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Growth of Human Gingival Epithelial Cells on Dental Restorative material: Zirconia Oxide Ceramic *in vitro*

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ABSTRACT

OBJECTIVES: To investigate the growth and attachment of primary human gingival epithelial (HGE) cells on zirconia oxide ceramic material (Cerec by Sirona) with different surface roughness Ra values.

METHODS: Zirconia oxide samples were either polished, glazed, or ground by diamond or carbide burs to produce material samples with different surface roughnesses (R_a), measured using a stylus-type profilometer. Pooled donor HGE cells were seeded on the surface and grown for 24, 72, or 144 hours. Growth on tissue culture plastic and on untreated zirconia oxide were used as controls. Cell growth was visualized under florescence microscopy after staining with ethidium bromide. Statistical analysis to determine growth patterns included field count variances, Shapiro-Wilk and D'Agostino and Pearson normality tests, two-way ANOVA and Non-linear regression. Graphpad Prism 6.0 Software was used for most of the statistical analysis.

RESULTS: Cells were initially plated (t=0) at a density of 500 cells/mm^2. The initial viability of the cell suspension was >95%, as determined by trypan blue staining. The overall mean for cells grown on the plastic control at 24 hours was 76.425 cells/mm^2, or a mean of 15.3% of the initial load. There was a modest increase in cell number between 24 and 72 hour and a lower rise through hour 144.

The mean field count coefficient of variance (CV) for the material samples (excluding plastic) was large, (Ground =43.2% and polished=43.8%) indicating heterogeneity in the plating density. There was an unevenness in growth distribution from 24 hour to 144 hour, with a positive skewness suggesting that the cells grew in patches on the sample surface. A two-way ANOVA test of the average CVs at each time point for each type of treatment (grinding, polishing) and the plastic and no treatment controls showed no significant effect (p=0.74, 0.31). Time showed a

modest significant effect (p=0.042) on cell growth. There was no clear trend in the cell growth on samples relative to the plastic controls across the Ra values tested.

CONCLUSIONS: Sample surface preparation of zirconia oxide ceramic material by grinding or polishing created an Ra test range of 0.027-1.678 μm. For HGE cell growth tested over a 144 hour period, there was no relationship between surface roughness (as measured by Ra) and epithelial cell growth (relative to growth on plastic). The Null Hypothesis of no relationship between Ra and growth was accepted.

IMPLICATIONS: Zirconia oxide proved to be equally biocompatible to the tissue grade plastic regardless of surface preparation and surface roughness, under the experimental conditions employed. These findings suggest that human gingival epithelial (HGE) cells will grow and attach to transmucosal zirconia oxide when used for an implant-supported restoration.

INTRODUCTION

Dental implants are being deployed more frequently to replace missing teeth. When planning and placing implants the dental team should think of implants as a prosthodontic problem with a surgical solution.[1] Thus, the restoring clinician needs to choose the best materials to provide a functional and esthetic prosthesis that will maintain both hard and soft tissues.[2]

Peri-implant mucosa, which develops after the transgingival abutment has been placed, contains a core of connective tissue covered by an outer surface of orthokeratinized epithelium.[3] (Fig. 1) In a study by Kawahara et al, three distinct soft tissue zones have been identified.[4] These zones have been labeled the plaque zone, the nude zone, and the epithelial cell attached zone.[1, 4] (Fig. 2) Animal histological studies demonstrate that the junctional epithelium extends approximately 2 mm apical from the peri-implant mucosal margin.[3, 5, 6] The epithelial cell attachment zone is located in the deepest area of the implant/epithelial tissue interface and has a stronger bond strength to the titanium implant surface than that of cell-cell attachment.[4] The epithelial cells bond to the titanium via hemidesmosomes and a basal lamina attachment mechanism.[6, 7, 8] (Fig. 3) This cell attachment becomes the first barrier of defense from micro-organism infiltration of the dental implant.[1] Within one week after abutment placement, the peri-implant mucosal tissue will be lined by oral epithelium that is continuous with the junctional epithelium.[4]

Possible transmucosal abutment material choices include: metal (gold or titanium), zirconia oxide, lithium disilicate, polyetheretherketone (PEEK), and polymer-infiltrated ceramic network (PICN) materials.[1] Zirconia is a commonly used material for custom transgingival implants, with comparable properties to titanium or gold regarding bone loss, soft tissue recession and biological complications. An advantage of zirconia over other materials for abutments is its superiority regarding soft tissue color. Once the abutment material has been

selected and placed into living tissue, an interaction with a complex biological system around it will occur.[9] Biocompatibility is the ability of a material to elicit an appropriate biological response.[9, 10] In the interaction between zirconia with gingiva, this would involve epithelial cell adhesion to the material surfaces, with subsequent proliferation and migration.

