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Surface properties correlated with the human gingival fibroblasts attachment on various materials for implant abutments: A multiple regression analysis

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Abstract

Objectives. To reveal the suitable surface condition of an implant abutment for fibroblast attachment, the correlation between the surface characteristics of various materials and the human gingival fibroblast (HGF-1) attachment to the surfaces were analyzed. ***Methods.*** Six kinds of surfaces comprised of machined titanium alloy (SM), machined Co-Cr-Mo alloy (CCM), titanium nitride coated titanium alloy (TiN), anodized titanium alloy (AO), composite resin coating on titanium alloy (R) and zirconia (Zr) were used. The measured surface parameters were S_a, S_q, S_z, S_{dr}, S_{dq}, S_{al}, S_{tr} and water contact angle (WCA). The HGF-1 cell attachment was investigated and the correlations were analyzed using a multiple regression analysis. ***Results.*** The HGF-1 cell attachment was greater in the SM, TiN and Zr groups than the other groups and smallest in the CCM group (p = 0.0096). From the multiple regression analysis, the HGF-1 cell attachment was significantly correlated with S_{dr}, S_{dq} and WCA. When the R group was excluded, only WCA showed significant correlation with the fibroblast attachment. ***Conclusions.*** Within the limitations of this study, the cell attachment of human gingival fibroblasts was correlated with WCA, developed interfacial area ratio and surface slope. When the surfaces with S_a values of ~ 0.2 μm or less were concerned, only WCA showed a correlation in a third order manner.

Key Words: *surface properties, wettability, fibroblasts, dental abutments, dental implants*

Introduction

For long-lasting implant-supported prostheses, it is essential to establish not only sound implant-to-hard tissue interfaces, but also a firm implant-to-soft tissue junction. Peri-implant soft tissues and the response of soft tissue cells to abutments made of different materials have previously been investigated [1–10]. According to those studies, peri-implant hard and soft tissue configurations were similar regardless of implant systems [3] and there was also no difference between the peri-implant tissues of submerged or non-submerged healing [2]. Meanwhile, the mucosal attachment and the marginal bone level at four different abutments including commercially pure (c.p.) titanium, gold, ceramic and short titanium abutment exhibited different configurations [1]. However, the subsequent studies on the different abutment materials reported inconsistent results among studies from similar hard and soft tissue integration [4,10] to different levels of inflammation [6]. Unfortunately, it is poorly understood why peri-implant soft tissues were influenced by changes in abutment materials.

Numerous studies on surface characteristics of implant fixtures and the *in vitro*/*in vivo* responses to the surfaces have been piled up and it is generally accepted that 'moderately rough' surfaces having average roughness between 1.0–2.0 μm induce a stronger bone response and have a tendency to produce better clinical results compared to turned surfaces [11]. For implant abutments, microbial attachment as well as fibroblasts or epithelial cells

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attachment should be considered. On microbial attachment, it was shown that the amount of developed biofilm became smaller until the surface roughness decreased to 0.2 μm and no further reduction in biofilm formation occurred below 0.2 μm [12–14]. On the other hand, there were few studies which explored the relationship between fibroblasts or epithelial cells attachment and surface properties, although the effecst of surface properties on cellular attachment were reported recently in several studies which compared the results from specific conditions such as anodized titanium disks or a thermally treated titanium surface [15,16].

Surface-free energy (SFE) can play an important role in protein adsorption and cell attachment or spreading [17]. It was shown with polymer surfaces that SFE became greater as surface roughness increased and more cell adhesion/migration was observed on the roughened surfaces [18]. Furthermore, the titanium surfaces with high surface energy showed more osteogenicity compared to those with low SFE [19]. While most of the studies on SFE and implant abutments focused on biofilm formation and reported a positive relationship between SFE and the amount of biofilm formation [20–22], a recent *in vivo* study using rats showed increases in the connective tissue attachment and the number of cells in contact with the surface and it was concluded that hydrophilicity influenced the connective tissue healing positively [23].

Parameters for areal surface texture are defined in the ISO 25178 standard. These parameters could be divided into sevaral groups such as parameters of height, spatiality, hybridness, functions and segmentation. Height parameters describe only the statistical distribution of height values along the z-axis and include arithmetical mean height of a surface (S_a), maximum height of a surface (S_z), valleys (S_v) and peaks (S_p), skewness (S_{sk}) and kurtosis (S_{ku}) of height distribution and root mean square (RMS) height of a surface (S_q). Spatial parameters involve the spatial periodicity of a surface, specifically its direction. Fastest decay auto-correlation rate (S_{al}), texture aspect ratio (S_{tr}) and texture direction (S_{td}) of a surface are spatial parameters. Hybrid parameters relate to the spatial shape of a surface. RMS gradient of a surface (S_{dq}) and developed area ratio (S_{dr}) belong to hybrid parameters. There are also other parameters such as functions and related parameters and parameters related to segmentation.

