Received: 28 November 2021 | Revised: 19 June 2022 | Accepted: 22 June 2022

DOI: 10.1111/clr.13973

ORIGINAL ARTICLE

CLINICAL ORAL IMPLANTS RESEARCH WILEY

Microleakage assessment of CAD-CAM Cobalt-Chrome and Zirconia abutments on a conical connection dental implant: A comparative in vitro study

Pedro Molinero-Mourelle[1,2] | Andrea Roccuzzo[3,4] | Burak Yilmaz[2,5] | Walter Yu Hang Lam[6] | Edmond H. N. Pow[6] | Jaime Del Río Highsmith[1] | Miguel Gómez-Polo[1]

[1]Department of Conservative Dentistry and Orofacial Prosthetics, Faculty of Dentistry, Complutense University of Madrid, Madrid, Spain

[2]Department of Reconstructive Dentistry and Gerodontology, School of Dental Medicine, University of Bern, Bern, Switzerland

[3]Department of Periodontology, School of Dental Medicine, University of Bern, Bern, Switzerland

[4]Department of Oral and Maxillofacial Surgery, Copenhagen University Hospital (Rigshospitalet), Copenhagen, Denmark

[5]Department of Preventive, and Pediatric Dentistry, School of Dental Medicine, University of Bern, Bern, Switzerland

[6]Prosthodontics, Restorative Dental Sciences, Faculty of Dentistry, The University of Hong Kong, Hong Kong Special Administrative Region, Hong Kong, China

Correspondence
Andrea Roccuzzo, Department of Periodontology, School of Dental Medicine, University of Bern, Freiburgstrasse 7, CH-3010 Bern, Switzerland.
Email: andrea.roccuzzo@zmk.unibe.ch

Abstract

Objective: To assess the marginal and bacterial microleakage in zirconia and CAD-CAM or cast Co-Cr implant abutments.

Methods: Sixty-four conical connection implants with their respective abutments were divided into four groups (Co-Cr (milled, laser-sintered, and cast) and Zirconia (milled)). All specimens were subjected to a chewing simulation and thermocycling. After aging process, specimens were submerged in a 0.2% methylene blue solution with *Porphyromonas gingivalis* (*P.g*) for 48 h. The marginal microleakage was measured using a 40× optical microscopy at the internal part of the implant, and when positive microleakage was observed, a DNA isolation with a polymerase chain reaction (PCR) test was used. The microbiological assessment was based on colony forming units (CFUs).

Results: Thirty (47%) implant-abutments presented microleakage and the PCR was performed on those specimens (1 Zirconia, 1 Co-Cr milled, 14 Co-Cr laser-sintered and 14 cast). Seven specimens (1 Co-Cr laser-sintered and six cast) presented values below the PCR detection limit (< 100 CFUs). The lowest CFUs count occurred in the Co-Cr milled group (5.17E+02 CFUs/ml) followed by zirconia (7.70E+03 CFUs/ml). The Co-Cr cast (9.39E+03 CFUs/ml) and laser-sintered (2.4E+05 CFUs/ml) groups had higher bacterial count. The CFU count comparison performed between Co-Cr cast and laser-sintered resulted in a statistically significant differences in favor of Co-CrCL ($p < .05$).

Conclusions: The abutment material and fabrication technique affected the implant-abutment microleakage. Although the CAD-CAM abutments presented favorable results, all tested groups presented microleakage.

KEYWORDS
dental implant abutment connection, dental implant abutment design, dental implants, implant supported dental prosthesis, microleakage, polymerase chain reaction

Pedro Molinero-Mourelle and Andrea Roccuzzo contributed equally to this manuscript and share first author position.

1 | INTRODUCTION

During last three decades, single implant-supported reconstructions have been widely studied and their performance improved, allowing clinicians to perform reliable oral rehabilitation with excellent long-term survival rates (Hjalmarsson et al., 2016). Nonetheless, these restorations are not free from biological complications (i.e., peri-implant mucositis, peri-implantitis) as reported in several systematic reviews (Jung et al., 2012) and cross-sectional studies (Rodrigo et al., 2018).

