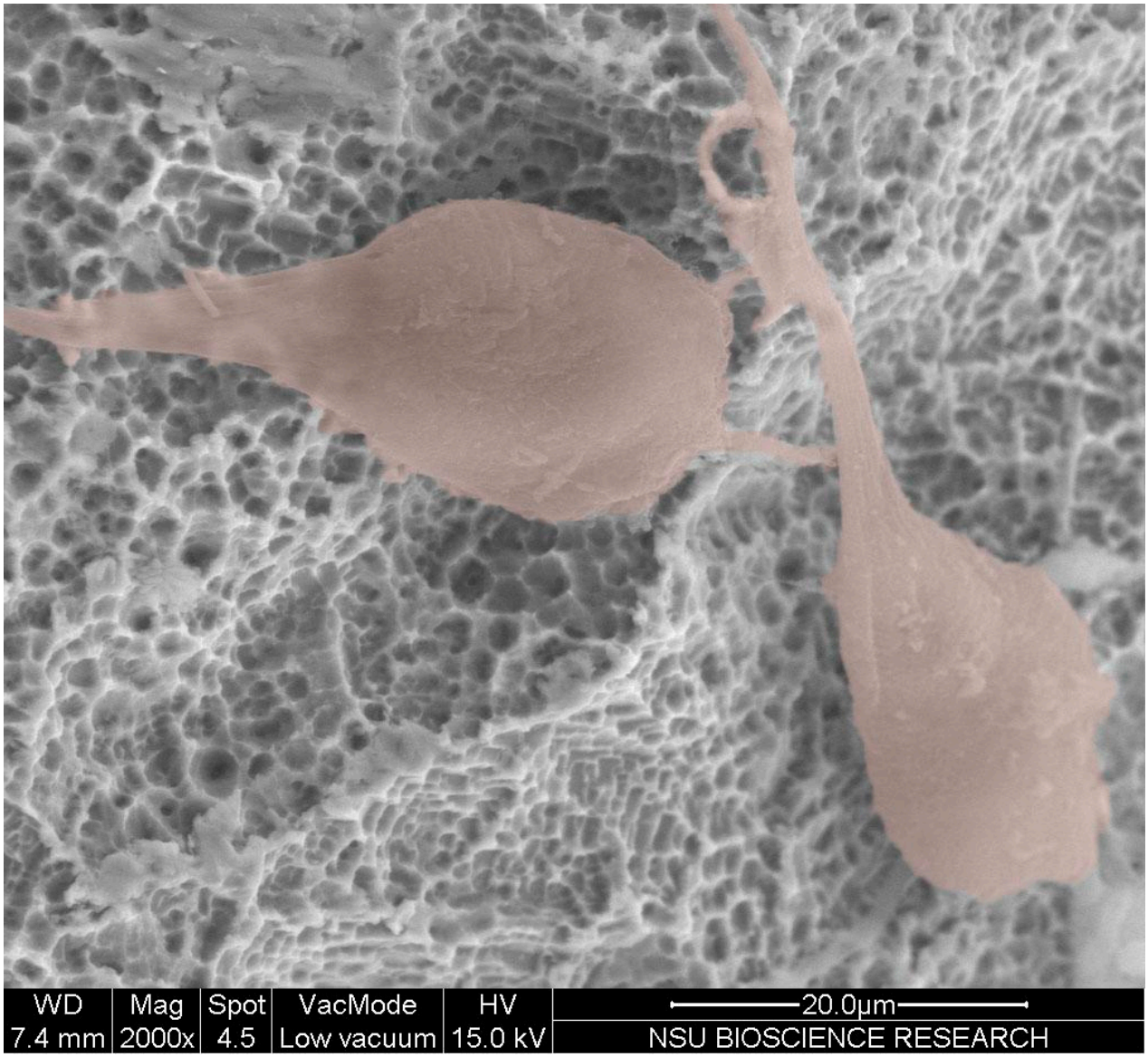
Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

Figure 24. Scanning electron micrograph of osteoblasts attached to 3iT3 dental implants with a DCD coating and also coated with Emdogain following disinfection with a Prophy Jet and $NaHCO_3$.



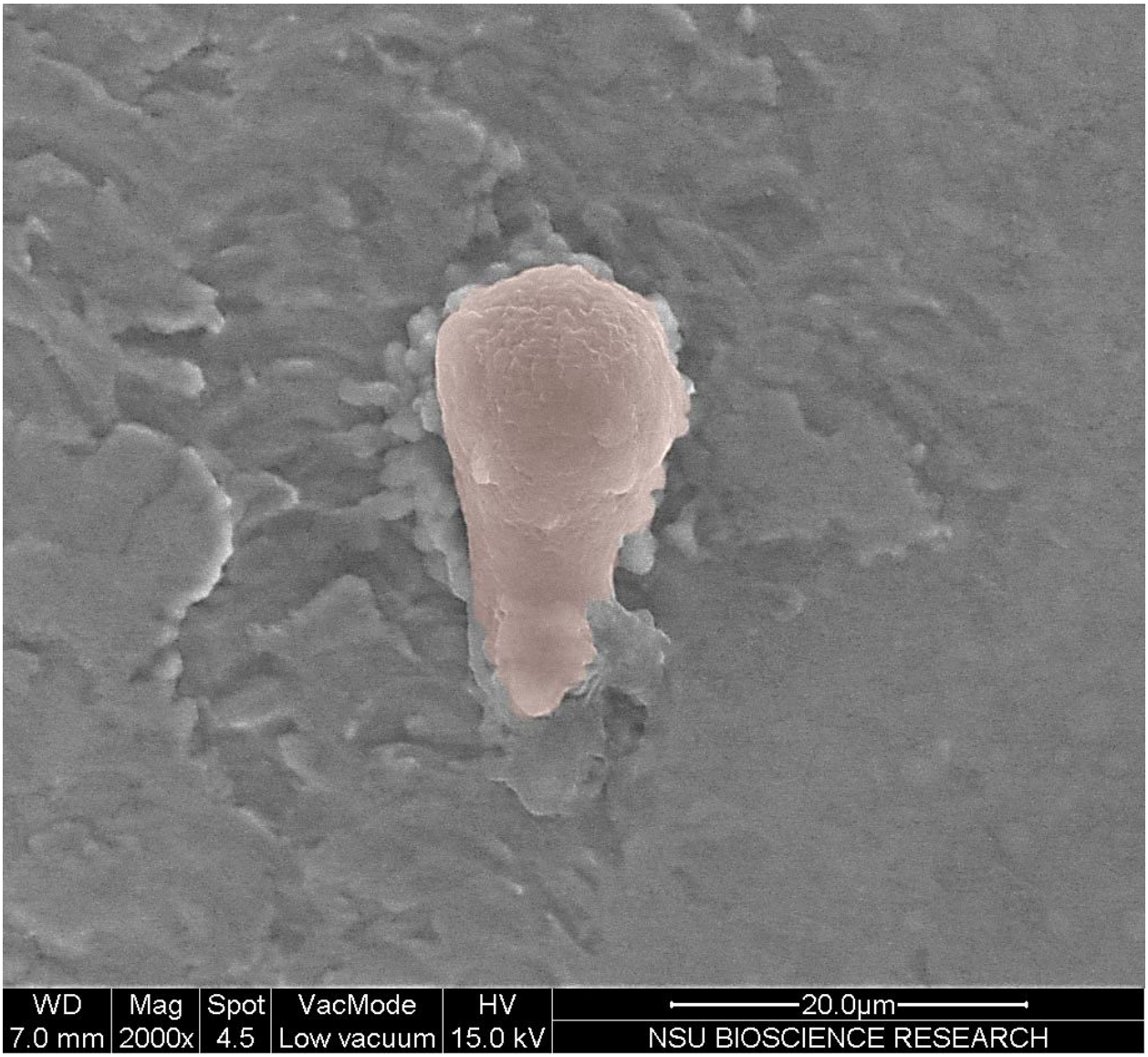
Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

Figure 25. Scanning electron micrograph of osteoblasts attached to disinfected 3iT3 dental implants with a DCD coating and also coated with GEM21s following Prophy Jet disinfection with $NaHCO_3$.



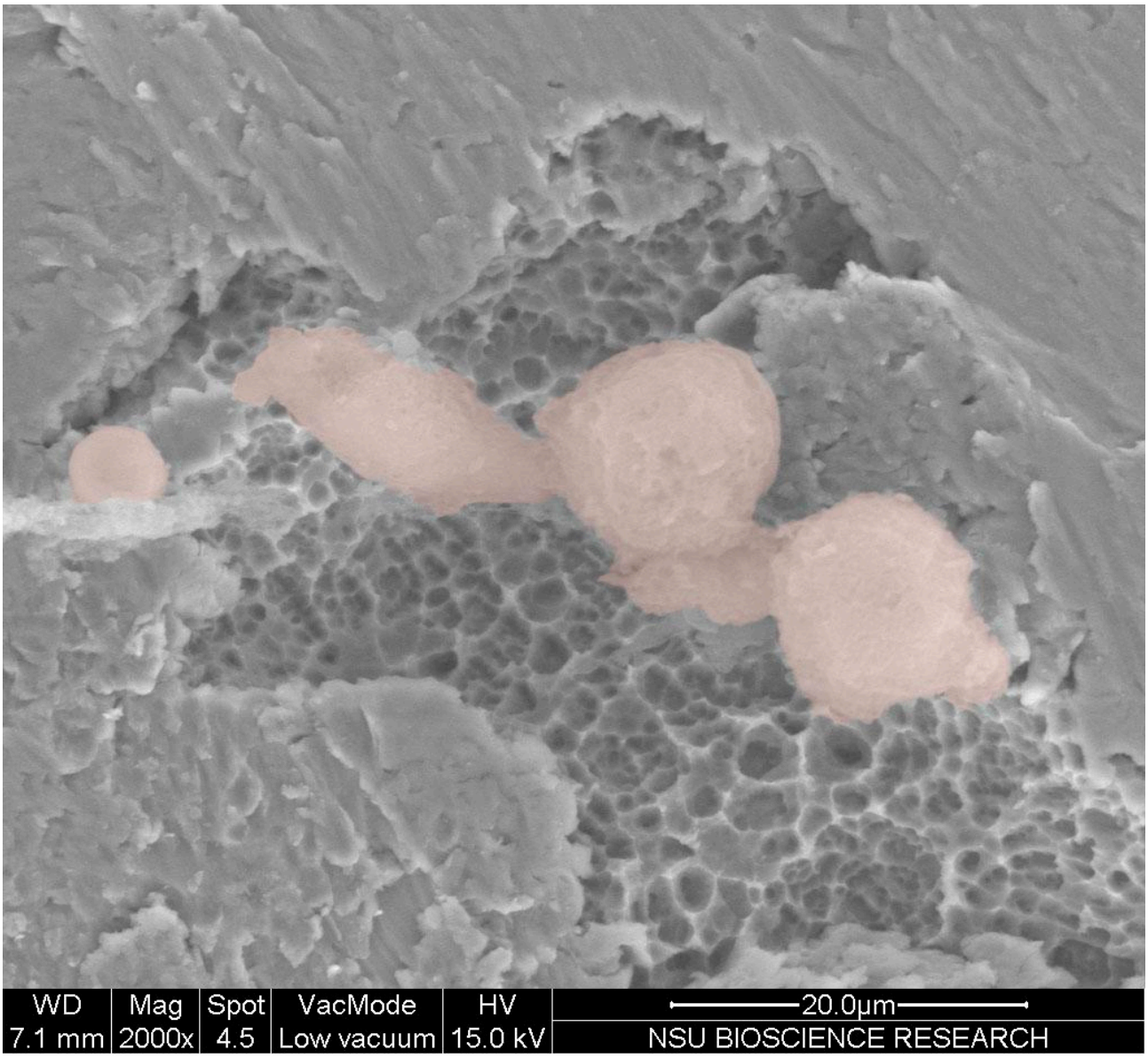
Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

Figure 26. Scanning electron micrograph of osteoblasts attached to 3iT3 dental implants with a DCD coating following ultrasonic hydrogen peroxide disinfection with $NaHCO_3$.



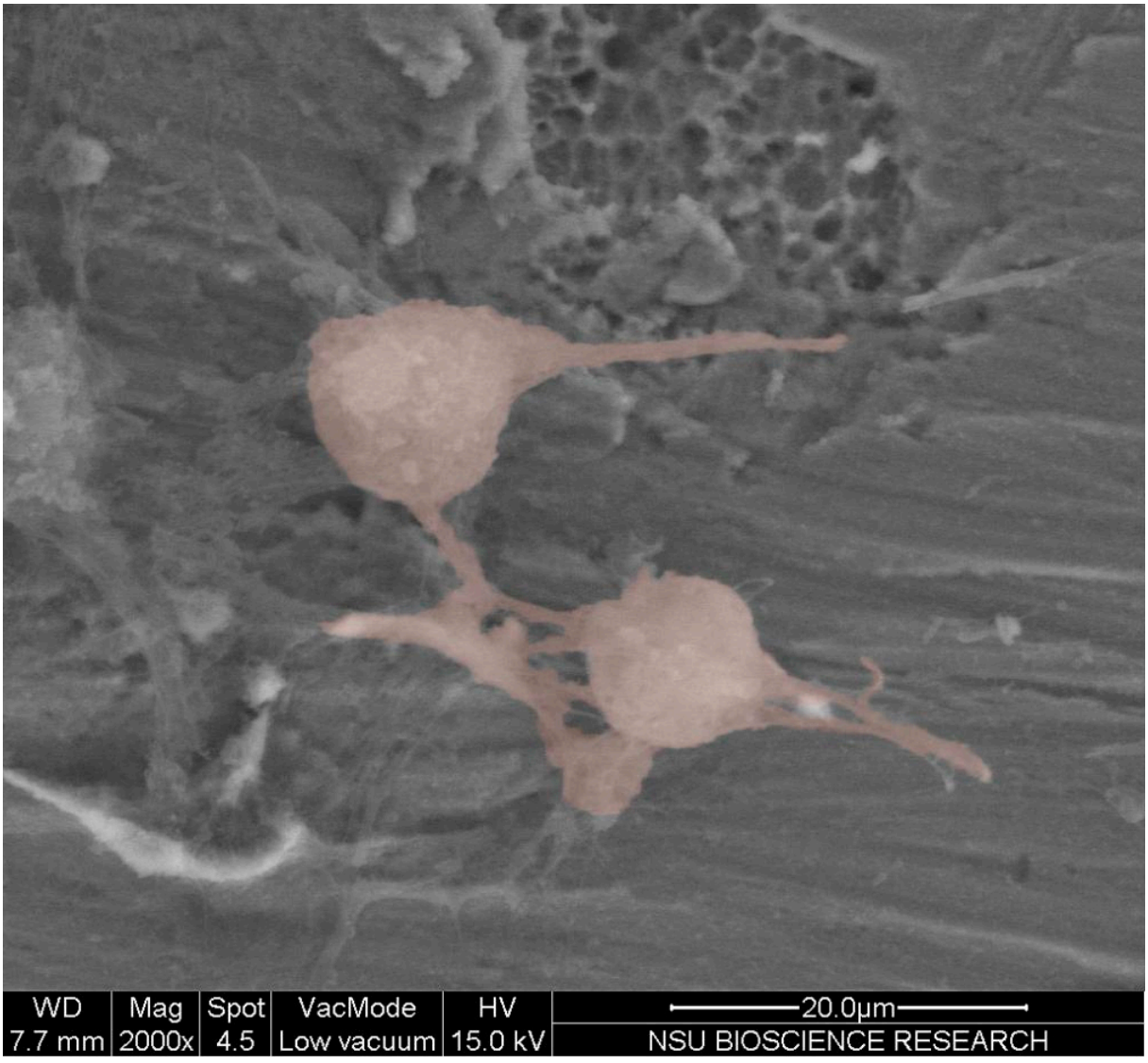
Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

Figure 27. Scanning electron micrograph of osteoblasts attached to 3iT3 dental implants with a DCD coating following ultrasonic chlorhexidine gluconate disinfection.



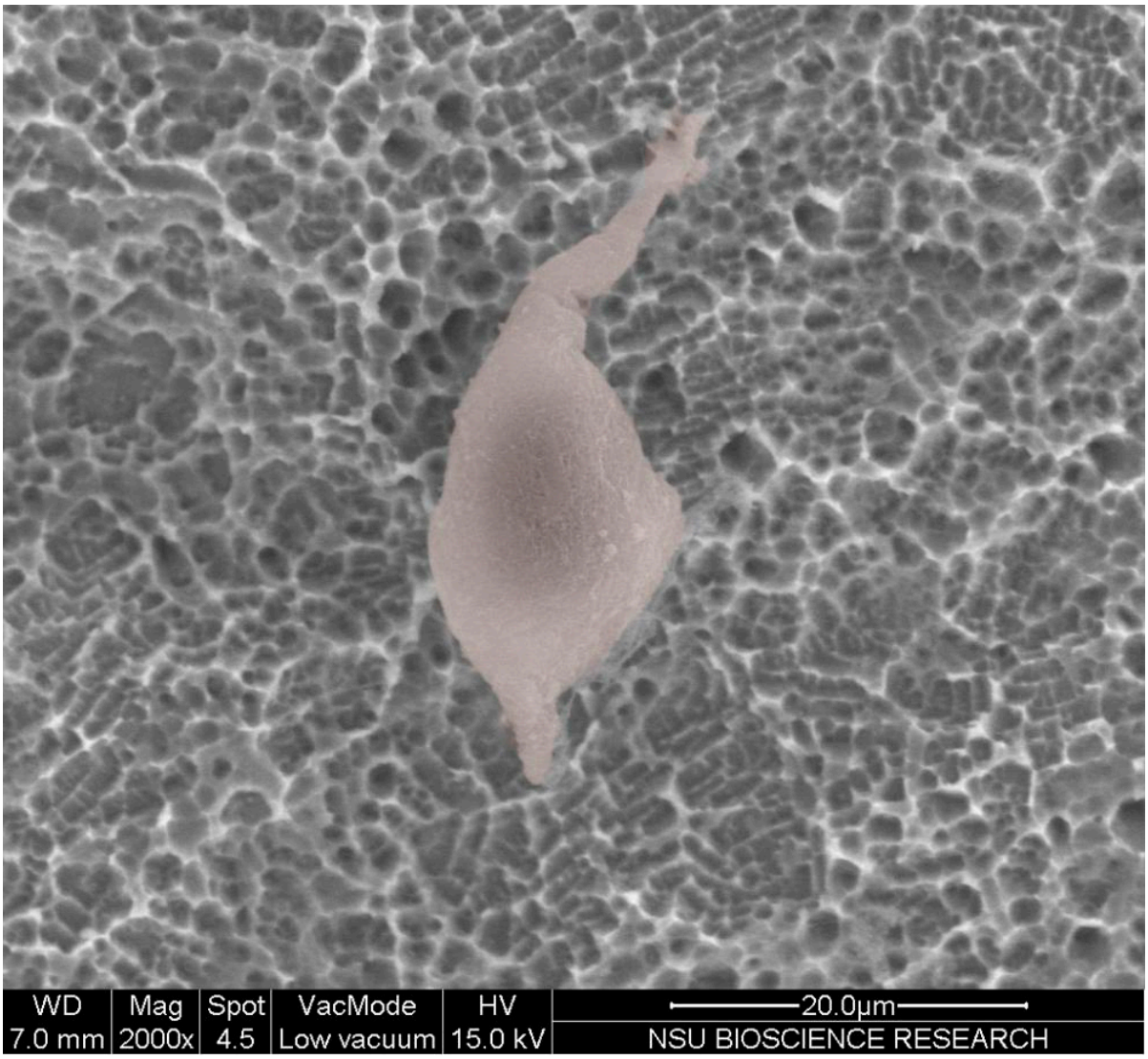
Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

Figure 28. Scanning electron micrograph of osteoblasts attached to 3iT3 dental implants with a DCD coating following brushing with hydrogen peroxide disinfection.



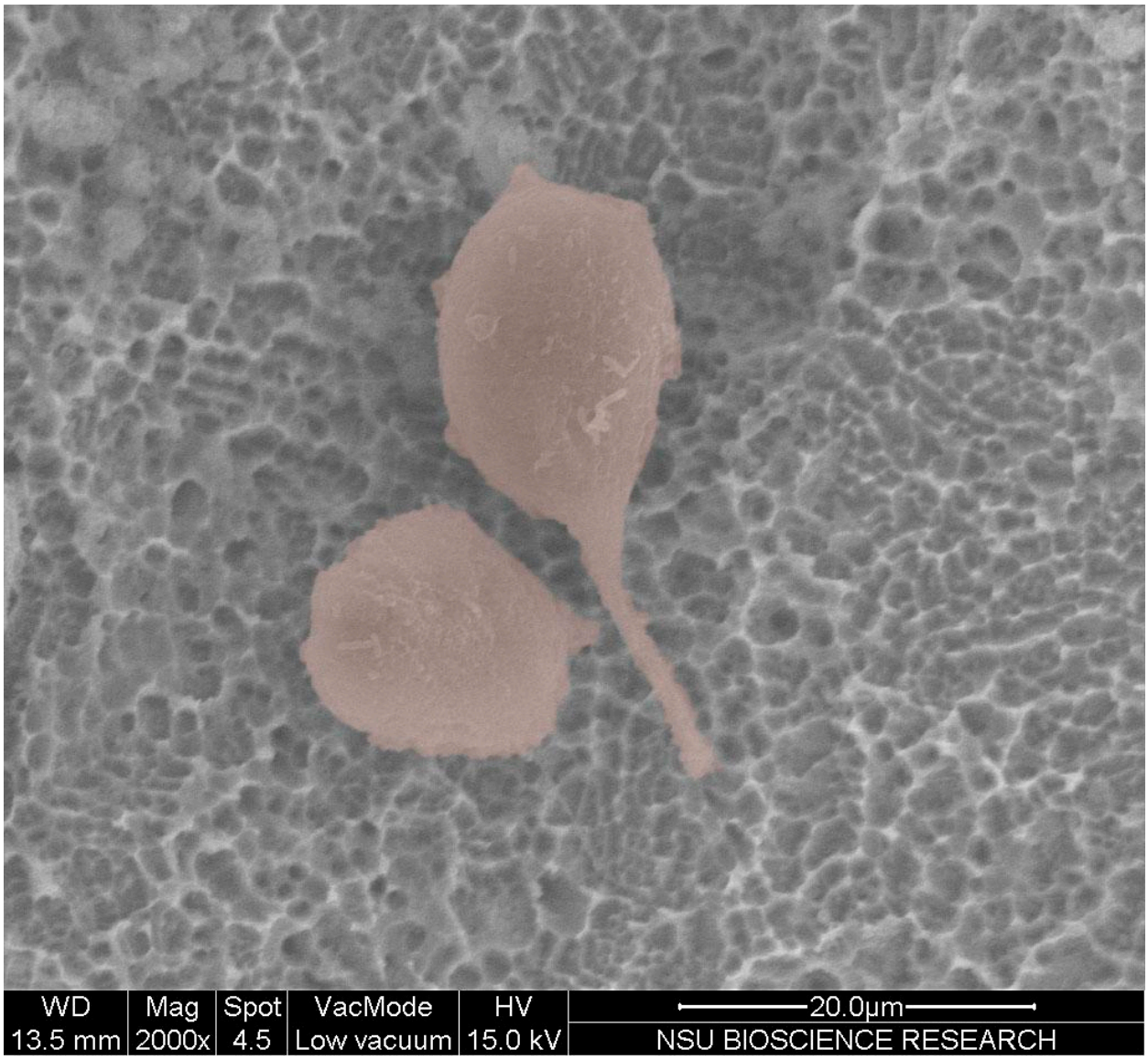
Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

Figure 29. Scanning electron micrograph of osteoblasts attached to 3iT3 dental implants with a DCD coating following brushing with chlorhexidine gluconate disinfection.



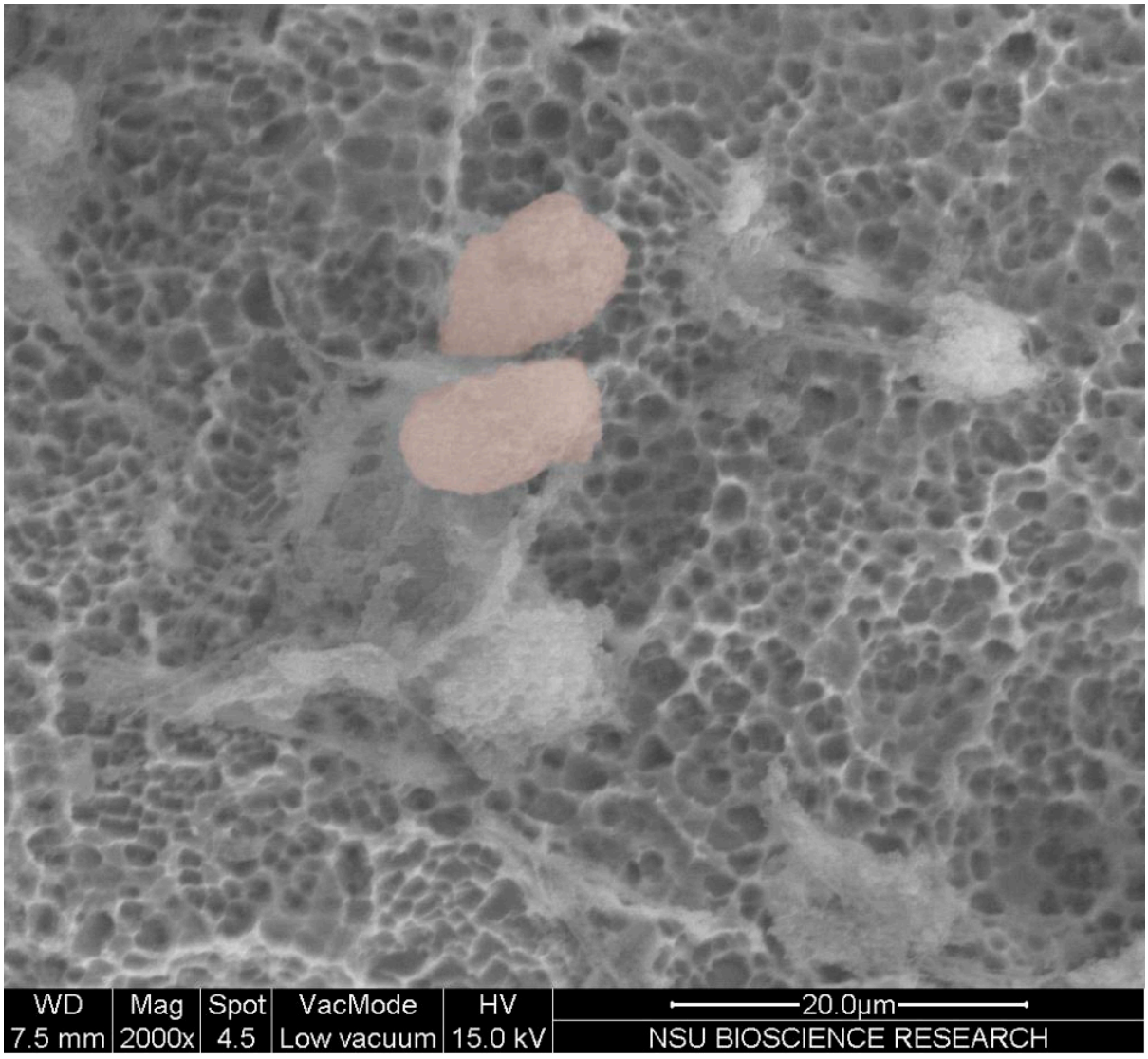
Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

Figure 30. Scanning electron micrograph of osteoblasts attached to 3iT3 dental implants with a DCD coating following chlorhexidine gluconate disinfection.



Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

Figure 31. Scanning electron micrograph of osteoblasts attached to 3iT3 dental implants with a DCD coating following Prophy Jet disinfection with sodium bicarbonate.



Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

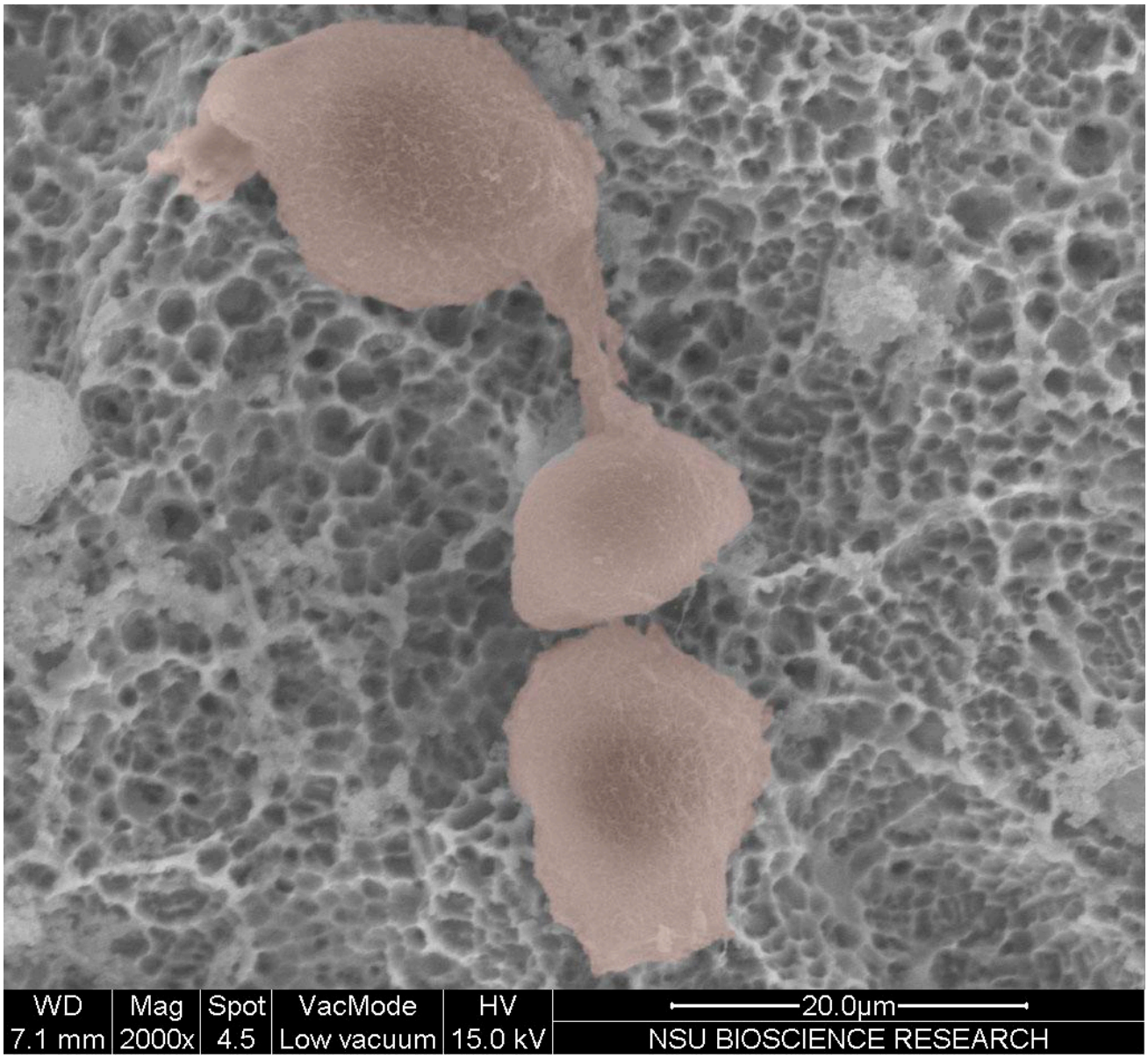
Osteoblasts were seen that had attached to 3iT3 dental implants with a DCD coating which were not infected with *P. gingivalis* and following Prophy Jet disinfection with sodium bicarbonate (Figure 32). Some osteoblasts had attached to the surface of 3iT3 dental implants with a DCD coating, which had no osteoblasts added, and following Prophy Jet disinfection with sodium bicarbonate (Figure 33). Osteoblasts had attached to the surface of 3iT3 dental implants with a DCD coating which were not infected with *P. gingivalis* and following Prophy Jet disinfection with sodium bicarbonate (Figure 34).

3.7. Analysis of osteoblast attachment to 3iT3 dental implant disks following the disinfection of *P. gingivalis*.

The numbers of osteoblasts were counted on scanning electron micrograph (SEM) that were seen to be attached to each disinfected dental implant surface. There were a mean number of 33 osteoblasts attached to the 3iT3 dental implant disk surfaces, and it was 9% higher at 36 osteoblasts per SEM attached to the 3iT3 implants with a DCD coating (Figure 35). Many of the disinfected implants had none or few osteoblasts attached, and this gave large standard deviations of the means (Figure 35). The DCD coating made little difference to the numbers of osteoblasts that attached to the 3iT3 implant surfaces (ANOVA P=0.7713, Power 0.059).

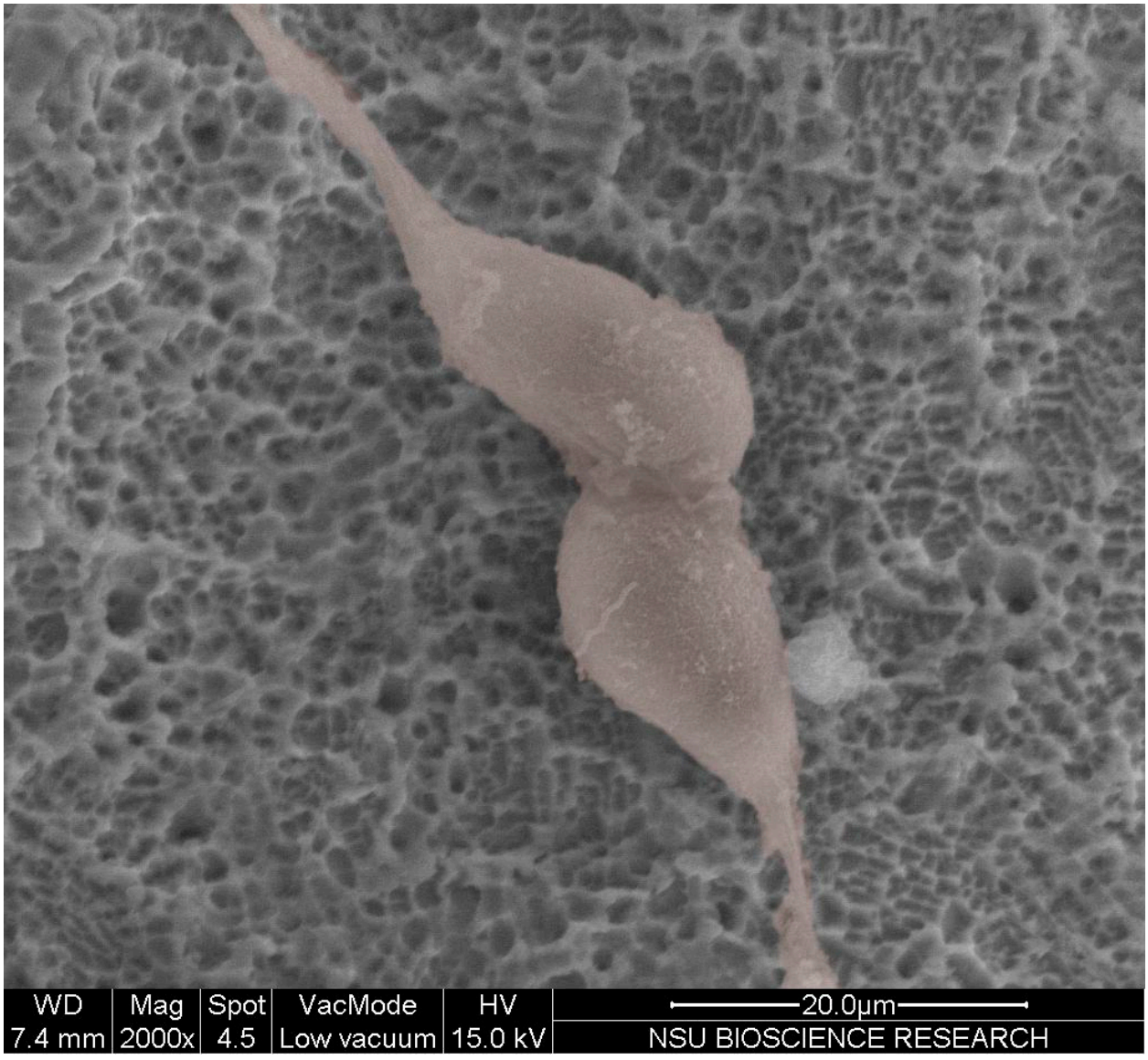
The numbers of osteoblasts attached to the 3iT3 dental implant disk surfaces appeared to be strongly affected by the method used for the physical disinfection of *P. gingivalis* (ANOVA P=0.0002, Power 0.988).

Figure 32. Scanning electron micrograph of osteoblasts attached to 3iT3 dental implants with a DCD coating which were not infected with *P. gingivalis* and following Prophy Jet disinfection with sodium bicarbonate.



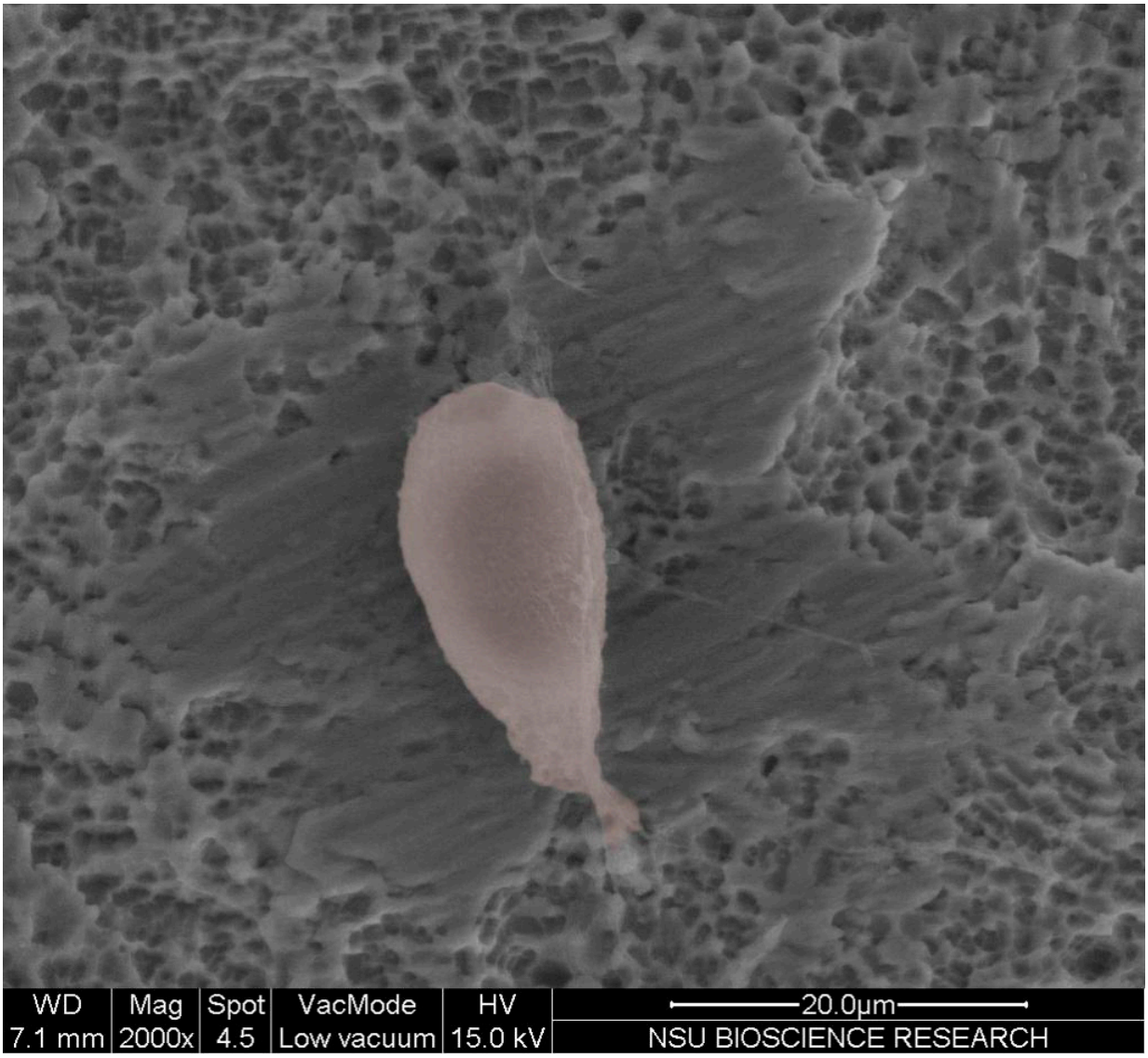
Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

Figure 33. Scanning electron micrograph of 3iT3 dental implants with a DCD coating which had no osteoblasts added and following Prophy Jet disinfection with sodium bicarbonate.



Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

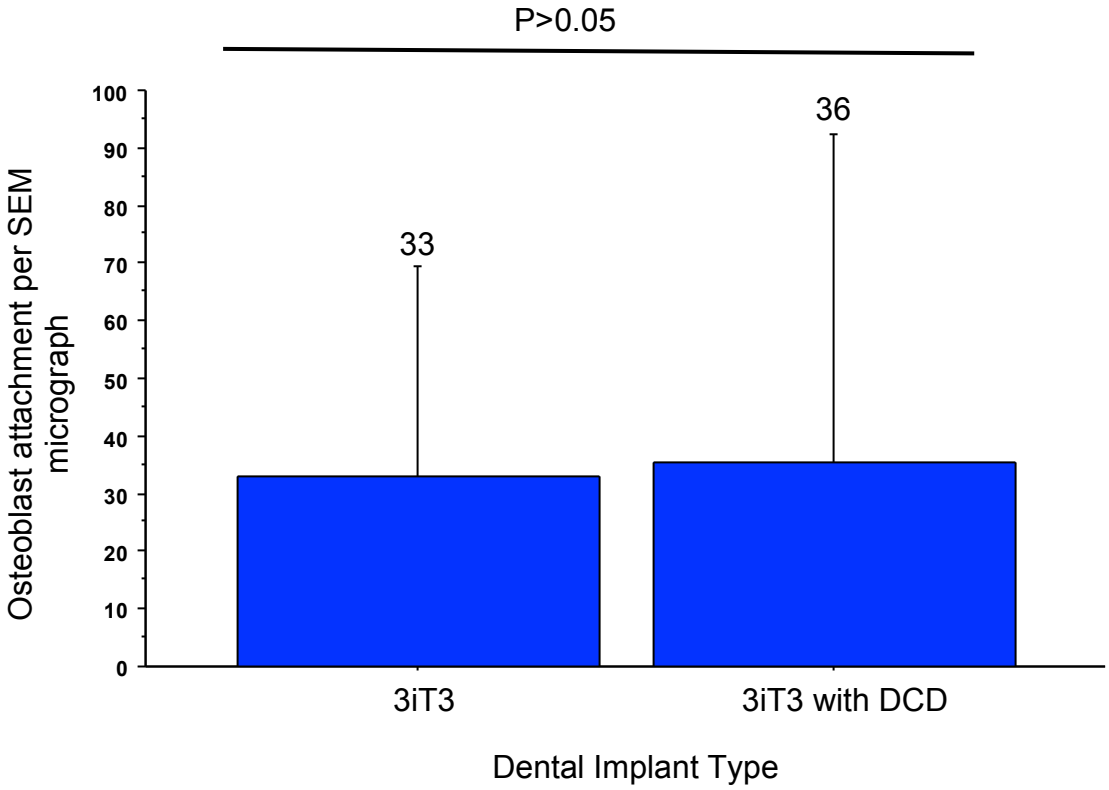
Figure 34. Scanning electron micrograph of osteoblasts attached to 3iT3 dental implants with a DCD coating which were not infected with *P. gingivalis* and following Prophy Jet disinfection with sodium bicarbonate.



Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

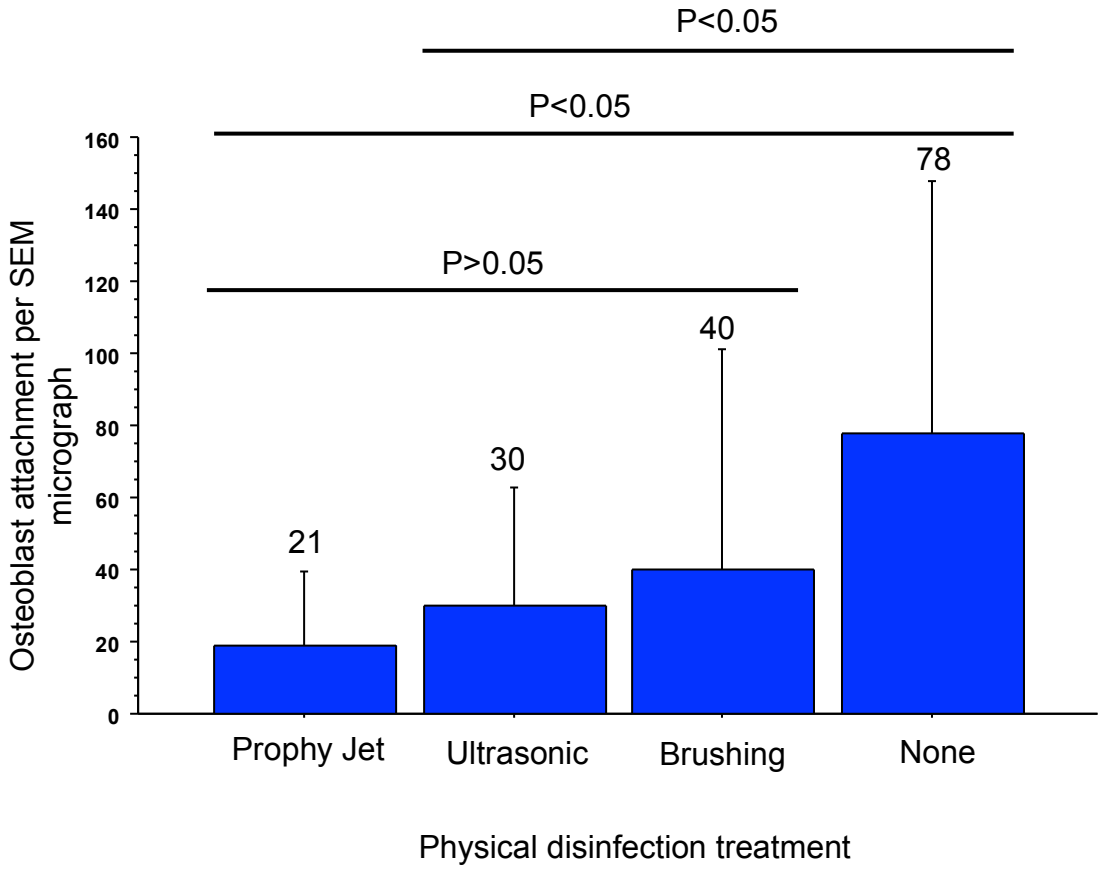
A comparison of the numbers of osteoblasts attached to the 3iT3 dental implant surfaces following disinfection with a Prophy Jet spray of Sodium bicarbonate, or the ultrasonic activation of Chlorhexidine gluconate or Hydrogen peroxide, or the brushing of Chlorhexidine gluconate or Hydrogen peroxide, or the use of Chlorhexidine gluconate without any physical disinfection produced some unexpected results. There was a mean of 21 osteoblasts attached to the 3iT3 dental implant surfaces following disinfection with a Prophy Jet spray of Sodium bicarbonate (Figure 36). In comparison to the Prophy Jet, there was a 43% increase in the mean number of 30 osteoblasts attached to the 3iT3 dental implant surfaces following disinfection with the ultrasonic activation of Chlorhexidine gluconate or Hydrogen peroxide (Figure 36). In comparison to the ultrasonic activation of disinfectants, there was a 33% increase in the mean number of 40 osteoblasts attached to the 3iT3 dental implant surfaces following disinfection by brushing with Chlorhexidine gluconate or Hydrogen peroxide (Figure 36). In comparison to the brushing of disinfectants, there was a 95% increase in the mean number of 78 osteoblasts attached to the 3iT3 dental implant surfaces that had no physical activation (Figure 36). There were large standard deviations between the means of the numbers of osteoblasts attached to the 3iT3 dental implant surfaces following the physical disinfection with a Prophy Jet, ultrasonic, brushing, and no activation, consequently there was little difference between these treatments (Scheffe post hoc ANOVA $P > 0.05$). There were differences between the mean numbers of osteoblasts attached without physical activation and all the other treatments except brushing (Scheffe post hoc ANOVA $P < 0.05$). The DCD coating on 3iT3 dental implant surfaces made little difference to the numbers of osteoblasts that attached to the implant surface following disinfection (ANOVA $P=0.5810$, Power 0.084) (Figure 37).

Figure 35. Bar chart of osteoblast attachment to 3iT3 disks versus 3iT3 disks with DCD following the physical disinfection of *P. gingivalis*.



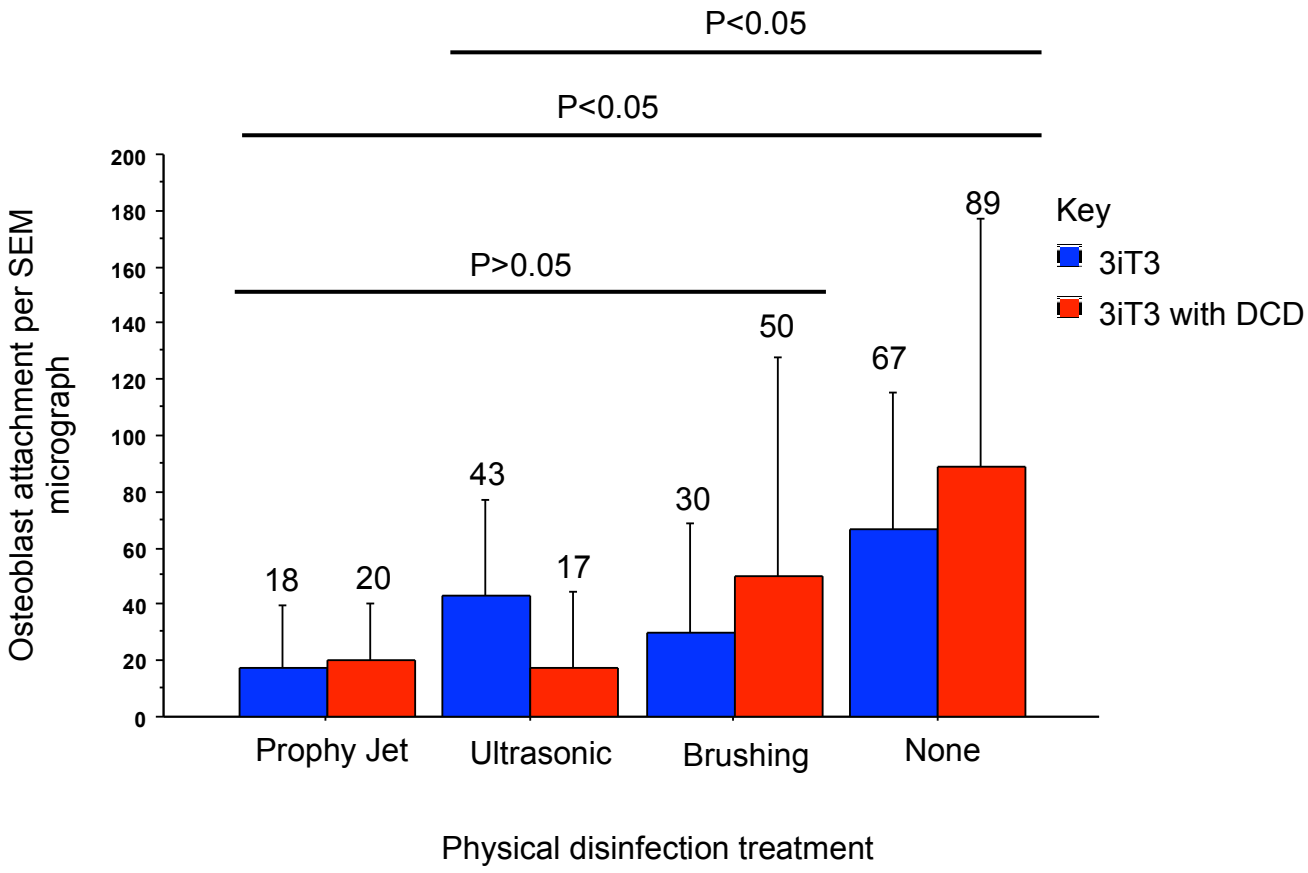
The bar char represents the means of each treatment group which contained 64 specimens. The standard error bars represent the standard deviation of the means. The numbers of osteoblasts attached to the implant surface were counted per scanning electron micrograph at a magnification of x2,000.