There are many kinds of implant abutments made of various materials. In the early days of implant development, abutments were made of titanium alloy, gold alloy or dental ceramic. Afterwards, other materials or surface treatments were employed to reduce cost and/or to promote color harmonization with surrounding mucosa. When an anodic oxidation is applied to a titanium surface, the surface shows a reddish color. The surface color after titanium nitride coating is yellowish, which resembles gold. Zirconia and resin can mimic the surface color of a natural tooth. As a result, clinicians may cope with cases of abutment exposure or transmission of abutment color through covering mucosa, especially in areas of esthetical demand.

Peri-implant soft tissues seem to be influenced by abutment material and its surface characteristics. However, most of the studies mentioned earlier focused on the small number of influencing factors such as materials, surface roughness or wettability, although there were other parameters of surface properties including S_{dq}, which is known to be related with wettability. For these reasons, there has been a lack of comprehensive understanding on the effective surface properties of implant abutments for enhancing soft tissue response. This study was performed to explore the correlations between the fibroblast attachment and the surface properties of implant abutment materials by investigating various materials with different surface properties and the human gingival fibroblast attachment to the surfaces.

Materials and methods

This study was an *in vitro* experiment using six groups of disks. The disks were made of machined titanium alloy (Ti6Al4V; SM), machined cobalt-chrome-molybdenum alloy (Co-Cr-Mo alloy; CCM), titanium nitride coating on a titanium alloy (TiN), surface treatment using anodic oxidation on a titanium alloy (AO), composite resin coating on a titanium alloy (R) and zirconia (Zr). A total of 152 specimens were prepared and 22 disks for each group were used: 10 disks for surface topography measurements, three for water contact angle (WCA) measurements and nine for the human gingival fibroblast attachment assay. Eight kinds of parameters for surface properties were measured and these parameters included surface roughness, developed interfacial area ratio, RMS surface slope and WCA. Following the investigation of the surface properties, the fibroblast attachment on the disks was observed indirectly using optical absorbance measurements. The detailed methods were described below.

Specimen preparation

Specimens were manufactured by a computerized numerical control lathe (Cincom L20-7M8, Mitsubishi, Tokyo, Japan) in the form of a disk. The disks were 6 mm in diameter and 1 mm in thickness. The disks for the SM group were made of titanium alloy (Carpenter, Wyomissing, PA) and machined for a smooth surface. The CCM group was comprised of disks manufactured by machining a Co-Cr-Mo alloy (Carpenter). The disks for the TiN group were prepared by coating titanium nitride onto machined titanium alloy disks. The AO group had an anodic oxidation treatment on titanium alloy disks at 60 V for 10 min in 1 M H_2SO_4 at room temperature using a DC power supply (Genesys 600-2, Densi-Lambda, TDK, Tokyo, Japan). The R group was prepared using a composite resin (Osstem, Busan, Korea) coating on both sides of the machined titanium alloy disks. After the composite resin was placed on the titanium alloy disk and cured for 40 s, the composite resin-coated surfaces were polished using SiC emery paper (Deerfos, Ridgefield, NJ) with 1000 grits. The disks of the Zr group were machined from zirconia blocks (Ssangyong Materials, Daegu, Korea) and polished. Following the completion of preparation, the disks were rinsed in distilled water and dried at 50°C for 24 h.

Surface properties measurement

The surface properties of prepared specimens were evaluated by the optical 3-dimensional (3D) profiling system (Wyko NT8000, Veeko, Plainview, NY). The measurement was performed at the center of each specimen, which had dimensions of 318×238 μm^2. The surface parameters obtained from the 3D profiling system were S_a, S_q, S_z, S_{dr}, S_{dq}, S_{al} and S_{tr}. The WCAs of all the specimens were measured using a video contact angle measuring system (DSA 10, Kruss, Hamburg, Germany) at room temperature. For WCA measurement, distilled water dropped on the specimens and the water deposited on the specimens were visualized with a camera and illumination system. The contact angle measurement was performed after 10 s of delay, which allowed for the droplet to become stable.