Currently, it is well documented that peri-implant mucositis and peri-implantitis are initiated by biofilm formation (Berglundh et al., 2018), where bacterial micro-organisms play a fundamental role (Belibasakis & Manoil, 2021). The bacterial colonization of a dental implant is an early event that starts immediately after implant placement (Van Winkelhoff et al., 2000). Thereafter, within the following 2 weeks, specific periodontopathogenic bacteria (i.e., *Porphyromonas gingivalis*, *Tannerella forsythia*) (Fürst et al., 2007) have been detected with no significant differences in terms of bacteria characteristics around healthy teeth and implants for the following 2 years (Salvi et al., 2008). Nonetheless, the presence of *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* around dental implants is not per se sufficient to enhance the development of a degenerative inflammatory response leading to implant loss, under optimal plaque control conditions (Socransky & Haffajee, 2005). In addition, it has been demonstrated that even though the presence of *Porphyromonas gingivalis* (*P.g*) was correlated with the peri-implant probing depth (Socransky et al., 1998), the architecture of the peri-implant microbiota is extremely complex and varies according to the structural and topographical differences among dental implants (Lafaurie et al., 2017).

Every time an implant is connected to an abutment, an interface between these two components is created (Liu & Wang, 2017). This microscopic chamber provides an ideal substrate, where microorganisms can adhere and proliferate and consequently constitute a bacteria reservoir activating an inflammatory host response around the abutment–implant interface (Koutouzis, 2019; Larrucea et al., 2014; Liu & Wang, 2017).

Historically, external hexagon connections were commonly used (Adell et al., 1981), however, due to technical disadvantages like relatively high frequency of screw-loosening, fractures, (Gracis et al., 2012) internal conical connections systems have been developed (Norton, 1997).

Different prosthetic materials have been recently proposed since precious alloys have been gradually relegated due to the increasing gold price and with the CAD-CAM technology developments (Edelhoff et al., 2019; Limones et al., 2020). Currently Titanium, Cobalt-Chrome (Co-Cr), or Zirconia (Zr) are some of the commonly used materials options by clinicians and patients due their accessibility, competitive price, and good clinical characteristics (Lemos et al., 2019; Molinero-Mourelle et al., 2021). Zirconia abutments have been a reliable alternative with clinical advantages regarding the soft tissue behavior especially in the anterior region (Fonseca et al., 2021; Naveau et al., 2019).

The integrity of implant–abutment interface plays an important role in long-term success and can be affected by the material type, the fabrication technique, and the processing method quality (Koutouzis, 2019; Pereira et al., 2017). Considering the sealing capacity and implant-abutment microleakage, most of the studies focused on implants or the materials for components, and in this respect, precious alloys and titanium abutments have been widely investigated, and although Co-Cr abutments present favorable properties, their seal capacity with respect to their fabrication technique has not been extensively studied; the number of published studies is limited (Jo et al., 2014; Koutouzis, 2019; Molinero-Mourelle et al., 2021).

In the last decade, a dramatic increase in the number of single implant reconstructions fabricated using conventional and digital workflows has occurred (Mello et al., 2019). However, studies, which evaluated the microleakage with zirconia and Co-Cr abutments using conventional and digital workflows fabrication techniques and inserted on conical connection implants are lacking (Molinero-Mourelle et al., 2021).

Therefore, the aim of the present in vitro study was to assess the microleakage at the implant–abutment interface when Co-Cr [Co-Cr (milled, laser-sintered, and cast) and zirconia (milled)] abutments were used on a conical connection-implant. The null hypothesis assessed was that there would be no difference in the microleakage (in terms of optical and bacterial assessment) among the Co-Cr (milled, cast, laser-sintered) and zirconia abutments.

2 | MATERIAL AND METHODS

2.1 | Study design

This comparative in vitro study was performed at the Department of Conservative Dentistry and Orofacial Prosthodontics, Faculty of Dentistry, Complutense University of Madrid and evaluated 64 one-piece implant abutments fabricated using four different techniques ($n = 16$). Since no human sample were used, ethics approval was not required from the Ethics Committee Research of Complutense University Hospitals for this in vitro study.