Surface roughness can affect host cellular adhesion and proliferation across a material surface. It has been recommended that all intraoral hard surfaces should approximate an Ra value of 0.20 μm or lower to inhibit plaque accumulation.[11] Studies have shown that reducing the surface roughness of various restorative materials below a threshold R_a of 0.20 μm would not have an impact on reducing bacterial adhesion.[11,12] Regarding soft tissue response to surface roughness, an *in vivo* study showed that different material types can affect the soft tissue health surrounding dental implants.[11,12] According to research performed by Okabe, et al. zirconia oxide can possible support binding of epithelial cells through hemidesmosomal attachment comparable to titanium.[8] More research is needed to understand the influence of ceramic materials on soft tissue growth and adhesion. This study is a continuation of the work performed by Smallidge et al, following the same cell culture protocol.[13]

The purpose of this study was to investigate, *in vitro*, the effect of surface roughness (R_a) of zirconia oxide on the growth and adhesion of pooled primary human gingival epithelial (HGE) cells. The effect of material surface roughness on cell growth was compared to cell growth on tissue culture plastic. The null hypothesis was that there is no relationship between cell attachment and surface roughness. A second null hypothesis was that growth on zirconia would be no different from growth on plastic.

MATERIAL AND METHODS

Pre-sintered green state zirconia oxide blocks were sectioned using a water-lubricated diamond cutting blade on a precision saw (Buhler; Uzwil, Switzerland), resulting in rectangular disks 1 mm thick, with a surface area of 14 x 12 mm =168 mm^2. The samples were then notched on the upper right to allow for easy identification of the prepared surface. The samples were then sintered in an HT-S Speed Sintering Furnace (Mihm-Vogt, Stutensee, Germany). In order to create a range of surface roughness values, samples were prepared with either a diamond bur (ISO color coding, Table 1), carbide bur (375R.016), polishing wheels or glazed. A total of 11 different surface roughness values were created. One sample (zirconia control) received no further treatment after sintering. For each diamond grit and carbide bur, the sample was held on a flat surface and the long side of the bur was brushed across the sample 5 times going length wise on the sample. The sample was then turned 90 degrees and the bur was brushed across the sample 5 times going width wise. This produced a homogeneous surface texture. The polished set of samples were polished according to the protocol described in Steiner et al, in which they were polished for 30 seconds in one direction and for another 30 seconds at 90 degrees to the first direction.[14] In order to have consistency in pressure each sample was placed in a putty matrix jig and placed on an electric scale. The scale was tared and the samples were then polished with a pressure range of 300-500 gm of pressure (2.94-4.90 N) with the hand piece set at 10,000 rpm. One sample was treated with just the grinding wheel (Dialite ZR extra oral), the next sample was polished using Dialite ZR extra oral polishing set, and the last sample was polished using the Garrison eZr intra oral set. The protocol was followed for each polishing wheel from course to fine. The final zirconia oxide sample was glazed using a low fusing universal glaze (IPS Empress universal glaze paste: Ivoclar Vivadent).

Primary cells age over time and become subject to senescence over a certain number of passages, (dependent on the cells, media, etc.), and the media can be unstable, resulting in poor growth. Cells grown on plastic should display exponential growth during at least part of the 144 hour growth period. The tissue culture plastic was used as the control and to determine the validity of the experiment design. The control was also used to assess the cells behavior on a test material.

A stylus-type profilometer (Mahr Perthometer M2, Providence, RI) was used to measure surface roughness, determined as R_a (μm) values. Three measurements were made per sample and the mean R_a calculated (Table 2).

To prepare the disk samples for tissue culture, the samples were steam-cleaned (Triton; BEGO GmbH & Co. KG, Bremen, Germany) for 10 seconds on each surface, and then sterilized in an autoclave. Disks were then placed in the wells of sterile 12-well tissue culture plates with the single polished surfaces facing upward. The same set of samples was cleaned and sterilized as described above, and re-used for replicate experiments. Fluorescence microscopy was used to confirm removal of epithelial cells after cleaning and sterilization in the autoclave.

Following the protocol of Smallidge et al, pooled-donor primary HGE cells (CELLnTEC, Bern, Switzerland) were used. Primary HGE cells (Image 1) were grown according to the source's instructions, and low passage numbers (3-6) were used for each experiment. Cells were grown in CnT-Prime epithelial culture media (CELLnTEC,) to a confluence of approximately 80% before passaging or harvesting. Passaging and harvesting were performed using Accutase (CELLnTEC) to dissociate cells from the culture surface. Cells were harvested by gently

removing the media from the tissue culture flask by aspiration, washing twice with pre-warmed calcium-free phosphate buffered saline (PBS), then cells were incubated at $37°C$ with 3.0 ml Accutase solution (CELLnTEC) for12 minutes with tapping bottom of the flask at the end of the incubation period. Detachment was observed under a light microscope (Carl Zeiss Vision Inc., Oberkochen, Germany). The cell suspension was removed from the flask and mixed with 10 ml media to stop digestion. After centrifugation at 700g for 10 minutes, cells were resuspended in 10 ml of media. A hemocytometer and trypan blue staining was used to determine live cell density.

Samples were seeded at a density of $2x10^5$ cells/mm onto three replicate 12- well tissue culture plates. Eleven wells each contained one disk sample prepared by a different method covering the range of Ra values. One well was used as a plastic surface positive control. The cells were incubated at 37ºC for 1, 3, or 6 days (24, 72 and 144 hours) prior to assay.