Cell attachment assay

The fibroblast attachment on each disk was investigated via an indirect method. The human gingival fibroblasts (HGF-1; ATCC CRL 2014, ATCC, Manassas, VA) were used in this study. Three specimens from each group were placed in each well of a 24-well plate with serum-free Dulbecco's modified Eagle's medium (DMEM). The HGF-1 cells were then loaded at a concentration of 1×10^5 cells/ml and the plate was placed in an incubator at 37°C, 5 % CO_2 condition for 1 h. After the cells reached ~ 80% confluence, the specimens were pulled out from the wells and were washed 3-times with phosphate-buffered saline (PBS) for the removal of unattached cells. The washed specimens were transferred to a new 24-well plate and the cells were fixed using 10% formaldehyde PBS solution at 4°C. After 1 h of fixation, the disks were rinsed 3-times with distilled water (DW). The attached cells on the specimens were stained using 0.04% cresyl violet in 20% methanol for 30 min. The specimens were dipped into DW for washing and the cresyl violet was eluted using 0.1 M citric acid in 50% ethanol; 200 μl of the eluted solution from each well was transferred to a 96-well plate and the colorimetric measurement of cresyl violet was performed on a DTX 880 (Beckman Coulter, Brea, CA) with an optical density reading at 590 nm.

Statistical analysis

The comparison of the means of the measured surface parameters among the groups was performed using one-way ANOVA with *post-hoc* comparison by the Tukey method. For the identification of a correlation between the surface parameters and the level of cell attachment, the 10 measured values for a certain surface parameter were reduced to three adjusted values by arithmetically averaging the first three values, the next three values and the seventh to ninth values while dropping the tenth value. The three adjusted values were then matched to the three measured values of absorbance from the cell attachment assay. Simple scatter plots with fit lines of 1st, 2nd and 3rd order were made to explore whether there was any trend of correlation. After deciding the order of relationship for each parameter, a multiple regression analysis was performed to estimate the correlation coefficients and linear model. The alpha error level was set to be 0.05. Statistical analysis was performed using PASW 18 (SPSS Inc., New York, NY).

Results

Surface characteristics and cell attachment assay

The surface characteristics of the selected materials are presented in Table I and Figure 1. In general, the Zr group exhibited exclusively smaller values than the other groups, except WCA, while the R group showed greater values for all surface parameters including WCA. Although all the groups had average roughness (S_a and S_q) smaller than 0.5 μm, the S_a of the R group was greater than 0.2 μm, while those of the rest groups were ~ 0.2 μm or less. On the parameters representing surface roughness such as S_a, S_q and S_z, there were significant differences among groups ($p < 0.001$) and the Zr group had the smallest roughness values, which were near zero. The S_{dr} and S_{dq} showed a similar pattern. The means of S_{dr} and S_{dq} ranged from 5–8% and from 18–22°, respectively, except the Zr group, which showed significantly smaller values (0.06% in S_{dr} and 1.91° in S_{dq}; $p < 0.001$). The mean of the S_{al} values was the highest in the R group and those of the rest groups were similarly smaller ($p < 0.001$). The SM, CCM and TiN groups had much smaller S_{tr} values of 0.05 compared to those of the AO, R and Zr groups (0.66, 0.69 and 0.22, respectively; $p < 0.001$). The WCA of all groups were greater than 40°. The SM, TiN, AO

Table I. Surface characteristics of the selected materials.

Parameter	*n*	SM Mean (SD)	CCM Mean (SD)	TiN Mean (SD)	AO Mean (SD)	R Mean (SD)	Zr Mean (SD)	Significance (ANOVA)
S_a (μm)	10	0.22^B (0.03)	0.21^B (0.05)	0.22^B (0.03)	0.23^B (0.09)	0.39^C (0.06)	0.019^A (0.00)	<0.001
S_q (μm)	10	0.27^B (0.04)	0.26^B (0.06)	0.27^B (0.03)	0.30^B (0.04)	0.51^C (0.07)	0.026^A (0.01)	<0.001
S_z (μm)	10	2.12^B (0.34)	2.14^B (0.56)	2.08^B (0.35)	2.35^B (0.13)	4.96^C (0.68)	0.24^A (0.10)	<0.001
S_{dr} (%)	10	7.43^B (2.77)	5.68^B (2.60)	7.43^B (3.47)	6.60^B (0.90)	7.59^B (1.99)	0.06^A (0.02)	<0.001
S_{dq} (°)	10	21.93^B (3.82)	18.84^B (5.38)	21.72^B (4.59)	20.40^B (1.32)	21.91^B (2.54)	1.91^A (0.41)	<0.001
S_{al} (μm)	10	2.40^A (0.54)	2.73^A (0.35)	2.23^A (0.35)	3.11^A (0.26)	16.20^B (11.46)	3.93^A (5.03)	<0.001
S_{tr}	10	0.05^A (0.02)	0.05^A (0.01)	0.05^A (0.02)	0.66^C (0.23)	0.69^C (0.16)	0.22^B (0.17)	<0.001
WCA (°)	3	58.5^A (4.9)	90.2^B (2.2)	63.5^A (1.0)	62.3^A (4.9)	110.0^C (3.4)	57.3^A (2.2)	<0.001

$^{A, B, C}$These superscript letters indicate the Tukey grouping.
SM, simply machined titanium alloy; CCM, cobalt-chrome-molybdenum alloy; TiN, titanium nitride coated titanium alloy; AO, titanium alloy with surface treatment using anodic oxidation; R, composite resin coated titanium alloy; Zr, zirconia; WCA, water contact angle.

and Zr groups exhibited similar and smaller WCA (~ 60°) than the CCM and R groups (90.2° and 110.0°, respectively; $p < 0.001$).

