The Effect of Implant Surface Bioactivation with PRGF on Cell Attachment in the Presence of Cigarette Smoke Extract

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Abstract

Background: This study was designed to examine the effect of implant surface bioactivation with plasma rich in growth factors (PRGF) in the presence of cigarette smoke extract (CSE) on the attachment of gingival fibroblasts to four different dental implant surfaces.

Methods: Gingival fibroblasts were exposed to 4 types of titanium implant surfaces and a cell attachment assays. For each surface, 4 samples were tested in both the experimental and control groups. Cell attachment assays were conducted in two phases: Phase I included 2 groups: experimental implants coated in PRGF; control implant surfaces alone. Phase II included 2 groups: experimental implants coated in PRGF in the presence of CSE; control implant surfaces alone in the presence of CSE. CyQUANT® dye was added to measure fluorescence using a fluorescence microplate reader. All data was collected and each group of four samples was averaged, with mean and standard deviation compared with the control value.

Results: There were no statistically significant differences in the number of cells attached to dental implants after 48 hours, regardless of implant surface bioactivation with PRGF. Mean cell attachment appeared to be higher in the control group for all implant surfaces, with the exception of the sand blasted, acid-etched surface (Surface 3). With exposure to CSE, when implants were coated in PRGF, there was no significant change in cellular attachment for either surface 1 or 4. However, a significantly lower number of cells were attached to surface 2 and 3 in the presence of CSE, as compared to surfaces 1 and 4. The addition of PRGF to these implant surfaces significantly increased the level of cell attachment on both surface 2 (p=0.031) and surface 3 (p=0.016)

Conclusion: In the presence of cigarette smoke extract; the overall attachment of gingival fibroblasts was reduced to various implant surfaces. When implants were placed in PRGF for 5 minutes prior to cell exposure, this reduction in cellular attachment was corrected.

KEYWORDS

Dental implants, smoking,

1 | INTRODUCTION

The role of cigarette smoking in the exacerbation of periodontal tissue destruction has been well documented in the literature(1)(2)(3) . Cigarette and cigar/pipe smokers have a higher prevalence of moderate and severe periodontitis, with increased attachment loss and gingival recession compared to non-smokers(2). Smokers have also been shown to have a poorer prognosis after periodontal treatment, and as a result, have a greater risk for tooth loss(4). In 2017, an estimated 34.3 million adults in the US smoked cigarettes (14.0%), which is a reduction from the estimated 45.3 million (19.3%) reported in 2011(5).

Replacement of missing teeth with dental implants has emerged as a reliable treatment option; however, in smokers the success rate of dental implants is diminished compared to the rates seen in non-smoking patients(4)(6)(7)(8)(9). A retrospective chart review found implants placed in smokers to be 2.6 times more likely to fail than implants placed in non-smokers(10). It is highly recommended that smokers should be informed of their increased risk for implant loss and peri-implantitis(11).

The mechanisms by which cigarette smoking impacts implant success are complex and have not been clearly identified. Cigarette smoke consists of a mixture of over 4,000 chemical compounds, all of which could have varying degrees of detrimental effects(12). Therefore, studies on nicotine alone do not provide a complete picture on the potential effects of cigarette smoking on dental implant outcomes. An *in vivo* study in rats illustrated the negative impact that nicotine plays on wound healing and osseointegration of titanium implants; however, the authors emphasized the need for further studies in order to understand how nicotine affects the process of osseointegration(13).

The use of plasma rich in growth factors (PRGF-Endoret®[*]) has emerged as a clinical adjunct with the potential to accelerate healing and stimulate periodontal tissue regeneration(14). The PRGF-Endoret® system involves a single spin centrifugation of 9mL tubes containing the patient's own blood. Through centrifugation, the blood is separated into its three basic components: plasma rich in growth factors; white blood cells or leukocytes (buffy coat); red blood cells. After centrifugation, two separate fractions are collected from the top layer of plasma. The fraction closest to the buffy coat (white blood

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cells and leukocytes) is considered to be the richest in growth factors. The use of calcium chloride as an activator stimulates release of growth factors and proteins from the platelets. An advantage with PRGF technology is that leukocytes (buffy coat) can removed from the preparation; thus eliminating their potential pro-inflammatory effects(14). *In vitro* studies have demonstrated that PRGF stimulates increased cellular proliferation, migration and chemotaxis of cell types including fibroblasts(15)(16)(17) and osteoblasts(18).

Implant surface bioactivation involves applying PRGF to the implant surface before final placement. Results from animal and human studies show that surface bioactivation influences cellular attachment, proliferation and differentiation, bone matrix deposition (17). Complete osseointegration in 99.6% of these implants was also reported (17). Animal studies have demonstrated higher bone-to-implant (BIC) when implants were bioactivated with PRGF prior to placement. Histological and histomorphometric studies have confirmed higher BIC at 12 weeks(19). Another study found that newly formed bone was found along the whole surface of the PRGF-treated implants, whereas it was only found in the upper half of control implants(20). Although bioactivated implants may lead to higher quality of bone, its use does not appear to increase implant success or survival rate in the reported literature(14).

