Influence of Modified Titanium Abutment Surface on Peri-implant Soft Tissue Behavior: A Systematic Review of In Vitro Studies

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Purpose: Abutment soft tissue integration in the transmucosal area supports the peri-implant tissues, improves esthetics, ensures a soft tissue seal against microorganisms, and preserves crestal bone level. The aim of this systematic review was to systematically analyze the cellular response of fibroblasts on different abutment materials and surface modifications in in vitro studies with a score-based reliability evaluation. ***Materials and Methods:*** A protocol aiming to answer the following focused question was developed: "What is the effect of different abutment materials and different surface modifications on in vitro cellular response of fibroblasts?" A search through three electronic databases (Medline/PubMed, EMBASE, and Web of Science) was performed using the following search terms: fibroblast, implant, abutment used as the main keywords, with AND/OR as Boolean operators. Only in vitro studies using machined titanium as control surface were included. A quality assessment of the selected studies was performed following the SciRAP method. ***Results:*** Out of a preliminary pool of 97 articles, 21 relevant articles were identified for final evaluation. Cellular response evaluation was investigated as follows: 10 studies compared two or more different materials, 7 assessed mechanical surface modification, 14 weighed chemical or biochemical surface modification, and 3 evaluated surfaces modified by a biophysical procedure. Rather than abutment bulk material, external surface features (collagen coating, electropolishing, plasma cleaning, and laser dimpling) were demonstrated to positively affect cell response (cell attachment, morphology and proliferations, expression of adhesion-related proteins and cytokines), mostly at the early stage. While sandblasting, acid etching, composite coating, nitride coating, and vitamin D presented lower results compared with machined, controversial results were demonstrated by anodization. The mean reporting quality SciRAP score was 78.17 ± 11.89, while the mean methodologic quality SciRAP score was 84.13 ± 12.35. Intrastudy comparison highlighted that the time after seeding chosen to evaluate the fibroblast response varies significantly and seems to deeply influence the response. In fact, due to heterogeneity in the study designs, statistical methods, and reported results, meta-analysis of the data was not possible. ***Conclusion:*** Within the limitation due to the design and heterogeneity of the included studies, it can be speculated that abutment material and its mechanical, physical, and chemical modification influence fibroblast response in vitro, especially in the earlier phases of contact with the abutment surface. *Int J Oral Maxillofac Implants 2020;35:503–519. doi: 10.11607/jomi.8110*

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Esthetic outcome, which is becoming more and more important in implant therapy, is related to the maintenance of peri-implant soft and hard tissues.

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While in the last decade, implant researchers have focused only on the implant survival, recently, attention has shifted to the relationship between abutment and soft tissue.[1] In fact, the soft tissue healing process around the abutment seems to deeply influence the peri-implant bone resorption, thanks to the seal realized by soft tissues around the abutment itself.[2]

Researchers have been focused on how to stabilize connective tissue cells on the abutment surface to increase the quality and quantity of the peri-implant connective tissue seal. In fact, incorporation of a foreign body (abutment) in a living tissue (peri-implant soft tissues) is a complex process involving both the foreign body characteristics and the living tissue healing potentialities.[2]

A modified abutment surface seems to promote the creation of a more robust perpendicular collagen fiber attached to the abutment, which is supposed to

Table 1 Full Search Strategy

Database	Search strategy	Results
PubMed	"fibroblasts"[MeSH Terms] OR "fibroblasts" [All Fields] OR "fibroblast"[All Fields]) AND implant[All Fields] AND abutment[All Fields]	48
Embase	'fibroblast implant abutment' OR (('fibroblast'/exp OR fibroblast) AND ('implant'/exp OR implant) AND abutment)	39
Web of Science	(TS=(Fibroblast OR fibroblasts) AND TS=(abutments OR implant abutment))	77

improve cell response, tissue healing, and implant stability.[3] At the same time, biomaterials with enhanced antibacterial properties are highly desirable for the prevention of implant-associated infection and promotion of soft tissue integration, even if different periimplantitis etiologies have been proposed.[4–6]

Evidence is categorical in clinical research. This has led to formulation of checklists for reporting clinical studies. These include: CONSORT guidelines for clinical trials,[7] STROBE guidelines for observational studies,[8] STRAD guidelines for studies involving diagnostic tests,[9] and PRISMA guidelines for meta-analysis and systematic review.[10] The introduction of these guidelines has improved the quality of reports of clinical trials and systematic reviews.[11] Guidelines or checklists for reporting in vitro studies, in particular, for the evaluation of risk of bias and quality of evidence, have not been certified. As a consequence of these considerations, in 2014, *The Journal of Conservative Dentistry* proposed undertaking the formation and validation of a Checklist for Reporting In vitro Study[12] (CRIS guidelines), which is actually in development (hiips://www.cris-statement.org/). Considering the typology of the included studies, based on cellular response to different abutment materials and surface treatments, the SciRAP method[13] has been adopted for the present study. SciRAP is a web-tool method developed to evaluate the reliability of in vitro toxicity studies, consisting of criteria for evaluating both the reporting quality and methodologic quality of studies, separately (hiip://www.scirap.org).

Although only human studies currently could really influence the everyday clinic, preclinical "in vivo animal" trials clearly explain the biologic scenario behind the material. However, both designs present a drawback represented by ethical and economic issues.

On the other hand, in vitro studies allow reaching a faster and wide overview of the new scientific tendencies.

The aim of the present systematic review was to systematically investigate the cellular response of fibroblasts on different abutment materials and surface modifications in in vitro studies with a score-based reliability evaluation.

MATERIALS AND METHODS

The present systematic review is reported in accordance with the guidelines of Transparent Reporting of Systematic Reviews and Meta-analyses (PRISMA statement.)[10,14]

The proposed focused question was: "What is the effect of different abutment materials and different surface modifications on in vitro cellular response of fibroblasts?"

The focused question was established according to the PICO strategy:

- Population: In vitro studies analyzing the fibroblast response to different materials used to produce implant abutments and/or surface modifications of that material
- Intervention: Any change in material and surface treatment (eg, decontamination, cleaning protocols, mechanical, chemical, or physical modifications)
- Comparison: Any type of machined titanium
- Outcomes: Cellular response (eg, proliferation, adhesion, morphology, expression of gene involved as extracellular matrix [ECM], cellular detachment force)

The inclusion criteria were:

- In vitro studies
- Studies investigating fibroblast cell response to abutment surface modification

The following studies were excluded:

- Studies investigating soft tissue response in vivo
- Studies investigating response of cells different from fibroblasts (eg, epithelial cells, osteoblasts)
- Studies not using the machined titanium surface as a control surface, considering that is the most common and studied abutment material
- Studies investigating only the response of bacterial cells to abutment surface properties

Search Strategy

An electronic search of studies published between January 2000 and July 2019 through three electronic databases (Medline/PubMed, EMBASE, and Web of Science) was achieved using the following search terms: fibroblast, implant, abutment used as the main keywords, with AND/OR as Boolean operators. More details regarding queries and their outputs for each database are shown in Table 1. No limits were applied regarding language and sample size.

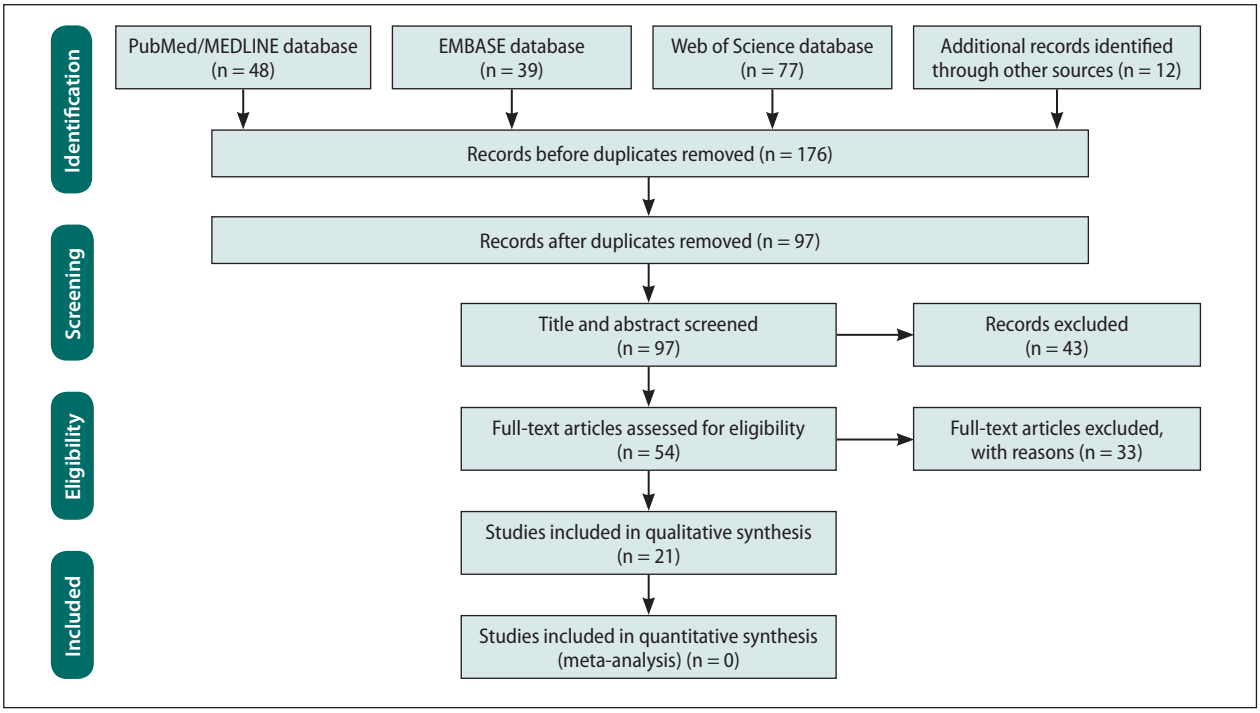

Fig 1 Flowchart depicting the search strategy and selection process.