The result of absorbance measurements for cell attachment was shown in Figure 2. The SM, TiN and Zr groups presented greater cell attachment than the other groups, while the CCM group exhibited the least cell attachment ($p < 0.001$).

Correlations between the parameters of surface properties and the cell attachment

To explore the correlation of each surface parameter and the level of cell attachment, simple scatter plots with fit lines of first, second and third order correlations were created (Figure 3). Among all the surface parameters, only WCA exhibited ~ 50% goodness of fit for second and third order correlations. In addition, S_{dr} and S_{dq} had R^2 values greater than 0.1 for second and third order relationships, while S_{al} and S_{tr} exhibited R^2 values greater than 0.1 for third order only. The parameters for surface roughness, including S_a, S_q and S_z, showed less than 10% goodness-of-fit for both the linear and polynomial correlations. A multiple linear regression analysis was performed with parameters showing the goodness of fit greater than 10% (Table II). S_{dr}, S_{dq}, WCA and WCA^3 were the effective parameters and the adjusted R^2 was 0.556 for the linear model when all six experimental groups were included (model 1 in Table II), while only WCA^3 was effective and the adjusted R^2 was 0.910 when the R group was excluded from the analysis (model 2 in Table II).

Discussion

This study performed analytic measurements of surface properties of six different materials for implant abutments and tried to explore the correlations between the surface properties and the fibroblasts attachment in order to reveal the effective surface parameters. There were six kinds of specimens. Titanium abutments and titanium nitride coated titanium abutments are available in the market and showed sufficient mechanical durability. Zirconia abutments are also easily obtainable in the market and they were often used in the anterior region. Anodized and oxidized titanium abutments were devised to harmonize with the surrounding mucosa because the surface was reddish. A cobalt-chrome-molybdenum alloy and composite resin has been widely used in restorative dentistry and these surfaces were employed for providing varieties in order to reveal more detailed correlation between surface properties and cell attachment.

Previously, few studies have investigated the effects of the various surface parameters of slightly different smooth surfaces on human gingival fibroblasts attachment and the correlations between cell attachment and the effective parameters. To seek the correlations and the effective parameters, in this study, the parameters representing surface roughness, surface texture and wettability were measured and cell attachment on the surfaces was investigated. Then, the correlations between the surface parameters and the level of cell attachment were explored. Usually, correlation analysis requires paired data or a series of data on each subject. Since surface contamination was unavoidable after a certain measurement in this study, the specimens used once were discarded to guarantee the same surface condition. As a result, the surface parameters obtained from the 3D profiling system, WCA and OD values for the cell attachment could not be collected on the same specimen. Despite of this, the exploration of the correlations between the surface parameters and the cell attachment could be justified because the specimens in each group were able to be considered as the same specimens in that the values of the surface parameters in each group were similar and the standard deviation was