Figure 36. Bar chart of osteoblast attachment to 3iT3 disks following the physical disinfection of *P. gingivalis*.



The bar char represents the means of each treatment group which contained 16 to 48 specimens. The standard error bars represent the standard deviation of the means. The numbers of osteoblasts attached to the implant surface were counted per scanning electron micrograph at a magnification of x2,000.

Figure 37. Bar chart of osteoblast viability on 3iT3 disks versus 3iT3 with DCD disks following the physical disinfection of *P. gingivalis*.



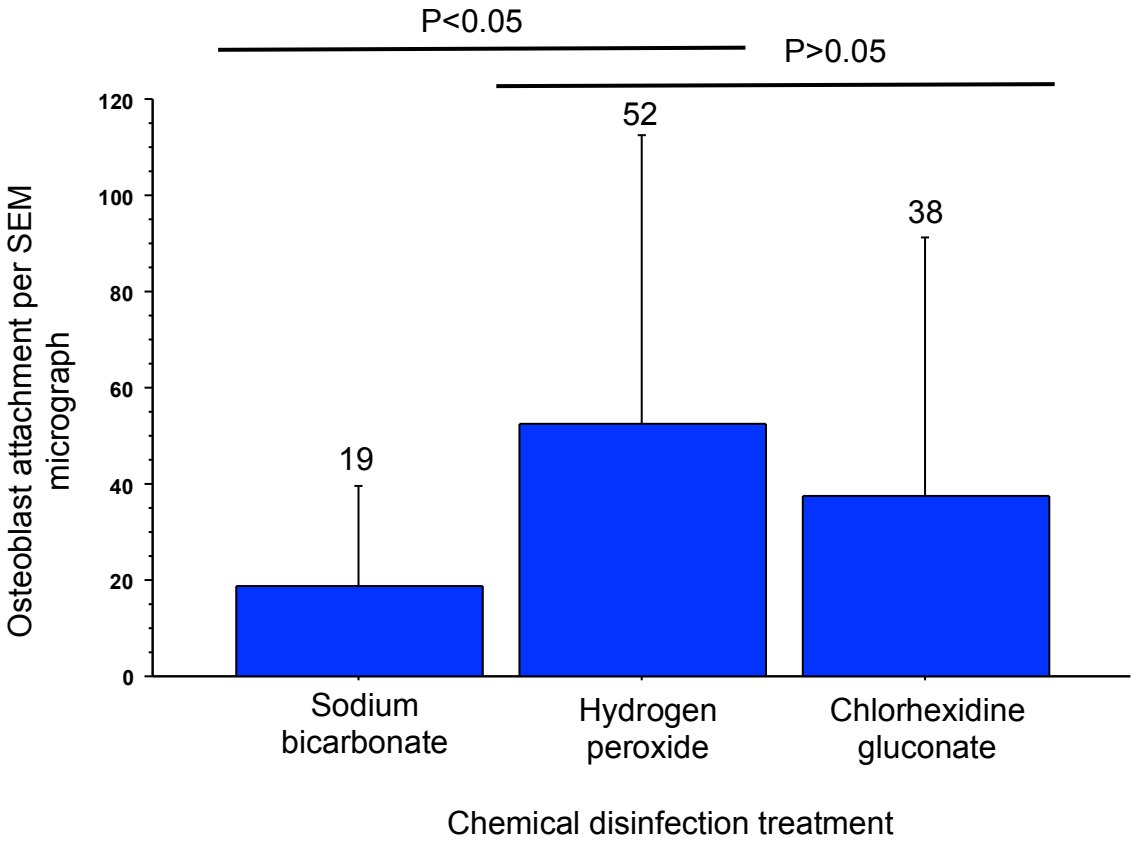
The bar char represents the means of each treatment group which contained 8 to 24 specimens. The standard error bars represent the standard deviation of the means. The numbers of osteoblasts attached to the implant surface were counted per scanning electron micrograph at a magnification of x2,000.

The chemicals used to disinfect the 3iT3 dental implant disks had an effect on the numbers of osteoblasts attached to their surface (ANOVA P=0.0064, Power 0.836). The lowest mean numbers of osteoblasts were the 19 attached to the implant surfaces following disinfection with Sodium bicarbonate (Figure 38). In comparison to the 3iT3 dental implant disks disinfected using Sodium bicarbonate, there was a 100% increase in the mean number of 38 osteoblasts attached to the implants that were disinfected using Chlorhexidine gluconate (Scheffe post hoc ANOVA P=0.1435) (Figure 38). In comparison to the 3iT3 dental implant disks disinfected using Chlorhexidine gluconate, there was a 37% increase in the mean number of 52 osteoblasts attached to the implants that were disinfected using Hydrogen peroxide (Scheffe post hoc ANOVA P=0.3838) (Figure 38). Due to the variability of the data due to the large standard deviations of the means, the greatest difference between the chemical treatments and the attachment of osteoblasts was between the 3iT3 dental implant disks disinfected with Sodium bicarbonate and Hydrogen peroxide (Scheffe post hoc ANOVA P=0.0077) (Figure 38).

A comparison of the effect of the chemicals used to disinfect the 3iT3 dental implant surfaces according to the surface type, without a coating or with a DCD coating, revealed that the implant surface coating had little effect on the numbers of attached osteoblasts (ANOVA P=0.8228, Power 0.056) (Figure 39).

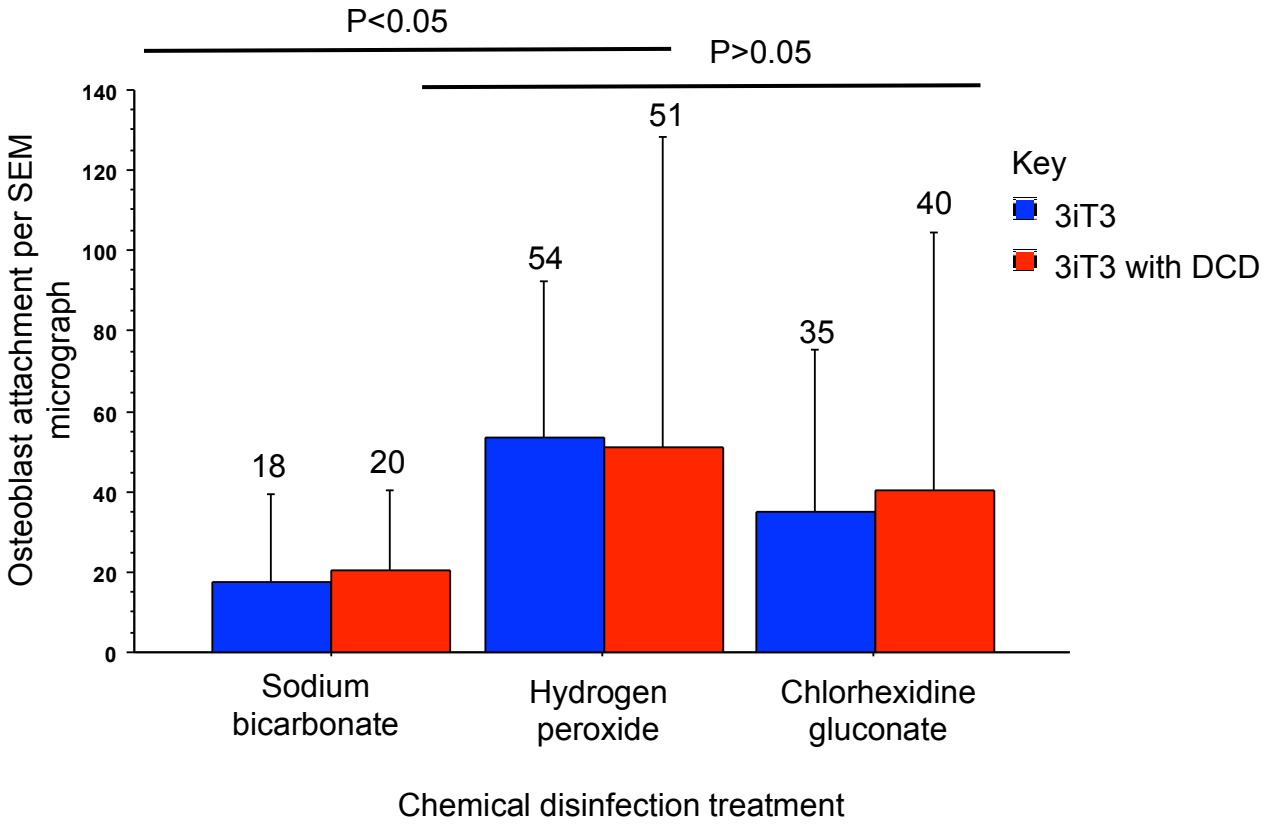
A comparison of the chemical and physical methods to disinfect the 3iT3 dental implant surfaces revealed that the disinfection methods had a powerful effect on the attachment of osteoblasts to the implant surface (ANOVA P=0.0001, Power 1.0) (Figure 40).

Figure 38. Bar chart of osteoblast attachment to 3iT3 disks following the chemical disinfection of *P. gingivalis*.



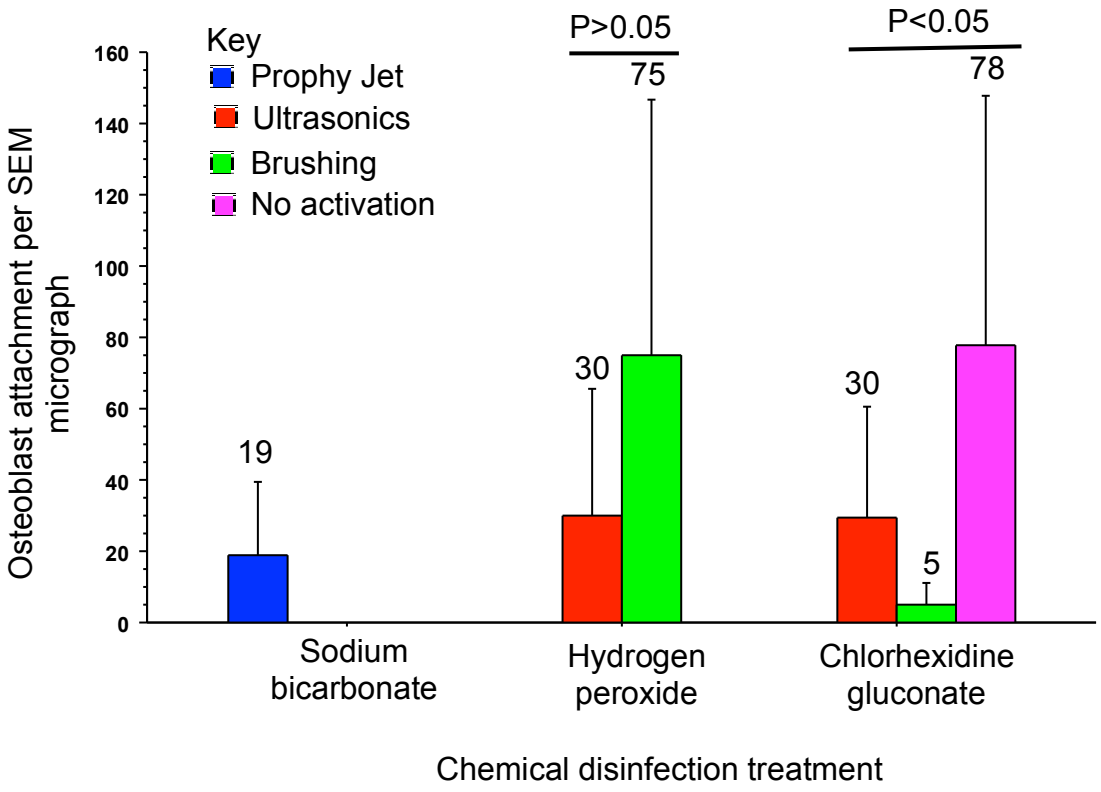
The bar char represents the means of each treatment group which contained 8 to 24 specimens. The standard error bars represent the standard deviation of the means. The numbers of osteoblasts attached to the implant surface were counted per scanning electron micrograph at a magnification of x2,000.

Figure 39. Bar chart of osteoblast attachment to 3iT3 disks versus 3iT3 disks with DCD following the chemical disinfection of *P. gingivalis.*



The bar char represents the means of each treatment group which contained 16 to 24 specimens. The standard error bars represent the standard deviation of the means. The numbers of osteoblasts attached to the implant surface were counted per scanning electron micrograph at a magnification of x2,000.

Figure 40. Bar chart of osteoblast attachment to 3iT3 disks following the chemical and physical disinfection of *P. gingivalis*.



The bar char represents the means of each treatment group which contained 8 to 12 specimens. The standard error bars represent the standard deviation of the means. The numbers of osteoblasts attached to the implant surface were counted per scanning electron micrograph at a magnification of x2,000.

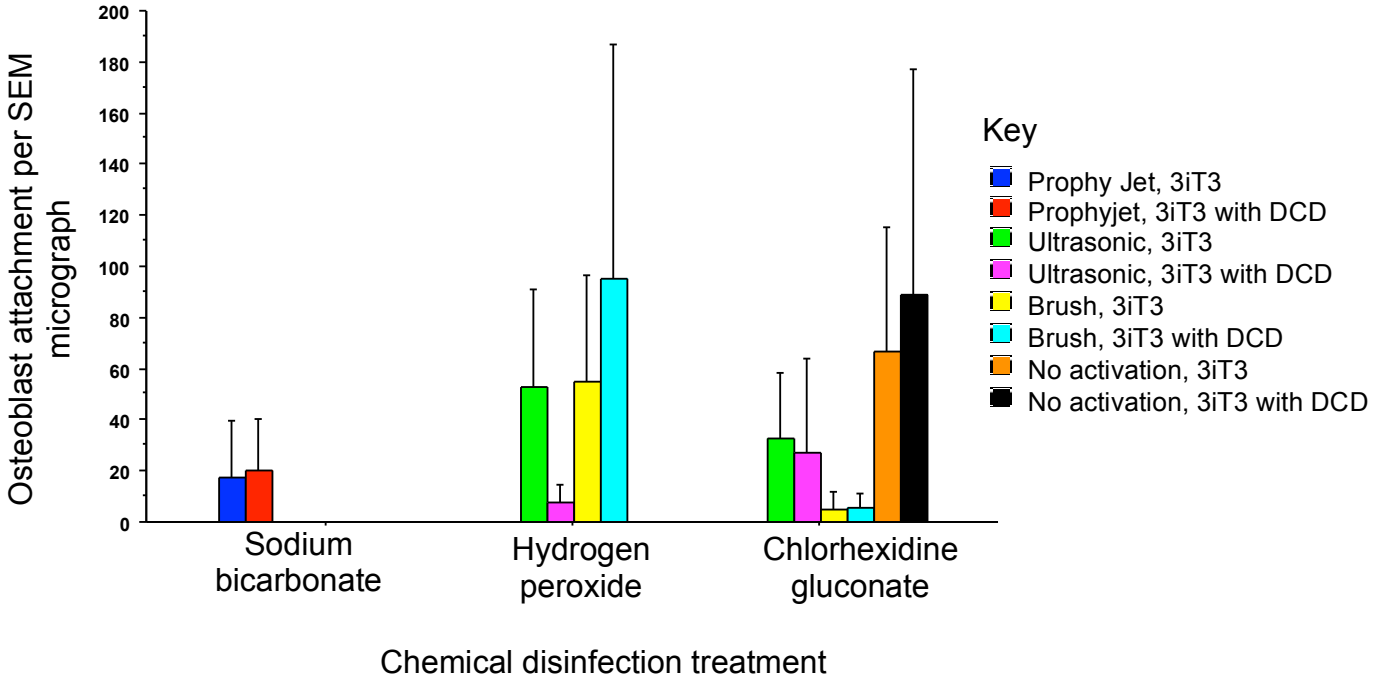
The highest mean numbers of 78 osteoblasts attached to the disinfected 3iT3 implant surface were observed following the non-physical activation of Chlorhexidine gluconate (Figure 40).

A comparison of the effect of the physical disinfection treatments according to the type of 3iT3 dental implant surface, with or without a DCD coating, found that the DCD coating made little difference to the numbers of osteoblasts that attached to the implant surface following disinfection (ANOVA P=0.7580, Power 0.061) (Figure 41).

The coating of the disinfected 3iT3 dental implant surfaces with growth factors; Emdogain or GEM 21s had a powerful effect on the numbers of osteoblasts that had attached (ANOVA P=0.0002, Power 0.990). Interestingly the effect of Emdogain and GEM 21s were very different on the numbers of osteoblasts that attached to the 3iT3 dental implant surfaces. The coating of the disinfected implant surface with Emdogain reduced the mean numbers of attached osteoblasts by 87% from a mean of 23 osteoblasts per implant surface without any growth factor, to 3 osteoblasts per SEM micrograph (Scheffe post hoc ANOVA P=0.094) (Figure 42). The coating of the 3iT3 implant surfaces with GEM21s increased the mean numbers of attached osteoblasts by 35% from a mean of 23 osteoblasts per implant surface without any growth factor, to 31 osteoblasts per SEM micrograph (Scheffe post hoc ANOVA P=0.4884) (Figure 42).

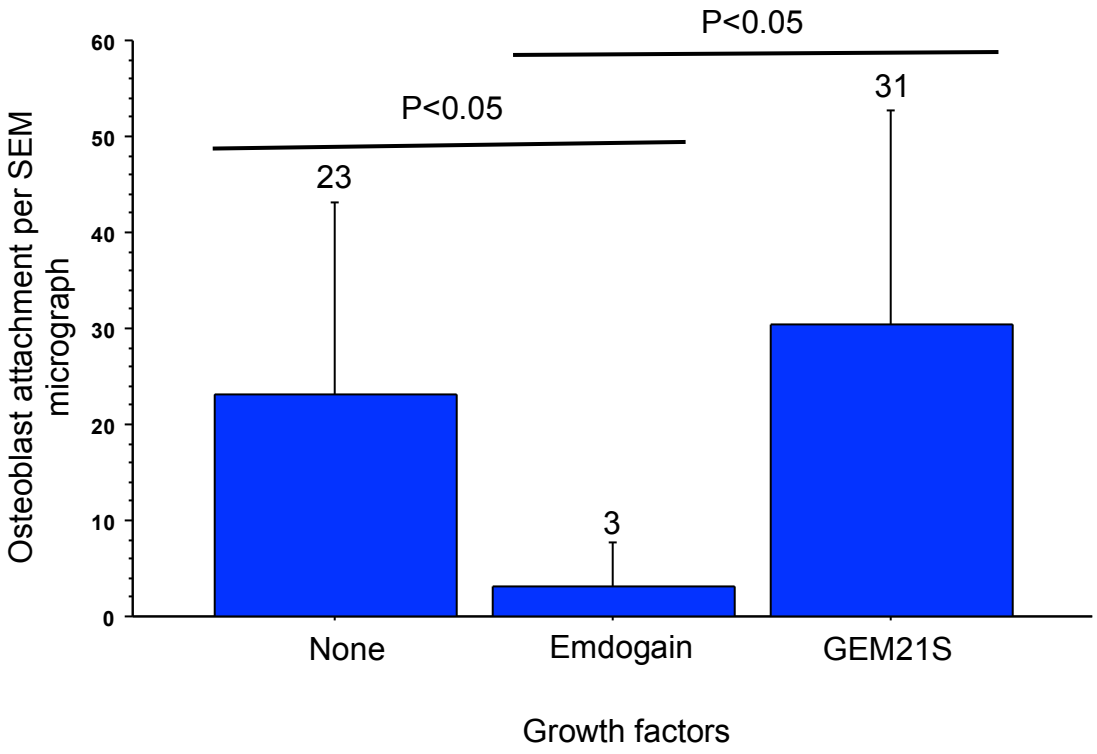
A comparison of the effect of growth factors treatments according to the type of 3iT3 dental implant surface, with or without a DCD coating, found that the DCD coating made little difference to the numbers of osteoblasts that attached to the implant surface following disinfection (ANOVA P=0.6065, Power 0.079) (Figure 43).

Figure 41. Bar chart of osteoblast attachment to 3iT3 disks versus 3iT3 disks with DCD following the chemical and physical disinfection of *P. gingivalis*.



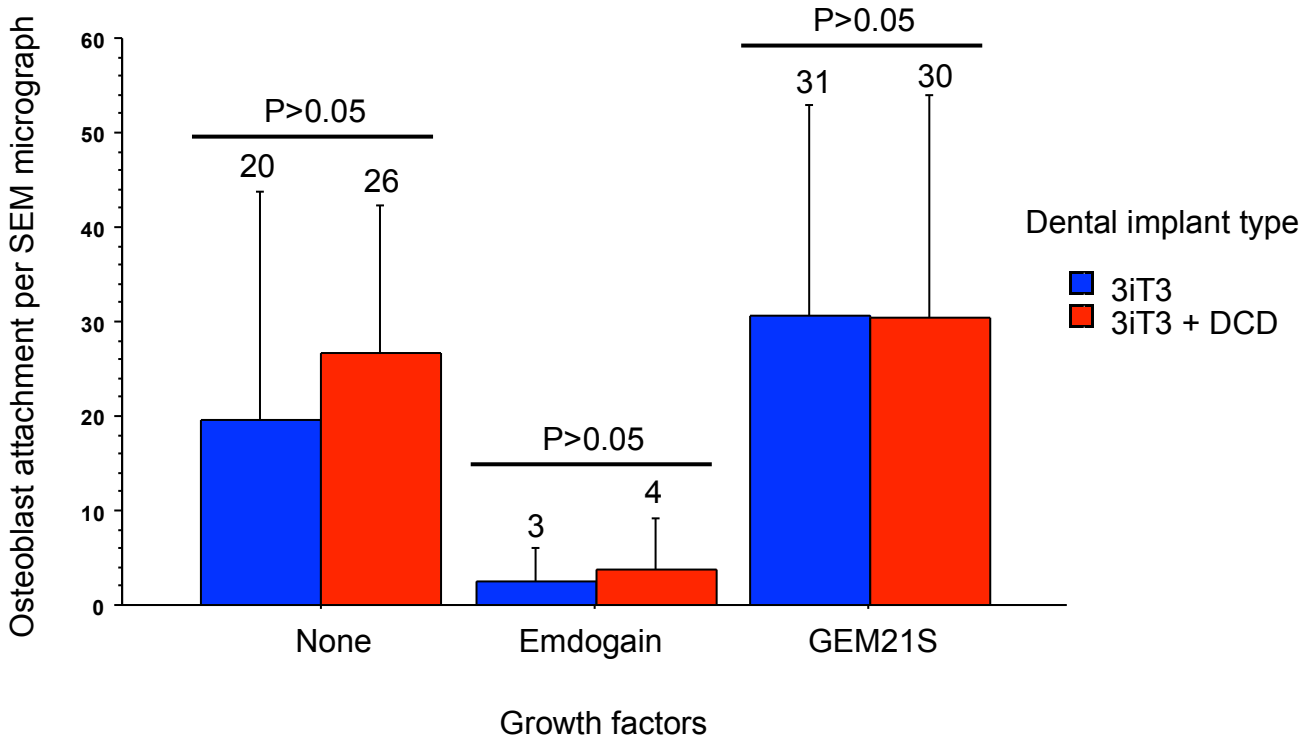
The bar char represents the means of each treatment group which contained 8 to 12 specimens. The standard error bars represent the standard deviation of the means. The numbers of osteoblasts attached to the implant surface were counted per scanning electron micrograph at a magnification of x2,000.

Figure 42. Bar chart of osteoblast attachment to 3iT3 disks treated with growth factors following the chemical and physical disinfection of *P. gingivalis*.



The bar char represents the means of each treatment group which contained 16 specimens. The standard error bars represent the standard deviation of the means. The numbers of osteoblasts attached to the implant surface were counted per scanning electron micrograph at a magnification of x2,000.

Figure 43. Bar chart of osteoblast viability on 3iT3 disks versus 3iT3 disks with DCD treated with growth factors following the chemical and physical disinfection of *P. gingivalis*.



The bar char represents the means of each treatment group which contained 8 specimens. The standard error bars represent the standard deviation of the means. The numbers of osteoblasts attached to the implant surface were counted per scanning electron micrograph at a magnification of x2,000.