At each time interval, one 12-well plate was removed for propidium iodide nuclear staining and cell counting. The media was gently removed from each well with a transfer pipette, and each well was washed twice with 2.0 ml of magnesium and calcium-free PBS. One milliliter of a propidium iodide staining solution consisting of 0.1% v/v TX-100, 100 µg/ml RNase A and 10µg/ml propidium iodide in PBS was added to each well and the plate incubated at 37ºC for 20 minutes. The solution was then removed and the wells containing material samples were washed twice with PBS. The samples were transferred to the new plate and placed cell surface down for microscopic observation.

Imaging of the disks and plastic controls was completed immediately following the staining procedure using fluorescence microscopy (Evos inverted microscope; Thermo Fisher Scientific, Waltham, MA), with a Texas red filter set (excitation 535 nm and emission 617 nm)

to visualize propidium iodide stained nuclei. Images were photographed at 10x magnification corresponding to a sample area of 1.0 mm^2 per image field (Image 1). Twenty images were taken of each disk and the control well, distributed over the entire material sample area in a 4 x 5 grid. The Evos imagining software Auto Count Tool (Thermo Fisher, Waltham, MA) was used to obtain stained nuclear counts for each image field after setting thresholds for brightness and particle area (Image 2). For each of the eleven samples and the plastic control at each incubation period, total cell number per field was determined by averaging the 20 field cell count (Table 3).

Non-linear regression modeling and other statistical tests were performed using statistical software (Graphpad Prism 6.0, Graphpad Software, Inc. La Jolla, CA), with alpha = 0.05.

RESULTS

The polishing, glazing and grinding protocols produced a range of R_a values (0.027-1.678 μm) (Table 2).

Growth on plastic controls

Cells were initially plated (t=0) at a density of 500 cells/mm^2. During this initial 24 hour period, cells would attach to the plastic surface. For each plastic control sample, the mean and standard deviation (SD) of the 20 field counts was determined (Table 3). The four replicate experiments gave a mean cell count on plastic at Day 1 of 76 cells$\pm$55.39 (SD) /mm^2, or a mean of 15.3% of the initial load. Thus, the initial plating density value of 500 cells/mm^2 could not be used as a day 0 value for regression analysis of growth curves due to the inefficient initial attachment.

The values for the field count coefficient of variance (CV) for the plastic samples (one well per plate) are shown in Table 4. The variance was relatively large (overall average 46.0%),

indicating considerable heterogeneity in the plating density at 24 hours and the unevenness of growth between days.

The results for statistical evaluation using D'Agostino and Pearson omnibus normality test, skewness using kurtosis values, and the ROUT algorithm to identify outliers are shown in Table 5. Values are shown for samples with all 20 values, no outliers removed. The p values for groups that failed the D'Agostino and Pearson omnibus normality test (significantly different from normal distribution) are shown. For the 11 values from growth on plastic (4 experiments, three time points; cells lost experiment #1 at 144 hour), 8/11 failed the D'Agostino and Pearson omnibus normality test (Table 5). Of the failing groups, 6/8 had a positive skewness value >1.0, and one had a value <-1.0. Five had a kurtosis value >1.0, and one had a value <-1.0. Identification of outliers using the ROUT algorithm with a relatively stringent Q value of 1% identified a total of 7 outliers in four of the non-normal distribution groups, and one outlier in a passing group. Removal of these outliers gave just 4 out of 12 groups failing the test for normality, with 3 out of 4 groups showing skewness and kurtosis values >1.0. This suggests that there were patches on the plastic surface with high numbers of cells.

Upon removal of outliers, 4 out of 12 groups still failed the test for normality, with 3 out of 4 groups showing skewness and kurtosis values >1.0. This suggested that the cells grew in patches with areas of high cell count plastic surface.

Experiment 1 showed a very large spread in the cells/mm^2 count at 72 hours, ranging from 11 to 1161, and at 144 hours, all cells were lost (Fig. 4A). This suggested an issue with the batch of cells, and this experiment was therefore excluded from further analysis.

Experiments 2-4 showed a modest increase in cell number between 24 and 72 hour, and then a lower rise through 144 hour. Experiments 2-4 showed similar cell count/ mm^2 through the

experimental period, indicating a similar rate of growth on plastic in the three experiments (Fig. 4B).

Growth on test samples

The mean field count coefficient of variance for the material samples (excluding plastic) in experiments 2-4 was also relatively large (overall average for Ground: 43.2%, for Polish: 43.8%), also indicating considerable heterogeneity in the plating density. Since the variances were comparable to those on plastic, there was no indication of a marked difference in heterogeneity of growth relative to plastic due to the material surfaces.

Evaluation of test samples and the effect of surface roughness Ra values on cell growth. The mean cells/mm^2 was determined at 24, 72, and 144 hours, using the same set of disks at each time point in the three replicated experiments, was consistent to the growth on the plastic controls in experiments 2-4. The mean roughness was used at each time point (Table 2). The cell counts at each time point were divided by the cell count for plastic at that time point to normalize for differences in cultures between experiments. As shown in figures 5 and 6, at each time point there was no clear trend in cell growth relative to plastic across the Ra range tested. For each time point, across the eight Ra values the relative density values had a normal distribution (Shapiro-Wilk and D'Agostino & Pearson normality tests; $p>0.42$). The overall means across all Ra values were 1.58+0.35 at 24 hours, 0.863+0.184 at 72 hours, and 1.29+0.16 at 144 hours.