Identifying potential therapies to increase dental implant success and survival in smokers is an important issue facing practitioners in order to provide more predictable long-term results. By looking at implant surface bioactivation *in vitro*, we can determine if this treatment modality could eventually prove beneficial in smokers. This study was designed to examine the effect of implant surface bioactivation in the presence of cigarette smoke extract (CSE) on the attachment of gingival fibroblasts to four different dental implant surfaces.

2 | MATERIALS AND METHODS

2.1 | Gingival Fibroblast Cell Lines

Gingival fibroblasts (GF) were previously established from patients with healthy gingiva who underwent oral surgery at the Louisiana State University School of Dentistry. The GF cell line used in this study was isolated from the gingival tissue of a 13 year old male donor with no systemic diseases who underwent periodontal surgery (32). Cells were maintained in minimum essential medium alpha (MEMα) containing 10% fetal calf serum (FCS) and 200 units/ml of penicillin and 200 µg/ml streptomycin (GIBCO

Grand Island, NY). Gingival fibroblasts between the 12th and 18th passage were deemed to be "rapidly dividing", while cells after the 33rd passage were deemed to be "senescent" for this study. After passage 30, these cells tend to slow their rate of proliferation, eventually becoming non-mitotic around passage 33. Gingival fibroblasts used in this study were passage 14.

2.2 | Dental Implants

Four different dental implant surfaces were utilized in all aspects of this study. Table 1 depicts the characteristics of these 4 surfaces. All implants were washed in distilled water and autoclaved. Implants were air-dried and then UV irradiated for 15 mins prior to use.

2.3 | PRGF Preparation

A single, healthy non-smoking female was recruited for the study and informed consent was obtained using a protocol approved by the LSUHSC-NO Institutional Review Board (IRB #9773). Peripheral blood samples were collected from the donor, using sterile technique. PRGF preparation was conducted according to the August 2016 manufacturer's guidelines f PRGF-Endoret®* Four 9ml tubes of blood were collected approximately 1 hour before implant surfaces were to be coated in the solution. The 9-ml tubes were centrifuged for 8 minutes. Fractionation using the Plasma Transfer Device (PTD) was completed to collect F1 and F2 plasma fractions. All F1 fractions were discarded and only the F2 fraction was utilized due to a higher platelet and growth factor content in F2. For the purpose of this study, F2 fractions were utilized for surface bioactivation. Implants in the experimental group were allowed to soak in PRGF for 5 minutes before placement in multi-well plates.

2.4 | Cigarette Smoke Extract (CSE)

CSE solutions were prepared using a previously reported protocol (22). Briefly, the smoke from a lit cigarette was drawn up through 10ml of MEMα media over 60 seconds. The solution generated was then filtered (0.22μ) to eliminate large particles. The resulting solution was designated as a 100% CSE solution. The CSE solution was stored at -80 C. A working dilution of 1.5% was made using MEMα media containing 10% fetal bovine serum (FBS). Immediately following smoking, nicotine levels were found to be 0.1 μM in serum, 1 μM in resting saliva, and up to 1mM in saliva. It was estimated that 5%

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CSE contains 13μM of cotinine, while 1.5% CSE contains about 4μM of continue (33). A dilution of 1.5% CSE solution was used for most experiments in the current study. This level reflects the salivary levels of CSE of resting saliva in an average smoker, and is significantly below the levels found immediately after smoking a single cigarette(23).

2.5 | Cell Attachment with and without PRGF Surface Bioactivation in the Presence of CSE

Cell attachment assays to the 4 types of titanium implant surfaces were conducted as previously described (24). For each surface, 4 samples were tested in both the experimental and control groups. Cell attachment assays were conducted in two phases: Phase I included 2 groups: experimental implants coated in PRGF; control implant surfaces alone. Phase II included 2 groups: experimental implants coated in PRGF in the presence of CSE; control implant surfaces alone in the presence of CSE.

Titanium dental implants in both experimental groups were soaked in PRGF for 5 mins prior to being placed at the bottom of 24-well plates. Approximately fifty thousand cells were added to each well containing the implants in a volume of 50 μL MEMα containing 10% fetal bovine serum (FBS). In Phase II, cells were exposed to CSE at the same time as the cells were added to the well plates containing the dental implants. Cells were allowed to adhere to the substrate for 24 hours. Next, the implants were moved to wells with fresh MEMα media with 10% serum and incubated for 48 hours. The implants were removed from the multi-well plates, placed in microfuge tubes and were then frozen at -20 degrees Celsius for a minimum of 2 hours.

Adherent cells were quantified fluorometrically using fluorescent dye*. The dye was prepared according to manufacturer's instructions, and this assay has a linear detection range extending from approximately 50 to 50,000 cells per microplate well (34). A 300-μl mixture of dye solution was added to each microfuge tube containing the dental implants and to the control tubes. All tubes were vortexed and centrifuged at 10,000 rpm for 3 min to release DNA content of frozen cells and allow nucleic acids bound to the dye to remain free in the supernatant. After centrifugation, 200 μl of supernatant was transferred from each tube into wells of a 48-well plate. Fluorescence was measured using a fluorescence microplate reader† with

* Cy- QUANT®, Molecular Probes Inc., Eugene, OR
† Bio-Tek Instruments FL500)

filters appropriate for 480 nm excitation and 520 nm emission maxima. All data was collected and each group of four samples was averaged, with mean and standard deviation compared with the control value.