Results of the electronic research were imported in software (Endnote, Clarivate Analytics) for exclusion of duplicates, and the last research was performed on July 31, 2019. Additionally, the references of all papers included in the systematic review were checked to select potentially relevant additional studies and to improve the sensitivity of the search. Two reviewers (E.M. and R.M.). did the primary search by independently screening titles and abstracts. The same reviewers evaluated the full text. Any disagreement was resolved by discussion with a third reviewer (E.C.). The interreviewer reliability was evaluated with percentages of agreement and kappa coefficients. The level of agreement was regarded as excellent when κ was > 0.80, fair to good when it was 0.40 to 0.80, and poor when it was < 0.40. No authors were contacted to obtain further information about their studies.

Data Extraction and Analysis

Data were extracted independently by the two reviewers using a Microsoft Excel spreadsheet specifically developed for this. The table included: article title, samples, material or surface treatment studied, number and types of samples, method of measuring variables, and results.

Quality Assessment of Individual Studies

As described in Molander et al, a quality assessment of the selected studies was performed following the SciRAP method.[13] Four criteria are present for the evaluation of relevance, but while the criteria for the evaluation of reporting and methodologic quality can be adopted in evaluation of any study focusing on cellular response to substances or materials, the relevance criteria, as proposed, in the authors' opinion, are strictly related to the evaluation of study on toxicity for the assessment of human health hazards or risk. Any criteria can be selected as "fulfilled," "partially fulfilled," or "not fulfilled." The score is the percentage of fulfilled and partially fulfilled criteria. The SciRAP score can have a value ranging from 0 (all criteria are judged as "not fulfilled") to 100 (all criteria are judged as "fulfilled").

Three criteria were removed from the reporting quality evaluation of all the studies, and three from the methodologic quality evaluation, because they were not applicable in the reviewed studies.

RESULTS

Search and Included Studies

From the initial search, 85 potentially relevant articles were found through database searching. An additional 12 articles were found reading the references of included studies. After reading titles, 25 were excluded, and after reading abstracts, a further 18 were not included for the full-text reading (agreement 93%, kappa .865). After full-text reading, 33 articles were excluded and 21 were included for qualitative synthesis. The flowchart of the included studies is reported in Fig 1. The excluded studies are presented in Table 2 with the main reason(s) for exclusion.

Table 2 Studies that Were Excluded from the Review in Alphabetical Order with Main Reason(s) for Exclusion

Study	Main reason for exclusion
Abdulmajeed et al,[15] 2014	No machined titanium as control
Atsuta et al,[16] 2019	No evaluation of fibroblast response
Fischer et al,[17] 2017	No machined titanium as control
Gómez-Florit et al,[18] 2014-1	No machined titanium as control
Groessner-Schreiber et al,[19] 2003	No machined titanium as control
Hoshi et al,[20] 2010	No machined titanium as control
Jeong et al,[21] 2018	No machined titanium as control
Jin et al,[22] 2012	No machined titanium as control
Kwon et al,[23] 2016	No abutment material evaluated
Lee DW et al,[24] 2015	No machined titanium as control
Lee EJ et al,[25] 2013	No machined titanium as control
Lee JJ et al,[26] 2015	No machined titanium as control
Linderback et al,[27] 2010	No evaluation of fibroblast response
Liu et al,[28] 2015	No machined titanium as control
Luo et al,[29] 2013	No evaluation of fibroblast response No machined titanium as control
Ma et al,[30] 2011	No machined titanium as control
Maeno et al,[31] 2017	No evaluation of fibroblast response
Marín-Pareja et al,[32] 2014	No machined titanium as control
Mehl at al,[33] 2017	No machined titanium as control
Mustafa et al,[34] 2005	No machined titanium as control
Nakajima et al,[35] 2017	No machined titanium as control
Pabst et al,[36] 2014	No machined titanium as control
Pansani et al,[37] 2019	No machined titanium as control
Rizo-Gorrita et al,[38] 2018	No machined titanium as control
Roffel et al,[39] 2019	No evaluation of fibroblast response
Rutkunas et al,[40] 2015	No machined titanium as control
Schierano et al,[41] 2001	No modification of the abutment material or surface
Shahramian et al,[42] 2017	No machined titanium as control
Shi et al,[43] 2015	No machined titanium as control
Sugawara et al,[44] 2016	No evaluation of fibroblast response
Xing et al,[45] 2014	No machined titanium as control
Xu et al,[46] 2017	No abutment material evaluated
Yang et al,[47] 2015	No machined titanium as control

Quality Assessment of the Included Studies

The quality of the included studies has been evaluated by the SciRAP method, a score-based web tool (Fig 2). Twenty out of 21 clearly defined a control sample, 15 described the number of replicates tested for each surface, and only 11 described the software used in the statistical evaluation. Only one study described the manufacturer and lot number of all the tested materials.

Table 3 Reporting and Methodologic Quality Score of the Studies Included Calculated with SciRAP Tool

	SciRAP score	
Study/year	Reporting quality	Methodologic quality
Al Mustafa et al,[48] 2015	71.05	59.09
Brunello et al,[49] 2018	47.37	66.67
Canullo et al,[68] 2013	84.21	95.45
Cho et al,[58] 2015	63.16	63.64
Dorkhan et al,[50] 2014	71.05	81.82
Esfahanizadeh et al,[59] 2016	78.95	95.45
Franková et al,[51] 2013	76.32	86.36
Gomez-Florit et al,[52] 2014-2	94.74	86.36
Guida et al,[53] 2013	86.84	86.36
Kim et al,[60] 2014	86.84	90.91
Kim et al,[61] 2015	63.16	86.36
Lee JH et al,[62] 2015	86.84	81.82
Mehl et al,[63] 2016	79.49	68.18
Meredith et al,[64] 2005	73.68	95.45
Moon et al,[54] 2013	82.05	100
Mussano et al,[66] 2018	92.31	100
Nothdurft et al,[65] 2015	69.23	72.73
Ponsonnet et al,[55] 2003	71.05	72.73
Ritz et al,[56] 2017	97.37	90.91
Satué et al,[57] 2016	81.58	90.91
Truc et al,[67] 2018	84.21	95.45
Mean	78.17	84.13
SD	11.89	12.35

No allocation concealment or blinding outcome assessment was described in the reviewed studies.

The mean reporting quality score was 78.17 (standard deviation [SD]: 11.89), while the mean methodologic quality SciRAP score was 84.13 (SD: 12.35). Details of quality evaluation are shown in Table 3.

Characteristics of the Included Studies

After full-text article review, the extreme heterogeneity in the study designs, measurement parameters, and data collection made performance of a meta-analysis impossible (Table 4).

The most frequent evaluated cell line was human gingival fibroblasts, obtained from donors[48–57] or cell collection,[58-67] while in one case, it was human dermal fibroblasts obtained from cell collection,[66] and in two cases, murine fibroblastic cells from cell collection.[67–68]

In 18 studies, the abutment material was tested in the form of disks,[48-52,54–66,68] in two studies in the form of plates,[53–63] and in one study in the form of strips.[67]

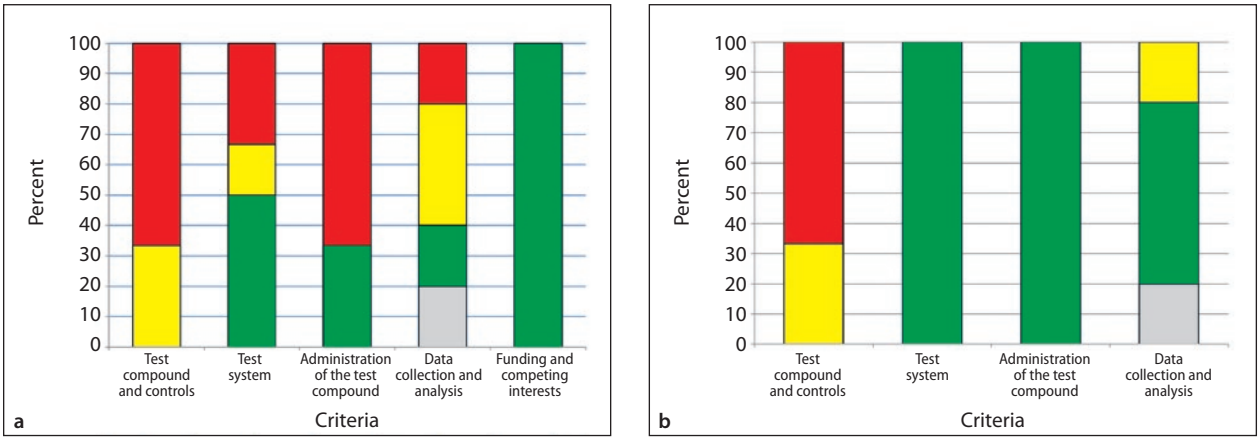

Fig 2 An example of a color profile generated with the SciRAP tool. In the color profile, the evaluations of *(a)* reporting quality and *(b)* methodologic quality are illustrated in bar charts, showing green for fulfilled criteria, yellow for partially fulfilled criteria, and red for criteria that were not fulfilled. Criteria that were "not determined" are shown as gray. The bar charts take the weights of criteria into account and do not include criteria that have been removed.