A minimum of 12 specimens per group were required with a power of 80% based on previous studies (Vélez et al., 2020) assuming a standard deviation of 3.5 using a two-group *t*-test with 0.05 two-sided significance level was determined using the nQuery Avisor Sample size software version 8.5.2 (Statistical Solutions Ltd).

2.2 | Study set up and specimen fabrication

For specimen fabrication, 64 implant sites (4.1×8.5 mm; Ocean, Avinent implant system, Santpedor, Barcelona, Spain, Ref. n° 1590) were prepared in polymethylmethacrylate resin blocks [1.8 mm length and 1.5 width, (Mechanical Workshop, Faculty of Physics,

Complutense University of Madrid)] with an elastic modulus of 3.000 MPa (Cancellous bone approximate module of 1.507 MPa) (Morgan et al., 2018). The osteotomies were performed using a parallelizing drill press to standardize the implant position/angle and the implants were placed following the manufacturer's protocol using a surgical handpiece (iChiropro) with Micro-Series CA 20: 1 L (Bien-Air Dental SA). The implants were tightened to 35 Ncm up to the implant resistance and with the implant system's rachet leaving the implant platforms at the resin level.

For the fabrication of each restorative group, an implant analog (4.1 mm) with an extraoral scan body (Core 3D centers; Dental Direkt GmbH) was scanned with a laboratory desktop scanner (Biomet Zfx, Zimmer ZFX; GmbH) and a standard triangle language (STL) file was generated and exported to the CAD software program (Exocad GmbH). A screw retained master abutment with a mandibular first premolar as the customized master abutment and one-piece crown configuration were designed and fabricated. Sixty-four identical abutments were fabricated in two different materials; milled 3 mol% Yttria-stabilized tetragonal zirconia polycrystalline doped with alumina (3Y-TZP-LA) and Cobalt-Chromium (Co-Cr) using different techniques.

The 3Y-TZP-LA (ZrCAD) abutment fabrication, a processing center with the implant manufacturer original components digital library carried out the process following the specifications of the International Organization Standard (ISO) 2013356:2015 on yttria-stabilized tetragonal zirconia in a 5-axis precision milling machine (Roland DWX-52 DC; Roland DG Deutschland GmbH, WillichGermany) (G1).

The Co-Cr abutments' groups were fabricated following the specifications of the ISO standard 583–12:2019 for Co-Cr using the milling, laser-sintered, and casting techniques. The milled group (G2) was fabricated by the aforementioned processing center in a 5-axis milling machine (DMG Sauer HSC 20 Linear (DMG MORI AKTIENGESELLSCHAFT). Laser-sintered implant abutments (G3) were fabricated following the same CAD design in a selective laser melting machine (SLM 125; SML Solutions Group AG). Finally, the cast group (G4), used a previously made framework introduced in an addition silicon block in its heavy form (Platinum 85 TOUCH, Zhermack SpA, Rome, Italy), to prepare the previous wax patterns for lost-wax technique. Once the wax patterns were obtained, the abutment shape were melted to a plastic castable abutment (Dental Smart Solutions, Terrats Medical S.L. Barberá del Valles, Spain), and cast Co-Cr abutments were fabricated using induction heated centrifugal casting in an ambient atmosphere. Material compositions and manufacturer information are displayed in Table 1.

2.3 | Aging process

The abutments were tightened to implants with 35 Ncm torque using implant system's brand-new wrench (Avinent implant system) and subjected to cyclic loading in a chewing simulation machine (Instron®, Euroortodoncia S.A. Zwick/Roell testXpert II Software). The loads were applied with a 2-mm Teflon cylinder to the occlusal surface of the abutments for 300,000 cycles under 200 N loads at 2 Hz and a 30° parallel angle following the ISO 14801:2016. Dentistry-Implants–Dynamic Loading Test for Endosseous Dental Implants recommendations. After cyclic loading, the specimens were thermocycled (10,000 cycles, 5 to 50°C, dwelling time 55 s) in a thermo-cycling machine (Complutense University of Madrid, Spain) in artificial saliva.