2.6 | Calcein staining of live gingival fibroblast cells

To examine cell morphology, cells were treated with the acetomethoxy derivate of calcein (calcein AM) for 1 hour. This dye is a non-fluorescent esterase substrate that is readily taken into live cells. Once internalized, this dye is cleaved by endogenous esterases and rendered fluorescent. Therefore, this dye readily labels the cytoplasm of live cells. This allowed for an examination of cell morphology using an inverted fluorescent microscope equipped with a high-resolution camera. Two implants of each surface were evaluated in all 4 of the groups examined in this study: 1) Control; 2) With PRGF; 3) CSE alone; 4) CSE + PRGF (23).

3 | RESULTS

3.1 | Cell Attachment to dental implants +/- PRGF Surface Bioactivation

In order to properly assess the impact of CSE on cell attachment to titanium dental implants, obtaining baseline measurements using gingival fibroblasts alone in the presence of PRGF was necessary. Overall, there were no statistically significant differences in the number of cells attached to dental implants after 48 hours, regardless of implant surface bioactivation with PRGF (table 2). Mean cell attachment appeared to be higher in the control group for all implant surfaces, with the exception of the sand blasted, acid-etched surface (Surface 3). The control group for the MTX surface (surface 4) had the highest mean cellular attachment of 31,509 cells (STD 5,928.09). In order to evaluate cell attachment as an overall percentage, the MTX control group value was set as the benchmark for a 100% attachment (Figure 1).

3.2 | Cell Attachment to dental implants +/- PRGF Surface Bioactivation in the Presence of CSE

The addition of CSE did not have a significant impact on the cellular attachment to both surface 1 (p=0.846) and surface 4 (p=0.650) (table 3) compared to controls. When implants were coated in PRGF, there was no significant change in cellular attachment for either surface 1 or 4. However, a significantly lower number of cells were attached to surface 2 and 3 in the presence of CSE, as compared to surfaces 1 and 4. The addition of PRGF to these implant surfaces significantly increased the level of cell

attachment on both surface 2 (p=0.031) and surface 3 (p=0.016). The same control value for the MTX surface in the control group was used to establish the 100% cell attachment value (Figure 2).

4 | DISCUSSION

It has been well documented that the success rate of dental implants is significantly lower in smokers (4)(6)(7)(8)(9). The rate of implant failure in smokers has been reported to be between 6.5% and 20%(25)(26). There is a clear dose-response relationship between implant failure and smoking, with consumption of greater than 20 cigarettes per day increasing the relative risk for loss from 10.8% to 30.8%(26). Patients that smoke who are planning to undergo dental implant therapy need to be made aware of the increased risk for failure.

Previous studies have attempted to identify the t mechanisms by which cigarette smoke negatively impacts the survival of dental implants. It has been shown *in vitro* that exposure of gingival fibroblasts to whole cigarette smoke resulted in reduced attachment to a collagen matrix (27). Another study demonstrated nicotine-induced cytotoxicity on PDL fibroblasts by inhibiting cell growth, protein synthesis and differentiation in a dose-dependent manner (28). It has been shown *in vitro* that CSE decreased the survival of PDL fibroblasts, as well as increased the expression of matrix metalloproteinases 1 and 3(23). A recent study by Rouabhia, examined the effects of cigarette smoke and E-cigarette vapor, with and without nicotine, on osteoblasts and dental implant disks(29). They demonstrated that cigarette smoke and nicotine rich e-cigarette vapor reduced osteoblast growth and downregulated osteoblast attachment(29).

Our study aimed to determine if soaking the dental implant in PRGF prior to placement would help to enhance the cellular attachment in the presence of CSE. In theory, if the addition of PRGF to the implant surface helps increase the cellular attachment, then it could potentially lead to less dental implant failures in smokers. Four different commercially available implant surfaces were used to see if variation existed between them in regards to cellular attachment. The study was conducted in two phases, first looking at gingival fibroblast attachment with or without PRGF, and then second phase with the addition of CSE.

Dental implants were placed in PRGF for 5 minutes prior to exposure to gingival fibroblasts. Five minutes was selected as an appropriate length of time for clinical relevance, because this is most likely

the maximum amount of time that most clinicians would wait when placing implants in practice. When CSE was added, there was a clear difference seen between the various groups. Implants with a MTX and Nanotite surfaces showed no significant variation in the gingival fibroblast attachment when PRGF was added in the presence of CSE. The amount of cellular attachment to the SLA and TiUnite surfaces was significantly reduced in the presence of CSE alone. When these implants were placed in PRGF, the negative effect of CSE on cellular attachment was corrected to levels similar seen on the MTX and Nanotite surfaces.