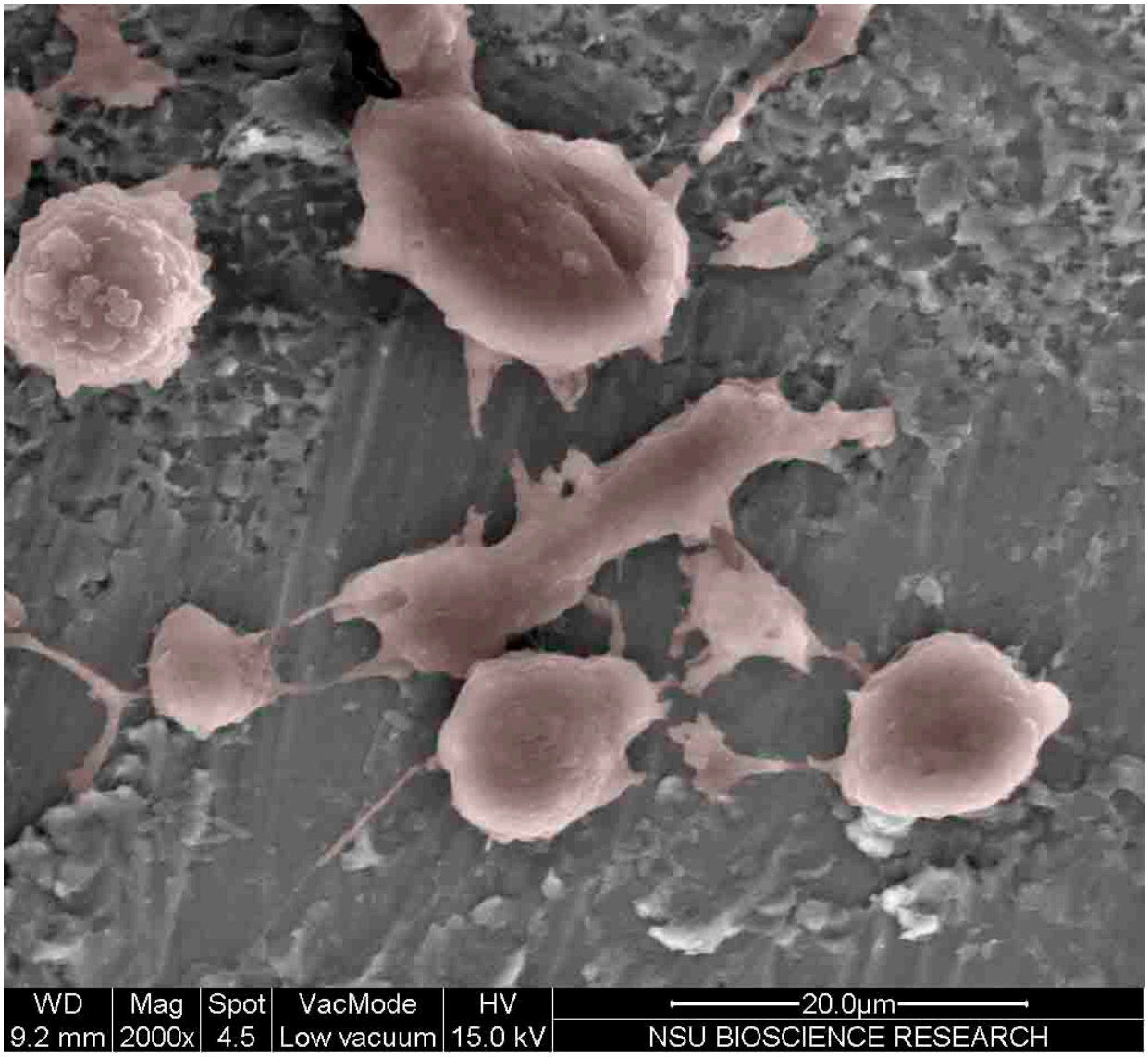
3.8. Osteoblast attachment to Osseotite dental implant disks following the disinfection of *P. gingivalis*

The osteoblasts were observed attached to the surfaces of the Osseotite dental implant disks with or without a NanoTite coating following disinfection treatments using scanning electron microscopy at a magnification of x2,000. Some osteoblasts were seen attached to the Osseotite dental implants disinfected with ultrasonics and chlorhexidine glucontate (Figure 44). Osteoblasts were seen attached to the Osseotite dental implants with a NanoTitle coating that were disinfected with ultrasonics and chlorhexidine glucontate (Figure 45). Some osteoblasts were attached to the surface of Osseotite dental implants that were disinfected with ultrasonics and citric acid (Figure 46). After disinfecting Osseotite dental implants by brushing them with chlorhexidine gluconate, some osteoblasts were seen attached to the implant surface (Figure 47). Some osteoblasts attached to the Osseotite dental implants that were not infected with *P. gingivalis* as a control group (Figure 48).

3.9. Analysis of osteoblast attachment to Osseotite dental implant disks following the disinfection of *P. gingivalis*

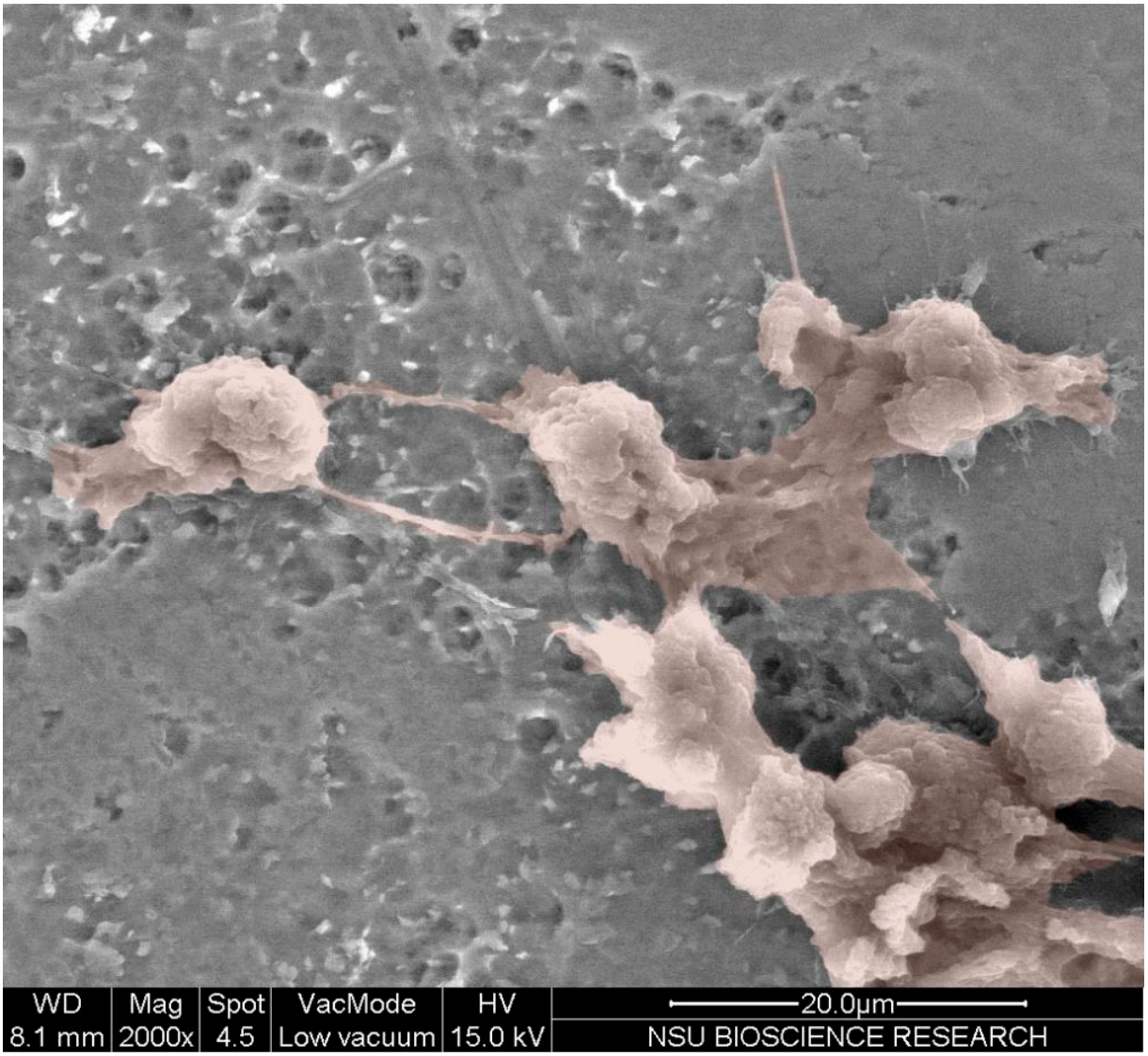
The numbers of osteoblasts were counted on scanning electron micrograph (SEM) that was attached to each disinfected dental implant surface. There were a mean number of 14 osteoblasts attached to the Osseotite dental implant disk surfaces, and it was 14% higher at the mean number of 12 osteoblasts per SEM attached to the Osseotite implants with a NanoTite coating (Figure 49). Many of the disinfected implants had none or few osteoblasts attached, and this gave large standard deviations of the means (Figure 49). The NanoTite coating made little difference to the numbers of osteoblasts that attached to the Osseotite implant surfaces (ANOVA P=0.6705, Power 0.069).

Figure 44. Scanning electron micrograph of osseotite dental implants disinfected with ultrasonics and chlorhexidine glucontate.



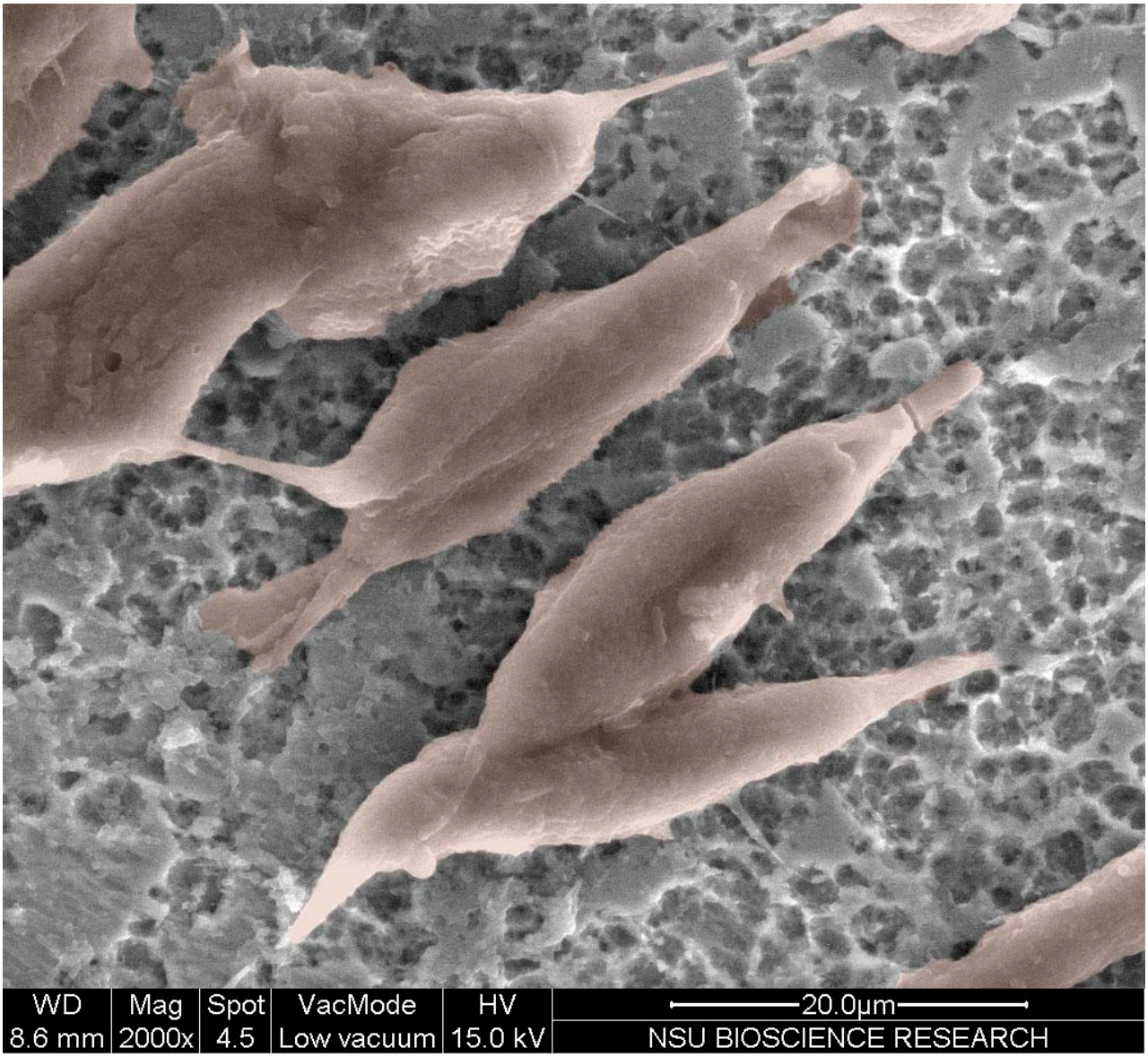
Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

Figure 45. Scanning electron micrograph of osteoblasts attached to osseotite dental implants with a NanoTite coating disinfected with ultrasonics and chlorhexidine glucontate.



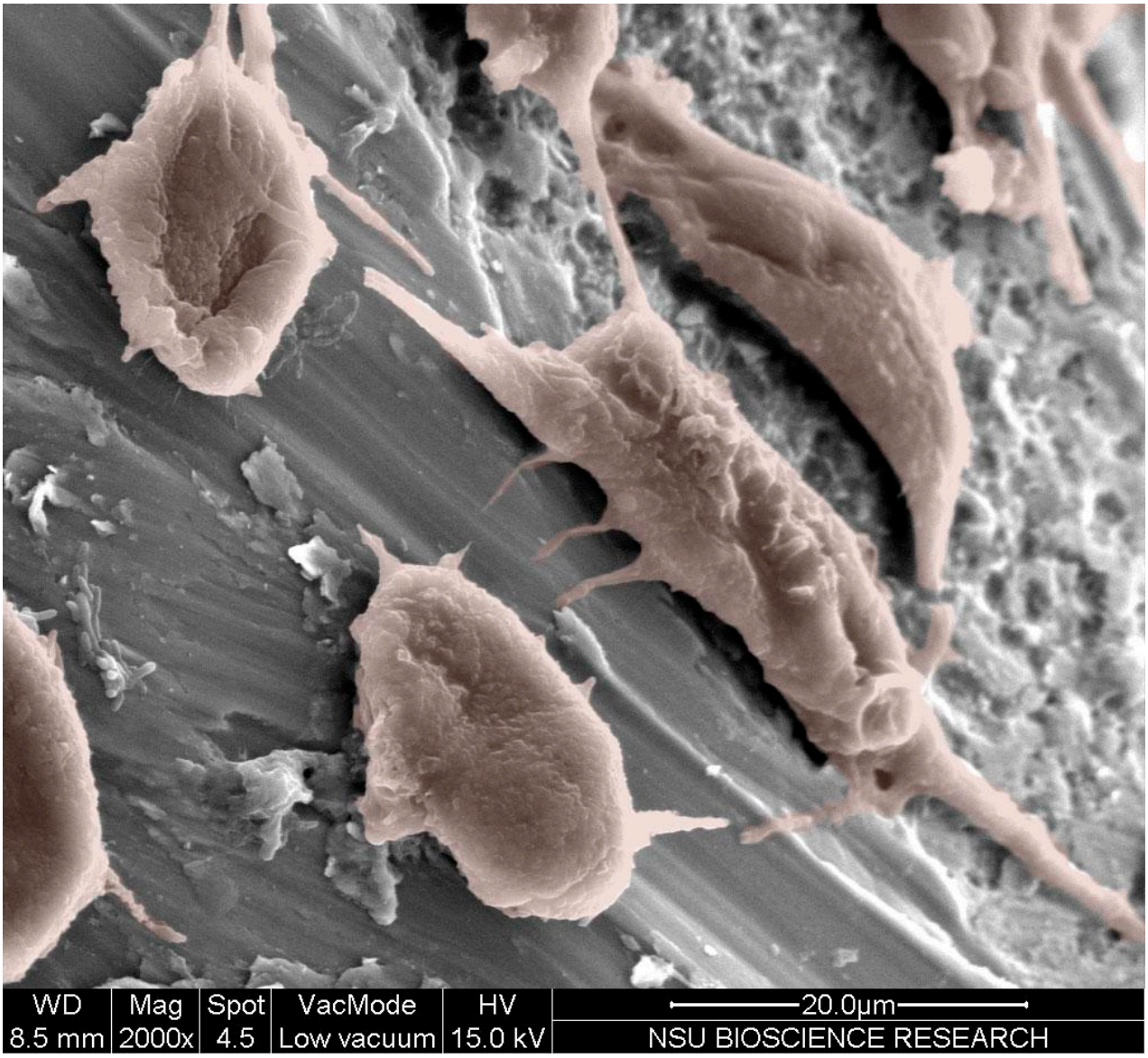
Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

Figure 46. Scanning electron micrograph of osteoblasts attached to osseotite dental implants disinfected with ultrasonics and citric acid.



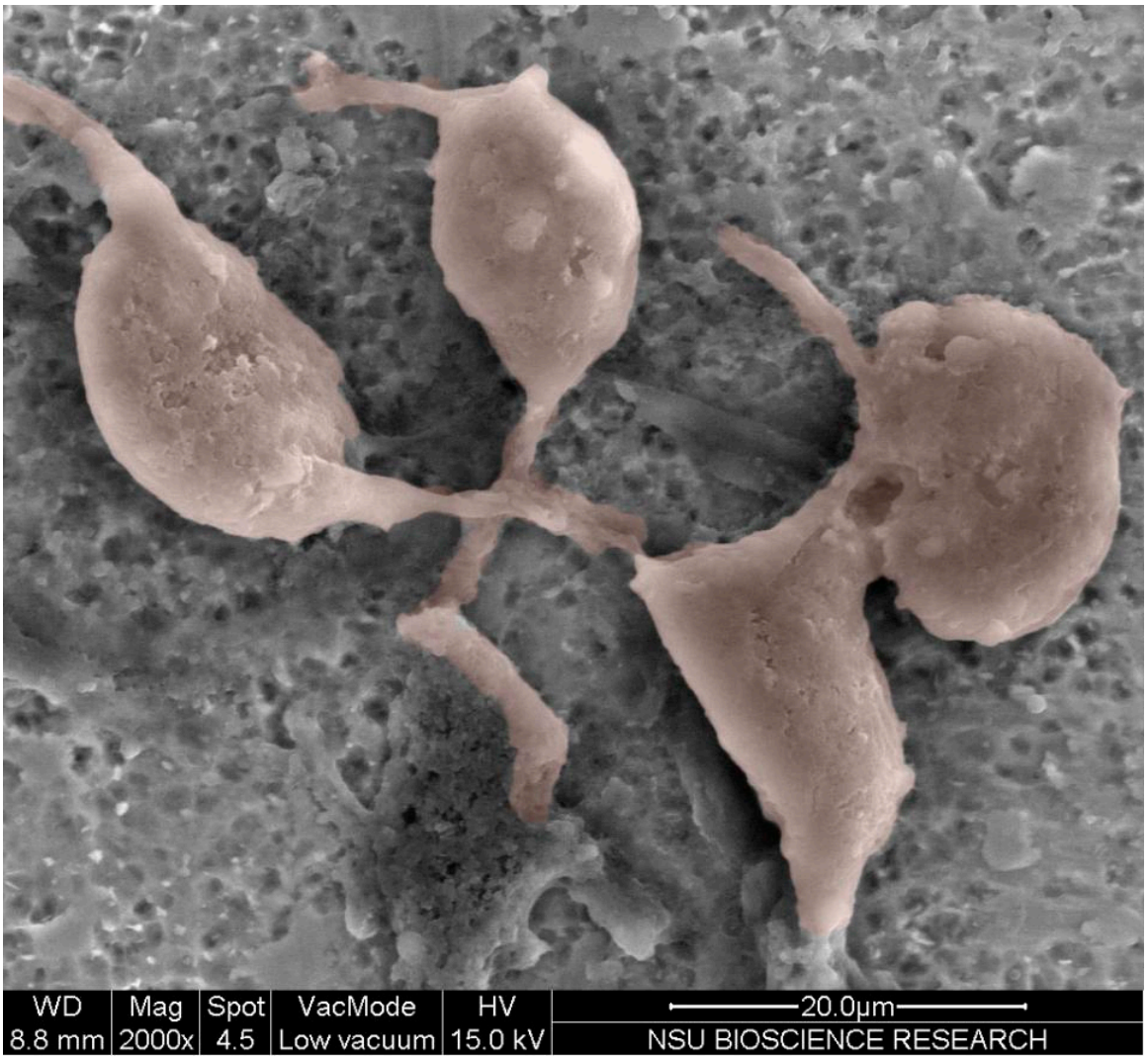
Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

Figure 47. Scanning electron micrograph of osteoblasts attached to osseotite dental implants disinfected with brushing and chlorhexidine gluconate.



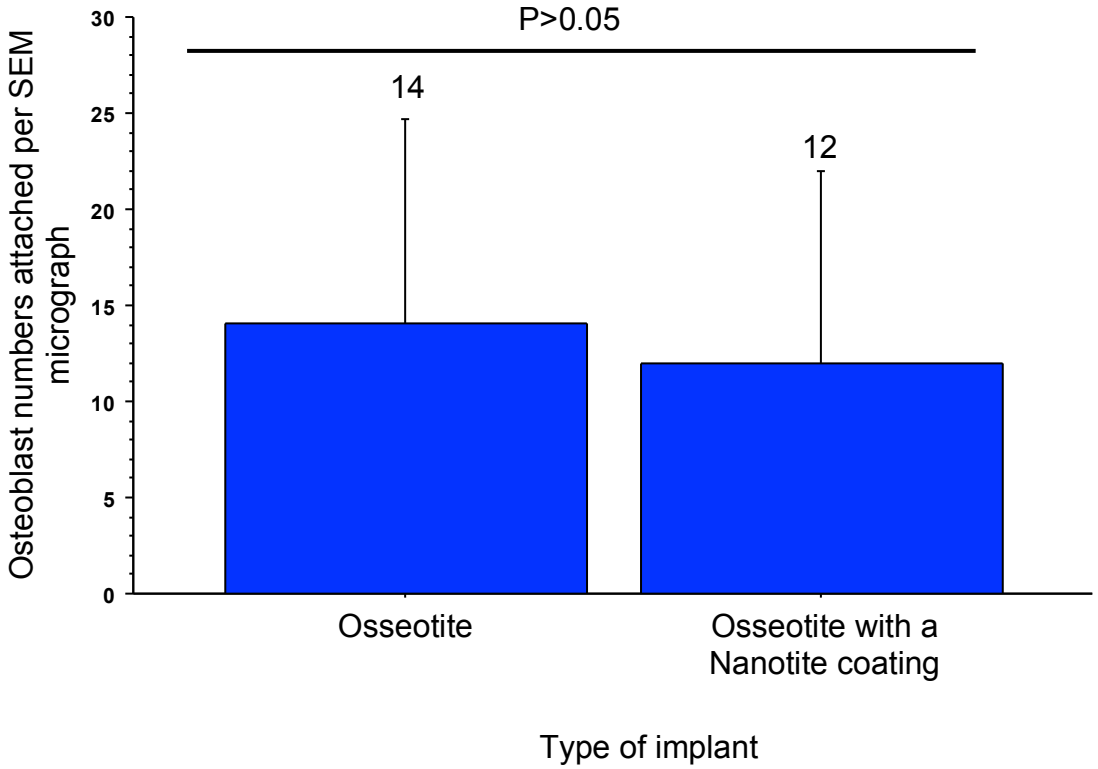
Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

Figure 48. Scanning electron micrograph of osteoblasts attached to osseotite dental implants which were not infected.



Scanning electron micrograph at x2,000 magnification of osteoblasts attached to the implant surface after dehydration and gold-palladium coating of specimens.

Figure 49. Bar chart of osteoblast attachment to Osseotite versus Osseotite with Nanotite disks.



The bar char represents the means of each treatment group which contained 6 to 21 specimens. The standard error bars represent the standard deviation of the means. The numbers of osteoblasts attached to the dental implant surfaces were counted per SEM micrograph. The vitality of the osteoblasts for each of the specimens was measured using the MTT assay at an absorbance at 600nm.

A comparison of the use of chemical and physical disinfection treatments found that they had little effect on the mean numbers of osteoblasts attached to the surface of the dental implant disks (ANOVA P=0.6308, Power 0.150) (Figure 50). There was little difference in the numbers of osteoblasts attached to the surface of the Osseotite implants following disinfection using Chlorhexidine gluconate with ultrasonic activation or brushing, or Citric acid with ultrasonic activation, and no infection treatment as a control (Figure 50).