Commercially available abutments were not used in any studies.

Ten authors compared two or more different materials such as zirconia,[54,58–61,64,66] titanium zirconium,[52] cobalt-chrome-molybdenum alloy,[60,61] lithium disilicate,[63] adhesive resin cement,[63] stainless steel,[64] titanium alloy,[64] and nickel-titanium alloy.[55] The zirconia surface seems to improve fibroblast adhesion,[58–60,64] whereas lithium disilicate and adhesive resin cement present similar adhesion values as machined titanium.[63] On the contrary, cobalt-chrome-molybdenum alloy presented worse adhesion than machined titanium.[60,61]

Seven authors compared the fibroblast response to mechanical modification of surfaces such as polishing[51,52,54,55,63,65] and sandblasting.[51,58,63,65] Polishing treatment does not seem to be able to modify cellular response,[51,52,54] even if in some cases it seems to be able to decrease cellular response.[63,65]

In 14 out of 21 studies, the response to chemical or biochemical surface modification was evaluated, for example, acid etching,[52] alkali-treatment,[48,51] anodization,[49,50,53,60,61,66] electropolishing,[64] titanium nitride coating,[49,54,56,61] zirconium nitride coating,[49,51] composite resin coating,[60,61] collagen coating,[56,67] vitamin D precursor, and vitamin E coating.[57] Surface modification can significantly influence cellular response. For example, titanium-aluminum-niobium alloy Ti6Al7Nb in standard form impairs fibroblast function, while if electropolished, induces increased cellular proliferation.[64] Most of the included work reported a worse behavior of fibroblast adhesion if an acid-etching or sandblasting treatment was employed on titanium,[52,58,65] zirconium,[58,63] or titanium zirconium when compared with machined surfaces.[52] Alkaline-induced hydrophilicity was also shown to stimulate the adhesion of blood cells. Ti6Al4V disks treated with sodium hydroxide show a more pronounced fibroblast adhesion compared with untreated titanium surfaces at 1 hour, but not at 3 and 24 hours.[48]

Three authors evaluated the cellular response to surfaces modified by a biophysical procedure such as plasma treatment[62,68] and laser dimpling,[59] showing increased cell adhesion. A summary of the main characteristics and main results of the included studies is shown in Tables 4 and 5, respectively.

DISCUSSION

The present systematic review aimed to measure the behavior of fibroblasts on different abutment materials and surface modifications.

In the included studies, several different methods and evaluation time (Tables 4 and 5) were used to study the cellular response, making it impossible to perform a meta-analysis.

In vitro studies, thereby, represent the major proportion of research carried out and published in dentistry.[12] Systematic reviews of in vitro studies can be performed to consolidate evidence about similar materials/techniques; however, lack of uniform methods and reporting may hamper the meaningful comparisons of these studies.

Dealing with methodology, the observation time varies among the included studies. For example, the surface adhesion has been evaluated in a range between 60 minutes[60,61] and 3 days.[59] This could explain contradictory results of some included studies. For the anodized titanium, for example, cellular adhesion evaluated at 10 minutes was greater than on machined titanium,[66] whereas after 24 hours, it was lower than on machined titanium.[50]

Table 4 Main Characteristics of the Reviewed Studies

Study/year	Type of sample	Material and surface treatment	Number of samples
Al Mustafa et al, 2015	Disks 13-mm diameter	Titanium alloy (Ti6AL4V) disks alkali treated with NaOH	3 independent measurements in each experiment
Brunello et al, 2018	Disks 13 mm × 3 mm	Disks of uncoated Ti6Al4V, anodized, and coated with titanium nitride or zirconium nitride	3 independent measurements in each experiment
Canullo et al, 2013	Disks 4-mm diameter	Grade 5 titanium alloy Ti6Al4V machined surfaced disks treated and not treated with argon plasma	60 total, divided in groups of 10
Cho et al, 2015	Disks 25 mm × 1 mm	Tetragonal zirconia polycrystal containing yttria and niobium oxide ((Y,Nb)-TZP) Zir, ((Y,Nb)-TZP) sandblasted Zir-R, Titanium Machined Ti-M and Titanium anodized, Ti-R	3 independent measurements in each experiment
Dorkhan et al, 2014	Disks 8 mm × 2 mm	Pure titanium disks (CpTi) two anodically oxidized surfaces N1 and N2	3 independent measurements in each experiment
Esfahanizadeh et al, 2016	Disks 9 mm × 1 mm	Laser-Lok titanium, titanium, zirconia disks	5 independent measurements in each experiment
Franková et al, 2013	Disks 6 or 15 mm in diameter	Six different chemically and physically modified titanium alloy Ti6Al4V: glazed (Tis-MALP); unglazed (Tis-O); unglazed and alkali-etched (Tis-OA); unglazed and coated with ZrN (Tis-OZ); unglazed, sandblasted, and acid-etched (Tis-OPAE); and unglazed, sandblasted, acid, and alkali etched (Tis-OPAAE)	3 independent measurements in each experiment
Gómez-Florit et al, 2014-2	Disks 4.3 mm × 2 mm	Coin-shaped samples of grade IV Ti and TiZr with different surfaces treatment: machined (M,) machined and acid-etched (ModMA), polished (P)	2 samples of each group
Guida et al, 2013	Plates 10 mm × 10 mm × 1 mm	Turned commercially pure titanium plates and oxidized titanium surfaces	Not specified
Kim et al, 2014	Disks of not-specified dimension	Disks of titanium alloy (SM), cobalt-chrome-molybdenum (CCM), titanium nitride-coated titanium (TiN), anodic-oxidized titanium (AO), composite resin-coated titanium (R), zirconia (Zr)	40 samples of each group: 10 for surface characterization 3 for contact angle measurement 9 for cell attachment 18 for cell proliferation
Kim et al, 2015	Disks 6 mm × 1 mm	Disks 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)	22 samples of each group: 10 for surface characterization 3 for contact angle measurement 9 for cell attachment
Lee JH et al, 2015	Disks 10 mm × 2 mm Osstem implant	Disks of titanium treated with different values of air atmospheric-pressure plasma-jet (AAPPJ)	5 replicates for each measurement
Mehl et al, 2016	Plates 10 mm × 10 mm × 1 mm	Disks of lithium disilicate (LS), zirconium dioxide (Zr), adhesive resin cement (AR), titanium (Ti), and human dentin (HD) with three different levels of surface roughness (rough, machined, and polished)	24 replicates for each group, 8 specimens per material and group

Cell line	Treatment before testing	Outcomes and Methodology
Human gingival fibroblast from biopsies	Cleaning with water and 70% ethanol, ultrasonic cleaning for 15 min in water	Cell adhesion was evaluated by scanning electron microscopy (SEM) Cell viability by Methyl Thiazolyl Tetrazolium (MTT) assay, protein synthesis, by 3[H] leucine labelling and proliferation. By 3[H]thymidine labellingß
Human gingival fibroblast from biopsies	Not specified	Cell proliferation by MTT Morphologic analysis by SEM Hemolysis test Gene expression by reverse-transcription PCR and quantitative real-time PCR (RT-qPCR)
Murine fibroblastic cells from cell collection	Not specified	Fibroblast adhesion and colonization by fluorescence microscopy
Human gingival fibroblasts from culture collection	Not specified	Average surface roughness (Ra) and topography by confocal laser microscopy (CLM) and atomic force microscope (AFM) Cell attachment and morphology at 6 and 24 h by CLM Proliferation at days 1, 4, and 7 by picogreen assay Expression of adhesion-related proteins 24, 48, and 72 h by RT-qPCR
Human gingival fibroblast from biopsies. Human oral keratinocytes from cell collection	Ultrasound cleaning in 70% ethanol for 2 × 10 minutes, washing with sterile ultrapure water for a further 2 × 10 minutes	Cell adhesion assay at 24 h by fluorescence microscope
Human gingival fibroblast from cell collection	Not specified	Surface topography, cell morphology, and cell attachment by SEM at 3 days Proliferation rate by MTT Expression of IL-10, TNFα, fibronectin, and integrin genes by RT-qPCR
Human gingival fibroblast from biopsies	The titanium disks were sterilized for 48 h in 70% ethanol and after that for 15 min by ultraviolet irradiation before use.	Surface microscopic characterization by AFM and SEM Expression of vinculin and a3β1 integrin by ELISA Collagen I production TNF-α and MMP-2 production Biofilm formation
Human gingival fibroblast from biopsies	Dipped in phosphate-buffered saline (PBS)	Cell number determination by DNA quantification Cell cytotoxicity by lactate dehydrogenase activity at 24 h Wound healing assay by scratch test Expression of gene involved as extracellular matrix (ECM) component, ECM turnover, cell adhesion, pro-inflammatory cytokine, anti-inflammatory cytokine wound healing fibrogenic by RT-qPCR
Human gingival fibroblast from biopsies	Rinsed in distilled water and in acetone, dried with nitrogen stream. Sterilized by autoclaving	Characterization of surface topography by SEM and AFM Cell adhesion and proliferation evaluation by MTT Cell adhesion and morphology evaluation by SEM and confocal laser scanning microscopy (CLSM) Type I collagen synthesis by ELISA
Human gingival fibroblast from cell collection	Rinsed in distilled water and dried at 50°C for 24 h	Water contact angle (WCA), surface characteristic measurements by optical three-dimensional profiling system, cell attachment, cell proliferation
Human gingival fibroblast from cell collection	Rinsed in distilled water and dried at 50°C for 24 h	Sa, Sq, Sz, Sdr, Sdq, Sal, Str by optical three-dimensional profiling system, water contact angle (WCA). Cell attachment assay by indirect method
Human gingival fibroblast from cell collection	Rinsed ultrasonically in acetone, ethanol, and distilled water for 10 min each, dried and sterilized with ethylene oxide gas	Surface analysis by optical three-dimensional surface profilometer Cell attachment by percentage of optical density, cell proliferation by BrdU test kit, vinculin and actin filaments by staining and CLM
Human gingival fibroblast from cell collection	Rinsed by air/water spray and ultrasonic cleaning for 10 min in deionized water	Surface analysis by 3D laser scanning microscope Contact angle measurements Cell detachment forces by single-cell force spectroscopy (SCFS)