The use of PRGF has widely been used in association with regenerative and soft tissue procedures. Previous in *vitro* studies have demonstrated that PRGF stimulates increased cellular proliferation, migration and chemotaxis of cell types including fibroblasts(15)(16)(17) and osteoblasts(18). This study showed that the addition of PRGF in the presence of CSE did have a positive impact on cellular attachment to both the SLA and TiUnite surfaces. While larger clinical studies are needed to confirm this finding, it is a promising indication that bioactive surface activation of dental implants could potentially improve the long-term survival and success of dental implants placed in smokers.

The findings of this study suggest that the MTX and Nanotite surfaces could potentially be more appropriate surfaces to be used in smokers. Both of these surfaces have been shown to have 7 year survival rates of 91% to 98.5%(30)(31). To our knowledge, no studies regarding survival of implants with MTX and Nanotite surfaces in smokers exists in the literature. Large scale studies examining various parameters are required to confirm the findings of this study in a clinical setting.

CONCLUSIONS

This study demonstrated that in the presence of cigarette smoke extract; the overall attachment of gingival fibroblasts to various implant surfaces was reduced. When implants were placed in PRGF for 5 minutes prior to cell exposure, this reduction in cellular attachment was corrected. Within the limitations of this study, this data provides a proof of concept that paves the way for additional, large-scale studies to evaluate the true efficacy of implant surface bioactivation with PRGF.

References

1 Bergström J, Eliasson S, Preber H. Cigarette Smoking and Periodontal Bone Loss. J Periodontol [Internet]. 1991 Apr [cited 2019 Apr 11];62(4):242–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2037954

2. Albandar JM, Streckfus CF, Adesanya MR, Winn DM. Cigar, Pipe, and Cigarette Smoking as Risk Factors for Periodontal Disease and Tooth Loss. J Periodontol [Internet]. 2000 Dec [cited 2019 Apr 11];71(12):1874–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11156044

3. Haffajee AD, Socransky SS. Relationship of cigarette smoking to the subgingival microbiota. J Clin Periodontol [Internet]. 2001 May [cited 2019 Apr 11];28(5):377–88. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11350499

4. Tonetti MS. Cigarette Smoking and Periodontal Diseases: Etiology and Management of Disease. Ann Periodontol [Internet]. 1998 Jul [cited 2019 Apr 11];3(1):88–101. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9722693

5. Wang TW, Asman K, Gentzke AS, Cullen KA, Holder-Hayes E, Reyes-Guzman C, et al. Tobacco Product Use Among Adults — United States, 2017. MMWR Morb Mortal Wkly Rep [Internet]. 2018 Nov 9 [cited 2019 Apr 11];67(44):1225–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30408019