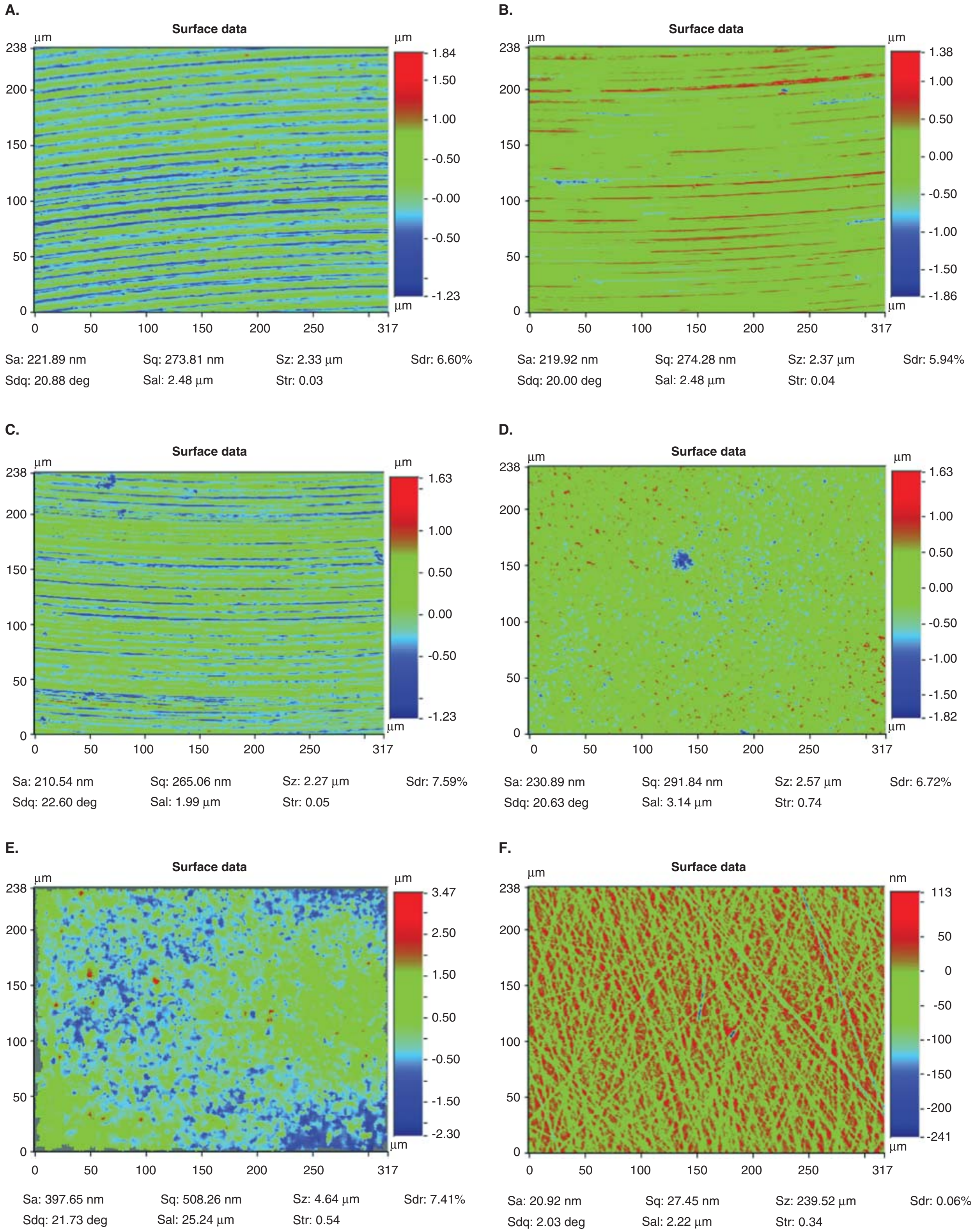

Figure 1. Images obtained from 3D profiling system. (A) Machined and polished titanium alloy (Ti_6Al_4V) disk (SM). (B) Cobalt-chrome-molybdenum alloy disk (CCM). (C) Titanium alloy disk with titanium nitride coating (TiN). (D) Titanium alloy disk with surface treatment using anodic oxidation (AO). (E) Composite resin coated titanium alloy disk (R). (F) Zirconia disk (Zr).