Non-linear regression fit to a linear model showed no significant difference from a fit to a line with zero slope (null hypothesis), $p>0.42$. That is, there was no significant relationship between growth relative to plastic and Ra value. Similarly, for each time point, there was no significant difference between the intercept on the Y axis and Y=1.00 ($p>0.091$), indicating that

at each time point, overall there was no difference between growth on zirconia and growth on plastic.

To further evaluate differences between relative growth and surface treatment, a two way ANOVA was performed with factors Treatment (7 levels including no surface treatment) and Time (three levels), with three replicate experiments, and time and materials treated as repeat measures (same cell culture). No significant effect for surface treatment ($p=0.085$), Time ($p=0.35$) or Interaction ($p=0.62$) was found (Fig. 7).

DISCUSSION

This study investigated the growth of primary HGE cells on zirconia oxide material surfaces. Samples were polished with a polishing wheel method, a diamond or carbide bur, and a glaze. Samples with a range of roughness values, quantified as R_a, (0.027-1.678 μm) were tested over a 144hr period. Growth on the samples were compared to the growth on tissue culture plastic, a control used routinely to characterize cell growth under laboratory conditions. HGE cell density on plastic over the six day experimental period was consistent with an exponential growth model.

The results of this study showed that cell growth on zirconia oxide material was not affected by surface roughness. In our research project, there was no observed change in cell growth compared to different surface roughness Ra values. The null hypothesis of no relationship between growth and surface roughness was accepted.

A limitation of this study regarding growth pattern was the same as Smallidge et al, in that sampling was confined to three time points, limiting the power of regression analysis. Further clarification of the specific effects of R_a on HGE cell growth would require more

frequent sampling. Another limitation of the current study is that it only described the topology of the experiment surfaces with a linear Ra value. A further limitation is that stylus-based measuring instruments, rather than optical instrumentation, were used to measure surface roughness. The size of the physical stylus limits the instruments scanning resolution and could possibly affect the measurements, especially on smoother surfaces. Average peak and valley heights to include area measurements like S_a could be used to better understand the cells interaction with material surface.

Surfaces polished using the extra oral Dialite eZr polishing kit showed R_a values ranging from 0.027-0.039 µm, all below the threshold value; those polished with the intra oral Garrison polishing kit had values between 0.228-0.242 µm. The glazed samples gave an R_a range from 0.464-0.525 µm. The surfaces treated with grinding gave a range of 0.245 µm for the carbide bur to a 1.677 µm for the super course. Even with such a small number of samples, following the manufacturers' recommendations for polishing still produced R_a values below the threshold for cell attachment and growth. It is likely that clinicians will be able to polish zirconia oxide to a surface smooth enough to maximize HGE growth.

The current study was done in a sterile environment and had an R_a at 0.027 µm much lower than the 0.2 µm previously suggested in the literature to be a minimal cut off for soft tissue attachment to implant abutments.[11] Although the results of this current study accepted the null hypothesis showing that Zirconia oxide is biocompatible at any R_a value, it is important to consider that microbial biofilm development is inhibited by smooth surfaces. Zirconia oxide should be polished to the lowest reasonable R_a value to maximize the desired outcome for hygiene, therefore the HGE will respond to the material and not to a build-up of biofilm.

CONCLUSIONS

There was no relationship between surface roughness (as measured by Ra) and epithelial cell growth (relative to growth on plastic), regardless of whether the surface was prepared by grinding or polishing, over the 0.027-1.678 μm range tested over a 144hr period. Zirconia, regardless of surface preparation, was as equally biocompatible as tissue grade plastic under the experimental conditions employed.

Overall conclusion: Zirconia is biocompatible, and the polishing method employed for the zirconia material would not be expected to affect the quality of the biological seal obtained by epithelial cell growth on the surface.

REFERENCES

1. Linkevicius T: Zero Bone Loss Concepts (ed 1). Batavia, Quintessence Publishing Co, Inc, 2019

2. Albrektsson T, Zarb G, Worthington P, Eriksson AR. The Long-Term Efficacy of Currently Used Dental Implants: A Review and Proposed Criteria of Success. JOMI, 1986; 1:11-25

3. Araujo MG, Lindhe J. Peri-implant Health. J Clin Periodontol. 2018;45(suppl 20):s230-s236. DOI: 10.1111/jcpe.12952

4. Kawahara H, Kawahara D, Mimura Y, Takashima Y, Ong JL. Morphologic studies on the biologic seal of titanium dental implants. Report II. In vivo study on the defending mechanism of epithelial adhesions/ attachment against invasive factors. Int J oral Maxillofac Implants 1998;13:465-473.

5. Vignoletti F. De Sanctis M, Berglundh T. Abrahamsson I, Sanz M. Early healing of implants placed into fresh extraction sockets: an experimental study in the beagle dog. II:ridge alterations. J Clin Perio 2009 36: 688-697.