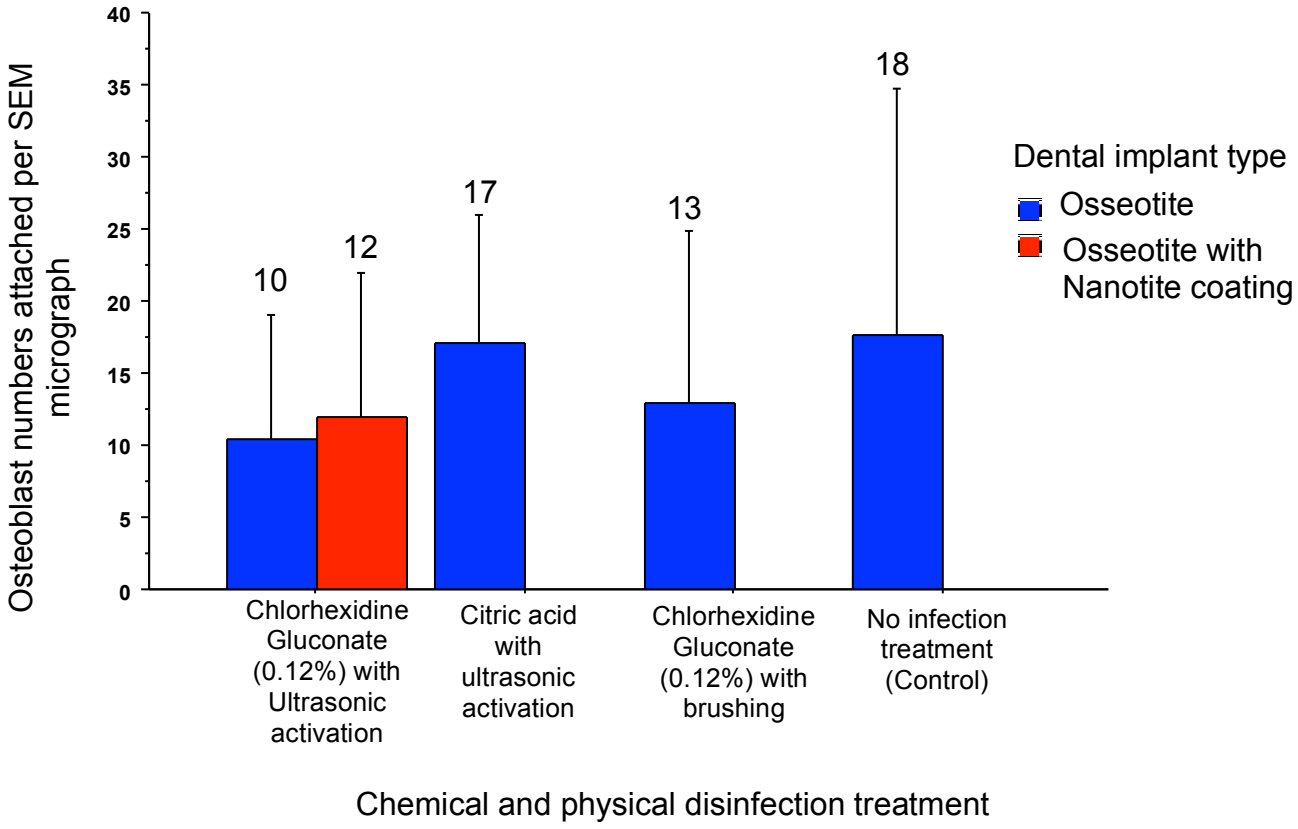
3.10. Analysis of osteoblast attachment morphology to 3iT3 dental implant disks following the disinfection of *P. gingivalis*

A comparison of the effect of osteoblast attachment morphology according to the type of 3iT3 dental implant surface, with or without a DCD coating, found that the DCD coating made little difference to the morphology of osteoblasts that attached to the implant surface following disinfection (Chi-square P=0.1116) (Figure 51).

A comparison of the effect of osteoblast attachment morphology according to the coating of the 3iT3 dental implant surfaces, with or without Emdogain or GEM21S growth factors, found that the addition of growth factors made little difference to the morphology of the osteoblasts that attached to the implant surface following disinfection (Chi-square P=0.1406) (Figure 52).

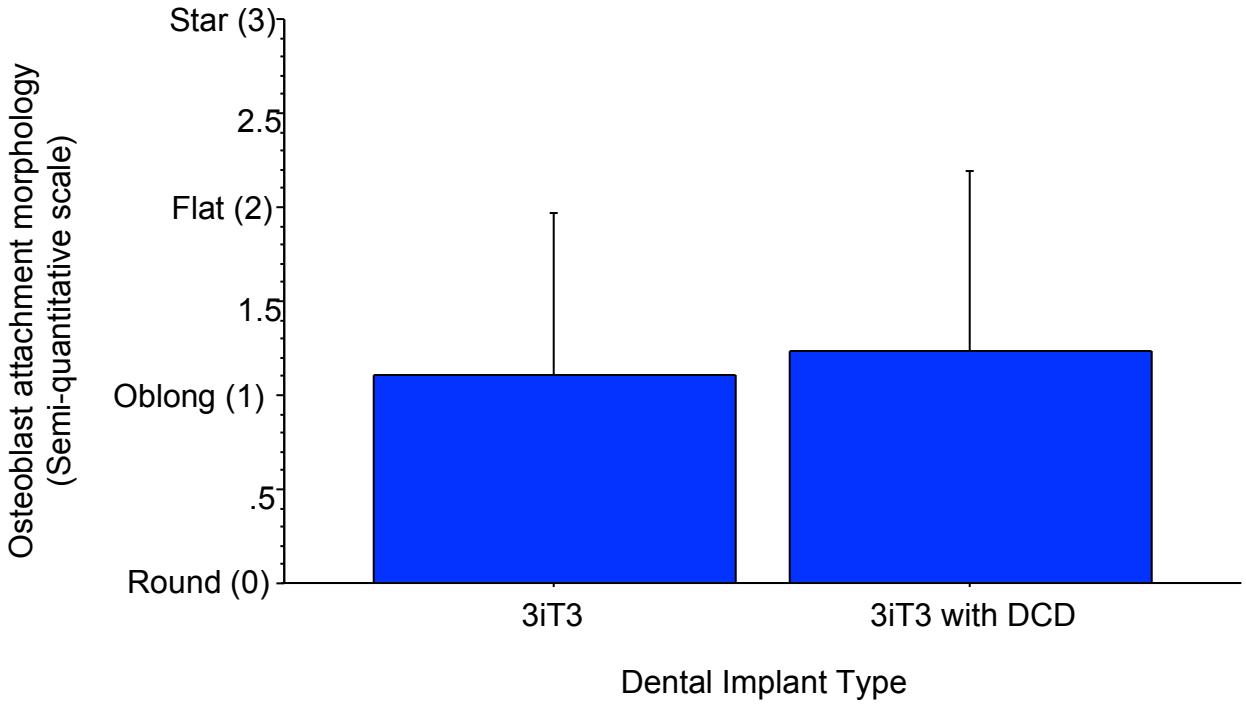
A comparison of the effect of osteoblast attachment morphology according to the type of 3iT3 dental implant surface, with or without a DCD coating, and the coating of the dental implant surfaces, with or without Emdogain or GEM 21s growth factors found that these variables had little effect (Chi-square P=0.2261) (Figure 53).

Figure 50. Bar chart of osteoblast attachment to Osseotite disks following disinfection.



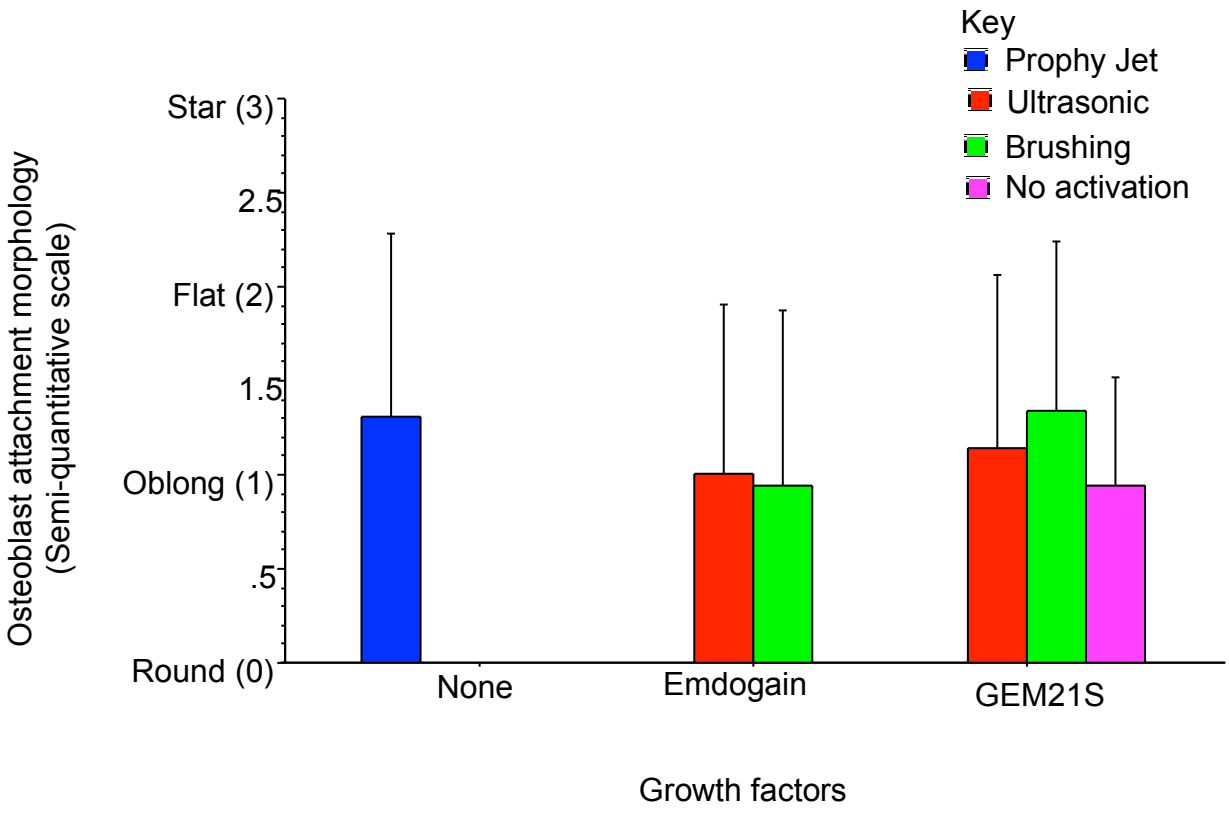
The bar char represents the means of each treatment group which contained 6 to 21 specimens. The standard error bars represent the standard deviation of the means. The numbers of osteoblasts attached to the dental implant surfaces were counted per SEM micrograph. The vitality of the osteoblasts for each of the specimens was measured using the MTT assay at an absorbance at 600nm.

Figure 51. Bar chart of osteoblast attachment morphology to 3iT3 versus 3iT3 with DCD dental implant disks.



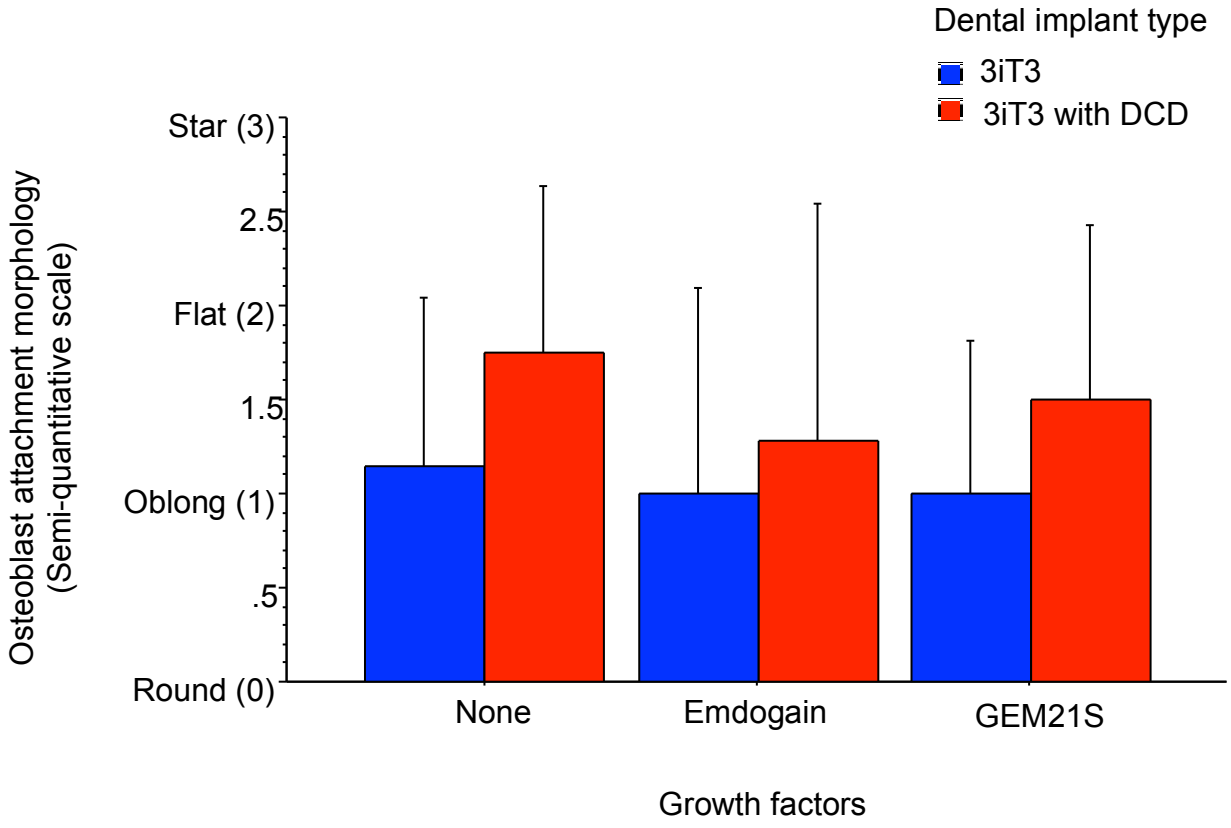
The bar char represents the means of each treatment group which contained 8 specimens. The standard error bars represent the standard deviation of the means. The morphology of osteoblasts attached to the dental implant surfaces were categorized according to their shape: Round (0), Oblong (1), Flat (2), and Star (3) seen on the SEM micrographs.

Figure 52. Bar chart of osteoblast attachment morphology to 3iT3 dental implant disks following physical and chemical disinfection.



The bar char represents the means of each treatment group which contained 8 specimens. The standard error bars represent the standard deviation of the means. The morphology of osteoblasts attached to the dental implant surfaces were categorized according to their shape: Round (0), Oblong (1), Flat (2), and Star (3) seen on the SEM micrographs.

Figure 53. Bar chart of osteoblast attachment morphology to 3iT3 dental implant disks coated with growth factors.



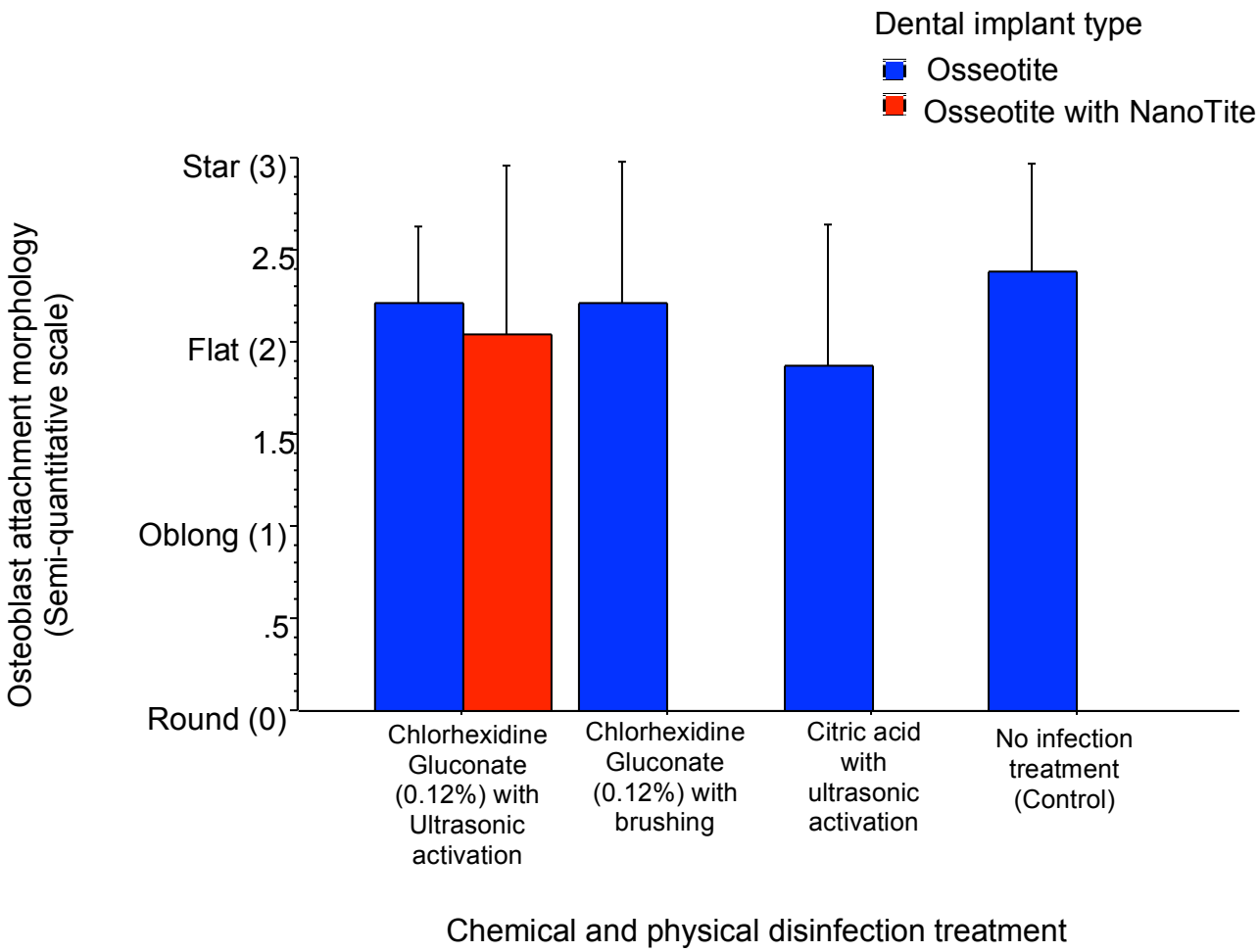
The bar char represents the means of each treatment group which contained 8 specimens. The standard error bars represent the standard deviation of the means. The morphology of osteoblasts attached to the dental implant surfaces were categorized according to their shape: Round (0), Oblong (1), Flat (2), and Star (3) seen on the SEM micrographs.

3.11. Analysis of osteoblast attachment morphology to Osseotite dental implant disks following the disinfection of *P. gingivalis*

A comparison of the effect of osteoblast attachment morphology according to the type of osseotite dental implant surface, with or without a Nanotite coating, found that the Nanotite coating made little difference to the morphology of osteoblasts that attached to the implant surface following disinfection (Chi-square P=0.4816) (Figure 54).

A comparison of the disinfection treatments found that they had little effect on the morphology of the osteoblasts attached to the surface of the dental implant disks (Chi-square P=0.958) (Figure 54). The morphology of the osteoblasts was flat and similar following osseotite implant disk disinfection with Chlorhexidine Gluconate (0.12%) with ultrasonic activation, Chlorhexidine Gluconate (0.12%) with brushing, Citric acid with ultrasonic activation, and disks which had no infection treatment (Figure 54). Most of the osteoblasts attached to the osseotite implant surfaces had a flat morphology (Figure 54), suggesting the implant surface is favorable for osteoblast attachment.

Figure 54. Bar chart of osteoblast attachment morphology to Osseotite dental implant disks.



The bar char represents the means of each treatment group which contained 6 specimens. The standard error bars represent the standard deviation of the means. The morphology of osteoblasts attached to the dental implant surfaces were categorized according to their shape: Round (0), Oblong (1), Flat (2), and Star (3) seen on the SEM micrographs.

4. DISCUSSION

4.1. Significance of this research

This is the first study to investigate the disinfection of *P. gingivalis* from dental implants by comparing the effectiveness of using three disinfection solutions, and three mechanical methods of applying the disinfection solutions. This is also the first study to measure the survival and attachment of osteoblasts to the dental implant disks according to the absence or presence of a NanoTite or a DCD coating, disinfection solution, mechanical methods of applying the disinfection solutions, and the additional coating of the dental implant disks with growth factors. There were ten phases in this research study: First, I measured the growth and disinfection of *P. gingivalis* in the presence of Biomet 3iT3™ dental implant disks with and without a DCD coating. Second, I measured the effectiveness of using Sodium BiCarbonate, 3% Hydrogen Peroxide, or a 0.12% CHX solution to disinfect *P. gingivalis* from Biomet 3iT3™ dental implant disks. Third, I compared the effectiveness of using Cavitron ProphyJet spraying, ultrasonic activation or brushing to disinfect *P. gingivalis* from the surfaces of Biomet 3iT3™ dental implant disks. Forth, I investigated osteoblast survival in cell culture with the disinfected Biomet 3iT3™ dental implant disks. Fifth, I counted the numbers of osteoblasts attached to the disinfected Biomet 3iT3™ implant disk surfaces, and Sixth, I investigated the effectiveness of using growth factors: Emdogain and Growth-factor Enhanced Matrix (GEM21S) to enhance osteoblast attachment to the Biomet 3iT3™ implant disk surface. Seventh, I investigated the growth and disinfection of *P. gingivalis* in the presence of Biomet 3i Osseotite dental implant disks with and without a NanoTite coating. Eighth, I compared the ability of Citric acid and Chlorhexidine to disinfect *P. gingivalis* from Osseotite dental implant disks. Ninth, I investigated osteoblast vitality in cell

culture with the disinfected Osseotite dental implant disks. Tenth, I counted the numbers of osteoblasts attached to the disinfected Biomet Osseotite implant disk surfaces to assess the effects of the disinfection treatments on osteoblast attachment. Compared to other studies of dental implants that used a total of 92 samples (79), my research used 207 samples, which suggests that I had created and analyzed a more complete research study compared to my peers.

4.2. Periimplantitis and dental implant failure

Each year, over three million Americans have dental implants to replace over five million missing and non-restorable teeth (33). In patients with healthy dental tissues, an osseointegrated dental implant not subjected to biomechanical overloads can have long term survival rate over a decade or longer of 77.7% to 100% (16,26-29). Although, the survival rate of dental implants is excellent, improvements to implantology treatments are needed which can more effectively disinfect dental implants and stimulate osteoblast survival and attachment to the dental implant surface, which can promote osseointegration.

The leading cause of dental implant failure is Periimplantitis (68,69). Periimplantitis is the inflammation of soft tissues adjacent to the dental implant, caused by a localized bacterial infection (68). The symptoms of Periimplantitis are bone loss around an osseointegrated implant in function, lack of implant stability, and an increase in tooth socket depth (69). Periimplantitis is treated by disinfecting the implant socket and the implant surface, to remove the bacterial infection, thereby allowing the regeneration of the alveolar bone for the osseointegration of the dental implant (79). A problem with selecting a dental implant decontamination procedure is the lack of consensus about which procedure or disinfectant is the most beneficial to remove the *P. gingivalis* (79). A benefit of this research is that it

could help dentists by guiding them to select the most effective disinfectants and disinfection methods to remove bacteria from dental implants and obtain maximal osteoblast attachment. This could also benefit patients through the development of more effective and successful procedures to disinfect dental implants.

4.3. *P. gingivalis* and dental infection

A leading cause of periimplantitis and the most serious threat to the success of a dental implant is an infection with an oral pathogen called *Porphyromona gingivalis* (*P. gingivalis*) (79). However, it must be recognized that a human mouth can contain more than 688 different species of bacteria (79). The presence of five to nineteen bacterial species has been identified in greater numbers from dental implants with Periimplantitis (79). This indicates that most perimplantitis infections contain multiple species of bacteria (79). My research focused on the disinfection of *P. gingivalis* from dental implants because it was the most important pathogen associated with perimplantitis. This study used a spectrophotometer at an absorbance of 600nm (97) to measure the growth of *P. gingivalis* to avoid any investigator bias during the collection of raw data. The *P. gingivalis* was supplied as a pure stock solution and was grown in isolation to prevent contamination of the bacteria. There was a problem with a contaminated stock of *P. gingivalis* supplied by a vendor, the results collected from the contaminated samples was discarded and the infection of the implants was repeated with a pure stock of *P. gingivalis* in order to obtain accurate data.

The scope of the present research was limited to *P. gingivalis* because of the limited number of dental implant disks (n=207), which were available to perform this research. If another five dental pathogens had been tested, it would have required over a thousand

samples, and given my budget and time constraints; increasing the numbers of pathogens was not possible. Clearly further research is needed to investigate the precise role of other pathogens in addition to *P. gingivalis* which cause perimplantitis in future studies.

4.4. Disinfection of 3iT3 dental implants

The 3iT3 dental implant disks contaminated with *P. gingivalis* were removed from the test tubes using an aseptic handling technique inside a laminar flow hood. The dental implant disks were randomly assigned to receive one of the treatments shown in Table 2. The disinfection of *P. gingivalis* was not very effective following most of the chemical treatments, because an average residual growth of 29% of *P. gingivalis* was observed from all the disinfected 3iT3 and 3iT3 with DCD dental implant disks. These results suggest the DCD coating has little effect on the ability of chemicals to disinfect *P. gingivalis* from the implants. A previous study by Lubin *et al.,*(79) showed that the Osseotite implant surface coating can alter the ability of chemicals to disinfect dental implants, probably because the surface coating increases the roughness of surface. Dental implants with a rougher surface, potentially can giving the *P. gingivalis* more places to attach, thereby making them more difficult to disinfect (102). *P. gingivalis* biofilm growth on moderately roughened dental implant disks was found not to enhance biofilm formation, but did reduce the efficacy of chlorhexidine disinfection on one implant disk type (103).

Among the chemical treatments, the Hydrogen peroxide (18% and 29%) was only slightly more effective than the Sodium bicarbonate (32%) and Chlorhexidine gluconate (23%, 30%, and 37%) (Figure 2). However, there was no significant difference between the three chemical treatments to disinfect the 3iT3 dental implant disks. In addition, the physical activation of the three chemical disinfectants by Prophy Jet, Ultrasonics, or Brushing, or with

no physical activation, was not found to have much effect on their ability to disinfect the dental implant disks. These results suggest that all the chemical and physical treatments had a similar effectiveness to disinfect *P. gingivalis* from the dental implant disks. Unfortunately, there have been no previous studies of the effectiveness of a Prophy Jet, Ultrasonics, or Brushing to disinfect *P. gingivalis* from dental implants (search of Pubmed on 3/13/15). The novel results of this current research; that these three physical treatments are similar in disinfection effectiveness to remove *P. gingivalis* from 3iT3 dental implants, suggests that no activation of the disinfection chemicals is needed to disinfect these type of dental implants.

4.5. Disinfection of Osseotite dental implants

The disinfection of *P. gingivalis* from the Osseotite and Osseotite with NanoTite dental implant disks was most effective using Chlorhexidine gluconate, which had very low residual amounts of *P. gingivalis* equal to a half of one percent (0.52-0.53%) (Figure 3). The least effective disinfection agent was Citric acid (51%) (Figure 3) (ANOVA, P=0.0025, Power 0.937). A comparison of the Chlorhexidine gluconate to disinfect Osseotite and Osseotite with NanoTite dental implant disks, found that the NanoTite coating had very little effect on the disinfection of *P. gingivalis* from the implant disks (0.52% versus 0.53%) (Figure 3). These disinfection results are similar to those of Dr. Lubins research using the same dental implant disks (79).