6. Bain CA, Moy PK. The association between the failure of dental implants and cigarette smoking.

Int J Oral Maxillofac Implants [Internet]. 1993 [cited 2019 Apr 11];8(6):609–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8181822
7. Galindo-Moreno P, Fauri M, Ávila-Ortiz G, Fernández-Barbero JE, Cabrera-León A, Sánchez-Fernández E. Influence of alcohol and tobacco habits on peri-implant marginal bone loss: a prospective study. Clin Oral Implants Res [Internet]. 2005 Oct [cited 2019 Apr 11];16(5):579–86. Available from: http://doi.wiley.com/10.1111/j.1600-0501.2005.01148.x
8. Nitzan D, Mamlider A, Levin L, Schwartz-Arad D. Impact of smoking on marginal bone loss. Int J Oral Maxillofac Implants [Internet]. [cited 2019 Apr 11];20(4):605–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16161745
9. Mundt T, Mack F, Schwahn C, Biffar R. Private practice results of screw-type tapered implants: survival and evaluation of risk factors. Int J Oral Maxillofac Implants [Internet]. [cited 2019 Apr 11];21(4):607–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16955613
10. Holahan CM, Koka S, Kennel KA, Weaver AL, Assad DA, Regennitter FJ, et al. Effect of osteoporotic status on the survival of titanium dental implants. Int J Oral Maxillofac Implants [Internet]. [cited 2019 Apr 11];23(5):905–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19014161
11. Heitz-Mayfield LJA, Huynh-Ba G. History of treated periodontitis and smoking as risks for implant therapy. Int J Oral Maxillofac Implants [Internet]. 2009 [cited 2019 Apr 11];24 Suppl:39–68. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19885434
12. Roemer E, Stabbert R, Rustemeier K, Veltel DJ, Meisgen TJ, Reininghaus W, et al. Chemical composition, cytotoxicity and mutagenicity of smoke from US commercial and reference cigarettes smoked under two sets of machine smoking conditions. Toxicology [Internet]. 2004 Jan 15 [cited 2019 Apr 11];195(1):31–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14698566
13. Berley J, Yamano S, Sukotjo C. The effect of systemic nicotine on osseointegration of titanium implants in the rat femur. J Oral Implantol [Internet]. 2010 Jun [cited 2019 Apr 11];36(3):185–93. Available from: http://www.joionline.org/doi/abs/10.1563/AAID-JOI-D-09-00050
14. Maney P, Amornporncharoen M, Palaiologou A. Applications of plasma rich in growth factors (PRGF) in dental surgery: a review. J West Soc Periodontol Periodontal Abstr [Internet]. 2013 [cited 2019 Apr 11];61(4):99–104. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24683761
15. Anitua E, Sánchez M, Zalduendo MM, de la Fuente M, Prado R, Orive G, et al. Fibroblastic response to treatment with different preparations rich in growth factors. Cell Prolif [Internet]. 2009 Apr [cited 2019 Apr 11];42(2):162–70. Available from: http://doi.wiley.com/10.1111/j.1365-2184.2009.00583.x
16. Sell SA, Wolfe PS, Spence AJ, Rodriguez IA, McCool JM, Petrella RL, et al. A preliminary study on the potential of manuka honey and platelet-rich plasma in wound healing. Int J Biomater [Internet]. 2012 [cited 2019 Apr 11];2012:313781. Available from: https://www.hindawi.com/journals/ijbm/2012/313781/
17. Anitua E, Sanchez M, De la Fuente M, Zalduendo MM, Orive G. Plasma rich in growth factors (PRGF-Endoret) stimulates tendon and synovial fibroblasts migration and improves the biological properties of hyaluronic acid. Knee Surg Sports Traumatol Arthrosc [Internet]. 2012 Sep 11 [cited 2019 Apr 11];20(9):1657–65. Available from: http://link.springer.com/10.1007/s00167-011-1697-4
18. Anitua E, Tejero R, Zalduendo MM, Orive G. Plasma rich in growth factors promotes bone tissue regeneration by stimulating proliferation, migration, and autocrine secretion in primary human osteoblasts. J Periodontol [Internet]. 2013 Aug [cited 2019 Apr 11];84(8):1180–90. Available from: http://doi.wiley.com/10.1902/jop.2012.120292
19. Birang R, Tavakoli M, Shahabouei M, Torabi A, Dargahi A, Soolari A. Investigation of peri-implant bone healing using autologous plasma rich in growth factors in the canine mandible after 12 weeks: a pilot study. Open Dent J [Internet]. 2011 Nov 4 [cited 2019 Apr 11];5(1):168–73. Available from: https://opendentistryjournal.com/VOLUME/5/PAGE/168/
20. Anitua E, Orive G, Pla R, Roman P, Serrano V, Andía I. The effects of PRGF on bone regeneration and on titanium implant osseointegration in goats: a histologic and histomorphometric study. J Biomed Mater Res A [Internet]. 2009 Oct [cited 2019 Apr 11];91(1):158–65. Available from: http://doi.wiley.com/10.1002/jbm.a.32217
21. Todisco M, Trisi P. Histomorphometric evaluation of six dental implant surfaces after early loading in augmented human sinuses. J Oral Implantol [Internet]. 2006 Aug [cited 2019 Apr 19];32(4):153–66. Available from: http://www.joionline.org/doi/abs/10.1563/812.1

22. Carnevali S, Nakamura Y, Mio T, Liu X, Takigawa K, Romberger DJ, et al. Cigarette smoke extract inhibits fibroblast-mediated collagen gel contraction. Am J Physiol Cell Mol Physiol [Internet]. 1998 Apr [cited 2019 Apr 11];274(4):L591–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9575878
23. Bulmanski Z, Brady M, Stoute D, Lallier TE. Cigarette smoke extract induces select matrix metalloproteinases and integrin expression in periodontal ligament fibroblasts. J Periodontol [Internet]. 2012 Jun [cited 2019 Apr 11];83(6):787–96. Available from: http://doi.wiley.com/10.1902/jop.2011.110395
24. Palaiologou A, Stoute D, Fan Y, Lallier TE. Altered cell motility and attachment with titanium surface modifications. J Periodontol [Internet]. 2012 Jan [cited 2019 Apr 11];83(1):90–100. Available from: http://doi.wiley.com/10.1902/jop.2011.100733
25. Levin L, Schwartz-Arad D. The effect of cigarette smoking on dental implants and related surgery. Implant Dent [Internet]. 2005 Dec [cited 2019 Apr 20];14(4):357–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16361886
26. Sánchez-Pérez A, Moya-Villaescusa MJ, Caffesse RG. Tobacco as a Risk Factor for Survival of Dental Implants. J Periodontol [Internet]. 2007 Feb [cited 2019 Apr 20];78(2):351–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17274726
27. Semlali A, Chakir J, Rouabhia M. Effects of whole cigarette smoke on human gingival fibroblast adhesion, growth, and migration. J Toxicol Environ Health A [Internet]. 2011 Jul [cited 2019 Apr 20];74(13):848–62. Available from: http://www.tandfonline.com/doi/abs/10.1080/15287394.2011.570230
28. Chang Y-C, Huang F-M, Tai K-W, Yang L-C, Chou M-Y. Mechanisms of cytotoxicity of nicotine in human periodontal ligament fibroblast cultures in vitro. J Periodontal Res [Internet]. 2002 Aug [cited 2019 Apr 20];37(4):279–85. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12200972
29. Rouabhia M, Alanazi H, Park HJ, Gonçalves RB. Cigarette Smoke and E-Cigarette Vapor Dysregulate Osteoblast Interaction With Titanium Dental Implant Surface. J Oral Implantol [Internet]. 2019 Feb [cited 2019 Apr 20];45(1):2–11. Available from: http://www.joionline.org/doi/10.1563/aaid-joi-D-18-00009
30. Ormianer Z, Palti A. Retrospective clinical evaluation of tapered screw-vent implants: results after up to eight years of clinical function. J Oral Implantol [Internet]. 2008 [cited 2019 Apr 20];34(3):150–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18616077
31. Browaeys H, Defrancq J, Dierens MCA, Miremadi R, Vandeweghe S, Van de Velde T, et al. A retrospective analysis of early and immediately loaded osseotite implants in cross-arch rehabilitations in edentulous maxillas and mandibles up to 7 years. Clin Implant Dent Relat Res [Internet]. 2013 Jun [cited 2019 Apr 20];15(3):380–9. Available from: http://doi.wiley.com/10.1111/j.1708-8208.2011.00367.
32. Palaiologou A, Yukna R, Moses R, et al. Gingival, Dermal, and Periodontal Ligament Fibroblasts Express Different Extracellular Matrix Receptors. J Periodontol [Internet]. 2001 June [cited 2019 Apr 11];72(6):798-807. Available from: http://doi.wiley.com/10.1902/jop.2001.72.6.798
33. Jarczyk L, Scherer G, Adlkofer F. Serum and saliva concentrations of cotinine in smokers and passive smokers. J Clinical Chemistry and Clinical Biochemistry. 1989 April [cited 2019 Apr 11]; 27(4):230-1. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2738526
34 Molecular Probes Inc. CyQUANT Cell Proliferation Assay Kit (C-7026). Product information, 1999