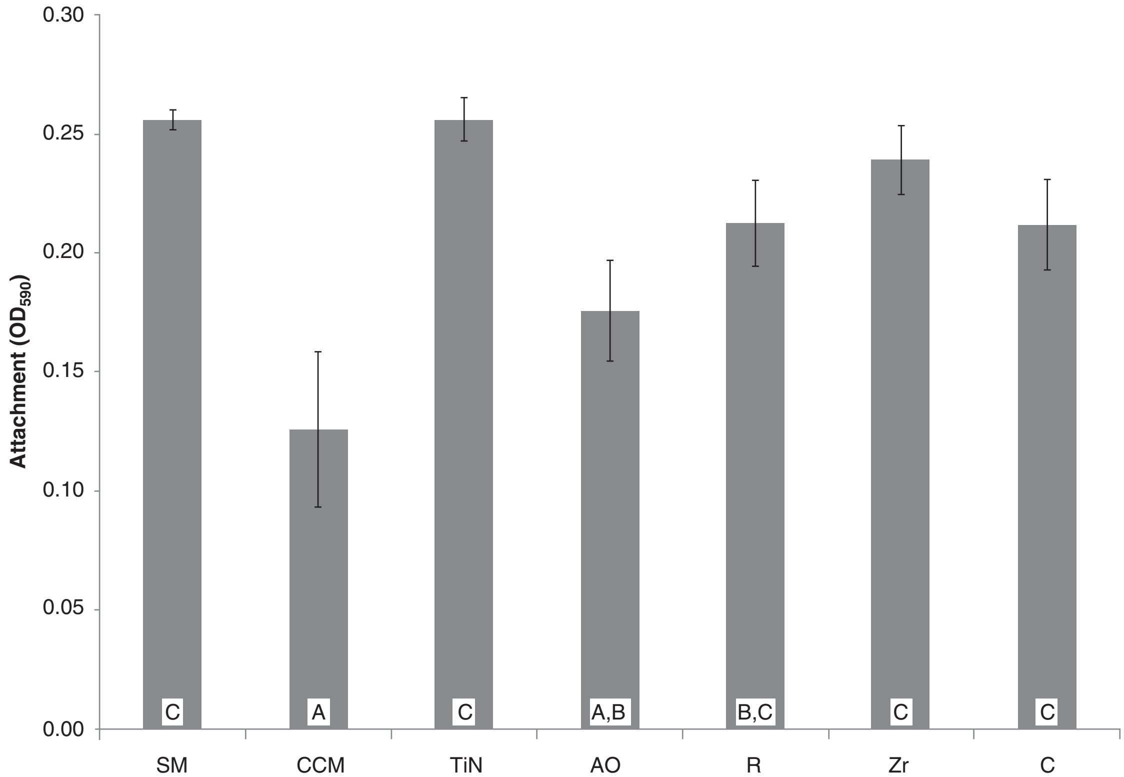

Figure 2. Absorbance measurements for the HGF-1 cell attachment. There were significant differences on the level of cell attachment among groups ($p < 0.001$, one-way ANOVA) and more fibroblasts attachment was observed in the SM, TiN and C groups. The serif-font letters A, B and C in the bars represented the Tukey grouping. SM, simply machined titanium alloy; CCM, cobalt-chrome-molybdenum alloy; TiN, titanium nitride coated titanium alloy; AO, titanium alloy with surface treatment using anodic oxidation; R, composite resin coated titanium alloy; Zr, zirconia.

sufficiently small compared to the mean value for each parameter (Table I).

When all the six groups were included in the multiple regression analysis, developed interfacial area ratio, RMS developed slope and wettability correlated with cell attachment with significance and the linear model 1 of '[Cell Attachment] = 0.817 - 0.190 * [S_{dr}] + 0.045 * [S_{dq}] 0.013 * [WCA] + 0.000 * [$S_{dr}{}^3$] + 3.882 * 10^5 * [$S_{dq}{}^3$] + 6.111 * 10^7 * [WCA^3]' ($p = 0.015$, adjusted $R^2 = 0.556$) was achieved. From the model 1, S_{dr} had a negative effect on cell attachment, while the increase of S_{dq} resulted in an increase of cell attachment. Furthermore, the least cell attachment may be expected at the WCA of ~ 72.5°. However, the explanation power of model 1 was relatively low (55.6%) and the interpretation of model 1 did not coincide with the currently accepted knowledge that cell attachment would increase as surface roughness increased and WCA decreased. This may be caused by the R group, which had a much rougher surface (0.39 ± 0.06 μm for S_a) and higher WCA than the other groups. So another multiple regression analysis with the exclusion of the R group was performed and the linear model 2 of '[Cell Attachment] = 0.38 0.086 * [S_{dr}] + 0.017 * [S_{dq}] 0.051 * [S_{al}] + 2.761 * 10^5 * [$S_{dq}{}^3$] + 0.001 * [$S_{al}{}^3$] – 0.06 * [$S_{tr}{}^2$] – 2.135 * 10^7 * [WCA^3]' ($p < 0.001$, adjusted $R^2 = 0.91$) was drawn. In model 2, only WCA^3 was a statistically significant parameter ($p < 0.001$) and the WCA increase in all the ranges from 0° to 180° resulted in a decrease of cell attachment, which was in agreement with the notion that WCA is inversely correlated with cell attachment.

Unlike an implant fixture, the implant abutment should have a smooth surface to prevent plaque accumulation [13,24] and all six types of surfaces in this study had S_a values less than 0.5 μm, which meant the surfaces were smooth [11]. This could be an explanation of no correlations between the parameters for surface roughness (S_a, S_q and S_z) and the cell attachment, even though these roughness parameters showed significant differences among the experimental groups. The previous studies using epithelial cells also exhibited similar cell attachment on the surfaces of different roughness [25,26].

It was interesting that the R group showed higher cell attachment even though it had an absolutely hydrophobic surface with a WCA of 110°. The R group had an ~ 2-fold rougher surface and more than 5-fold wider peak-to-peak distance than the SM, CCM, TiN and AO groups. According to previous studies [12–14], plaque formation decreased until surface roughness reached 0.2 μm. When combining