6. Gould TR, Westbury L, Brunette DM. Ultrastructural study of the attachment of human gingiva to titanium in vivo. J Prosthet Dent 1984;52:418–420.

7. Cochran DL, Herman JS, Schenk RK, Higginbottom FL, Buser D. Biologic width around titanium implants. A histometric analysis of the implant-gingival junction around unladed and loaded nonsubmerged implants in the canine mandible. J Periodontol 1997; 68:186-198.

8. Okabe E, Ishihara Y, Kikuchi T, et al. Adhesion Properties of Human Oral Epithelial-Derived Cells to Zirconia. Clinical Implant Dentistry and Related Research, Volume 18, Number 5, 2016. DOI 10.1111/cid.12369

9. Wataha JC. Principles of biocompatibility for dental practitioners. J. Prosthet Dent 2001;86: 203-9.

10. Williams, DF. On the mechanisms of biocompatibility. Biomaterials 2008; 1-13. doi:10.1016/j.biomaterials.2008.04.023.

11. Bollen C, Lambrechts P, and Quirynen M. Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: A review of the literature. Dent Mater 1997; 13:258-269.

12. Bollen CML, Papaioannou W, Van Eldere J, Quirynen M, Van Steenberghe D. The influence of abutment surface roughness on plaque accumulation and peri-implant mucositis. Clin Oral Implants Res 1996; 7:201-11.

13. Smallidge MJ, Sabol JV, Aita-Holmes C, Chuang H, Dickinson D. Human Gingival Epithelial Growth In Vitro on a Polymer-Infiltrated Ceramic Network Restorative Material. J Prosthodontics. 28, 2019; 541-546. doi: 10.1111/jopr.13017

14. Steiner R, Beier US, Heiss-kisielewsky I, Engelmeier R, Dumfahrt H, Dhima M. Adjusting Dental Ceramics: An In Vitro Evaluaton of the Ability of Verious Ceramic Polishing Kits to Mimic Glazed Dental Ceramic Surface. J of Prosthetic Dent. 2015; 616-622.

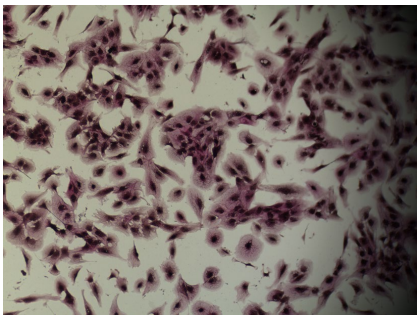

Image 1: Human gingival epithelial (HGE) cells stained with H and E stain and view under 10X magnification.

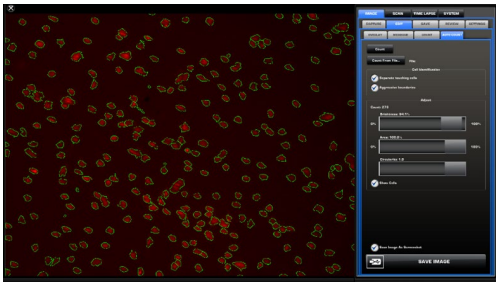

Image 2: human gingival epithelial (HGE) cells visualized under florescence microscopy after staining with ethidium bromide with Texas red filter

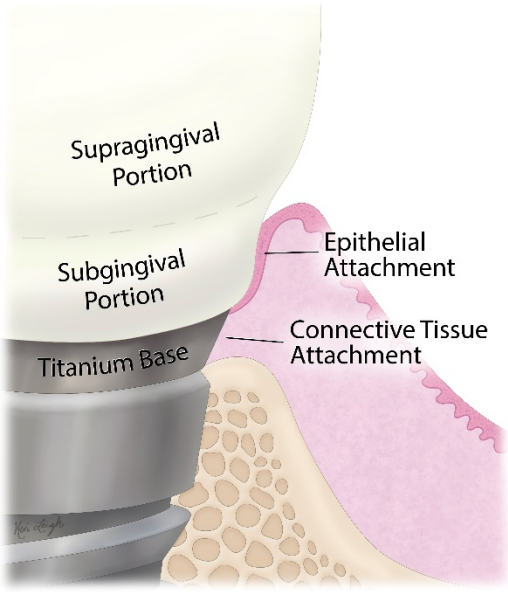

Figure 1, Illustrations by Keri Leigh Jones, MSMI, CMI at Dwight D. Eisenhower Army Medical Center

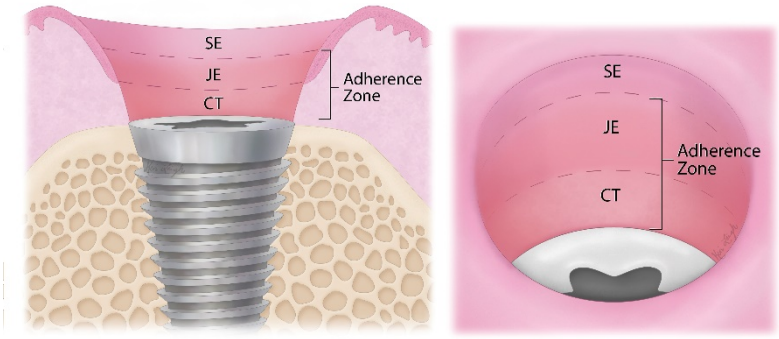

Figure 2, Illustrations by Keri Leigh Jones, MSMI, CMI at Dwight D. Eisenhower Army Medical Center