Table 4 Main Characteristics of the Reviewed Studies *(continued)*

Study/year	Type of sample	Material and surface treatment	Number of samples
Meredith et al, 2005	Disks of 12-mm diameter for morphology and qualitative analysis Disks of 49 mm for quantitative analysis	Disks stainless steel (SS), commercially pure titanium (CpTi), and titanium alloyTi6Al7Nb (TAN) standard and electropolished	2 replicates for morphology and qualitative analysis 3 replicates for quantitative analysis
Moon et al, 2013	Disks 15 mm × 1 mm	Disks of commercially pure grade III titanium and zirconia with machined, polished and unpolished surface, and titanium coated with titanium nitride	9 for each group
Mussano et al, 2018	Disks 8 mm × 3 mm	Disks of titanium alloy (Ti-Al-V), machined titanium (Ti), and anodized titanium (AnoTi)	Not specified
Nothdurft et al, 2015	Disks 5 mm × 3.5 mm	Cylinders of TiAl6V4 and Zir, machined, polished and sandblasted	Not specified
Ponsonnet et al, 2003	Disks 1.13 cm^2 in surface area and 2 mm thick	Square samples of NiTi in three roughnesses (NiTi 80, NiTi 400, NiTi 2400, and disks of commercially pure titanium (cp-Ti) and titanium alloy (Ti6Al4V)	Not specified
Ritz et al, 2017	Disks 10 mm × 2 mm	Titanium alloy grade V Ti6Al4V, TiN-coated grade V titanium disks and disks with collagen immobilization by dip coating and anodic immobilization, with or without additional EDC carbodiimide cross-linking	Not specified
Satué et al, 2016	Disks 6.2 mm x 2 mm	Disks of grade 4 commercially pure (cp) Ti coated with ultraviolet-irradiated vitamin D precursor and vitamin E	Not specified
Truc et al, 2018	Strips 10 mm × 8 mm × 1.45 mm	Plates of Ti, TiO_2, and collagen I coated titanium by electrochemical deposition	2 replicates of each group

Moon et al[54] evaluated cell behavior on Ti and ceramics, machined and polished, respectively, at 1 and 5 days of culture, disclosing that several materials and surface conditions only slightly influence human gingival fibroblast (HGF) survival and adhesion. However, it is important to note that differences in adhesion and proliferation rates were statistically significant in the first 24 hours after fibroblast seeding.[52,63] In most of the studies included in the present research, the quantity of HGF growth on different surfaces was not statistically different among them after 48 hours of the seeding. Likewise, the strength of the adhesion of cells to the abutment material surfaces seems to be closely related to the contact time. This could be the result of the increased expression of focal adhesion local proteins (FALPs) during the time of exposition.

Ponsonnet et al[55] studied commercially pure titanium, titanium alloy, and nickel-titanium alloy with different surface roughnesses showing that peak-to-valley roughness (Rz) values for all the substrates were associated with cell proliferation: higher roughness, lower cell proliferation, low polar component. It was suggested that a roughness between 0.08 and 1 nm might represent the threshold over which cell proliferation became difficult. This is in accord with the result of Grössner-Schreiber et al,[19] suggesting that an average of 30 to 90 nm might represent the most correct roughness to accomplish a stable soft tissue sealing around abutments.

Abutment surface can also be modified with physical procedures to improve cellular adhesion. Plasma treatment has been adopted to remove organic contamination from metallic surfaces. The term "plasma" indicates

Cell line	Treatment before testing	Outcomes and Methodology
Human gingival fibroblast from cell collection	Not specified	Surface characterization by AFM, with noncontact "white-light" profilometer and field emission scanning electron microscope (FESEM) Qualitative cell growth and morphology by SEM Cell quantification by DNA quantification
Human gingival fibroblast from biopsies	Ultrasonic cleaning in methanol, water, and autoclave sterilization	Cell morphologies by SEM. Biocompatibility and focal adhesion were evaluated by ultrasonic wave application and cell viability assay (MTT). FALPs expression levels were assessed by RT-PCR and western blot.
Fibroblastic cell line Normal human dermal fibroblasts (NHDF)	Acetone cleaning, 70% isopropanol rinsing, ultrasonic cleaning in isopropanol, and rinsing in Milli-Q Water	Surface characterization by SEM and XPS Contact angle and surface energy evaluation by optical contact angle measurements Cell adhesion by rhodamine-phalloidine staining and dapi staining Cell viability by luminescent cell viability assay Cell morphology and focal adhesion quantification by Ti-E microscope
Human gingival fibroblast cell line (HGF1) from cell collection	Steam blasting, ultrasonic cleaning, and degreasing with isopropanol autoclave sterilization	Surface characterization by SEM and energy-dispersive x-ray spectroscopy (EDX), topographic analysis by a profilometer, water contact angle measurements by sessile drop Cell counting at 24 h and 72 h by DAPI staining Vinculin distribution by immunofluorescence staining
Human gingival fibroblast from biopsies	Ultrasonic cleaning in acetone, 70% ethanol, rinsing with distillated water and g-ray sterilization	Surface characterization by SEM and profilometry-atomic force microscopy surface free energy (SFE) Proliferation activity by MTT
Human gingival fibroblast from biopsies	Ultrasonics cleaning in propanol and g-ray sterilization	Cell morphology by CLSM Cell adhesion by fluorescence intensity Cell viability and proliferation by semi-quantitative colorimetric assay focal cell adhesion by CLSM Gene expression by RT-qPCR
Human gingival fibroblast from biopsies	Not specified	Cytotoxicity analysis by LDH activity Cell morphology analysis by DAPI staining and CLM Gene expression of ECM related proteins, proinflammatory and anti-inflammatory cytokine, bone resorption–related proteins, wound healing–related proteins by RT-qPCR Wound-healing assay by scraping assay
Murine fibroblastic cells from cell collection	Ultrasonic cleaning in ethanol. UV sterilization	Cell adhesion/spreading and cell viability/proliferation by MTT assay

the partially ionized state of gas, extensively adopted for different industrial uses.[69] Canullo at al[68] compared fibroblast response to titanium alloy smooth-surfaced disks treated with plasma of argon. Statistical analysis showed more fibroblast adherence on a surface treated with plasma of argon than on control at 2 hours of observation time, with a cellular morphology revealing advanced cellular adhesion. Statistically significant differences with more fibroblasts adherent on treated surfaces were found at the 8-hour interval as well, while no quantitative and qualitative (morphology) differences were presented in terms of adherent cells at 48 hours of observation time due to saturation effect. Lee et al[62] addressed the effects of atmospheric-pressure plasma-jet technology (AAPPJ) at 250, 500, or 1,000 SCCM for 10 seconds on a machined titanium disk. Contact angle decreased as the flow rate increased. At the same time, TiO_2 peak, wettability, and cell adhesion increased, without a change in terms of surface roughness. It is interesting to observe how despite not changing the surface roughness, but only decontaminating it with an AAPPJ treatment, the fibroblast response could increase.

Laser ablation has been newly used to modify the implant and abutment microtopography, providing identical three-dimensional microgrooves on the surfaces. Esfahanizadeh et al[59] found significant differences in terms of cell proliferation, integrin gene expression, and fibronectin between the Laser-Lok and zirconia, and Laser-Lok and titanium, despite its higher Sa (1.33 µm). The authors explained this result was thanks to the surface microstructure that presents a repeating nano-morphology, which is aimed to maximize the contact area with cells and collagen microfibrils.