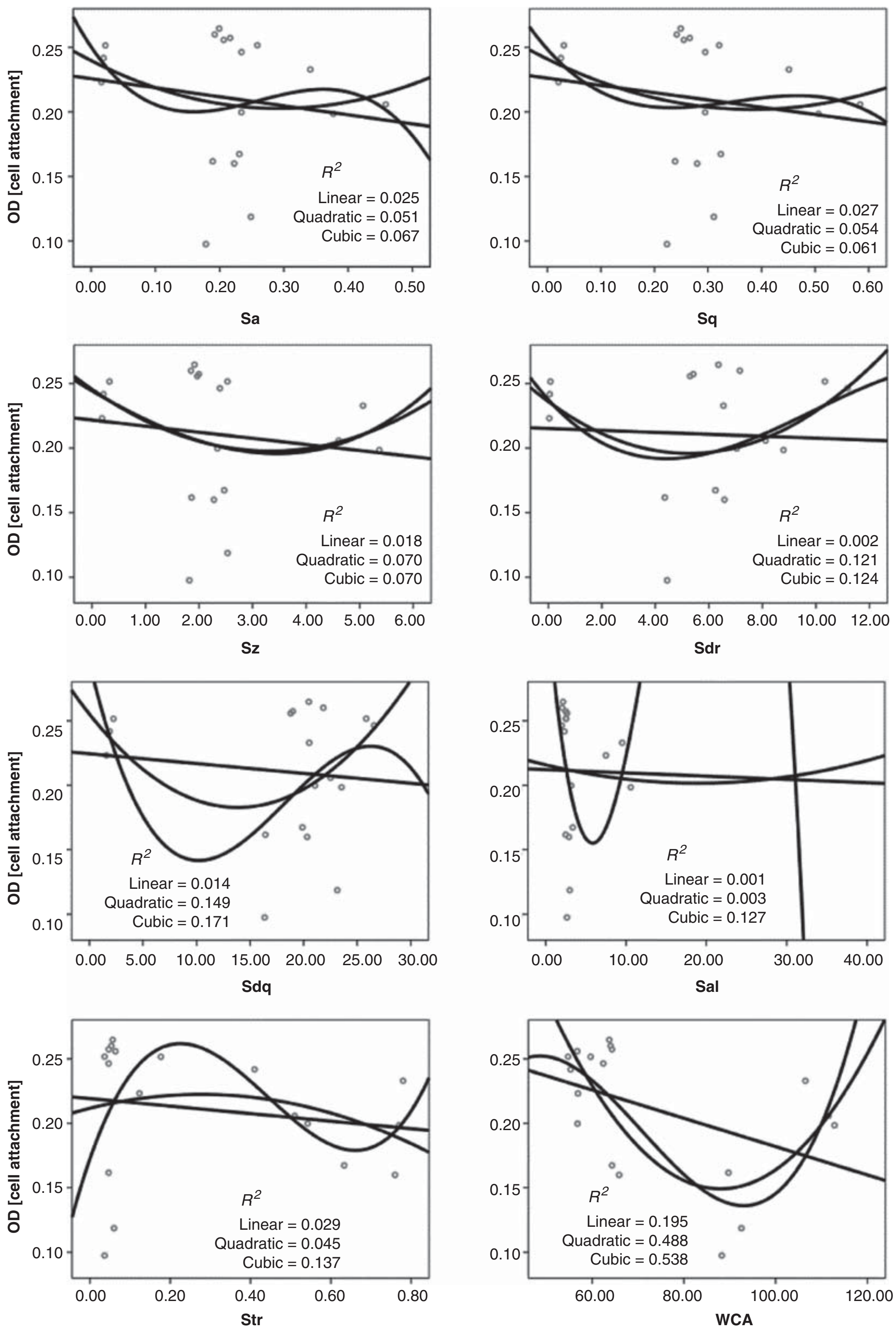

Figure 3. Scatter plots of fibroblasts attachment (OD values at 590 nm) vs surface parameters with fit lines of linear and polynomial correlations. Second and third order correlations with R^2 values of ~0.5 were observed only between cell attachment and WCA. S_{dr}, S_{dq}, S_{al} and S_{tr} exhibited polynomial correlations with goodness of fit between 10–20%. S_a, 3D arithmetic average roughness (μm); S_q, 3D root mean square roughness (μm); S_z, maximum height of surface (μm); S_{dr}, developed interfacial area ratio (%); S_{dq}, root mean square surface slope (°); S_{al}, auto-correlation length (μm); S_{tr}, texture aspect ratio; WCA, water contact angle (°).

Table II. Linear models of the highest goodness of fit from multiple linear regression analyses.