4.6. Osteoblast viability to 3iT3 disks following the disinfection of *P. gingivalis*

Some studies of osteoblast viability to dental implants have used fibroblasts instead of osteoblast cells (104), or rodent osteoblast cell lines (103). Clearly the most clinically

applicable cells are human osteoblasts (93,94), hence the selection of a human osteoblast cell line (CRL-1427) supplied from the American Tissue and Cell Culture Collection (ATCC, Manassas, VA) for use in this research.

The viability of osteoblasts was 13% higher in the presence of 3iT3 dental implant disks (85%) compared to the 3iT3 with DCD dental implant disks (72%) (Figure 4). The use of four physical disinfection treatments: Prophy Jet, Ultrasonic activation, brushing, and no activation, had an effect on the viability of osteoblasts. The lack of physical activation of the chemical disinfectants to remove *P. gingivalis* from the dental implant disk had the lowest amount of osteoblast vitality at 70% of the control (Figure 5). Brushing the implant surfaces with a chemical disinfectant had an osteoblast vitality at 73% of the control (Figure 5). Both the Prophy Jet and ultrasonic activation allowed the highest amount of osteoblast vitality at 81% of the control (Figure 5). These results suggest that the physical decontamination method for disinfecting dental implants can influence osteoblast vitality by up to 11%. The modification of dental implant surfaces with a Prophy Jet was found to reduce fibroblast cell proliferation (103), indicating the effect that modifying the surface of dental implants can alter the local cellular responses. This helps to explain why there are so many different coatings and modifications to dental implant surfaces in order to optimize osseointegration (38).

A comparison of the osteoblast vitality on 3iT3 dental implant disks versus the 3iT3 with DCD dental implant disks for each of the four physical disinfection treatments found that osteoblast vitality was higher between 28% and 8% on the 3iT3 dental implant disks (Figure 6). The physical decontamination of the 3iT3 with DCD dental implant disks always gave a lower amount of osteoblast vitality compared to the 3iT3 dental implant disks. The combined effects of the implant type and physical activation of chemical disinfectants, found that

because osteoblast vitality was always highest on the 3iT3 dental implant disks, that overall effect of the physical decontamination procedures on osteoblast vitality was relatively much weaker. The lower osteoblast viability in the presence of 3iT3 implants coated with DCD, suggests that the osteoblasts prefer to live in the presence of 3iT3 implants without DCD, which indicates that the normal 3iT3 surface coating is adequate to promote osteoblast viability.

The vitality of osteoblasts on 3iT3 dental implant disks was also measured following the use of the chemicals; Sodium bicarbonate, Hydrogen peroxide, and Chlorhexidine gluconate to disinfect *P. gingivalis*. The type of chemical used to disinfect *P. gingivalis* from the 3iT3 implants had an effect on the vitality of osteoblasts. The use of Hydrogen peroxide had the highest osteoblast vitality at 86%, sodium bicarbonate had an osteoblast vitality of 81%, and the lowest osteoblast vitality of 68% was found following the use of Chlorhexidine gluconate (Figure 7). These results suggest the substantive toxicity of the disinfection chemicals can have an effect on the vitality of the osteoblasts following dental implant disinfection: Where more toxic chemicals will cause a greater loss of osteoblast cell viability. The low osteoblast vitality following the disinfection of the dental implant disks with Chlorhexidine, confirms that this chemical is highly toxic to cells in a dose- and time-dependent manner (105). A suggestion to optimize the viability of osteoblasts is to rinse away the disinfection chemical with saline that was used to disinfect the dental implant in order to reduce any residual chemical which maybe toxic to osteoblasts.

The vitality of osteoblasts on 3iT3 dental implant disks and 3iT3 dental implant disks with a DCD coating were highest after the disks had been decontaminated with Hydrogen peroxide which had been brushed (90%) and ultrasonically activated (82%) (Figure 9). The

vitality of osteoblasts on the dental implant disks was 81% following Prophy Jet disinfection with Sodium bicarbonate (Figure 9). The vitality of osteoblasts in the presence of dental implant disks disinfected with Chlorhexidine gluoconate was highest following ultrasonic activation (80%), less without any activation (70%) and worst when used with brushing (56%) (Figure 9). These results suggest that brushing the surface of the dental implant had a negative impact on the subsequent viability of osteoblasts, and the reason for this maybe that the brush modified the implant surface roughness and chemistry. This study was not able to measure the roughness of the implant surface before and after the disinfection treatments, but it is likely that brushing the implant surface caused the most severe abrasion and scratches, which may help explain why it was the least favorable for osteoblast viability. In order to avoid changing the surface roughness of dental implants during disinfection with metal ultrasonic tips (106) or titanium brushes, plastic ultrasonic trips and plastic brushes are recommended.

Growth factors around injured bone can recruit osteoprogenitor cells and modulate inflammatory cells to regenerate bone (61). Bone formation and osseointegration is necessary for the clinical success of disinfected dental implants (60). An increase in the proliferation and differentiation of undifferentiated mesenchymal cells, osteoprogenitor cells, and preosteoblasts into osteoblasts may improve bone response and subsequently osseointegration of Ti implants (63). Previous studies have coated the surfaces of dental implants with Emdogain (64) or Growth-factor Enhanced Matrix [GEM21S] (65) to enhance the natural responses of osteoprogenitor cells to remodel bone and to accelerate osseointegration. However, the present research discovered that the coating of the disinfected 3iT3 dental implant disks with Emdogain (64) or Growth-factor Enhanced Matrix [GEM21S] (65) had little effect on the vitality of osteoblasts. The coating of the 3iT3 dental

implant disks with GEM21S increased the vitality of osteoblasts by 6% compared to the control (absence of growth factors), and the addition of Emdogain reduced the vitality of the osteoblasts by 4% compared to the control (Figure 11). However, a further investigation of the results discovered that the 3iT3 implants coated with DCD did benefit from the addition of Emdogain and GEM21S because it increased osteoblast vitality by 13% and 18% in comparison with the non-growth factor 3iT3 implants coated with DCD (Figure 12). These results indicate that adding growth factors to dental implants is not always beneficial and that the surface modification may influence the effectiveness of growth factors to promote osteoblast vitality and healing. These results may explain why growth factors are sometimes not beneficial for dental implant osseointegration (107) and are not always needed.

4.7. Osteoblast viability to Osseotite disks following the disinfection of *P. gingivalis*

The vitality of osteoblasts in the presence of Osseotite dental implant disks and Osseotite dental implant disks with a NanoTite coating were similar at 98% and 96% respectively (Figure 13). These results are slightly different to the 87% and 93% osteoblast viability reported by using the same Osseotite dental implant disks by Lubin *et al.,* (79). However, the previous study by Lubin *et al.,* (79) investigated different disinfection treatments including a laser, which appears to have reduce the osteoblast viability in the previous study compared to the current research results.

A comparison of the effect of chemical and physical disinfection of Osseotite disks found that osteoblast vitality was similar with all the disinfection treatments (ANOVA, P=0.0676, Power 0.581) (Figure 14). The low power of the P value suggests that if the sample numbers had been increased that a significant difference may be found. The vitality of

osteoblasts was highest at 113% following the disinfection of Osseotite implant disks with Chlorhexidine gluconate with ultrasonic activation (Figure 14). The vitality of the osteoblasts in the presence of the Osseotite dental implant disks for all the dental implant types and disinfection treatments were similar. The brushing of Osseotite dental implant disks with Chlorhexidine gluconate gave the lowest osteoblast vitality at 89% (Figure 14). This confirms the high toxicity of Chlorhexidine (105), and the need to rinse all the disinfectants from the implants with saline in order to remove residual chemicals, which may prove to be toxic to osteoblasts.

4.8. Osteoblast attachment to 3iT3 dental implant disks following the disinfection of *P. gingivalis*

A major reason why implants can fail is because of a lack of osseointegration (67). Improvements to implantology treatments are needed which can more effectively disinfect dental implants and stimulate osteoblast attachment to the dental implant surface, which can promote osseointegration (79). In this research, the osteoblasts were observed attached to some of the surfaces of the 3iT3 dental implant disks following disinfection treatments using scanning electron microscopy at a magnification of x2,000. Some osteoblasts were seen attached to at least one 3iT3 dental implant per group of implants following disinfection (Figures 15 to 34) suggesting that the osteoblasts will attach to all the implant surfaces following disinfection treatments. After adding Emdogain to the 3iT3 dental implants following disinfection with a Prophy Jet and Sodium bicarbonate, some osteoblasts were attached to the surfaces (Figure 16). Osteoblasts were seen attached to the surface of 3iT3 dental implants that had been coated with GEM21S after disinfection with a Prophy Jet and Sodium bicarbonate

(Figure 17). A few osteoblasts were seen attached to the surface of 3iT3 dental implants following disinfection with ultrasonically activated hydrogen peroxide (Figure 18). After disinfecting 3iT3 dental implants with ultrasonically activated Chlorhexidine gluconate, some osteoblasts were observed to have attached to the implant surface (Figure 19). Two osteoblasts had attached to the surface of 3iT3 dental implants following brushing with hydrogen peroxide disinfection (Figure 20). After disinfecting 3iT3 dental implants by brushing them with Chlorhexidine gluconate, some osteoblasts were seen attached to the implant surface (Figure 21). Some osteoblasts were observed attached to the surface of 3iT3 dental implants following Chlorhexidine gluconate disinfection (Figure 22).

The osteoblasts were observed attached to the surfaces of the 3iT3 dental implant disks with a DCD coating following disinfection treatments using scanning electron microscopy at a magnification of x2,000. Some osteoblasts were seen attached to the 3iT3 dental implants with a DCD coating following disinfection with a Prophy Jet and Sodium bicarbonate (Figure 23). After adding Emdogain to the 3iT3 dental implants with a DCD coating following disinfection with a Prophy Jet and Sodium bicarbonate, some osteoblasts were attached to the surfaces (Figure 24). Osteoblasts were seen attached to the surface of 3iT3 dental implants with a DCD coating that had also been coated with GEM21S after disinfection with a Prophy Jet and Sodium bicarbonate (Figure 25). A few osteoblasts were seen attached to the surface of 3iT3 dental implants with a DCD coating following disinfection with ultrasonically activated hydrogen peroxide (Figure 26). After disinfecting 3iT3 dental implants with a DCD coating using ultrasonically activated chlorhexidine gluconate, some osteoblasts were observed to have attached to the implant surface (Figure 27). Two osteoblasts had attached to the

surface of 3iT3 dental implants with a DCD coating following brushing with hydrogen peroxide disinfection (Figure 28). After disinfecting 3iT3 dental implants with a DCD coating by brushing them with chlorhexidine gluconate, some osteoblasts were seen attached to the implant surface (Figure 29). Some osteoblasts were observed attached to the surface of 3iT3 dental implants with a DCD coating following chlorhexidine gluconate disinfection (Figure 30). The attachment of osteoblasts was observed to the surface of a 3iT3 dental implant with a DCD coating following Prophy Jet disinfection with sodium bicarbonate (Figure 31). Osteoblasts were seen that had attached to 3iT3 dental implants with a DCD coating which were not infected with *P. gingivalis* and following Prophy Jet disinfection with sodium bicarbonate (Figure 32). Some osteoblasts had attached to the surface of 3iT3 dental implants with a DCD coating, which had no osteoblasts added, and following Prophy Jet disinfection with sodium bicarbonate (Figure 33). Osteoblasts had attached to the surface of 3iT3 dental implants with a DCD coating which were not infected with *P. gingivalis* and following Prophy Jet disinfection with sodium bicarbonate (Figure 34). These results suggest that osteoblasts can readily attach to most dental implant surfaces, following all of the chemical and physical disinfection treatments, however on many implant surfaces zero osteoblasts were seen, which highlights the importance of investigating osteoblast attachment to ensure the surface is optimal for osseointegration.

4.9. Analysis of osteoblast attachment to 3iT3 dental implant disks following the disinfection of *P. gingivalis*

The numbers of osteoblasts were counted on scanning electron micrograph (SEM) that was attached to each disinfected dental implant surface. There were a mean number of 33

osteoblasts attached to the 3iT3 dental implant disk surfaces, and it was 9% higher at 36 osteoblasts per SEM attached to the 3iT3 implants with a DCD coating (Figure 35). Many of the disinfected implants had none or few osteoblasts attached, and this gave large standard deviations of the means (Figure 35). The DCD coating made little difference to the numbers of osteoblasts that attached to the 3iT3 implant surfaces. These *in vitro* results may not be the same as the clinical results. A recent animal study of 3iT3 implants with a DCD coating found that it had a similar healing pattern to another type of dental implant, but was less effective for bone formation, as less bone density was observed (108). These results indicate that further research is needed to optimize the DCD coating in terms of its ability to promote osteoblast attachment and osseointegration.

The numbers of osteoblasts attached to the 3iT3 dental implant disk surfaces appeared to be strongly affected by the method used for the physical disinfection of *P. gingivalis*. A comparison of the numbers of osteoblasts attached to the 3iT3 dental implant surfaces following disinfection with a Prophy Jet spray of Sodium bicarbonate, or the ultrasonic activation of Chlorhexidine gluconate or Hydrogen peroxide, or the brushing of Chlorhexidine gluconate or Hydrogen peroxide, or the use of Chlorhexidine gluconate without any physical disinfection produced some unexpected results. There was a mean of 21 osteoblasts attached to the 3iT3 dental implant surfaces following disinfection with a Prophy Jet spray of Sodium bicarbonate (Figure 36). In comparison to the Prophy Jet, there was a 43% increase in the mean number of 30 osteoblasts attached to the 3iT3 dental implant surfaces following disinfection with the ultrasonic activation of Chlorhexidine gluconate or Hydrogen peroxide (Figure 36). In comparison to the ultrasonic activation of disinfectants, there was a 33% increase in the mean number of 40 osteoblasts attached to the 3iT3 dental implant surfaces following disinfection by brushing with Chlorhexidine

gluconate or Hydrogen peroxide (Figure 36). In comparison to the brushing of disinfectants, there was a 95% increase in the mean number of 78 osteoblasts attached to the 3iT3 dental implant surfaces that had no physical activation (Figure 36). There were large standard deviations between the means of the numbers of osteoblasts attached to the 3iT3 dental implant surfaces following the physical disinfection with a Prophy Jet, ultrasonic, brushing, and no activation, consequently there was little difference between these treatments. There were differences between the mean numbers of osteoblasts attached without physical activation and all the other treatments except brushing. These results indicate that a lack of physical disinfection may be optimal to promote osteoblast attachment to disinfected 3iT3 dental implants, one reason maybe because the lack of physical disinfection does not modify the surface characteristics of the implant surface. The surface characteristics of the implants may already be optimal; hence any change might make the surface sub-optimal for osteoblast attachment. Clearly the implant surface roughness is important, but it also appears important to avoid abrasion and scratching the implant surface in order to obtain optimal osteoblast attachment similar to the results of studies which investigated other types of cell attachment to implants (104,106).

The chemicals used to disinfect the 3iT3 dental implant disks had an effect on the numbers of osteoblasts attached to their surface. The lowest mean numbers of osteoblasts were attached to the implant surfaces following disinfection with Sodium bicarbonate (Figure 38). In comparison to the 3iT3 dental implant disks disinfected using Sodium bicarbonate, there was a 100% increase in the mean number of 38 osteoblasts attached to the implants that were disinfected using Chlorhexidine gluconate (Figure 38). In comparison to the 3iT3 dental implant disks disinfected using Chlorhexidine gluconate, there was a 37% increase in the mean number of 52 osteoblasts attached to the implants that were disinfected using

Hydrogen peroxide (Figure 38). Due to the variability of the data and the large standard deviations of the means, the greatest difference between the chemical treatments and the attachment of osteoblasts was between the 3iT3 dental implant disks disinfected with Sodium bicarbonate and Hydrogen peroxide (Figure 38). These results indicate that Hydrogen peroxide is likely to be the best chemical for the disinfection of *P. gingivalis* from dental implants, and to accomplish the subsequent attachment of osteoblasts for osseointegration. The results also indicate that the use of the Prophy jet and Sodium bicarbonate is the worst treatment for the disinfection of dental implants to accomplish the subsequent attachment of osteoblasts. The Prophy Jet has many useful applications in dentistry, but the mess it makes with the spray, and its lack of effectiveness in this research means that it is not recommended for the disinfection of dental implants.

A potential problem with adding growth factors is that their cellular activity is concentration-dependent, so at one concentration the Emdogain and GEM21S could promote osteoblast attachment, while at another concentration they could repel osteoblasts and prevent them from attaching to the implant surface by promoting migration (109). The coating of the disinfected 3iT3 dental implant surfaces with growth factors; Emdogain or GEM21S had a powerful effect on the numbers of osteoblasts that had attached. Interestingly the effect of Emdogain and GEM21S were very different on the numbers of osteoblasts that attached to the 3iT3 dental implant surfaces. The coating of the disinfected implant surface with Emdogain reduced the mean numbers of attached osteoblasts by 87% from a mean of 23 osteoblasts per implant surface without any growth factor, to 3 osteoblasts per SEM micrograph (Figure 42). The coating of the 3iT3 implant surfaces with GEM21S increased the mean numbers of attached osteoblasts by 35% from a mean of 23 osteoblasts per implant surface without any growth factor, to 31 osteoblasts per SEM

micrograph (Figure 42). In this research study the stock doses of Emdogain of GEM21S were used to avoid problems about selecting the most suitable concentration for testing, however the results for the Emdogain suggest that its concentration was too powerful or too weak and further research is needed to optimize its effects.

4.10. Osteoblast attachment to Osseotite dental implant disks following the disinfection of *P. gingivalis*

The osteoblasts were observed attached to the surfaces of the Osseotite dental implant disks with or without a NanoTite coating following disinfection treatments using scanning electron microscopy at a magnification of x2,000. Some osteoblasts were seen attached to the Osseotite dental implants disinfected with ultrasonics and chlorhexidine glucontate (Figure 44). Osteoblasts were seen attached to the Osseotite dental implants with a NanoTitle coating that were disinfected with ultrasonics and chlorhexidine glucontate (Figure 45). Some osteoblasts were attached to the surface of Osseotite dental implants that were disinfected with ultrasonics and citric acid (Figure 46). After disinfecting Osseotite dental implants by brushing them with chlorhexidine gluconate, some osteoblasts were seen attached to the implant surface (Figure 47). Some osteoblasts attached to the Osseotite dental implants that were not infected with *P. gingivalis* as a control group (Figure 48).

The numbers of osteoblasts were counted on scanning electron micrograph (SEM) that were seen to be attached to each disinfected dental implant surface. There were a mean number of 14 osteoblasts attached to the Osseotite dental implant disk surfaces, and it was 14% higher at the mean number of 12 osteoblasts per SEM attached to the Osseotite implants with a NanoTite coating (Figure 49). Many of the disinfected implants had none or few osteoblasts attached, and this gave large standard deviations of the means (Figure 49).

The NanoTite coating made little difference to the numbers of osteoblasts that attached to the Osseotite implant surfaces.

A comparison of the use of chemical and physical disinfection treatments found that they had little effect on the mean numbers of osteoblasts attached to the surface of the dental implant disks (Figure 50). There was little difference in the numbers of osteoblasts attached to the surface of the Osseotite implants following disinfection using Chlorhexidine gluconate with ultrasonic activation or brushing, or Citric acid with ultrasonic activation, and no infection treatment as a control (Figure 50).

4.11. Analysis of osteoblast attachment morphology to 3iT3 dental implant disks following the disinfection of *P. gingivalis*

A comparison of the effect of osteoblast attachment morphology according to the type of 3iT3 dental implant surface, with or without a DCD coating, found that the DCD coating made little difference to the morphology of osteoblasts that attached to the implant surface following disinfection (Figure 51).

A comparison of the effect of osteoblast attachment morphology according to the coating of the 3iT3 dental implant surfaces, with or without Emdogain or GEM 21s growth factors, found that the addition of growth factors made little difference to the morphology of the osteoblasts that attached to the implant surface following disinfection (Figure 52).

A comparison of the effect of osteoblast attachment morphology according to the type of 3iT3 dental implant surface, with or without a DCD coating, and the coating of the dental implant surfaces, with or without Emdogain or GEM 21s growth factors found that these variables had little effect (Figure 53).

4.12. Analysis of osteoblast attachment morphology to Osseotite dental implant disks following the disinfection of *P. gingivalis*

A comparison of the effect of osteoblast attachment morphology according to the type of osseotite dental implant surface, with or without a Nanotite coating, found that the Nanotite coating made little difference to the morphology of osteoblasts that attached to the implant surface following disinfection (Figure 54).

A comparison of the disinfection treatments found that they had little effect on the morphology of the osteoblasts attached to the surface of the dental implant disks (Figure 54). The morphology of the osteoblasts was flat and similar following osseotite implant disk disinfection with Chlorhexidine Gluconate (0.12%) with ultrasonic activation, Chlorhexidine Gluconate (0.12%) with brushing, Citric acid with ultrasonic activation, and disks which had no infection treatment (Figure 54). Most of the osteoblasts attached to the osseotite implant surfaces had a flat morphology (Figure 54), suggesting the implant surface is favorable for osteoblast attachment.

4.13. Conclusions and future research directions

Several interesting results were observed for the first time in this study. This is the first study to measure the survival and attachment of osteoblasts to 3iT3 and osseotite dental implant disks according to the absence or presence of a DCD or NanoTite coating, disinfection solution, mechanical methods of applying the disinfection solutions, and the additional coating of the dental implant disks with growth factors. This is also the first study to investigate the disinfection of *P. gingivalis* from dental implants by comparing the effectiveness of using three disinfection solutions, and three mechanical methods of

applying the disinfection solutions. My conclusions for the ten phases in this research study are:

First, I measured the growth and disinfection of *P. gingivalis* in the presence of Biomet 3iT3™ dental implant disks with and without a DCD coating. The results from this part of the study found that the DCD coating made little difference to the growth or ability of the disinfection chemicals or physical activation to disinfect the *P. gingivalis* from the implant disks.

Second, I measured the effectiveness of using Sodium BiCarbonate, 3% Hydrogen Peroxide, or a 0.12% CHX solution to disinfect *P. gingivalis* from Biomet 3iT3™ dental implant disks. These chemicals all had a similar effectiveness to disinfect *P. gingivalis* from the implant disks.

Third, I compared the effectiveness of using Cavitron ProphyJet spraying, ultrasonic activation or brushing to disinfect *P. gingivalis* from the surfaces of Biomet 3iT3™ dental implant disks. These physical activation methods for the chemicals used to disinfect *P. gingivalis* from the implant disks, all had a similar effectiveness

Forth, I investigated osteoblast vitality in cell culture with the disinfected Biomet 3iT3™ dental implant disks. The 3iT3 dental implant disks without the DCD coating had 13% more osteoblast vitality compared to the 3iT3 dental implant disks with the DCD coating. The results showed that the DCD coating was less favorable for osteoblast vitality. The lack of physical activation of the disinfection chemicals gave the highest osteoblast vitality compared to activation of the chemicals using brushing, ultrasonics or spraying with a Prophy Jet. The vitality of the osteoblasts was least (68%) following the disinfection of the

3iT3 dental implant disks with Chlorhexidine gluconate, suggesting it is more toxic to osteoblasts compared to Hydrogen peroxide and Sodium bicarbonate. The coating of the disinfected 3iT3 dental implant surfaces with GEM21S or Emdogain had little effect on the vitality of osteoblasts suggesting that adding growth factors to the surface of disinfected dental implants is not beneficial for enhancing osteoblast vitality.

Fifth, I counted the numbers of osteoblasts attached to the disinfected Biomet 3iT3™ implant disk surfaces. There were a similar number of osteoblasts attached to the 3iT3 dental implants with or without a DCD coating, suggesting the DCD coating does not promote osteoblast attachment. The lack of physical activation of disinfection chemicals greatly enhanced the attachment of osteoblasts to the 3iT3 dental implant disk surfaces, suggesting that the use of a Prophy Jet, ultrasonics, alter the implant surface making them less optimal for osteoblast attachment. The highest numbers of attached osteoblasts were seen on dental implant surfaces that were disinfected with Hydrogen peroxide, suggesting it is less toxic compared to Chlorhexidine gluconate or Sodium bicarbonate, and that it provided a more biocompatible environment for osteoblast attachment.

Sixth, I investigated the effectiveness of using growth factors: Emdogain and Growth-factor Enhanced Matrix (GEM21S) to enhance osteoblast attachment to the Biomet 3iT3™ implant disk surface. Surprisingly, the coating of the implant surfaces with Emdogain appeared to promote the migration of osteoblasts way from the implant surfaces, because very few osteoblasts were attached. Many more osteoblasts were attached to the implant surfaces that had been coated with GEM21S, but because of the high variability of osteoblast attachment the effectiveness of GEM21S were similar to the implants without any growth factors.

Seventh, I investigated the growth and disinfection of *P. gingivalis* in the presence of Biomet 3i Osseotite dental implant disks with and without a NanoTite coating. The NanoTite coating made little difference to the disinfection of *P. gingivalis* from the Osseotite dental implant disks.

Eighth, I compared the ability of Citric acid and Chlorhexidine to disinfect *P. gingivalis* from Osseotite dental implant disks. The most effective disinfectants were Chlorhexidine gluconate with ultrasonic activation or with brushing using a titanium brush; the least effective disinfection treatment was Citric acid with ultrasonic activation. These results suggest that Citric acid is the worst choice of treatment for the disinfection of Osseotite dental implants, and that Chlorhexidine should be used to ensure that the implant has been adequately disinfected.