Table 5 Summary of the Results of the Reviewed Studies on the Basis of the Abutment Material and Surface Treatments

Material/surface treatment	Study	Surface roughness Ra or Sa mm (SD)	Outcomes	Results compared with machined titanium (MT)
Different materials				
Zirconia (Zr)				
	Cho et al, 2015	Ra 0.092 0.01	Cell attachment and morphology at 6 and 24 h	At 6 h, better results on Ti; at 24 h similar results for Ti and Zr
			Cell proliferation at 1, 4, and 7 d	Similar at day 1, increased at 4 d until 7 d
			Expression of adhesion-related proteins at 24, 48, 72 h	Zr led to significantly high mRNA expression in integrin α2 at 24 and 48 h, and integrin β1 at every time point
	Esfahanizadeh et al, 2016	Ra 0.40 ± 0.05	Cell attachment and morphology at 3 d	Cells were round without pseudopod-like processes similar to titanium
			Cell proliferation at 3 d	No difference
			Expression of adhesion-related proteins and cytokines at 4 d	Expression of IL-10, TNFα, fibronectin, and integrin was higher than in titanium group
	Kim et al, 2014	Sa 0.019 (0.00)	Cell attachment at 1 h	Lower than MT
	Kim et al, 2015	Sa 0.019 (0.00)	Cell attachment at 1 h	Lower than MT
			Cell proliferation at 3 d and 7 d	At 3 d, higher than MT
				At 7 d, higher than MT and higher than all the compared surfaces
	Mehl et al, 2016	Ra 0.13 (0.02)	Cell detachment force on cantilever at 15 m	No difference
	Moon et al, 2013	Not evaluted	Cell morphology at 1 and 5 d	At 1 d, cell density was lower
			Cell proliferation at 1, 3, 5, and 7 d	No differences
			Expression of adhesion-related proteins at 24, 48, 72 h	Expression increased in a time-dependent manner irrespective of the culture conditions used
	Nothdurft et al, 2015	Ra 0.199 (0.016)	Cells grown at 24 and 72 h	Cell proliferation rate on zirconia was higher than on the titanium alloy
			Vinculin distribution	Zr presented more favorable adhesion properties compared with titanium
	Gómez-Florit et al, 2014-2	Sa 0.084 (0.004)	Cell number at 48 h	Higher on TiZr
			Cytotoxicity at 24 h	No difference
			Cell morphology at 2 and 14 d	No difference
			Wound healing assay	No difference
			Expression of adhesion-related proteins, ECM components, and cytokines at 14 d	Integrin β3 upregulated on all TiZr surface compared to Ti. Expression of others gene varied on the basis of the surface treatment
Lithium disilicate (LDis)				
	Mehl et al, 2016	0.13 (0.02)	Cell detachment force on cantilever at 15 mo	No difference
Cobalt-chrome-molybdenum alloy (CCM)				
	Kim et al, 2014	Sa 0.21 (0.05)	Cell attachment at 1 h	Lower than machined titanium and lower than all the compared surfaces
	Kim et al, 2015	Sa 0.21 (0.05)	Cell attachment at 1 h	Lower than machined titanium and lower than all the compared surfaces
			Cell proliferation at 3 d and 7 d	At 3 d, lower than machined titanium and lower than all the compared surfaces; at 7 d, lower than machined titanium and lower than all the compared surfaces except resin-coated titanium
Stainless steel				
	Meredith et al, 2005	Ra 0.19 (0.022)	Cell morphology at 24 h, 5 d, and 10 d	Similar to machined titanium
			Cell number	Similar to machined titanium
Titanium–aluminum–niobium alloy Ti–6Al–7Nb				
	Meredith et al, 2005	Ra 0.77 (0.076)	Cell morphology at 24 h, 5 d, and 10 d	Impaired cellular function
			Cell number	Impaired cellular function

Table 5 Summary of the Results of the Reviewed Studies on the Basis of the Abutment Material and Surface Treatments *(continued)*

Material/surface treatment	Study	Surface roughness Ra or Sa mm (SD)	Outcomes	Results compared with machined titanium (MT)
Nickel–titanium alloy				
	Ponsonnet et al, 2003	NiTi 80 Ra 1.02 (0.17) NiTi 400 Ra 0.15 (0.015) NiTi 2400 Ra 0.057 (0.009)	Cell proliferation at 2 d, 4 d, and 7 d	Ni-Ti with roughness comparable to MT showed higher proliferation rate
Titanium-aluminium-vanadium alloy (Ti6Al4V)				
	Ponsonnet et al, 2003	Ra 0.068 (0.007)	Cell proliferation at 2 d, 4 d, and 7 d	Proliferation rate similar to MT
Mechanical modification				
Polishing				
	Franková et al, 2013	Ti Ra 0.13	Detection of vinculin and $\alpha_3\beta_1$ integrin at 6 h and 24 h	No difference at 24 h for both and at 6 h for vinculin. Increased expression of $\alpha_3\beta_1$ integrin at 6 h
			Collagen I production at 24 h and 72 h	No difference at 24 h. Increased expression at 72 h
			TNF-α and MMP-2 production at 24 h and 72 h	No difference in production of MMP-2
	Gómez-Florit et al, 2014-2	Ti Sa 0.025 (0.002) TiZr Sa 0.031 (0.0015)	Cell number at 48 h	Increased on Ti and TiZr
			Cytotoxicity at 24 h	No difference
			Cell morphology at 2 and 14 d	Cell grew disorderly
			Wound healing assay at 2 d	Similar to MT
			Expression of adhesion-related proteins, ECM components, and cytokines at 14 d	Upregulation on TiZr
	Mehl et al, 2016	Ti Ra 0.1(0.02) Zir Ra 0.04 (0.01) LDis Ra 0.03(0.01)	Cell detachment force on cantilever at 15 mo	Reduced detachment force of Ti, Zr, Ar, and Ls
	Moon et al, 2013	Not evaluated	Cell morphology at 1 and 5 d	No difference
			Cell proliferation at 1, 3, 5, and 7 d	No difference
			Expression of adhesion-related proteins at 24, 48,72 h	No difference
	Nothdurft et al, 2015	Ti Ra 0.01 (0.0015)	Cells grown at 24 h and 72 h Vinculin distribution	On titanium increased cell number, on zirconia decreased
				Less favorable distribution on Ti at days 1 and 3, on Zr at day 1
	Ponsonnet et al, 2003	NiTi 80 Ra 1.02 (0.17) NiTi 400 Ra 0.15 (0.015) NiTi 2400 Ra 0.057 (0.009)	Cell proliferation at 2 d, 4 d, and 7 d	For Ni-TI alloy, the higher the roughness, the lower the cell proliferation
Sandblasting				
	Cho et al, 2015	Zir 0.739 (0.05)	Cell attachment and morphology at 6 and 24 h	Decreased cell attachment on Zr
			Cell proliferation at 1, 4, and 7 d	Decreased on Ti and Zr
			Expression of adhesion-related proteins at 24 h, 48 h,72 h	Decreased
	Mehl et al, 2016	Ti Ra 1.1 (0.03) Zir Ra 0.47 (0.04	Cell detachment force on cantilever at 15 mo	Reduced detachment force of Ti, Zr, Ar except Ls
	Franková et al, 2013	Ldis Ra 1 (0.08)	Detection of vinculin and $\alpha_3\beta_1$ integrin at 6 h and 24 h	No difference at 24 h for both and at 6 h for vinculin. Increased expression of $\alpha_3\beta_1$ integrin at 6 h
			Collagen I production at 24 h and 72 h	No difference at 24 h. Reduced expression at 72 h
			TNF-α and MMP-2 production at 24 h and 72 h	TNF-α at 24 h was increased. Not detectable at 72 h. No difference in MMP-2 production

Table 5 Summary of the Results of the Reviewed Studies on the Basis of the Abutment Material and Surface Treatments *(continued)*

Material/surface treatment	Study	Surface roughness Ra or Sa mm (SD)	Outcomes	Results compared with machined titanium (MT)
Sandblasting				
	Nothdurft et al, 2015	Ti Ra 1.514 (0.045) Zir Ra 1.021 (0.043	Cells grown at 24 h and 72 h Vinculin distribution	On zirconia increased cell number, on Ti decreased More favorable distribution for Zir and Ti
Chemical modification				
	Gómez-Florit et al, 2014-2	Ti Sa 1.040 (0.017)	Cell number at 48 h Cytotoxicity at 24 h Cell morphology at 2 and 14 d Wound healing assay at 2 d Expression of adhesion-related proteins, ECM components and cytokines at 14 d	Decreased Decreased on Ti and TiZr Round-shaped morphology, indicating poor cell attachment Not healed both Ti and TiZr MMP1/TIMP1 mRNA ratio statistically increased
Alkali treatment				
	Al Mustafa et al, 2015	Not evaluted	Cell adhesion at 1, 3, and 24 h Cell viability at 24 h Protein synthesis at 24 h Cell proliferation at 24 h	Enhanced at 1 h No difference No difference No difference
	Franková et al, 2013	Ra 0.21	Detection of vinculin and $\alpha_3\beta_1$ integrin at 6 h and 24 h Collagen I production at 24 h and 72 h TNF-α and MMP-2 production at 24 h and 72 h	No difference at 24 h for both and at 6 h for vinculin. Increased expression of $\alpha_3\beta_1$ integrin at 6 h No difference at 24 h. Reduced expression at 72 h TNF-α at 24 h was increased. Not detectable at 72 h
Anodization				
	Brunello et al, 2018	Ti Ra 0.113 (0.004)	Cell proliferation at 3, 7, 14, and 21 d Expression of cell adhesion molecules at 21 d Vinculin distribution at 14 d	No difference No difference No difference
	Cho et al, 2015	Ti Ra 0.689 (0.04)	Cell attachment and morphology at 6 and 24 h Cell proliferation at 1, 4, and 7 d Expression of adhesion-related proteins at 24, 48,72 h	Well attached at 6 h, no difference at 24 h Reduced Reduced expression of type I collagen
	Dorkhan et al, 2014	Ti6Al4V Sa 0.21 CpTi Sa 0.17	Cell adhesion at 24 h	Reduced number of adherent cells on commercially pure anodized titanium, no influence on titanium alloy anodized. No difference in adherent strength
	Guida et al, 2013	CpTi Sa 0.22	Cell adhesion at 6 h Cell proliferation at 48 h and 7 d Type I collagen synthesis at 6 h, 48 h, and 7 d	Increased Increased Increased
	Kim et al, 2014	Ti6Al4V Sa 0.23 (0.09)	Cell attachment at 1 h	Lower than machined titanium
	Kim et al, 2015	Ti6Al4V Sa 0.23 (0.09)	Cell attachment at 1 h Cell proliferation at 3 and 7 d	Lower than machined titanium Higher than MT
	Mussano et al, 2018	Not evaluated	Cell adhesion at 10 mo Cell viability at 1, 2, and 3 d Focal adhesion quantification at 24 h	Manifold increased Increased at 2 d and 3 d Increased
Collagen coating				
	Ritz et al, 2017	Not evaluated	Cell adhesion at 2 and 4 h Cell viability and proliferation at 1, 3, and 7 d Expression of ECM proteins, fibroblast differentiation markers, adhesion markers, cytoskeletal marker at 1 d and 7 d	Increased Increased Increased