Before the microleakage assessment, the specimens were visually inspected for failure before the fit analysis with ×2.5 magnification loupes (ExamVision ApS) and tactile motion test was applied with dental pliers. The specimens were cleaned using a polishing set for metal and ceramic (Komet Dental; Gebr. Brasseler GmbH & Co. KG) and were stored in an airtight chamber before the evaluation.

2.4 | Microleakage evaluation

For the microleakage assessment, a medium based on 20 ml BHI (Brain Heart Infusion), *Porphyromonas gingivalis* (*P.g*) and 5 ml of methylene blue, at a concentration of 0.2% were prepared as staining agent. The ATCC 33277 (ATCC = American Type Culture Collection) was used in cryovials at a stable temperature of −80°C. Two tests were performed on blood agar with 5% Hemin-Menadione before including a preinoculum in 10 ml BHI (BHI2) for 24 hours 37°C in

TABLE 1 Study material groups ZrCAD, Cr-CrMill, co-CrLS, co-CrCL

Group	Material composition	Material manufacturer	Prosthetic manufacturer
ZrCAD (G1)	3 mol% yttria stabilized tetragonal zirconia polycrystal (3Y-TZP-A)	Dental Direkt GmbH. Spenge, Germany	CORE 3D PROTECH, S.L.U. Santpedor. Spain
Co-CrMill (G2)	63% Co, 29% Cr, 6% Mo, <1% Nb, Si, Mn, Fe	Dental Direkt GmbH. Spenge, Germany	CORE 3D PROTECH, S.L.U. Santpedor. Spain
Co-CrLS (G3)	59% Co, 25% Cr, 9,5% W, 3,5% M, 1% Si	S & S Scheftner GmbH – dental alloys. Mainz, Germany	Prótesis S.A. Madrid. Spain
Co-CrCL(G4)	61% Co, 24% Cr, 8% W, 2,5% Mo, 1% Nb, 1% Mn, 1% Si, 1% Fe	Dentalforschung Schleicher GmbH. Riedenburg Germany	Riosa Laboratory Pozuelo de Alarcón. Spain.

Abbreviations: 3Y-TZP-A, Yttria-stabilized tetragonal zirconia polycrystalline doped with alumina; Co-CrCL, cobalt-chromium frameworks with cast abutments; Co-CrMill, cobalt-chromium framework milled; Co-CrLS, cobalt-chromium framework laser-sintered fabricated; ZrCAD, CAD-CAM-fabricated zirconia frameworks.

anaerobiosis and finally the Optical Density is measured and adjusted to 1×10^6 CFU/ml.

Once the medium was prepared, all specimens were subjected to the staining agent at 37°C for 48 h in anaerobiosis condition. After the immersion in the agent, the specimens were unscrewed and the microleakage was assessed.

2.4.1 | Marginal microleakage evaluation

The marginal microleakage was determined using an optical microscope [Optical microscope: stereomicroscope m-80 (Leica) (40× Magnification) charge-coupled device camera (Hitachi cctv hv-720e; Hitachi Ltd)] analyzed, and image analysis software Leica application suite (Leica) to determine the penetration of the agent in the implant–abutment interface. The assigned assessment levels are represented by the scale presented in Figure 1. The microscope was operated by one of the researchers (PMM) who was previously trained and calibrated taking three digital pictures in order to avoid examination bias.

The marginal microleakage was defined as the presence of methylene blue in any surface of the stated implant levels. After the optical assessment, the pictures taken were evaluated by two independent calibrated examiners (MGP, JRH) in order to avoid the interobserver bias. To be considered as positive microleakage, the specimens subjected to the staining process were compared with a brand-new implant that was considered as a negative control. Once the marginal microleakage was directly determined, the microbiological assessment of the bacterial microleakage at interior of the implant was performed using a DNA isolation with a polymerase chain reaction (PCR) test for the positive microleakage specimens.

2.4.2 | Bacterial microleakage evaluation

Before the bacterial microleakage DNA isolation, the exterior area of all the specimens were sequentially rinsed in sodium hypochlorite with a concentration of 5% three times, in order to eliminate the not adherents' bacteria. To ensure that the implant connection surface was not washed, the process was carried out using a microbrush applicator (Microbrush International) carefully on the surface of the block.