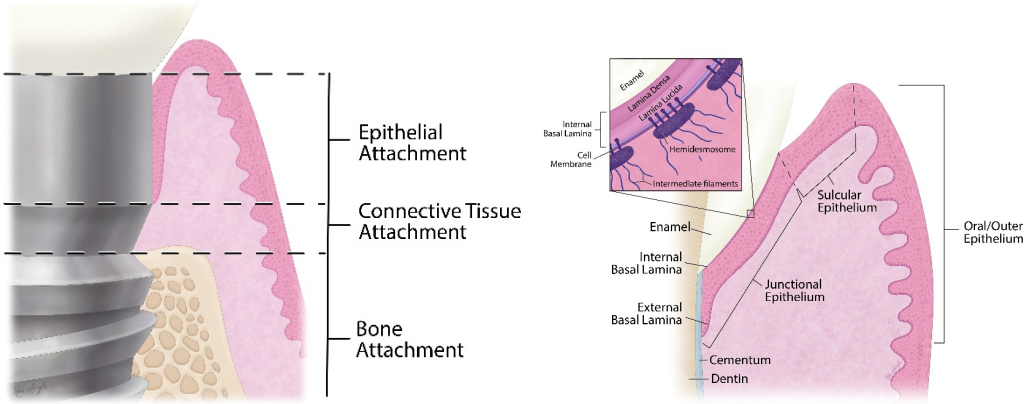

Figure 3, Illustrations by Keri Leigh Jones, MSMI, CMI at Dwight D. Eisenhower Army Medical Center

Color code	Bur type	Grit size
Black	Super Course	181 µm
Green	Course	125-150 µm
Blue	Standard	106-125 µm
Red	Fine	53-63 µm
Yellow	Extra Fine	20-30 µm

Table 1: ISO Dental diamond burs used to create different surface Ra Values

1	No Treatment	0.261 µm
2	Super Course	1.677 µm
3	Course	1.619 µm
4	Standard	1.196 µm
5	Fine	0.691 µm
6	Extra Fine	0.564 µm
7	Carbide	0.245 µm
8	Grinding Wheel	0.901 µm
9	Dialite Extra oral	0.027 µm
10	Garrison intra oral	0.242 µm
11	Empress Glaze	0.525 µm
12	Control	

Table 2: Sample mean Ra values

Experiment	Mean cell count/SD 24hrs	24hr % initial load
1	148.8/106.4	29.76
2	15.85/19.94	3.17
3	59.90/21.09	11.98
4	81.15/20.95	16.23
Overall Mean/SD	76.425/55.39	15.285/11.08

Table 3: Mean and SD of 24 hour field counts of plastic samples. For each Plastic control sample, the mean and SD of the 20 field counts was determined. Overall means and SD values across the four experiments (n=4) are shown. The % of initial load was determined as a proportion of 500 cells/mm2 loaded at time =0.

Experiment	24 hour	72 hour	144 hour
1	71.51	71.12	Cells lost
2	75.82	60.47	78.28
3	20.98	60.80	52.23
4	13.56	37.87	23.07
Overall mean	45.47	57.56	51.19

Table 4: Coefficients of variance for field counts of plastic samples. For each sample, the mean and SD of the 20 field counts was used to determine a % CV value.

Run	1			2			3			4		
Time (Hr)	24	72	144	24	72	144	24	72	144	24	72	144
P Value	0.012		Cells lost	<0.0001	0.0002	0.005	0.003		0.022	0.0002	0.013	
Skewness	1.218	0.526		3.161	1.386	0.6685	1.571	0.5297	1.144	-2.41	1.223	-1.247
Kurtosis	1.1236	-0.6239		11.94	0.594	1.101	2.735	0.4602	2.793	8.896	0.918	2.504

Table 5: Normality of distribution, skewness and kurtosis of cell counts of plastic samples. Values are shown for samples with all 20 values, no outliers removed. The p values for groups that failed the D'Agostino and Pearson omnibus normality test (significantly different from normal distribution) are shown. The results suggests that the cells grew in patches on the plastic surface.