Table 5 Summary of the Results of the Reviewed Studies on the Basis of the Abutment Material and Surface Treatments *(continued)*

Material/surface treatment	Study	Surface roughness Ra or Sa mm (SD)	Outcomes	Results compared with machined titanium (MT)
Collagen coating				
	Truc et al, 2018	Not evaluated	Cell adhesion at 1 h	Increased
			Cell viability at 1, 3, and 5 d	Increased
Composite resin coating (R)				
	Kim et al, 2014	Sa 0.39 (0.06)	Cell attachment 1 h	Lower than machined titanium, higher than CCM and R
	Kim et al, 2015	Sa 0.39 (0.06)	Cell attachment 1 h	Lower than machined titanium, higher than CCM and R
			Cell proliferation at 3 and 7 d	At 3 d, higher than machined titanium At 7 d, lower than machined titanium and lower than all the compared surfaces
Electropolishing				
	Meredith et al, 2005	Ti Ra 0.19 (0.030) TAN Ra 0.18 (0.037)	Cell morphology at 24 h, 5 d, and 10 d	Very well spread, both commercially pure titanium and titanium alloy (Ti6Al7Nb)
			Cell number	Significantly increased cell proliferation of commercially pure titanium and titanium alloy (Ti6Al7Nb), which in standard form impair fibroblast function
Titanium nitride coating (TiN)				
	Brunello et al, 2018	Ra 0.115 (0.022)	Cell proliferation at 3 d, 7 d, 14 d, 21 d	No difference
			Expression of cell adhesion molecules at 21 d	No difference
			Vinculin distribution at 14 d	No difference
	Kim et al, 2014	Sa 0.22 (0.03)	Cell attachment 1 h	Similar to machined titanium
	Kim et al, 2015	Sa 0.22 (0.03)	Cell attachment 1 h	Similar to machined titanium
			Cell proliferation at 3 d and 7 d	Higher than MT
	Moon et al, 2013	Not evaluated	Cell morphology at 1 and 5 d	No difference
			Cell proliferation at 1, 3, 5, and 7 d	No difference
			Expression of adhesion-related proteins at 24 h, 48 h, 72 h	No difference
	Ritz et al, 2017	Not evaluated	Cell adhesion at 2 h and 4 h	Less adherent cell
			Cell viability and proliferation at 1 d, 3 d, and 7 d	No difference
			Expression of ECM proteins, fibroblast differentiation markers, adhesion markers, cytoskeletal marker at 1 d and 7 d	Data not available
Zirconium nitride coating (ZrN)				
	Brunello et al, 2018	Ra 0.067 (0.008)	Cell proliferation at 3 d, 7 d, 14 d, 21 d	No difference
			Expression of cell adhesion molecules at 21 d	Increased expression
			Vinculin distribution at 14 d	No difference
	Franková et al, 2013	Ra 0.28	Detection of vinculin and $\alpha_3\beta_1$ integrin at 6 h and 24 h	No difference at 24 h and 6 h
			Collagen I production at 24 h and 72 h	No difference at 24 h Reduced expression at 72 h
			TNF-α and MMP-2 production at 24 h and 72 h	TNF-α at 24 h was increased Not detectable at 72 h No influence on production of MMP-2
Vitamin D precursor and vitamin E coating				
	Satué et al, 2016	Not evaluated	Cytotoxicity at 3 d	No difference
			Cell morphology at 3 d	No difference
			Expression of adhesion-related proteins and cytokines at 3 d and 14 d	Upregulation of COL3A1 and FN1 Decreased interleukin-8 Increased TIMP-1
			Wound healing assay	No difference

Table 5 Summary of the Results of the Reviewed Studies on the Basis of the Abutment Material and Surface Treatments *(continued)*

Material/surface treatment	Study	Surface roughness Ra or Sa mm (SD)	Outcomes	Results compared with machined titanium (MT)
Plasma treatment				
	Canullo et al, 2013	Not evaluated	Cell number at 2 h, 8 h, and 48 h	Increased at 2 h and 8 h, no difference at 48 h
			Cell adhesion at 2 h, 8 h, and 48 h	Increased at 2 h and 8 h, no difference at 48 h
	Lee et al, 2015	Ra 0.132 (0.023) Sa 0.129 (0.020)	Cell attachment at 4 h	Increased
			Cell proliferation at 4 h	No difference
			Detection of vinculin and actin filaments	Increased
Laser dimpling				
	Esfahanizadeh et al, 2016	Ra 1.33 (0.03)	Cell attachment and morphology at 3 d	Increased
			Cell proliferation at 3 d	Increased
			Expression of adhesion-related proteins and cytokines at 4 d	Increased expression of IL-10, increased expression of integrin

Dorkhan et al[50] compared anodically oxidized surfaces of commercially pure titanium (CpTi) and titanium alloy (Ti6Al4V) with commercially pure titanium as control. In this study, gingival fibroblasts presented lower adhesion ability to CpTi anodically oxidized surfaces than to Ti6Al4V and control surfaces, although they failed to show any significant difference in terms of gingival fibroblast adhesion strength between CpTi and nano-structured surfaces. Kim et al[60] found that Ti6Al4V treated with anodic oxidation in H_2SO_4 solution showed the higher proliferation rate of fibroblasts at the seventh day of proliferation compared with machined Ti6Al4V. Mussano et al[66] found that anodized Ti increased manifold the adhesion of fibroblasts and epithelial cells. Guida et al[53] found that the fibroblast adhesion and the proliferation rate significantly improved on oxidized nano-structured surfaces compared with machined disks, in a time-dependent manner. On the other hand, oxidized surfaces displayed higher levels of type I collagen synthesis.

Titanium nitride coating TiN seemed to not alter the original texture of the surface. Kim et al[60] found that titanium nitride coating on a titanium alloy showed a higher proliferation rate of fibroblast cells compared with machined Ti6Al4V. Brunello et al[49] found that the highest levels of collagen type I, FGFs, and integrin-related proteins were displayed on Ti disks coated with zirconium nitride.

Gómez-Florit et al[52] compared the response of HGF to titanium (Ti) and titanium-zirconium (Ti-Zr) with different surface treatments: machined, polished, and machined + acid-etched in HCl/H_2SO_4. For machined + acid-etched surfaces, scanning electron microscope images showed that Ti-Zr preserved the original grooved morphology of machined and was less roughened than machined + acid-etched Ti. The acid-etched surface reduced the cytotoxicity of both materials, but the fibroblast adherence was better in the non-acid-etched surface, and wound healing closure could not be assessed in wound healing assay. The expression of genes involved in extracellular matrix turnover, cell adhesion, and wound healing was significantly downregulated in Ti-Zr compared with Ti. Although modified surfaces were not cytotoxic, initial cell attachment, cell survival, and growth were reduced. This is due to the improved roughness of the acid-etched surface.

Abutment surfaces can be biochemically modified by coating with collagen[56,67] or other molecules.[57] Collagen-coated surfaces are associated with higher speed and capacity of cell spreading,[67] and the thickness of collagen coatings seems to influence the fibroblast response.[56] Interestingly, especially thinner collagen coatings of pure Ti were associated with significantly and consistently higher expression levels of factors that are involved in attachment, migration, and signal transduction of fibroblasts. Satué et al[57] tested the effect of coating with the precursor of vitamin D (7-DHC) and irradiation with ultraviolet (UV) light on commercially pure titanium implants, suggesting a decrease in the matrix metalloproteinase (MMP)-related breakdown of ECM and a beneficial effect on soft tissue integration.

It is important to evaluate that although biofilm formation and bacterial adhesion were not the object of the present review, different abutment materials or surface treatments, in addition to influencing gingival cell response, as described earlier, can affect microbial colonization and biofilm formation. While the in vitro formation and dynamics of biofilm on titanium and zirconium can be similar, significant differences for the biofilm thickness and three-dimensional structure were noticed.[70,71] Biofilms on zirconium surfaces were significantly thinner than on titanium and hydroxyapatite surfaces.[71] The tridimensional structure also

presented dissimilarities both in the deposition of the extracellular polysaccharides (EPS) matrix and in the association of the bacterial cells. Titanium presented a clear identification of the bacterial stacks and the circulation channels. Conversely, the biofilm on zirconium presented a cobweb morphology,[71] a less-structured form of aggregation.