DNA were isolated from all specimens using a commercial kit (MolYsis Complete5; Molzym GmbH & CoKG), following the manufacturer's instructions. The 5' nuclease assay PCR method with hydrolysis probes was used to detect and quantify bacterial DNA. Primers and probes were obtained from Life Technologies Invitrogen, Applied Biosystems and Roche (Roche Diagnostic GmbH) (Sánchez et al., 2014). The PCR detection limit was 100 colony forming units (CFUs) per specimen. For the quantitative analysis the values below 100 CFUs could not be quantified, therefore, these specimens were given a value of 0.

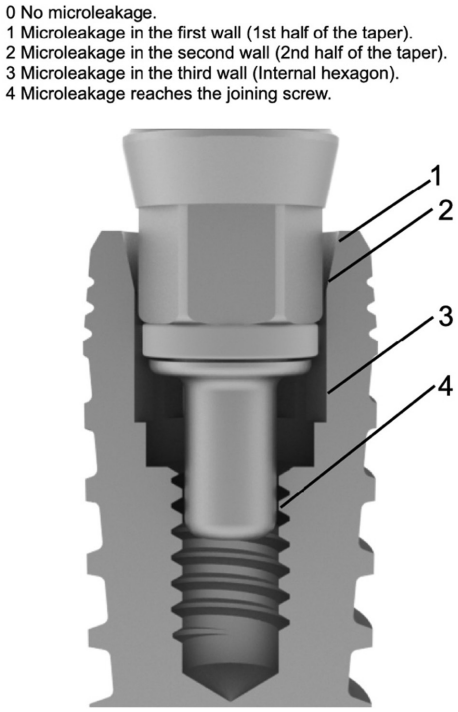

FIGURE 1 Schematic representation of the microleakage levels

2.5 | Statistical analysis

Data analysis was performed using a statistical software program (SPSS V27.0; IBM Corp). For the group comparisons, the Co-Cr milled group was considered as the control. The marginal optical microleakage level was described by percentages and compared per group using Fisher–Freeman–Halton Exact Test. The microbiological microleakage assessment was based on colony forming units (CFUs); the differences among groups were compared using Mann–Whitney's *U* test. All multiple comparisons were corrected by Bonferroni's criteria. *p* values smaller than .05 were considered statistically significant.

3 | RESULTS

During the chewing simulation and thermocycling process, no specimen presented failures or complications and the interobserver microleakage optical assessment was concordant; both examiners agreed on all the assessments for all pictures and measured levels of all the specimens.

3.1 | Marginal microleakage

The descriptive analysis showed marginal microleakage in all assessed groups in at least one of the optical evaluated levels (Figure 2). From these findings, the CAD-CAM groups obtained better results being the ZrCAD the best group, showed microleakage in one specimen at the first level, followed by Co-CrMill group ($n = 1$) presenting methylene blue at the first and second measured levels.

The Co-CrCL group had optical microleakage in 14 specimens at the four proposed levels with nine specimens reaching the second level, five the third and one the fourth level.

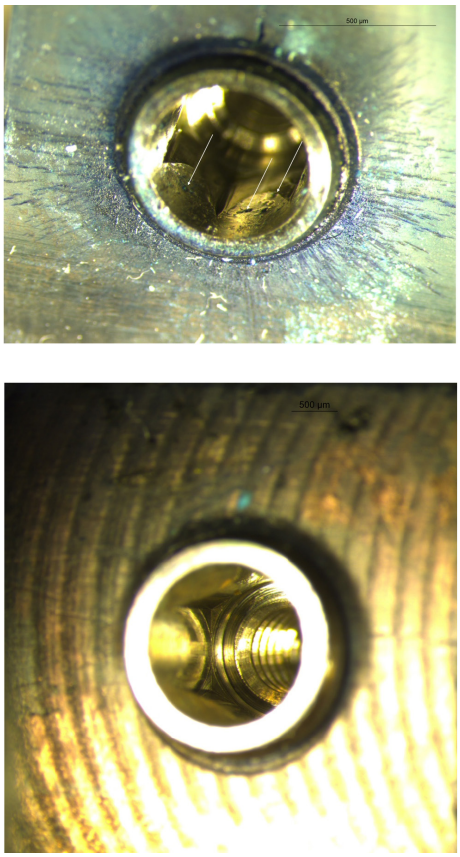

FIGURE 2 Optical Microscopical image of the liquid penetration (a) and control (b) at the first level of assessment