FIGURES AND TABLES

TABLE 1 Surface Characteristics of Study Dental Implants(21)

Surface Number	**Surface Brand Name**	**Processing**
Surface 1	Osseotite*	Acid etched
Surface 2	TiUnite†	Oxidization
Surface 3	SLA‡	Sandblasted and acid etched
Surface 4	MTX§	Grit blasted with hydroxyapatite (HA)

TABLE 2 Mean Cellular Attachment levels of gingival fibroblasts with or without the addition of PRGF to implant surfaces

Implant Surface	**Mean Cellular Attachment**	
	Control Group (SD)	*PRGF Group (SD)*
Surface 1	26,908 (±10,873.9)	21,610 (±3,126.03)

* 3i Zimmer Biomet Inc, Carlsbad, Calif.
† Nobel Biocare, Yorba Linda, Calif.
‡ Straumann USA, Andover, Mass
§ Zimmer Biomet Inc, Carlsbad, Calif.

Surface 2	28,768 (±9,708.53)	22,032 (±7,800.79)
Surface 3	23,106 (±5,047.50)	26,983(±2,318.63)
Surface 4	31,509 (±8,938.61)	27,859(±8,938.61)

Surface 1:Nanotite by 3i; Surface 2: by Nobel; Surface 3: SLA by Straumann; Surface 4: MTX by Zimmer. The mean values of cells attached to the various implant surfaces in the control group were found to be higher than the value of attached cells in the PRGF group for all surfaces except Surface 3. There was no statistically significant difference noted between control or PRGF group for any implant surface.