In vitro preclinical research is essential to develop new dental materials and techniques. It can deliver vital data for future tests of therapeutic methodologies in clinical trials.

In vitro studies are relatively simple to perform; however, lack of certain methodologic rigor makes the comparison of results between studies difficult or impossible.[12] For example, as reported by Menezes-Silva et al in a review on restorative glass-ionomer cements,[72] authors affirmed to follow valid standardized protocols, although they altered different variables, therefore making direct comparisons with other studies impossible. Another problem is the lack of quality on reporting procedure and data, with insufficient details to make a study replica possible.

Until now, a validated checklist for reporting the risk of bias of in vitro studies in dentistry has not been available. To overcome this problem and to increase the quality of the present review, the authors decided to adopt the SciRAP method to evaluate the studies reviewed. Although it was developed to evaluate the reliability of in vitro toxicity studies, it was easily adapted to evaluate different types of laboratory research on cellular response to a chemical substance or material. The aim of the SciRAP method is to offer a possible guideline and a structured methodology for defining the significance of in vitro studies. Following this approach, it is possible to evaluate the quality of a single article on the basis of a detailed checklist and to compare the score of two or more articles.

The introduction of a standardized criterion of evaluation, similar to the one adopted for clinical trials, could standardize the reporting of in vitro experimental studies in implant dentistry, increasing the transparency and quality of these studies, as occurred for clinical trials.[11]

CONCLUSIONS

Within the limitations of this systematic review, it can be speculated that abutment material and its mechanical, physical, or chemical modification influence fibroblast response in vitro, in terms of adhesion, proliferation, cell morphology, and expression of extracellular matrix–related protein, especially in the earlier phases of contact with the abutment surface. In fact, it was highlighted that zirconia, collagen coating, electropolishing, plasma cleaning, and laser dimpling allowed better cell behavior compared with machined titanium.

On the other hand, sandblasting, acid-etching, composite coating, nitride coating, and vitamin D presented lower results compared with machined titanium. Anodization presented controversial results.

Additionally, the adoption of a score-based method allowed discriminatation of the real effectiveness of the included studies.

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REFERENCES

1. Patil R, Gresnigt MMM, Mahesh K, Dilbaghi A, Cune MS. Esthetic evaluation of anterior single-tooth implants with different abutment designs—patients' satisfaction compared to dentists' observations. J Prosthodont 2017;26:395–398.
2. Sculean A, Gruber R, Bosshardt DD. Soft tissue wound healing around teeth and dental implants. J Clin Periodontol 2014;41(suppl 15):s6–s22.
3. Ricci J, Alexander H. Cell response to surfaces: A concise summary. Int J Periodontics Restorative Dent 2016;36(suppl):s39–s46.
4. Pesce P, Menini M, Tealdo T, Bevilacqua M, Pera F, Pera P. Peri-implantitis: A systematic review of recently published papers. Int J Prosthodont 2014;27:15–25.
5. Canullo L, Schlee M, Wagner W, Covani U; Montegrotto Group for the Study of Peri-implant Disease. International brainstorming meeting on etiologic and risk factors of peri-implantitis, Montegrotto (Padua, Italy), August 2014. Int J Oral Maxillofac Implants 2015;30:1093–1104.
6. Pesce P, Canullo L, Grusovin MG, de Bruyn H, Cosyn J, Pera P. Systematic review of some prosthetic risk factors for periimplantitis. J Prosthet Dent 2015;114:346–350.
7. Moher D, Hopewell S, Schulz KF, Montori V, Gøtzsche PC, Devereaux PJ, et al. CONSORT 2010 Explanation and Elaboration: Updated guidelines for reporting parallel group randomised trials. BMJ 2010;340:c869.
8. von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: Guidelines for reporting observational studies. J Clin Epidemiol 2008;61:344–349.
9. Bossuyt PM, Reitsma JB; Standards for Reporting of Diagnostic Accuracy. The STARD initiative. Lancet 2003;361:71.
10. Moher D, Liberati A, Tetzlaff J, Altman DG; The PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. BMJ 2009;339:b2535.
11. Hopewell S, Dutton S, Yu LM, Chan AW, Altman DG. The quality of reports of randomised trials in 2000 and 2006: Comparative study of articles indexed in PubMed. BMJ 2010;340:c723.
12. Krithikadatta J, Gopikrishna V, Datta M. CRIS Guidelines (Checklist for Reporting In-vitro Studies): A concept note on the need for standardized guidelines for improving quality and transparency in reporting in-vitro studies in experimental dental research. J Conserv Dent 2014;17:301–304.
13. Molander L, Hanberg A, Rudén C, Ågerstrand M, Beronius A. Combining web-based tools for transparent evaluation of data for risk assessment: Developmental effects of bispenol A on the mammary gland as a case study. J Appl Toxicol 2017;37:319–330.
14. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: Explanation and elaboration. BMJ 2009;339:b2700.