Parameters	Unstandardized coefficients B	SE	Standardized coefficients β	Significance	95% CI for B Lower bound	Upper bound
Model 1. All six groups were included (p = 0.015, adjusted R^2 = 0.556)						
(Constant)	0.817	0.153		0.000	0.480	1.155
S_{dr}*	0.190	0.069	12.033	0.018	0.341	0.040
S_{dq}*	0.045	0.018	6.927	0.027	0.006	0.084
WCA*	0.013	0.003	5.411	0.002	0.021	0.006
$S_{dr}{}^3$	0.000	0.000	1.548	0.207	0.000	0.001
$S_{dq}{}^3$	3.882E-5	0.000	4.075	0.078	0.000	0.000
WCA^3*	6.111E-7	0.000	5.256	0.003	0.000	0.000
Model 2. The R group was excluded and the remaining five groups were included (p < 0.001, adjusted R^2 = 0.910)						
(Constant)	0.380	0.048		0.000	0.267	0.493
S_{dr}	0.086	0.039	5.206	0.066	0.179	0.007
S_{dq}	0.017	0.007	2.527	0.056	0.001	0.034
S_{al}	0.051	0.023	1.222	0.057	0.105	0.002
$S_{dq}{}^3$	2.761E-5	0.000	2.805	0.084	0.000	0.000
$S_{al}{}^3$	0.001	0.000	1.086	0.090	0.000	0.001
$S_{tr}{}^2$	0.060	0.044	0.195	0.219	0.165	0.045
WCA^3*	2.135E-7	0.000	0.827	0.000	0.000	0.000

On the multiple linear regression analyses, initially all the parameters showing a goodness of fit more than 10% from the scatter plots with fit lines were entered and then the ineffective parameters were excluded using a backward elimination method. The entered parameters were S_{dr}, $S_{dr}{}^2$, $S_{dr}{}^3$, S_{dq}, $S_{dq}{}^2$, $S_{dq}{}^3$, S_{al}, $S_{al}{}^2$, $S_{al}{}^3$, S_{tr}, $S_{tr}{}^2$, $S_{tr}{}^3$, WCA, WCA^2 and WCA^3.
*These parameters showed statistical significance.

the results of the present study and the findings of the previous studies, it seemed that the cell attachment may be influenced by surface roughness above 0.2 μm and wettability may play a major role in the surface roughness range of ~ 0.2 μm or less. Another interpretation on the R group could be that it was caused by the nature of the material. Raisanen et al. [27] also reported different epithelial cell attachment on metallic and porcelain surfaces and suggested that the possible causes were the oxide layer and/or surface roughness.

The other parameters correlated with cell attachment were developed interfacial area ratio and RMS surface slope. Although these two parameters had no statistical significance in model 2, they caused a larger change in cell attachment in both models, which was shown by the larger beta values from the multiple linear regression analyses. The Zr group exhibited markedly smaller S_{dr} and S_{dq} values. The previous studies [6,10,28] reported similar or superior soft tissue responses to the zirconia abutments. The results from the present study were consistent with those from the previous studies and the S_{dr} and S_{dq} parameters could influence the attachment of gingival fibroblasts in the context of the Zr group results. It could be suggested that the evener a surface was the greater number of cells attached on the surface.

A surface with high SFE absorbs more proteins and facilitates cell attachment. SFE can be calculated from liquid contact angles, which are polar/non-polar and/or acidic/basic [18]. Although a positive correlation between SFE and cell attachment is commonly agreed, there is no consensus on the critical SFE value for hydrophobicity or hydrophilicity. For this reason, WCA of distilled water was used for the parameter of wettability in this study. Previous studies reported that fibroblast attachment and spreading increased as the wettability increased [18,29,30]. Since surfaces with a WCA less than 40° were considered hydrophilic [31], all the surfaces investigated in the present study were hydrophobic. From the results, cell attachment was superior in the SM, TiN and Zr groups, with comparatively smaller contact angles of 58.5°, 63.5° and 57.3°, respectively. These results were in agreement with the study by Kim et al. [32], which demonstrated the highest fibroblast adhesion and proliferation at a WCA of 50–60°.

This study had some weaknesses. According to linear model 1, only the condition for minimal cell attachment could be obtained. This was partly caused from the fact that a mixed model analysis could not be completed due to the small number of observations. Since the model was drawn from the regression analysis, the interactions between the parameters could not be considered. There was also a possibility that cell attachment could be influenced by other surface parameters not investigated in the present study as well as by the chemical surface characteristics, which could play a role in cell attachment. Taking these drawbacks into account, future studies on the condition for the most/least cell attachment of a certain material to promote cell attachment or to inhibit microbial adhesion are necessary. Despite of the weaknesses of this study, it seems certain that a surface with lower WCA promotes more cell attachment and is considered to be more biocompatible and it is suggested that manufacturers of implant systems should provide WCA values of their products for dental professionals.

Conclusions

Within the limitations of this *in vitro* study, it can be concluded that the human gingival fibroblast attachment to the implant abutment surfaces was influenced by wettability, especially on the surfaces having roughness of ~ 0.2 μm or less and may also be affected by the surface roughness in the range of 0.2–0.5 μm. It is recommended that any implant abutment should have a smooth and hydrophilic surface with a S_a value of ~ 0.2 μm or less and water contact angle as small as possible.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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