Finally Co-CrLS abutments had the highest marginal microleakage, showed the presence of the staining liquid in 14 specimens, reaching all of them the first level, 13 the second, 11 the third and finally nine specimens the fourth level of the implants (Table 2).

Within the marginal microleakage global comparison, the Chi-squared test showed significant differences between the ZrCAD group and the Co-CrLS and Co-CrCL groups ($p < .001$). The mean microleakage values between Co-CrMill and Co-CrLS, and the comparison between Co-CrMill and Co-CrCL were significantly different in favor of the milled group ($p < .001$).

Comparing the marginal microleakage at the measured levels, there were significant differences among all groups ($p < .001$). At the individual level assessment among the groups, the results showed significant differences between ZrCAD compared to Co-CrLS and Co-CrCL and between the Co-CrMill in comparison with Co-CrLS and Co-CrCL at the first and second level.

At the third level comparison, the analysis showed significant differences between the ZrCAD and Co-CrLS and the Co-CrCL and when these two groups were together compared. Finally, regarding the fourth level comparison, there were significant differences among the Co-CrLS and the rest of the groups.

3.2 | Bacterial microleakage

From the 64 implant-supported reconstructions, 30 of those presented a certain degree of marginal microleakage and therefore underwent a PCR analysis. More specifically, seven of these 30 specimens (1 Co-CrLS and 6 Co-CrCL) presented values below the PCR detection limit (<100 CFUs) and consequently were not included in

TABLE 2 Descriptive and comparative analysis of the marginal microleakage levels according abutment material and fabrication technique

	Abutment material and fabrication technique					
Microleakage results (levels)	ZrCAD ($n = 16$)	Co-CrMill ($n = 16$)	Co-CrLS ($n = 16$)	Co-CrCL ($n = 16$)	p-Value	Total ($n = 64$)
Microleakage (n specimens)	a 1 (6.3%)	a 1 (6.3%)	b 14 (87.5%)	b 14 (87.5%)	<.001	30 (46.9%)
No Microleakage	0	0	0	0	–	0
Microleakage in the 1st wall (1st half of the taper)	a 1 (6.3%)	a 1 (6.25%)	b 14 (87.5%)	b 14 (87.5%)	<.001	30 (46.87%)
Microleakage in the 2nd wall (2nd half of the taper)	a 0	a 1 (6.25%)	b 13 (81.3%)	b 9 (56.3%)	<.001	23 (35.93%)
Microleakage in the 3rd wall (Internal hexagon)	a 0	a 0	b 11 (68.8%)	c 5 (31.3%)	<.001	16 (25%)
Microleakage reaches the implant joining screw	a 0	a 0	b 9 (56.3%)	a 1 (6.25%)	<.001	10 (15.62%)

Note: a, b, c. Each subscript letter denotes a subset of groups whose column proportions do not differ significantly from each other at the .05 level, with Bonferroni's correction.

Abbreviations: 3Y-TZP-A, Yttria-stabilized tetragonal zirconia polycrystalline doped with alumina; Co-CrCL, cobalt-chromium frameworks with cast abutments; Co-CrMill: cobalt-chromium framework milled; Co-CrLS, cobalt-chromium framework laser-sintered fabricated; ZrCAD, CAD-CAM-fabricated zirconia frameworks.

the qualitative analysis. The presented data were gathered from the remaining 23 specimens.