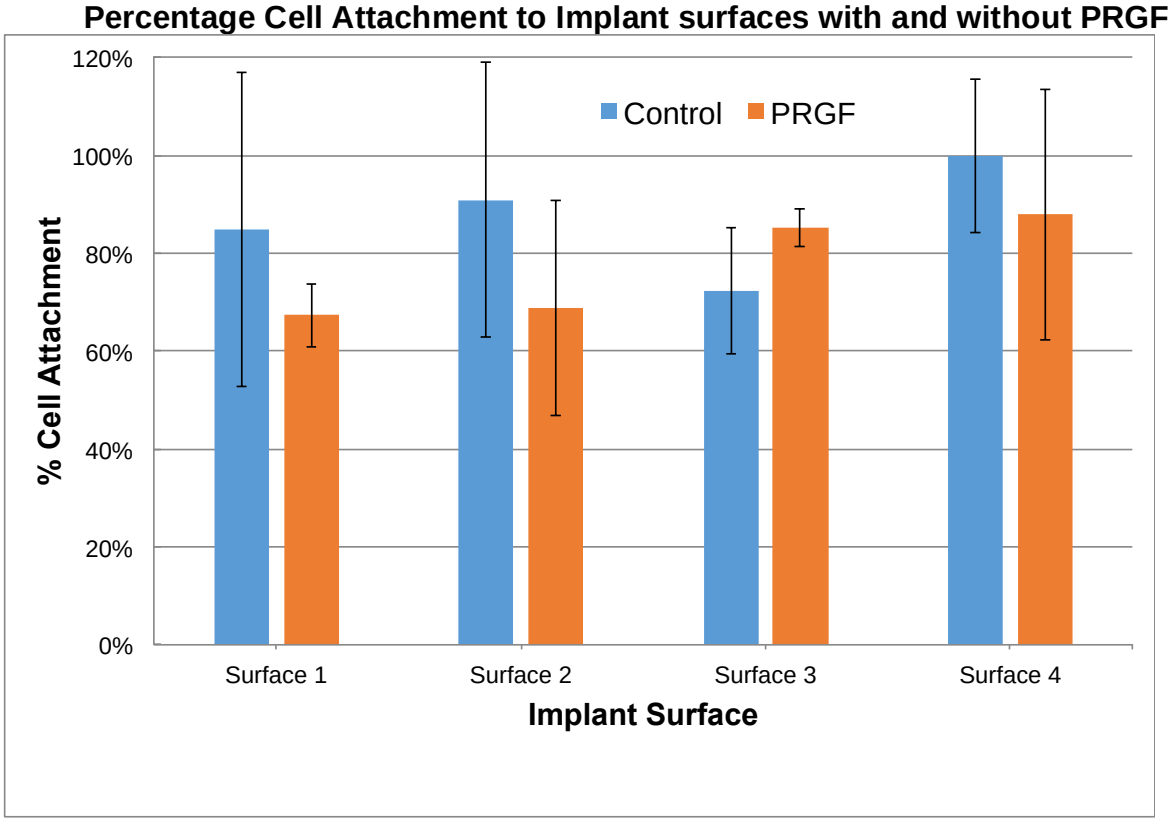

Figure 1: Surface 1:Nanotite by 3i; Surface 2: TiUnite by Nobel; Surface 3: SLA by Straumann; Surface 4: MTX by Zimmer. Percentages of attachment are a representation of the percentage of attachment compared to the control attachment of surface 4, which displayed the highest mean attachment values.

TABLE 3 Mean Cellular Attachment levels of gingival fibroblasts to dental implants coated in PRGF with or without the addition of CSE

Implant Surface	**Mean Cellular Attachment**	
	CSE Alone Group (SD)	*CSE + PRGF Group (SD)*
Surface 1	12,034 (±3,624.77)	11,594 (±2,321.38)
Surface 2	5,872 (±1,506.55)	13,319 (±4,159.53)
Surface 3	5,682 (±1,999.37)	13,662 (±3,773.63)
Surface 4	10,968 (±2,374.82)	11,890 (±3,036.68)

Surface 1:Nanotite by 3i; Surface 2: TiUnite by Nobel; Surface 3: SLA by Straumann; Surface 4: MTX by Zimmer. The difference in mean cellular attachment between the CSE Alone Group and CSE+PRGF group were statistically significant for both Surface 2 and 3.

Comparison of Percentage Cell Attachment to implant surfaces with and without PRGF in the Presence of CSE