Ninth, I investigated osteoblast vitality in cell culture with the disinfected Osseotite dental implant disks. The vitality of osteoblasts was similar following disinfection with Citric acid and Chlorhexidine gluconate, and the NanoTite coating on some of the Osseotite disks did not enhance osteoblast attachment, compared to the control osteoblast attachment was 18% less in the disks with a NanoTite surface coating.

Tenth, I counted the numbers of osteoblasts attached to the disinfected Biomet Osseotite implant disk surfaces. There were a similar number of osteoblasts attached to the Osseotite dental implants with or without a NanoTite coating, suggesting the NanoTite coating did not promote osteoblast attachment. There was very little difference in the numbers of osteoblasts attached to the surfaces of the Osseotite dental implant disks following disinfection with Citric acid or Chlorhexidine gluconate. These results suggest these disinfection treatments had a similar effect on osteoblast attachment, however

because Citric acid was less effective for disinfecting *P. gingivalis* from the implants it cannot be recommended for disinfecting dental implants.

The results of this study has provided several avenues for further investigation which includes analyzing the effect of the physical activation of disinfection chemicals on the surface roughness properties of dental implants. The surface roughness of dental implants is a kay factor in promoting osseointegration hence any treatment which influences roughness could impact the clinical performance of the implants. Another key variable that was identified for further investigation is the toxicity and biocompatibility of the disinfection chemicals to osteoblasts, clearly the use of more toxic chemicals could reduce the ability of implants to heal and cause complications. For this reason it is recommended that all chemicals used to disinfect dental implants should be rinsed away from the dental implant to avoid toxic reactions. The standard concentration of Emdogain used in this study appeared to promote osteoblast migration away from the dental implant surface, which is a deleterious reaction. This suggests there is a need to investigate the optimal doses of Emdogain and GEM21S that will enhance osteoblast attachment and osseointegration of the disinfected dental implants.

My hope is that dentists and patients will benefit from my study data. Dentists can used my data to help guide them to select the most effective disinfectants and disinfection methods to remove bacteria from dental implants and obtain maximal osteoblast attachment. Dental patients will benefit from more successful procedures to disinfect dental implants.

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STATISTICS APPENDIX

Figure 1. Statistics for the effectiveness of disinfection of *P. gingivalis* from 3iT3 versus 3iT3 with DCD dental implants.

ANOVA Table for Disinfection (%)
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Implant Type	1	1.253	1.253	.003	.9593	.003	.050
Residual	125	59751.438	478.012				

Means Table for Disinfection (%)
Effect: Implant Type
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Count	Mean	Std. Dev.	Std. Err.
a 3iT3	63	28.996	22.815	2.874
b 3iT3 + Nano	64	29.194	20.885	2.611

Figure 2. Statistics for the effectiveness of chemicals and physical disinfection treatments to disinfect *P. gingivalis* from 3iT3 dental implant disks.

ANOVA Table for Disinfection (%)
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Chemical decontam	2	1511.228	755.614	1.609	.2043	3.218	.323
Residual	124	58241.462	469.689				

Means Table for Disinfection (%)
Effect: Chemical decontam
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Count	Mean	Std. Dev.	Std. Err.
a NaHCO3	48	31.838	20.729	2.992
b H202	32	23.269	19.436	3.436
c CHX	47	30.262	23.927	3.490

Scheffe for Disinfection (%)
Effect: Chemical decontam
Significance Level: 5 %
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean Diff.	Crit. Diff	P-Value
a NaHCO3, b H202	8.569	12.254	.2270
a NaHCO3, c CHX	1.576	11.019	.9391
b H202, c CHX	-6.992	12.306	.3742

ANOVA Table for Disinfection (%)
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Physical decontam	3	2389.570	796.523	1.708	.1689	5.124	.427
Residual	123	57363.121	466.367				

Means Table for Disinfection (%)
Effect: Physical decontam
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Count	Mean	Std. Dev.	Std. Err.
a Prophyjet	48	31.838	20.729	2.992
b Ultrasonic	31	24.010	17.978	3.229
c Brush	32	26.084	19.196	3.393
d None	16	36.746	32.709	8.177

Scheffe for Disinfection (%)
Effect: Physical decontam
Significance Level: 5 %
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean Diff.	Crit. Diff	P-Value
a Prophyjet, b Ultrasonic	7.828	14.105	.4825
a Prophyjet, c Brush	5.755	13.970	.7146
a Prophyjet, d None	-4.908	17.671	.8917
b Ultrasonic, c Brush	-2.073	15.427	.9859
b Ultrasonic, d None	-12.736	18.844	.3041
c Brush, d None	-10.663	18.743	.4604

ANOVA Table for Disinfection (%)
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Decontamination TX	5	3919.005	783.801	1.699	.1400	8.493	.565
Residual	121	55833.685	461.435				

Means Table for Disinfection (%)
Effect: Decontamination TX
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Count	Mean	Std. Dev.	Std. Err.
a Prophyjet	48	31.838	20.729	2.992
b H202 Ultrasonic	16	17.751	15.128	3.782
c CHX Ultra	15	30.687	18.837	4.864
d H2O2 Brush	16	28.788	22.065	5.516
e CHX Brush	16	23.379	16.095	4.024
f CHX	16	36.746	32.709	8.177

Scheffe for Disinfection (%)
Effect: Decontamination TX
Significance Level: 5 %
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean Diff.	Crit. Diff	P-Value
a Prophyjet, b H202 Ultrasonic	14.087	20.979	.4019
a Prophyjet, c CHX Ultra	1.152	21.497	>.9999
a Prophyjet, d H2O2 Brush	3.051	20.979	.9986
a Prophyjet, e CHX Brush	8.459	20.979	.8669
a Prophyjet, f CHX	-4.908	20.979	.9865
b H202 Ultrasonic, c CHX Ultra	-12.936	26.119	.7293
b H202 Ultrasonic, d H2O2 Brush	-11.036	25.694	.8324
b H202 Ultrasonic, e CHX Brush	-5.628	25.694	.9900
b H202 Ultrasonic, f CHX	-18.995	25.694	.2897
c CHX Ultra, d H2O2 Brush	1.899	26.119	>.9999
c CHX Ultra, e CHX Brush	7.307	26.119	.9700
c CHX Ultra, f CHX	-6.059	26.119	.9870
d H2O2 Brush, e CHX Brush	5.408	25.694	.9917
d H2O2 Brush, f CHX	-7.959	25.694	.9535
e CHX Brush, f CHX	-13.367	25.694	.6851

Figure 3. Statistics for the effectiveness of chemicals and physical disinfection treatments to disinfect *P. gingivalis* from Osseotite and Osseotite with NanoTite dental implants.

ANOVA Table for Percentage dis %

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Group	2	11461.232	5730.616	8.068	.0025	16.137	.937
Residual	21	14915.552	710.264				

Descriptive Statistics
Split By: Group

	Percentage dis %, Total	Percentage dis %, a CHX Ultra	Percentage dis %, b CHX brush	Percentage dis %, c Citric acid Ultra
Mean	13.135	.515	.526	50.986
Std. Dev.	33.865	.196	.249	54.617
Std. Error	6.913	.057	.102	22.297
Count	24	12	6	6
Minimum	.066	.197	.066	.526
Maximum	104.336	.854	.723	104.336
# Missing	0	0	0	0

Scheffe for Abs 600 nm
Effect: Group
Significance Level: 5 %

	Mean Diff.	Crit. Diff	P-Value	
a CHX Ultra, b CHX brush	-1.667E-4	.534	>.9999	
a CHX Ultra, c Citric acid Ultra	-.768	.534	.0042	S
b CHX brush, c Citric acid Ultra	-.768	.617	.0130	S

ANOVA Table for Abs 600 nm

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Implant type	1	.296	.296	1.119	.3017	1.119	.165
Residual	22	5.814	.264				

Descriptive Statistics
Split By: Implant type

	Percentage dis %, Total	Percentage dis %, a Osseotite	Percentage dis %, b Oss + Nano
Mean	13.135	17.346	.504
Std. Dev.	33.865	38.425	.142
Std. Error	6.913	9.057	.058
Count	24	18	6
Minimum	.066	.066	.329
Maximum	104.336	104.336	.723
# Missing	0	0	0

Figure 4. Statistics for osteoblast viability on 3iT3 disks versus 3iT3 disks with DCD following the physical disinfection of *P. gingivalis*.

ANOVA Table for Osteoblast vitality (100%)
Row exclusion: Flavia data 3 5 15 MTT first set of implants.svd

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Implant Type	1	5560.451	5560.451	26.786	<.0001	26.786	1.000
Residual	117	24287.905	207.589				

Descriptive Statistics
Split By: Implant Type
Row exclusion: Flavia data 3 5 15 MTT first set of implants.svd

	Osteoblast vitality (100%), Total	Osteoblast vitality (100%), a 3iT3	Osteoblast vitality (100%), b 3iT3 + Nano
Mean	78.851	85.296	71.601
Std. Dev.	15.904	15.255	13.389
Std. Error	1.458	1.922	1.789
Count	119	63	56
Minimum	48.367	53.171	48.367
Maximum	131.633	131.220	131.633
# Missing	0	0	0

Figure 5. Statistics for osteoblast viability on 3iT3 disks following the physical disinfection of *P. gingivalis*.

ANOVA Table for Osteoblast vitality (100%)
Row exclusion: Flavia data 3 5 15 MTT first set of implants.svd

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Physical decontam	3	2288.837	762.946	2.967	.0346	8.902	.689
Residual	123	31623.404	257.101				

Descriptive Statistics
Split By: Physical decontam
Row exclusion: Flavia data 3 5 15 MTT first set of implants.svd

	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum	# Missing
Osteoblast vitality (100%), Total	77.425	16.406	1.456	127	46.327	131.633	0
Osteoblast vitality (100%), a Prophyjet	80.579	13.123	1.894	48	59.756	115.610	0
Osteoblast vitality (100%), b Ultrasonic	80.799	15.109	2.714	31	61.224	131.220	0
Osteoblast vitality (100%), c Brush	73.072	20.674	3.655	32	48.367	131.633	0
Osteoblast vitality (100%), d None	70.127	15.126	3.781	16	46.327	89.024	0

Scheffe for Osteoblast vitality (100%)
Effect: Physical decontam
Significance Level: 5 %
Row exclusion: Flavia data 3 5 15 MTT first set of implants.svd

	Mean Diff.	Crit. Diff	P-Value
a Prophyjet, b Ultrasonic	-.220	10.473	>.9999
a Prophyjet, c Brush	7.506	10.373	.2453
a Prophyjet, d None	10.452	13.121	.1706
b Ultrasonic, c Brush	7.727	11.454	.3058
b Ultrasonic, d None	10.672	13.991	.2029
c Brush, d None	2.945	13.916	.9482

Figure 6. Statistics for osteoblast viability on 3iT3 disks versus 3iT3 with DCD disks following the physical disinfection of *P. gingivalis*.

ANOVA Table for Osteoblast vitality (100%)
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Implant Type	1	8201.424	8201.424	43.101	<.0001	43.101	1.000
Physical decontam	3	2354.824	784.941	4.125	.0080	12.375	.847
Implant Type * Physical decontam	3	1178.934	392.978	2.065	.1085	6.196	.508
Residual	119	22643.812	190.284				

Descriptive Statistics
Split By: Implant Type, Physical decontam
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum	# Missing
Osteoblast vitality (100%), Total	77.425	16.406	1.456	127	46.327	131.633	0
Osteoblast vitality (100%), a 3iT3, a Prophyjet	87.907	12.605	2.573	24	64.390	115.610	0
Osteoblast vitality (100%), a 3iT3, b Ultrasonic	90.618	15.602	4.029	15	74.146	131.220	0
Osteoblast vitality (100%), a 3iT3, c Brush	77.012	19.246	4.811	16	53.171	113.171	0
Osteoblast vitality (100%), a 3iT3, d None	84.055	3.974	1.405	8	78.293	89.024	0
Osteoblast vitality (100%), b 3iT3 + Nano, a Prophyjet	73.251	8.997	1.837	24	59.756	88.980	0
Osteoblast vitality (100%), b 3iT3 + Nano, b Ultrasonic	71.594	6.524	1.631	16	61.224	84.082	0
Osteoblast vitality (100%), b 3iT3 + Nano, c Brush	69.133	21.905	5.476	16	48.367	131.633	0
Osteoblast vitality (100%), b 3iT3 + Nano, d None	56.199	5.573	1.970	8	46.327	61.837	0

Row exclusion: Flavia 3iT3 attachment and viability

	Mean Diff.	Crit. Diff	P-Value	
a 3iT3, b 3iT3 + Nano	14.655	6.363	<.0001	S

Row exclusion: Flavia 3iT3 attachment and viability

	Mean Diff.	Crit. Diff	P-Value	
a 3iT3, b 3iT3 + Nano	19.023	8.683	.0001	S

Cell: c Brush
Row exclusion: Flavia 3iT3 attachment and viability

	Mean Diff.	Crit. Diff	P-Value
a 3iT3, b 3iT3 + Nano	7.880	14.887	.2883

Cell: d None
Row exclusion: Flavia 3iT3 attachment and viability

	Mean Diff.	Crit. Diff	P-Value	
a 3iT3, b 3iT3 + Nano	27.856	5.190	<.0001	S

Figure 7. Statistics for osteoblast viability on 3iT3 disks following the chemical disinfection of *P. gingivalis*.

ANOVA Table for Osteoblast vitality (100%)
Row exclusion: Flavia data 3 5 15 MTT first set of implants.svd

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Chemical decontam	2	6723.008	3361.504	15.331	<.0001	30.661	1.000
Residual	124	27189.233	219.268				

Descriptive Statistics
Split By: Chemical decontam
Row exclusion: Flavia data 3 5 15 MTT first set of implants.svd

	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum	# Missing
Osteoblast vitality (100%), Total	77.425	16.406	1.456	127	46.327	131.633	0
Osteoblast vitality (100%), a NaHCO3	80.579	13.123	1.894	48	59.756	115.610	0
Osteoblast vitality (100%), b H202	86.030	17.549	3.102	32	62.041	131.633	0
Osteoblast vitality (100%), c CHX	68.344	14.407	2.102	47	46.327	98.293	0

Scheffe for Osteoblast vitality (100%)
Effect: Chemical decontam
Significance Level: 5 %
Row exclusion: Flavia data 3 5 15 MTT first set of implants.svd

	Mean Diff.	Crit. Diff	P-Value	
a NaHCO3, b H202	-5.451	8.373	.2759	
a NaHCO3, c CHX	12.235	7.529	.0005	S
b H202, c CHX	17.686	8.408	<.0001	S

Figure 8. Statistics for osteoblast viability on 3iT3 disks versus 3iT3 disks with DCD following the chemical disinfection of *P. gingivalis.*

ANOVA Table for Osteoblast vitality (100%)
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Implant Type	1	7516.442	7516.442	46.512	<.0001	46.512	1.000
Chemical decontam	2	6583.808	3291.904	20.371	<.0001	40.741	1.000
Implant Type * Chemical decontam	2	29.552	14.776	.091	.9127	.183	.064
Residual	121	19553.717	161.601				

Descriptive Statistics
Split By: Implant Type, Chemical decontam
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum	# Missing
Osteoblast vitality (100%), Total	77.425	16.406	1.456	127	46.327	131.633	0
Osteoblast vitality (100%), a 3iT3, a NaHCO3	87.907	12.605	2.573	24	64.390	115.610	0
Osteoblast vitality (100%), a 3iT3, b H202	94.573	14.912	3.728	16	79.268	131.220	0
Osteoblast vitality (100%), a 3iT3, c CHX	76.119	13.417	2.798	23	53.171	98.293	0
Osteoblast vitality (100%), b 3iT3 + Nano, a NaHCO3	73.251	8.997	1.837	24	59.756	88.980	0
Osteoblast vitality (100%), b 3iT3 + Nano, b H202	77.487	16.076	4.019	16	62.041	131.633	0
Osteoblast vitality (100%), b 3iT3 + Nano, c CHX	60.893	11.162	2.278	24	46.327	84.082	0

Cell: a NaHCO3

	Mean Diff.	Crit. Diff	P-Value	
a 3iT3, b 3iT3 + Nano	14.655	6.363	<.0001	S

Cell: b H202

	Mean Diff.	Crit. Diff	P-Value	
a 3iT3, b 3iT3 + Nano	17.086	11.195	.0040	S

Cell: c CHX

	Mean Diff.	Crit. Diff	P-Value	
a 3iT3, b 3iT3 + Nano	15.226	7.238	.0001	S

Figure 9. Statistics for 3iT3 versus 3iT3 Nanotite disk chemical and mechanical disinfection and osteoblast viability

ANOVA Table for Osteoblast vitality (100%)
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Decontamination TX	5	11682.210	2336.442	12.717	<.0001	63.587	1.000
Residual	121	22230.030	183.719				

Descriptive Statistics
Split By: Chemical decontam, Physical decontam
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum	# Missing
Osteoblast vitality (100%), Total	77.425	16.406	1.456	127	46.327	131.633	0
Osteoblast vitality (100%), a NaHCO3, a Prophyjet	80.579	13.123	1.894	48	59.756	115.610	0
Osteoblast vitality (100%), b H202, b Ultrasonic	81.891	19.067	4.767	16	62.041	131.220	0
Osteoblast vitality (100%), b H202, c Brush	90.170	15.375	3.844	16	70.816	131.633	0
Osteoblast vitality (100%), c CHX, b Ultrasonic	79.635	9.841	2.541	15	61.224	98.293	0
Osteoblast vitality (100%), c CHX, c Brush	55.975	4.829	1.207	16	48.367	64.146	0
Osteoblast vitality (100%), c CHX, d None	70.127	15.126	3.781	16	46.327	89.024	0

Scheffe for Osteoblast vitality (100%)
Effect: Decontamination TX
Significance Level: 5 %
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean Diff.	Crit. Diff	P-Value	
a Prophyjet, b H202 Ultrasonic	-1.312	13.238	.9998	
a Prophyjet, c CHX Ultra	.944	13.565	>.9999	
a Prophyjet, d H2O2 Brush	-9.591	13.238	.3125	
a Prophyjet, e CHX Brush	24.604	13.238	<.0001	S
a Prophyjet, f CHX	10.452	13.238	.2193	
b H202 Ultrasonic, c CHX Ultra	2.256	16.481	.9989	
b H202 Ultrasonic, d H2O2 Brush	-8.279	16.213	.7024	
b H202 Ultrasonic, e CHX Brush	25.916	16.213	<.0001	S
b H202 Ultrasonic, f CHX	11.764	16.213	.3108	
c CHX Ultra, d H2O2 Brush	-10.535	16.481	.4607	
c CHX Ultra, e CHX Brush	23.660	16.481	.0006	S
c CHX Ultra, f CHX	9.508	16.481	.5790	
d H2O2 Brush, e CHX Brush	34.194	16.213	<.0001	S
d H2O2 Brush, f CHX	20.043	16.213	.0055	S
e CHX Brush, f CHX	-14.152	16.213	.1296	

Figure 10. Statistics for osteoblast viability on 3iT3 disks versus 3iT3 disks with DCD following the chemical and physical disinfection of *P. gingivalis*.

ANOVA Table for Osteoblast vitality (100%)
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Decontamination TX	5	11709.881	2341.976	20.887	<.0001	104.436	1.000
Implant Type	1	6782.405	6782.405	60.490	<.0001	60.490	1.000
Decontamination TX * Implant Type	5	1551.113	310.223	2.767	.0213	13.834	.816
Residual	115	12894.391	112.125				

Descriptive Statistics
Split By: Implant Type, Physical decontam, Chemical decontam
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum	# Missing
Osteoblast vitality (100%), Total	77.425	16.406	1.456	127	46.327	131.633	0
Osteoblast vitality (100%), a 3iT3, a Prophyjet, a NaHCO3	87.907	12.605	2.573	24	64.390	115.610	0
Osteoblast vitality (100%), a 3iT3, b Ultrasonic, b H202	94.726	19.711	6.969	8	79.268	131.220	0
Osteoblast vitality (100%), a 3iT3, b Ultrasonic, c CHX	85.923	8.155	3.082	7	74.146	98.293	0
Osteoblast vitality (100%), a 3iT3, c Brush, b H202	94.421	9.374	3.314	8	82.439	113.171	0
Osteoblast vitality (100%), a 3iT3, c Brush, c CHX	59.604	3.622	1.281	8	53.171	64.146	0
Osteoblast vitality (100%), a 3iT3, d None, c CHX	84.055	3.974	1.405	8	78.293	89.024	0
Osteoblast vitality (100%), b 3iT3 + Nano, a Prophyjet, a NaHC...	73.251	8.997	1.837	24	59.756	88.980	0
Osteoblast vitality (100%), b 3iT3 + Nano, b Ultrasonic, b H202	69.056	3.733	1.320	8	62.041	72.041	0
Osteoblast vitality (100%), b 3iT3 + Nano, b Ultrasonic, c CHX	74.133	7.909	2.796	8	61.224	84.082	0
Osteoblast vitality (100%), b 3iT3 + Nano, c Brush, b H202	85.918	19.426	6.868	8	70.816	131.633	0
Osteoblast vitality (100%), b 3iT3 + Nano, c Brush, c CHX	52.347	2.600	.919	8	48.367	56.531	0
Osteoblast vitality (100%), b 3iT3 + Nano, d None, c CHX	56.199	5.573	1.970	8	46.327	61.837	0

	Mean Diff.	Crit. Diff	P-Value	
a Prophyjet, b H202 Ultrasonic	-1.312	13.238	.9998	
a Prophyjet, c CHX Ultra	.944	13.565	>.9999	
a Prophyjet, d H2O2 Brush	-9.591	13.238	.3125	
a Prophyjet, e CHX Brush	24.604	13.238	<.0001	S
a Prophyjet, f CHX	10.452	13.238	.2193	
b H202 Ultrasonic, c CHX Ultra	2.256	16.481	.9989	
b H202 Ultrasonic, d H2O2 Brush	-8.279	16.213	.7024	
b H202 Ultrasonic, e CHX Brush	25.916	16.213	<.0001	S
b H202 Ultrasonic, f CHX	11.764	16.213	.3108	
c CHX Ultra, d H2O2 Brush	-10.535	16.481	.4607	
c CHX Ultra, e CHX Brush	23.660	16.481	.0006	S
c CHX Ultra, f CHX	9.508	16.481	.5790	
d H2O2 Brush, e CHX Brush	34.194	16.213	<.0001	S
d H2O2 Brush, f CHX	20.043	16.213	.0055	S
e CHX Brush, f CHX	-14.152	16.213	.1296	

Figure 11. Statistics for osteoblast viability on 3iT3 disks treated with growth factors following the chemical and physical disinfection of *P. gingivalis*.

ANOVA Table for Osteoblast vitality (100%)
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Growth factor	2	735.514	367.757	2.249	.1172	4.498	.422
Residual	45	7358.084	163.513				

Descriptive Statistics
Split By: Growth factor
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum	# Missing
Osteoblast vitality (100%), Total	77.425	16.406	1.456	127	46.327	131.633	0
Osteoblast vitality (100%), a None	79.695	19.496	4.874	16	59.756	115.610	0
Osteoblast vitality (100%), b Emdogain	76.288	5.681	1.420	16	64.390	88.537	0
Osteoblast vitality (100%), c GEM 21	85.753	8.842	2.211	16	72.041	109.024	0

Results for totals may not agree with results for individual cells because of missing values for split variables.

Figure 12. Statistics for osteoblast viability on 3iT3 disks versus 3iT3 disks with DCD treated with growth factors following the chemical and physical disinfection of *P. gingivalis.*

ANOVA Table for Osteoblast vitality (100%)
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Growth factor	2	735.514	367.757	6.383	.0038	12.765	.891
Implant Type	1	2577.334	2577.334	44.731	<.0001	44.731	1.000
Growth factor * Implant Type	2	2360.760	1180.380	20.486	<.0001	40.972	1.000
Residual	42	2419.990	57.619				

Descriptive Statistics
Split By: Growth factor, Implant Type
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum	# Missing
Osteoblast vitality (100%), Total	77.425	16.406	1.456	127	46.327	131.633	0
Osteoblast vitality (100%), a None, a 3iT3	96.524	12.779	4.518	8	82.927	115.610	0
Osteoblast vitality (100%), a None, b 3iT3 + Nano	62.866	1.946	.688	8	59.756	65.610	0
Osteoblast vitality (100%), b Emdogain, a 3iT3	76.402	6.812	2.408	8	64.390	88.537	0
Osteoblast vitality (100%), b Emdogain, b 3iT3 + Nano	76.173	4.767	1.685	8	69.796	83.878	0
Osteoblast vitality (100%), c GEM 21, a 3iT3	90.793	8.169	2.888	8	81.707	109.024	0
Osteoblast vitality (100%), c GEM 21, b 3iT3 + Nano	80.714	6.541	2.312	8	72.041	88.980	0

Results for totals may not agree with results for individual cells because of missing values for split variables.

Scheffe for Osteoblast vitality (100%)
Effect: Growth factor
Significance Level: 5 %
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean Diff.	Crit. Diff	P-Value	
a None, b Emdogain	3.407	6.810	.4535	
a None, c GEM 21	-6.058	6.810	.0903	
b Emdogain, c GEM 21	-9.466	6.810	.0043	S

Scheffe for Osteoblast vitality (100%)
Effect: Implant Type
Significance Level: 5 %
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean Diff.	Crit. Diff	P-Value	
a 3iT3, b 3iT3 + Nano	14.655	4.422	<.0001	S

Figure 13. Statistics for osteoblast vitality on Osseotite versus Osseotite with Nanotite disks.

ANOVA Table for Osteo vit (%)

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Disc type	1	49.127	49.127	.216	.6453	.216	.073
Residual	34	7746.019	227.824				

Descriptive Statistics
Split By: Disc type

	Osteo vit (%), Total	Osteo vit (%), a Osseotite	Osteo vit (%), b Nanotite
Mean	97.663	98.288	95.478
Std. Dev.	14.924	14.522	17.121
Std. Error	2.487	2.744	6.053
Count	36	28	8
Minimum	73.529	73.529	78.824
Maximum	144.706	144.706	126.765
# Missing	0	0	0

Figure 14. Statistics for osteoblast vitality on Osseotite disks following disinfection.