Within the 23 quantitatively measurable specimens, the lowest CFUs count occurred in the Co-CrMill group (5.17E+02 CFUs/ml), followed by ZrCAD (7.70E+03 CFUs/ml). The Co-CrCL (9.39E+03 CFUs/ml) and Co-CrLS (2.4E+05 CFUs/ml) groups had the higher bacterial count. The CFU count comparison was performed between Co-CrCL and Co-CrLS groups since the Co-CrMill and ZrCAD presented microleakage in only one specimen per group. Mann–Whitney's U test showed significant differences in the bacterial count between the Co-CrCL and Co-CrLS groups ($p < .05$) (Table 3).

4 | DISCUSSION

The results of this study have shown how material type and fabrication technique had a significant impact on implant-abutment microleakage after artificial chewing simulation. When the materials and techniques used were considered, ZrCAD and Co-CrMill showed the most favorable microleakage results compared to the Co-CrLS and Co-CrCL. In detail, statistically significant differences between the Co-CrCL and Co-CrLS groups were found in the PCR assessment. The null hypothesis was rejected since significant differences in microleakage values were detected among groups.

Despite the lack of consensus on a reliable technique to investigate the implant-abutment marginal microleakage, a review by Koutouzis, concluded that in vitro studies which evaluated the implant–abutment connection bacterial penetration showed that the connection design have an influence on the bacterial penetration, and although it included in vitro studies with bacterial medium and evaluated by PCR, the material of the abutments was not exhaustively mentioned (Koutouzis, 2019). On the in vitro microleakage assessment, a systematic review by Misrha et al. (Mishra et al., 2017) of 30 studies, reported that, despite the high methodological variability, most of the studies used bacterial cultures, only two reported staining agents, and finally one used water and resin acrylic, concluding that microbiological cultures might present a valid method (Mishra et al., 2017).

With respect to different abutment materials, Şen et al. (2019) evaluating the sealing capacity of two materials (i.e., titanium and zirconia) in conical and external connection implants by evaluating bacterial turbidity of PG, reported the best results in conical connection in zirconia and titanium abutments. Although this study did not use the same methodology and without Co-Cr abutments, the microleakage results were like those described in this study.

The use of methylene blue as a staining agent for microleakage evaluation has been previously described (Larrucea et al., 2014; Martin-Gili et al., 2015; Ortega-Martínez et al., 2020). Larrucea et al, investigated with a very similar methodology (i.e., methylene blue and optical microscopy in the implant connection), the microleakage at different levels in external hex and conical connection implants. Although this study did not include different abutments materials and PCR analysis, the results from conical implants can be compared with those obtained in our study with the Co-Cr milled and zirconia. Nevertheless, our results seem more favorable to those presented by Larrucea Verdugo and co-workers who detected a microleakage visible only up to the second wall in the Co-Cr milled group (Larrucea et al., 2014).

Similar results have been reported by Martin-Gili et al. (2015) who investigated the microleakage with methylene blue in conical, external, and internal connection implants without material comparison, (Martin-Gili et al., 2015) and showed a significant greater microleakage in the external connection implants compared to the conical connection in terms of bacterial concentration measured by the spectrophotometer and although the methodology used differs from that used in this study, there was microleakage for conical connection implants.

Ortega-Martinez et al. (2020) evaluated two abutment materials (i.e., titanium and PEEK) under static and dynamic conditions using methylene blue. An optical analysis of the staining medium reported the most favorable results in the titanium abutments, which only showed microleakage in two of 24 abutments. In contrast, when focusing on the microleakage level, the PEEK abutments and those with loading obtained more unfavorable results

TABLE 3 Descriptive and comparative analysis for PCR values

Manufacturing material and technique	ZrCAD ($n = 1$)	Co-CrMill ($n = 1$)	Co-CrLS ($n = 14$)	Co-CrCL ($n = 14$)	Total ($n = 30$)
PG Total quantification	7.70E+03 CFUs/ml	5.17E+02 CFUs/ml	2.23E+05 CFUs/ml	9.39E+03 CFUs/ml	2.4E+05 CFUs/ml
U-Mann test					
Manufacturing material and technique	**Mean**	**Median**	**IQR**	**Minimum**	**Maximum**
CoCrLS ($n = 14$)	16440.1 CFUs/ml	18900.0 CFUs/ml	28744.0 CFUs/ml	0.0	42000.0 CFUs/ml
CoCrCL ($n = 14$)	670.5 CFUs/ml	785,0 CFUs/ml	1235.0 CFUs/ml	0.0	2470.0 CFUs/ml

Abbreviations: Co-CrCL, cobalt-chromium frameworks with cast abutments; Co-CrLS, cobalt-chromium framework laser-sintered fabricated; Co-CrMill, cobalt-chromium framework milled; IQR, Interquartile range; PG, Porphyromonas Gingivalis; Sig, Significance <.001 Mann–Whitney's U test; ZrCAD, CAD-CAM-fabricated zirconia frameworks.