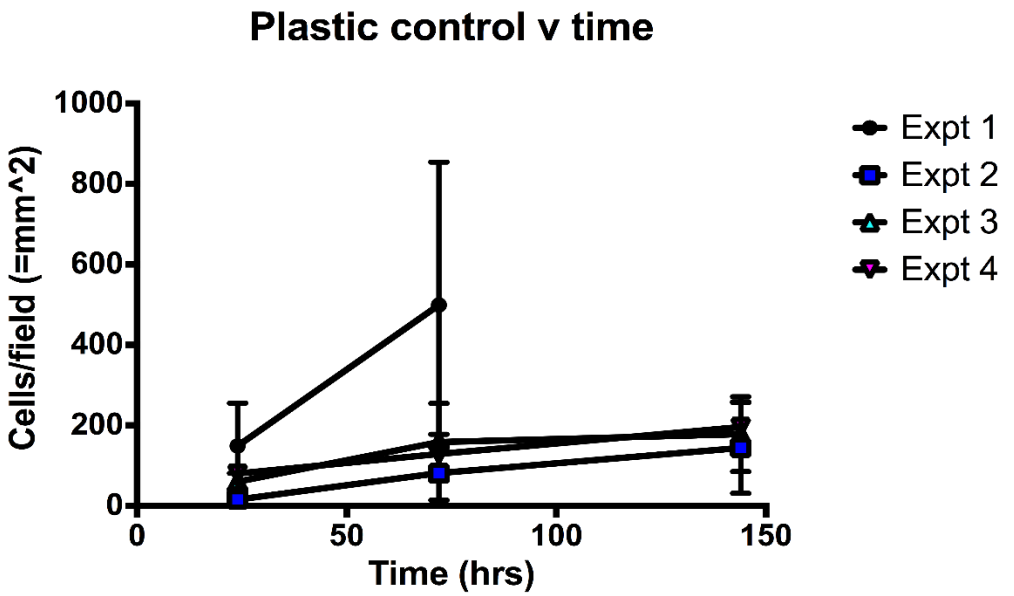

Figure 4A: Graph of plastic mean cell count/mm^2 during experimental period. For each sample, the mean and SD of the 20 field counts are shown.

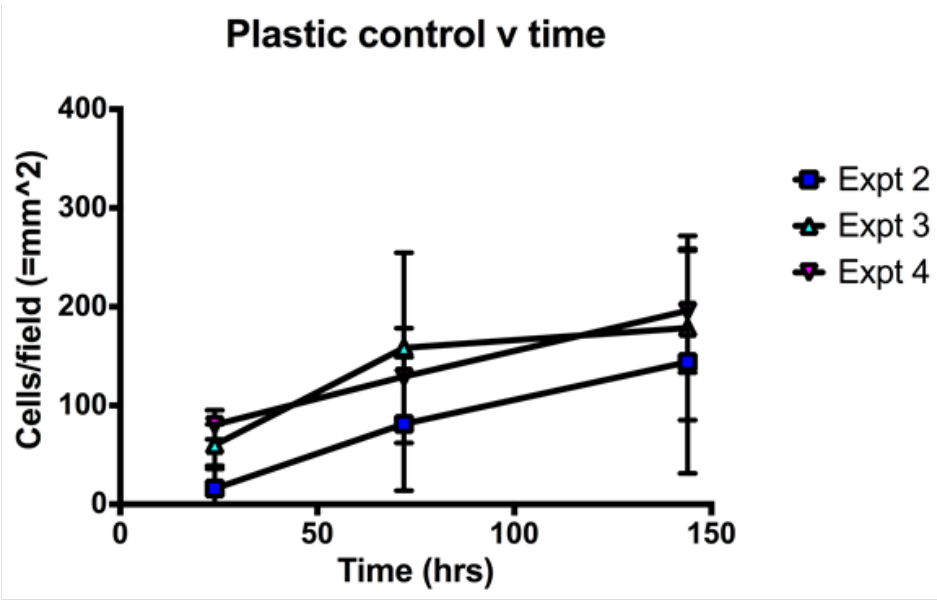

Figure 4B: Graph of plastic mean cell count/mm^2 during experimental period. For each sample, the mean and SD of the 20 field counts are shown excluding experiment 1.

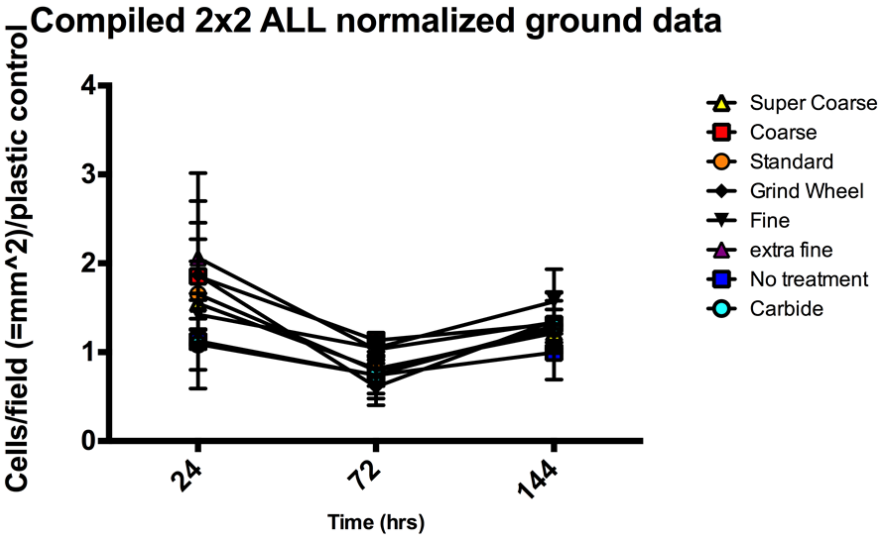

Figure 5: Graph of normalized ground material cell counts at each treatment grouped by time For each point, the mean and SEM of experiments 2-4 are shown.