ANOVA Table for Osteo vit (%)

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Groups	3	1537.782	512.594	2.621	.0676	7.864	.581
Residual	32	6257.364	195.543				

Descriptive Statistics
Split By: Groups, Disc type

	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum	# Missing
Osteo vit (%), Total	97.663	14.924	2.487	36	73.529	144.706	0
Osteo vit (%), a 1 Ultr + CHX, a Osseotite	113.493	16.388	5.794	8	97.353	144.706	0
Osteo vit (%), a 1 Ultr + CHX, b Nanotite	95.478	17.121	6.053	8	78.824	126.765	0
Osteo vit (%), c 3 Citric + Ultra, a Osseotite	95.662	8.439	2.984	8	75.882	102.059	0
Osteo vit (%), d 4 Brush + CHX, a Osseotite	88.640	7.391	2.613	8	73.529	96.471	0
Osteo vit (%), e 5 Control, a Osseotite	92.426	6.986	3.493	4	86.176	100.588	0

Figures 15 to 34. Scanning electron micrograph images of osteoblast attachment to implant surfaces.

No statistics were calculated for these images.

Figure 35. Statistics for osteoblast attachment to 3iT3 disks versus 3iT3 disks with DCD following the physical disinfection of *P. gingivalis*.

ANOVA Table for Osteo attached per SEM
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Implant Type	1	193.838	193.838	.085	.7713	.085	.059
Residual	125	285522.228	2284.178				

Means Table for Osteo attached per SEM
Effect: Implant Type
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Count	Mean	Std. Dev.	Std. Err.
a 3iT3	63	33.060	36.352	4.580
b 3iT3 + Nano	64	35.531	56.847	7.106

Figure 36. Statistics for osteoblast attachment to 3iT3 disks following the physical disinfection of *P. gingivalis*.

ANOVA Table for Osteo attached per SEM
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Physical decontam	3	43225.170	14408.390	7.308	.0002	21.925	.988
Residual	123	242490.896	1971.471				

Means Table for Osteo attached per SEM
Effect: Physical decontam
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Count	Mean	Std. Dev.	Std. Err.
a Prophyjet	48	18.925	20.650	2.981
b Ultrasonic	31	29.852	32.830	5.896
c Brush	32	39.956	61.413	10.856
d None	16	77.775	69.855	17.464

Scheffe for Osteo attached per SEM
Effect: Physical decontam
Significance Level: 5 %
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean Diff.	Crit. Diff	P-Value	
a Prophyjet, b Ultrasonic	-10.927	29.000	.7674	
a Prophyjet, c Brush	-21.031	28.723	.2356	
a Prophyjet, d None	-58.850	36.332	.0002	S
b Ultrasonic, c Brush	-10.105	31.718	.8456	
b Ultrasonic, d None	-47.923	38.743	.0082	S
c Brush, d None	-37.819	38.536	.0567	

Figure 37. Statistics for osteoblast viability on 3iT3 disks versus 3iT3 with DCD disks following the physical disinfection of *P. gingivalis*.

ANOVA Table for Osteo attached per SEM
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Physical decontam	3	43113.185	14371.062	7.371	.0001	22.113	.989
Implant Type	1	597.181	597.181	.306	.5810	.306	.084
Physical decontam * Implant Type	3	10274.587	3424.862	1.757	.1592	5.270	.438
Residual	119	232011.270	1949.675				

Means Table for Osteo attached per SEM
Effect: Physical decontam * Implant Type
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Count	Mean	Std. Dev.	Std. Err.
a Prophyjet, a 3iT3	24	17.583	21.725	4.435
a Prophyjet, b 3iT3 + Nano	24	20.267	19.891	4.060
b Ultrasonic, a 3iT3	15	43.240	33.630	8.683
b Ultrasonic, b 3iT3 + Nano	16	17.300	27.436	6.859
c Brush, a 3iT3	16	29.787	38.634	9.659
c Brush, b 3iT3 + Nano	16	50.125	77.983	19.496
d None, a 3iT3	8	66.950	48.092	17.003
d None, b 3iT3 + Nano	8	88.600	88.747	31.377

Scheffe for Osteo attached per SEM
Effect: Physical decontam
Significance Level: 5 %
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean Diff.	Crit. Diff	P-Value	
a Prophyjet, b Ultrasonic	-10.927	28.853	.7644	
a Prophyjet, c Brush	-21.031	28.577	.2312	
a Prophyjet, d None	-58.850	36.148	.0002	S
b Ultrasonic, c Brush	-10.105	31.557	.8434	
b Ultrasonic, d None	-47.923	38.546	.0078	S
c Brush, d None	-37.819	38.341	.0548	

Scheffe for Osteo attached per SEM
Effect: Implant Type
Significance Level: 5 %
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean Diff.	Crit. Diff	P-Value
a 3iT3, b 3iT3 + Nano	-2.471	15.517	.7531

Figure 38. Statistics for osteoblast attachment to 3iT3 disks following the chemical disinfection of *P. gingivalis*.

ANOVA Table for Osteo attached per SEM
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Chemical decontam	2	22353.919	11176.960	5.262	.0064	10.525	.836
Residual	124	263362.147	2123.888				

Means Table for Osteo attached per SEM
Effect: Chemical decontam
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Count	Mean	Std. Dev.	Std. Err.
a NaHCO3	48	18.925	20.650	2.981
b H202	32	52.381	60.219	10.645
c CHX	47	37.706	53.346	7.781

Scheffe for Osteo attached per SEM
Effect: Chemical decontam
Significance Level: 5 %
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean Diff.	Crit. Diff	P-Value	
a NaHCO3, b H202	-33.456	26.058	.0077	S
a NaHCO3, c CHX	-18.781	23.431	.1435	
b H202, c CHX	14.675	26.169	.3838	

Figure 39. Statistics for osteoblast attachment to 3iT3 disks versus 3iT3 disks with DCD following the chemical disinfection of *P. gingivalis.*

ANOVA Table for Osteo attached per SEM
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Chemical decontam	2	22335.003	11167.502	5.141	.0072	10.281	.825
Implant Type	1	109.437	109.437	.050	.8228	.050	.056
Chemical decontam * Implant Type	2	310.413	155.207	.071	.9311	.143	.061
Residual	121	262866.189	2172.448				

Means Table for Osteo attached per SEM
Effect: Chemical decontam * Implant Type
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Count	Mean	Std. Dev.	Std. Err.
a NaHCO3, a 3iT3	24	17.583	21.725	4.435
a NaHCO3, b 3iT3 + Nano	24	20.267	19.891	4.060
b H202, a 3iT3	16	53.650	38.673	9.668
b H202, b 3iT3 + Nano	16	51.112	77.429	19.357
c CHX, a 3iT3	23	34.887	40.470	8.439
c CHX, b 3iT3 + Nano	24	40.408	64.104	13.085

Scheffe for Osteo attached per SEM
Effect: Chemical decontam
Significance Level: 5 %
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean Diff.	Crit. Diff	P-Value	
a NaHCO3, b H202	-33.456	26.363	.0086	S
a NaHCO3, c CHX	-18.781	23.705	.1499	
b H202, c CHX	14.675	26.475	.3921	

Scheffe for Osteo attached per SEM
Effect: Implant Type
Significance Level: 5 %
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean Diff.	Crit. Diff	P-Value
a 3iT3, b 3iT3 + Nano	-2.471	16.377	.7657

Figure 40. Statistics for osteoblast attachment to 3iT3 disks following the chemical and physical disinfection of *P. gingivalis*.

ANOVA Table for Osteo attached per SEM
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Decontamination TX	5	81910.024	16382.005	9.726	<.0001	48.630	1.000
Residual	121	203806.042	1684.347				

Descriptive Statistics
Split By: Physical decontam, Chemical decontam
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean	Std. Dev.	Std. E...	Count	Minimum	Maxi...	# ...
Osteo attached per SEM, Total	34.306	47.619	4.226	127	0.000	262	0
Osteo attached per SEM, a Prophyjet, a NaHC...	18.925	20.650	2.981	48	0.000	68.000	0
Osteo attached per SEM, b Ultrasonic, b H202	30.038	35.378	8.844	16	.600	123	0
Osteo attached per SEM, b Ultrasonic, c CHX	29.653	31.121	8.035	15	1.400	107	0
Osteo attached per SEM, c Brush, b H202	74.725	71.956	17.989	16	10.600	262	0
Osteo attached per SEM, c Brush, c CHX	5.188	6.159	1.540	16	0.000	19.600	0
Osteo attached per SEM, d None, c CHX	77.775	69.855	17.464	16	3.000	231	0

Scheffe for Osteo attached per SEM
Effect: Decontamination TX
Significance Level: 5 %
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean Diff.	Crit. Diff	P-Value	
a Prophyjet, b H202 Ultrasonic	-11.112	40.082	.9711	
a Prophyjet, c CHX Ultra	-10.728	41.072	.9778	
a Prophyjet, d H2O2 Brush	-55.800	40.082	.0010	S
a Prophyjet, e CHX Brush	13.738	40.082	.9293	
a Prophyjet, f CHX	-58.850	40.082	.0004	S
b H202 Ultrasonic, c CHX Ultra	.384	49.902	>.9999	
b H202 Ultrasonic, d H2O2 Brush	-44.688	49.091	.0997	
b H202 Ultrasonic, e CHX Brush	24.850	49.091	.7102	
b H202 Ultrasonic, f CHX	-47.737	49.091	.0624	
c CHX Ultra, d H2O2 Brush	-45.072	49.902	.1050	
c CHX Ultra, e CHX Brush	24.466	49.902	.7378	
c CHX Ultra, f CHX	-48.122	49.902	.0665	
d H2O2 Brush, e CHX Brush	69.538	49.091	.0007	S
d H2O2 Brush, f CHX	-3.050	49.091	>.9999	
e CHX Brush, f CHX	-72.587	49.091	.0003	S

Figure 41. Statistics for osteoblast attachment to 3iT3 disks versus 3iT3 disks with DCD following the chemical and physical disinfection of *P. gingivalis*.

ANOVA Table for Osteo attached per SEM
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Decontamination TX	5	81884.864	16376.973	10.069	<.0001	50.345	1.000
Implant Type	1	155.169	155.169	.095	.7580	.095	.061
Decontamination TX * Implant Type	5	16555.178	3311.036	2.036	.0787	10.179	.659
Residual	115	187045.266	1626.481				

Means Table for Osteo attached per SEM
Effect: Decontamination TX * Implant Type
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Count	Mean	Std. Dev.	Std. Err.
a Prophyjet, a 3iT3	24	17.583	21.725	4.435
a Prophyjet, b 3iT3 + Nano	24	20.267	19.891	4.060
b H202 Ultrasonic, a 3iT3	8	52.700	38.218	13.512
b H202 Ultrasonic, b 3iT3 + Nano	8	7.375	6.885	2.434
c CHX Ultra, a 3iT3	7	32.429	26.061	9.850
c CHX Ultra, b 3iT3 + Nano	8	27.225	36.612	12.944
d H2O2 Brush, a 3iT3	8	54.600	41.739	14.757
d H2O2 Brush, b 3iT3 + Nano	8	94.850	91.800	32.456
e CHX Brush, a 3iT3	8	4.975	7.003	2.476
e CHX Brush, b 3iT3 + Nano	8	5.400	5.668	2.004
f CHX, a 3iT3	8	66.950	48.092	17.003
f CHX, b 3iT3 + Nano	8	88.600	88.747	31.377

Scheffe for Osteo attached per SEM
Effect: Implant Type
Significance Level: 5 %
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean Diff.	Crit. Diff	P-Value
a 3iT3, b 3iT3 + Nano	-2.471	14.178	.7306

Scheffe for Osteo attached per SEM
Effect: Decontamination TX
Significance Level: 5 %
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean Diff.	Crit. Diff	P-Value	
a Prophyjet, b H202 Ultrasonic	-11.113	39.422	.9688	
a Prophyjet, c CHX Ultra	-10.728	40.396	.9760	
a Prophyjet, d H2O2 Brush	-55.800	39.422	.0007	S
a Prophyjet, e CHX Brush	13.738	39.422	.9241	
a Prophyjet, f CHX	-58.850	39.422	.0003	S
b H202 Ultrasonic, c CHX Ultra	.384	49.080	>.9999	
b H202 Ultrasonic, d H2O2 Brush	-44.687	48.282	.0891	
b H202 Ultrasonic, e CHX Brush	24.850	48.282	.6943	
b H202 Ultrasonic, f CHX	-47.738	48.282	.0548	
c CHX Ultra, d H2O2 Brush	-45.072	49.080	.0940	
c CHX Ultra, e CHX Brush	24.466	49.080	.7230	
c CHX Ultra, f CHX	-48.122	49.080	.0585	
d H2O2 Brush, e CHX Brush	69.537	48.282	.0005	S
d H2O2 Brush, f CHX	-3.050	48.282	>.9999	
e CHX Brush, f CHX	-72.588	48.282	.0003	S

Figure 42. Statistics for osteoblast attachment to 3iT3 disks treated with growth factors following the chemical and physical disinfection of *P. gingivalis*.

ANOVA Table for Osteo attached per SEM
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Growth factor	2	6367.715	3183.858	10.477	.0002	20.955	.990
Residual	45	13674.575	303.879				

Means Table for Osteo attached per SEM
Effect: Growth factor
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Count	Mean	Std. Dev.	Std. Err.
a None	16	23.062	20.033	5.008
b Emdogain	16	3.212	4.398	1.100
c GEM 21	16	30.500	22.158	5.539

Scheffe for Osteo attached per SEM
Effect: Growth factor
Significance Level: 5 %
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean Diff.	Crit. Diff	P-Value	
a None, b Emdogain	19.850	15.602	.0094	S
a None, c GEM 21	-7.438	15.602	.4884	
b Emdogain, c GEM 21	-27.288	15.602	.0003	S

Figure 43. Statistics for osteoblast viability on 3iT3 disks versus 3iT3 disks with DCD treated with growth factors following the chemical and physical disinfection of *P. gingivalis*.

ANOVA Table for Osteo attached per SEM
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Growth factor	2	6367.715	3183.858	9.927	.0003	19.854	.985
Implant Type	1	86.403	86.403	.269	.6065	.269	.079
Growth factor * Implant Type	2	117.752	58.876	.184	.8330	.367	.076
Residual	42	13470.420	320.724				

Means Table for Osteo attached per SEM
Effect: Growth factor * Implant Type
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Count	Mean	Std. Dev.	Std. Err.
a None, a 3iT3	8	19.550	24.125	8.530
a None, b 3iT3 + Nano	8	26.575	15.804	5.587
b Emdogain, a 3iT3	8	2.575	3.488	1.233
b Emdogain, b 3iT3 + Nano	8	3.850	5.326	1.883
c GEM 21, a 3iT3	8	30.625	22.370	7.909
c GEM 21, b 3iT3 + Nano	8	30.375	23.487	8.304

Scheffe for Osteo attached per SEM
Effect: Growth factor
Significance Level: 5 %
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean Diff.	Crit. Diff	P-Value	
a None, b Emdogain	19.850	16.068	.0121	S
a None, c GEM 21	-7.438	16.068	.5072	
b Emdogain, c GEM 21	-27.288	16.068	.0005	S

Scheffe for Osteo attached per SEM
Effect: Implant Type
Significance Level: 5 %
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	Mean Diff.	Crit. Diff	P-Value
a 3iT3, b 3iT3 + Nano	-2.683	10.433	.6065

Figures 44 to 48. Scanning electron micrographs of osteoblasts attached to osseotite implant surfaces.

No data was analyzed for these figures.

Figure 49. Statistics for osteoblast attachment to Osseotite versus Osseotite with Nanotite disks.

ANOVA Table for # Cells attached SEM

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Disc type	1	20.301	20.301	.185	.6705	.185	.069
Residual	25	2737.806	109.512				

Descriptive Statistics
Split By: Disc type

	# Cells attached SEM, Total	# Cells attached SEM, a Osseotite	# Cells attached SEM, b Nanotite
Mean	13.622	14.086	12.000
Std. Dev.	10.300	10.582	9.984
Std. Error	1.982	2.309	4.076
Count	27	21	6
Minimum	.200	.600	.200
Maximum	36.000	36.000	29.000
# Missing	9	7	2

Figure 50. Statistics for osteoblast attachment to Osseotite disks following disinfection.

ANOVA Table for # Cells attached SEM

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Groups	3	195.610	65.203	.585	.6308	1.756	.150
Residual	23	2562.497	111.413				

Descriptive Statistics
Split By: Groups, Disc type

	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum	# Missing
# Cells attached SEM, Total	13.622	10.300	1.982	27	.200	36.000	9
# Cells attached SEM, a 1 Ultr + CHX, a Osseotite	10.433	8.638	3.526	6	1.600	25.800	2
# Cells attached SEM, a 1 Ultr + CHX, b Nanotite	12.000	9.984	4.076	6	.200	29.000	2
# Cells attached SEM, c 3 Citric + Ultra, a Osseotite	17.133	8.811	3.597	6	3.000	27.400	2
# Cells attached SEM, d 4 Brush + CHX, a Osseotite	12.900	11.933	4.872	6	.600	30.600	2
# Cells attached SEM, e 5 Control, a Osseotite	17.667	17.081	9.862	3	2.200	36.000	1

Figure 51. Statistics for osteoblast attachment morphology to 3iT3 versus 3iT3 with DCD dental implant disks.

Summary Table for Osteo Cell Shape, Implant Type
Row exclusion: Flavia 3iT3 attachment and viability data.svd

Num. Missing	0
DF	4
Chi Square	6.868
Chi Square P-Value	.1430
G-Squared	7.504
G-Squared P-Value	.1116
Contingency Coef.	.227
Cramer's V	.233

Observed Frequencies for Osteo Cell Shape, Implant Type
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	a 3iT3	b 3iT3 + Nano	Totals
Flat	20	17	37
No cells	5	1	6
Oblongue	20	24	44
Round	17	16	33
Star	1	6	7
Totals	63	64	127

Percents of Column Totals for Osteo Cell Shape, Implant Type
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	a 3iT3	b 3iT3 + Nano	Totals
Flat	31.746	26.563	29.134
No cells	7.937	1.563	4.724
Oblongue	31.746	37.500	34.646
Round	26.984	25.000	25.984
Star	1.587	9.375	5.512
Totals	100.000	100.000	100.000

Percents of Row Totals for Osteo Cell Shape, Implant Type
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	a 3iT3	b 3iT3 + Nano	Totals
Flat	54.054	45.946	100.000
No cells	83.333	16.667	100.000
Oblongue	45.455	54.545	100.000
Round	51.515	48.485	100.000
Star	14.286	85.714	100.000
Totals	49.606	50.394	100.000

Figure 52. Statistics for osteoblast attachment morphology to 3iT3 dental implant disks following physical and chemical disinfection.

Summary Table for Osteo Cell Shape, Physical decontam
Row exclusion: Flavia 3iT3 attachment and viability data.svd

Num. Missing	0
DF	12
Chi Square	17.246
Chi Square P-Value	.1406
G-Squared	•
G-Squared P-Value	•
Contingency Coef.	.346
Cramer's V	.213

Observed Frequencies for Osteo Cell Shape, Physical decontam
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	a Prophyjet	b Ultrasonic	c Brush	d None	Totals
Flat	16	10	9	2	37
No cells	5	0	1	0	6
Oblongue	12	10	11	11	44
Round	11	10	9	3	33
Star	4	1	2	0	7
Totals	48	31	32	16	127

Percents of Row Totals for Osteo Cell Shape, Physical decontam
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	a Prophyjet	b Ultrasonic	c Brush	d None	Totals
Flat	43.243	27.027	24.324	5.405	100.000
No cells	83.333	0.000	16.667	0.000	100.000
Oblongue	27.273	22.727	25.000	25.000	100.000
Round	33.333	30.303	27.273	9.091	100.000
Star	57.143	14.286	28.571	0.000	100.000
Totals	37.795	24.409	25.197	12.598	100.000

Percents of Column Totals for Osteo Cell Shape, Physical decontam
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	a Prophyjet	b Ultrasonic	c Brush	d None	Totals
Flat	33.333	32.258	28.125	12.500	29.134
No cells	10.417	0.000	3.125	0.000	4.724
Oblongue	25.000	32.258	34.375	68.750	34.646
Round	22.917	32.258	28.125	18.750	25.984
Star	8.333	3.226	6.250	0.000	5.512
Totals	100.000	100.000	100.000	100.000	100.000

Percents of Overall Total for Osteo Cell Shape, Physical decontam
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	a Prophyjet	b Ultrasonic	c Brush	d None	Totals
Flat	12.598	7.874	7.087	1.575	29.134
No cells	3.937	0.000	.787	0.000	4.724
Oblongue	9.449	7.874	8.661	8.661	34.646
Round	8.661	7.874	7.087	2.362	25.984
Star	3.150	.787	1.575	0.000	5.512
Totals	37.795	24.409	25.197	12.598	100.000

Summary Table for Osteo Cell Shape, Chemical decontam
Row exclusion: Flavia 3iT3 attachment and viability data.svd

Num. Missing	0
DF	8
Chi Square	12.755
Chi Square P-Value	.1206
G-Squared	•
G-Squared P-Value	•
Contingency Coef.	.302
Cramer's V	.224

Observed Frequencies for Osteo Cell Shape, Chemical decontam
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	a NaHCO3	b H202	c CHX	Totals
Flat	16	9	12	37
No cells	5	0	1	6
Oblongue	12	10	22	44
Round	11	12	10	33
Star	4	1	2	7
Totals	48	32	47	127

Percents of Row Totals for Osteo Cell Shape, Chemical decontam
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	a NaHCO3	b H202	c CHX	Totals
Flat	43.243	24.324	32.432	100.000
No cells	83.333	0.000	16.667	100.000
Oblongue	27.273	22.727	50.000	100.000
Round	33.333	36.364	30.303	100.000
Star	57.143	14.286	28.571	100.000
Totals	37.795	25.197	37.008	100.000

Percents of Overall Total for Osteo Cell Shape, Chemical decontam
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	a NaHCO3	b H202	c CHX	Totals
Flat	12.598	7.087	9.449	29.134
No cells	3.937	0.000	.787	4.724
Oblongue	9.449	7.874	17.323	34.646
Round	8.661	9.449	7.874	25.984
Star	3.150	.787	1.575	5.512
Totals	37.795	25.197	37.008	100.000

Percents of Column Totals for Osteo Cell Shape, Chemical decontam
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	a NaHCO3	b H202	c CHX	Totals
Flat	33.333	28.125	25.532	29.134
No cells	10.417	0.000	2.128	4.724
Oblongue	25.000	31.250	46.809	34.646
Round	22.917	37.500	21.277	25.984
Star	8.333	3.125	4.255	5.512
Totals	100.000	100.000	100.000	100.000

Figure 53. Statistics for osteoblast attachment morphology to 3iT3 dental implant disks coated with growth factors.

Summary Table for Osteo Cell Shape, Growth factor
Row exclusion: Flavia 3iT3 attachment and viability data.svd

Num. Missing	79
DF	8
Chi Square	10.589
Chi Square P-Value	.2261
G-Squared	•
G-Squared P-Value	•
Contingency Coef.	.425
Cramer's V	.332

Percents of Row Totals for Osteo Cell Shape, Growth factor
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	a None	b Emdogain	c GEM 21	Totals
Flat	31.250	37.500	31.250	100.000
No cells	20.000	60.000	20.000	100.000
Oblongue	50.000	0.000	50.000	100.000
Round	18.182	54.545	27.273	100.000
Star	50.000	25.000	25.000	100.000
Totals	33.333	33.333	33.333	100.000

Observed Frequencies for Osteo Cell Shape, Growth factor
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	a None	b Emdogain	c GEM 21	Totals
Flat	5	6	5	16
No cells	1	3	1	5
Oblongue	6	0	6	12
Round	2	6	3	11
Star	2	1	1	4
Totals	16	16	16	48

Percents of Column Totals for Osteo Cell Shape, Growth factor
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	a None	b Emdogain	c GEM 21	Totals
Flat	31.250	37.500	31.250	33.333
No cells	6.250	18.750	6.250	10.417
Oblongue	37.500	0.000	37.500	25.000
Round	12.500	37.500	18.750	22.917
Star	12.500	6.250	6.250	8.333
Totals	100.000	100.000	100.000	100.000

Percents of Overall Total for Osteo Cell Shape, Growth factor
Row exclusion: Flavia 3iT3 attachment and viability data.svd

	a None	b Emdogain	c GEM 21	Totals
Flat	10.417	12.500	10.417	33.333
No cells	2.083	6.250	2.083	10.417
Oblongue	12.500	0.000	12.500	25.000
Round	4.167	12.500	6.250	22.917
Star	4.167	2.083	2.083	8.333
Totals	33.333	33.333	33.333	100.000

Figure 54. Statistics for osteoblast attachment morphology to Osseotite dental implant disks.

Summary Table for Osteo morphology, Group

Num. Missing	0
DF	4
Chi Square	1.010
Chi Square P-Value	.9082
G-Squared	.958
G-Squared P-Value	.9161
Contingency Coef.	.201
Cramer's V	.145

Observed Frequencies for Osteo morphology, Group

	a CHX Ultra	b CHX brush	c Citric acid Ultra	Totals
Flat	7	3	3	13
Oblong	2	1	2	5
Star	3	2	1	6
Totals	12	6	6	24

Summary Table for Osteo morphology, Implant type

Num. Missing	0
DF	2
Chi Square	1.463
Chi Square P-Value	.4811
G-Squared	1.461
G-Squared P-Value	.4816
Contingency Coef.	.240
Cramer's V	.247

Observed Frequencies for Osteo morphology, Implant type

	a Osseotite	b Oss + Nano	Totals
Flat	11	2	13
Oblong	3	2	5
Star	4	2	6
Totals	18	6	24