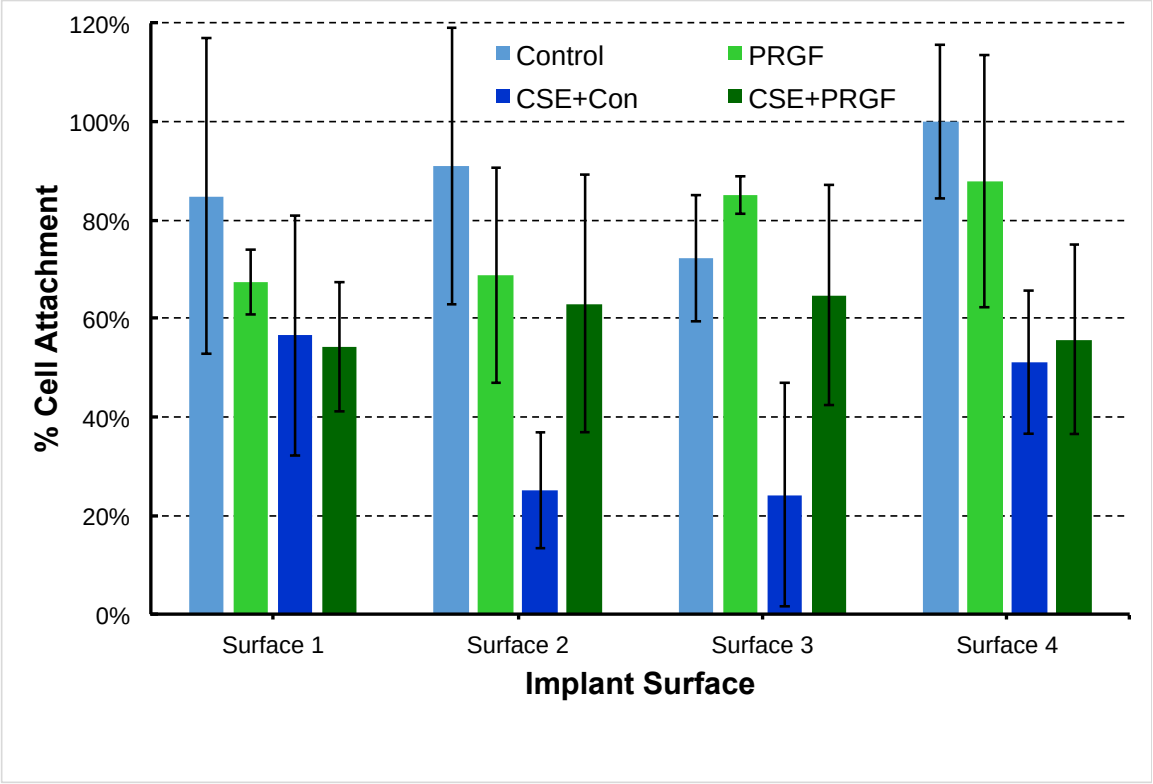

Figure 2: Surface 1:Nanotite by 3i; Surface 2: by Nobel; Surface 3: SLA by Straumann; Surface 4: MTX by Zimmer. Percentages of attachment are a representation of the percentage of attachment compared to the control attachment of surface 4, which displayed the highest mean attachment values.

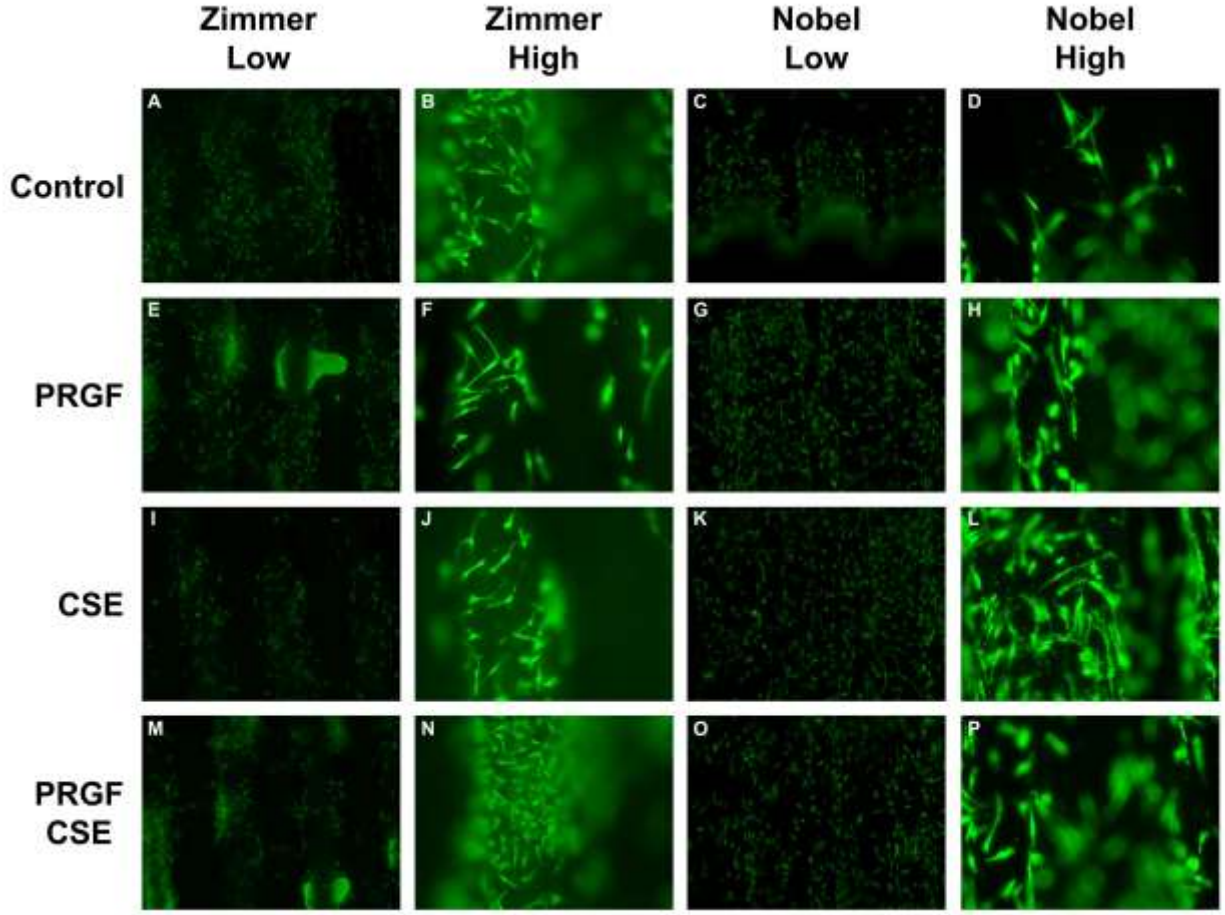

Figure 3: Comparison of Surface 4 and surface 2 when using calcein fluorescent staining. It was noted that there were no real differences seen in the amount of cellular attachment between the two implant surfaces in any of the test groups. This was in opposition to what was seen when the Cyquant fluorescence was used.