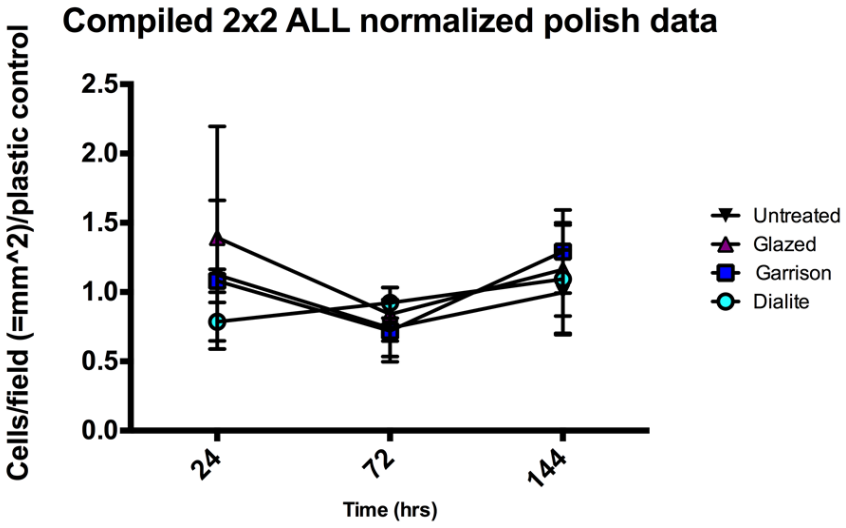

Figure 6: Graph of normalized polished material cell counts at each treatment grouped by time For each point, the mean and SEM of experiments 2-4 are shown.

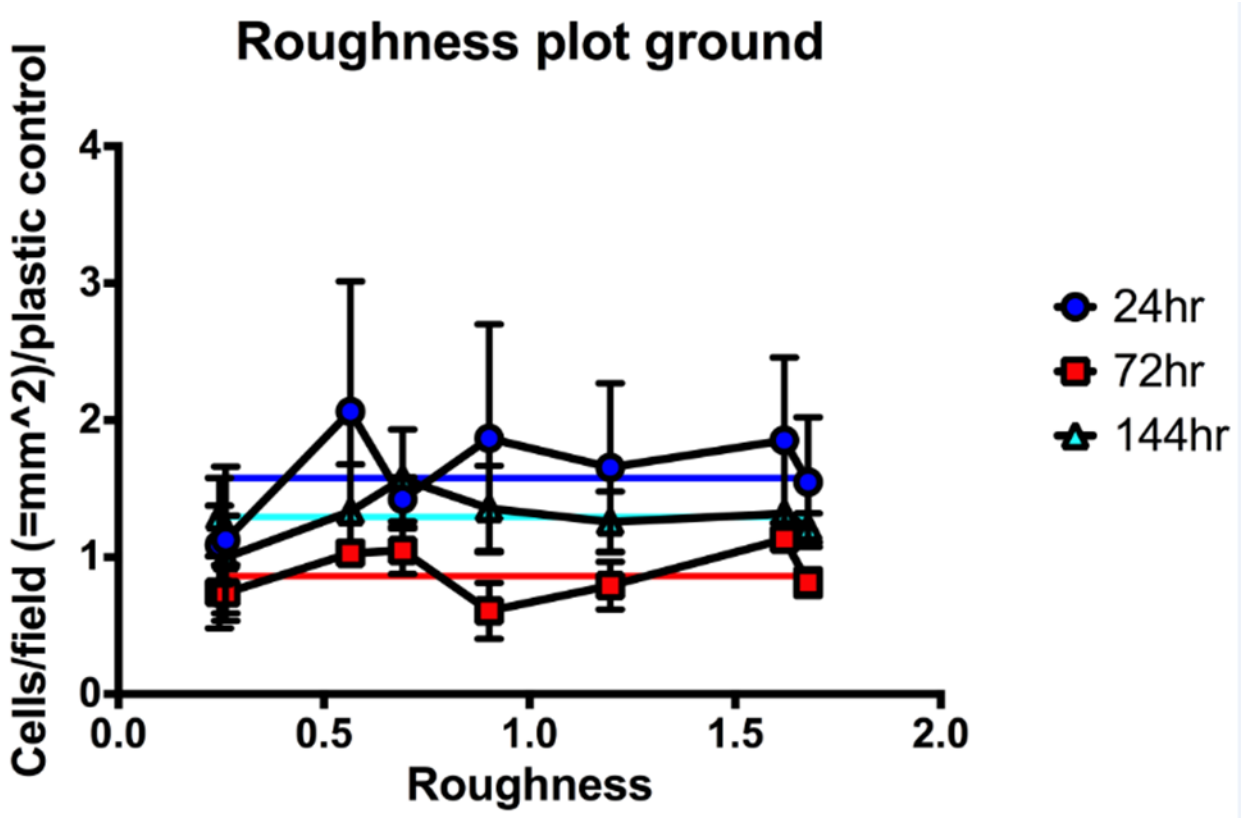

Figure 7: Graph of material cell count/mm^2 normalized to plastic growth against mean material Ra value during experimental period**.** For each sample, the mean and SEM of experiments 2-4 are shown. Colored lines show non-linear regression fit to a linear model for each time point.