15. Abdulmajeed AA, Kokkari AK, Käpylä J, et al. In vitro blood and fibroblast responses to BisGMA-TEGDMA/bioactive glass composite implants. J Mater Sci Mater Med 2014;25:151–162.
16. Atsuta I, Ayukawa Y, Furuhashi A, et al. Epithelial sealing effectiveness against titanium or zirconia implants surface. J Biomed Mater Res A 2019;107:1379–1385.
17. Fischer NG, Wong J, Baruth A, Cerutis DR. Effect of clinically relevant CAD/CAM zirconia polishing on gingival fibroblast proliferation and focal adhesions. Materials (Basel) 2017;10:e1358.
18. Gómez-Florit M, Xing R, Ramis JM, et al. Human gingival fibroblasts function is stimulated on machined hydrided titanium zirconium dental implants. J Dent 2014;42:30–38.
19. Groessner-Schreiber B, Neubert A, Müller WD, Hopp M, Griepentrog M, Lange KP. Fibroblast growth on surface-modified dental implants: An in vitro study. J Biomed Mater Res A 2003;64:591–599.
20. Hoshi N, Negishi H, Okada S, Nonami T, Kimoto K. Response of human fibroblasts to implant surface coated with titanium dioxide photocatalytic films. J Prosthodont Res 2010;54:185–191.
21. Jeong WS, Kwon JS, Choi EH, Kim KM. The effects of non-thermal atmospheric pressure plasma treated titanium surface on behaviors of oral soft tissue cells. Sci Rep 2018;8:15963.
22. Jin C, Ren LF, Ding HZ, Shi GS, Lin HS, Zhang F. Enhanced attachment, proliferation, and differentiation of human gingival fibroblasts on titanium surface modified with biomolecules. J Biomed Mater Res B Appl Biomater 2012;100:2167–2177.
23. Kwon JS, Kim YH, Choi EH, Kim CK, Kim KN, Kim KM. Non-thermal atmospheric pressure plasma increased mRNA expression of growth factors in human gingival fibroblasts. Clin Oral Investig 2016;20: 1801–1808.
24. Lee DW, Kim JG, Kim MK, et al. Effect of laser-dimpled titanium surfaces on attachment of epithelial-like cells and fibroblasts. J Adv Prosthodont 2015;7:138–145.
25. Lee EJ, Kwon JS, Uhm SH, et al. The effects of non-thermal atmospheric pressure plasma jet on cellular activity at SLA-treated titanium surfaces. Curr Appl Phys 2013;13(suppl):s36–s41.
26. Lee JJ, Song KY, Ahn SG, Choi JY, Seo JM, Park JM. Evaluation of effect of galvanic corrosion between nickel-chromium metal and titanium on ion release and cell toxicity. J Adv Prosthodont 2015;7:172–177.
27. Linderbäck P, Harmankaya N, Askendal A, Areva S, Lausmaa J, Tengvall P. The effect of heat- or ultra violet ozone-treatment of titanium on complement deposition from human blood plasma. Biomaterials 2010;31:4795–4801.
28. Liu M, Zhou J, Yang Y, Zheng M, Yang J, Tan J. Surface modification of zirconia with polydopamine to enhance fibroblast response and decrease bacterial activity in vitro: A potential technique for soft tissue engineering applications. Colloids Surf B Biointerfaces 2015;136:74–83.
29. Luo Q, Huang Y, Deng X, Zhang J, Li X, Zhao S, Li X. Polyelectrolyte multilayer coating with two regulatory molecules on titanium: Construction and its biological effects. Nanomedicine (Lond) 2013;8: 739–755.
30. Ma Q, Mei S, Ji K, Zhang Y, Chu PK. Immobilization of Ag nanoparticles/FGF-2 on a modified titanium implant surface and improved human gingival fibroblasts behavior. J Biomed Mater Res A 2011; 98:274–286.
31. Maeno M, Lee C, Kim DM, et al. Function of platelet-induced epithelial attachment at titanium surfaces inhibits microbial colonization. J Dent Res 2017;96:633–639.
32. Marín-Pareja N, Salvagni E, Guillem-Marti J, Aparicio C, Ginebra MP. Collagen-functionalised titanium surfaces for biological sealing of dental implants: Effect of immobilisation process on fibroblasts response. Colloids Surf B Biointerfaces 2014;122:601–610.
33. Mehl C, Kern M, Zimmermann A, Harder S, Huth S, Selhuber-Unkel C. Impact of cleaning procedures on adhesion of living cells to three abutment materials. Int J Oral Maxillofac Implants 2017;32:976–984.
34. Mustafa K, Odén A, Wennerberg A, Hultenby K, Arvidson K. The influence of surface topography of ceramic abutments on the attachment and proliferation of human oral fibroblasts. Biomaterials 2005;26:373–381.
35. Nakajima K, Odatsu T, Shinohara A, Baba K, Shibata Y, Sawase T. Effects of cleaning methods for custom abutment surfaces on gene expression of human gingival fibroblasts. J Oral Sci 2017;59:533–539.
36. Pabst AM, Walter C, Grassmann L, et al. Influence of CAD/CAM all-ceramic materials on cell viability, migration ability and adenylate kinase release of human gingival fibroblasts and oral keratinocytes. Clin Oral Investig 2014;18:1111–1118.
37. Pansani TN, Basso FG, Souza IDR, Hebling J, de Souza Costa CA. Characterization of titanium surface coated with epidermal growth factor and its effect on human gingival fibroblasts. Arch Oral Biol 2019;102:48–54.
38. Rizo-Gorrita M, Luna-Oliva I, Serrera-Figallo MÁ, Gutiérrez-Pérez JL, Torres-Lagares D. Comparison of cytomorphometry and early cell response of human gingival fibroblast (HGFs) between zirconium and new zirconia-reinforced lithium silicate ceramics (ZLS). Int J Mol Sci 2018;19:e2718.
39. Roffel S, Wu G, Nedeljkovic I, Meyer M, Razafiarison T, Gibbs S. Evaluation of a novel oral mucosa in vitro implantation model for analysis of molecular interactions with dental abutment surfaces. Clin Implant Dent Relat Res 2019;21(suppl 1):25–33.
40. Rutkunas V, Bukelskiene V, Sabaliauskas V, Balciunas E, Malinauskas M, Baltriukiene D. Assessment of human gingival fibroblast interaction with dental implant abutment materials. J Mater Sci Mater Med 2015;26:169.
41. Schierano G, Bellone G, Manzella C, Preti G, Emanuelli G. In vitro effect of transforming growth factor-beta on adhesion molecule expression by human gingival fibroblasts cultured in the presence of a titanium abutment. J Periodontol 2001;72:1658–1665.
42. Shahramian K, Leminen H, Meretoja V, et al. Sol-gel derived bioactive coating on zirconia: Effect on flexural strength and cell proliferation. J Biomed Mater Res B Appl Biomater 2017;105:2401–2407.
43. Shi X, Xu L, Munar ML, Ishikawa K. Hydrothermal treatment for TiN as abrasion resistant dental implant coating and its fibroblast response. Mater Sci Eng C Mater Biol Appl 2015;49:1–6.
44. Sugawara S, Maeno M, Lee C, et al. Establishment of epithelial attachment on titanium surface coated with platelet activating peptide. PLoS One 2016;11:e0164693.
45. Xing R, Salou L, Taxt-Lamolle S, Reseland JE, Lyngstadaas SP, Haugen HJ. Surface hydride on titanium by cathodic polarization promotes human gingival fibroblast growth. J Biomed Mater Res A 2014;102:1389–1398.
46. Xu X, Wang L, Luo Z, et al. Facile and versatile strategy for construction of anti-inflammatory and antibacterial surfaces with polydopamine-mediated liposomes releasing dexamethasone and minocycline for potential implant applications. ACS Appl Mater Interfaces 2017;9:43300–43314.
47. Yang Y, Zhou J, Liu X, Zheng M, Yang J, Tan J. Ultraviolet light-treated zirconia with different roughness affects function of human gingival fibroblasts in vitro: The potential surface modification developed from implant to abutment. J Biomed Mater Res B Appl Biomater 2015;103:116–124.
48. Al Mustafa M, Agis H, Müller HD, Watzek G, Gruber R. In vitro adhesion of fibroblastic cells to titanium alloy discs treated with sodium hydroxide. Clin Oral Implants Res 2015;26:15–19.
49. Brunello G, Brun P, Gardin C, et al. Biocompatibility and antibacterial properties of zirconium nitride coating on titanium abutments: An in vitro study. PLoS One 2018;13:e0199591.
50. Dorkhan M, Yücel-Lindberg T, Hall J, Svensäter G, Davies JR. Adherence of human oral keratinocytes and gingival fibroblasts to nanostructured titanium surfaces. BMC Oral Health 2014;14:75.
51. Franková J, Pivodová V, Růžička F, et al. Comparing biocompatibility of gingival fibroblasts and bacterial strains on a different modified titanium discs. J Biomed Mater Res A 2013;101:2915–2924.
52. Gómez-Florit M, Ramis JM, Xing R, et al. Differential response of human gingival fibroblasts to titanium- and titanium-zirconium-modified surfaces. J Periodontal Res 2014;49:425–436.
53. Guida L, Oliva A, Basile MA, Giordano M, Nastri L, Annunziata M. Human gingival fibroblast functions are stimulated by oxidized nano-structured titanium surfaces. J Dent 2013;41:900–907.
54. Moon YH, Yoon MK, Moon JS, et al. Focal adhesion linker proteins expression of fibroblast related to adhesion in response to different transmucosal abutment surfaces. J Adv Prosthodont 2013;5:341–350.
55. Ponsonnet L, Reybier K, Jaffrezic N, Comte V, Lagneau C, Lissac M, Martelet C. Relationship between surface properties (roughness, wettability) of titanium and titanium alloys and cell behaviour. Mater Sci Engin: C 2003;23:551–560.

56. Ritz U, Nusselt T, Sewing A, et al. The effect of different collagen modifications for titanium and titanium nitrite surfaces on functions of gingival fibroblasts. Clin Oral Investig 2017;21:255–265.
57. Satué M, Gómez-Florit M, Monjo M, Ramis JM. Improved human gingival fibroblast response to titanium implants coated with ultraviolet-irradiated vitamin D precursor and vitamin E. J Periodontal Res 2016;51:342–349.
58. Cho YD, Shin JC, Yoon HI, et al. Characterization of human gingival fibroblasts on zirconia surfaces containing niobium oxide. Materials (Basel) 2015;8:6018–6028.
59. Esfahanizadeh N, Motalebi S, Daneshparvar N, Akhoundi N, Bonakdar S. Morphology, proliferation, and gene expression of gingival fibroblasts on Laser-Lok, titanium, and zirconia surfaces. Lasers Med Sci 2016;31:863–873.
60. Kim YS, Ko Y, Kye SB, Yang SM. Human gingival fibroblast (HGF-1) attachment and proliferation on several abutment materials with various colors. Int J Oral Maxillofac Implants 2014;29:969–975.
61. Kim YS, Shin SY, Moon SK, Yang SM. Surface properties correlated with the human gingival fibroblasts attachment on various materials for implant abutments: A multiple regression analysis. Acta Odontol Scand 2015;73:38–47.
62. Lee JH, Kim YH, Choi EH, Kim KM, Kim KN. Air atmospheric-pressure plasma-jet treatment enhances the attachment of human gingival fibroblasts for early peri-implant soft tissue seals on titanium dental implant abutments. Acta Odontol Scand 2015;73:67–75.
63. Mehl C, Kern M, Schütte AM, Kadem LF, Selhuber-Unkel C. Adhesion of living cells to abutment materials, dentin, and adhesive luting cement with different surface qualities. Dent Mater 2016;32:1524–1535.
64. Meredith DO, Eschbach L, Wood MA, Riehle MO, Curtis AS, Richards RG. Human fibroblast reactions to standard and electropolished titanium and Ti-6Al-7Nb, and electropolished stainless steel. J Biomed Mater Res A 2005;75:541–555.
65. Nothdurft FP, Fontana D, Ruppenthal S, et al. Differential behavior of fibroblasts and epithelial cells on structured implant abutment materials: A comparison of materials and surface topographies. Clin Implant Dent Relat Res 2015;17:1237–1249.
66. Mussano F, Genova T, Laurenti M, et al. Early response of fibroblasts and epithelial cells to pink-shaded anodized dental implant abutments: An in vitro study. Int J Oral Maxillofac Implants 2018;33:571–579.
67. Truc NT, Minh HH, Khanh LL, et al. Modification of type I collagen on TiO_2 surface using electrochemical deposition. Surf Coat Technol 2018;344:664–672.
68. Canullo L, Cassinelli C, Götz W, Tarnow D. Plasma of argon accelerates murine fibroblast adhesion in early stages of titanium disk colonization. Int J Oral Maxillofac Implants 2013;28:957–962.
69. Kalghatgi S, Kelly CM, Cerchar E, et al. Effects of non-thermal plasma on mammalian cells. PLoS One 2011;6:e16270.
70. de Avila ED, de Molon RS, Palomari Spolidorio DM, de Assis Mollo F Jr. Implications of surface and bulk properties of abutment implants and their degradation in the health of periodontal tissue. Materials (Basel) 2013;6:5951–5966.
71. Sánchez MC, Llama-Palacios A, Fernández E, et al. An in vitro biofilm model associated to dental implants: Structural and quantitative analysis of in vitro biofilm formation on different dental implant surfaces. Dent Mater 2014;30:1161–1171.
72. Menezes-Silva R, Cabral RN, Pascotto RC, et al. Mechanical and optical properties of conventional restorative glass-ionomer cements—a systematic review. J Appl Oral Sci 2019;27:e2018357.