(Ortega-Martínez et al., 2020). Although a direct comparison may not be possible, the use of titanium abutments can be compared with those of Co-Cr milled abutments and therefore, microleakage was similar in this study since only one specimen presented staining liquid in the first upper level, in the second and in the third level, however in accordance with Ortega-Martínez et al, no methylene blue was observed in the last level. Considering this study results, the use of conical connection implants and machined abutments seem to be the most favorable combination to avoid possible bacterial microleakage.

With respect to the relationship between the implant abutment connection and its potential link with peri-implant conditions, a systematic review on human studies with at least 1 year of functional load revealed a higher bacterial count at sites with peri-implantitis compared to healthy sites. This review included a total of 14 articles and 1126 implants, including conical connection and PCR evaluation among others, concluding that bacteria could easily colonize within the implant–abutment interface, and therefore the interior of the implant and prosthetic component should always be considered contaminated, even under clinically healthy conditions (Tallarico et al., 2017). When clinical implications are considered, the use of conical connection implants and original and machined prosthetic components may present a better implant-abutment fit, potentially minimizing related technical and biological complications. In addition to provide an optimal fit, zirconia abutments do present some advantages (Şen et al., 2019).

This study has certain limitations: first due the in vitro design, the obtained results might not be directly compared with a clinical scenario. Nevertheless, the marginal microleakage assessment can be performed by unscrewing the reconstruction allowing the visual analysis and the direct the sample collection. Secondly, in this study, only two materials (Co-Cr and Zr) have been analyzed due the economical and feasibility reasons; nevertheless, it has to be mentioned that inclusion of additional materials (i.e., Titanium and Precious alloys) could have provided additional relevant results.

Within the limitations, it can be concluded that the use of zirconia and milled Co-Cr abutments may lead to more favorable long-term results because these groups presented lower microleakage.

AUTHOR CONTRIBUTIONS

P.M-M. was involved in the concept and design of the study, data collection, analyzed and interpreted data, drafted the article, and approved the final version of the manuscript; A.R, B.Y. W.Y.H.L. were involved article draft and critical revision of the manuscript; E.H.N.P, J.R.H., and M.G-P. were involved in the concept and design of the study, data analysis, critical revision of the manuscript. All authors approved the final version.

ACKNOWLEDGEMENTS

The authors thank the Microbiology and Oral Biology laboratories Faculty of Dentistry, Complutense University of Madrid for the microbiological assistance and Dr. Pedro Cuesta for conducting the statistical analysis. Open access funding provided by Universitat Bern. Open access funding provided by Universitat Bern.

CONFLICT OF INTEREST

The study was partially funded by Spanish Society of Prosthodontics Research Scholarship; The authors declare no potential conflict of interests with respect to this study. A.R. was the recipient of a 3-year scholarship from the Clinical Research Foundation (CFR) for the Promotion of Oral Health, Brienz, Switzerland. A.R. is the recipient of a 1-year scholarship from the International Team of Implantology (ITI).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are partially available on request from the corresponding author.

ORCID

Pedro Molinero-Mourelle https://orcid.org/0000-0001-5493-0295
Andrea Roccuzzo https://orcid.org/0000-0002-8079-0860
Edmond H. N. Pow https://orcid.org/0000-0003-2640-8437

REFERENCES

Adell, R., Lekholm, U., Rockler, B., & Brånemark, P. I. (1981). A 15-year study of osseointegrated implants in the treatment of the edentulous jaw. *International Journal of Oral Surgery*, *6*, 387–416. https://doi.org/10.1016/s0300-9785(81)80077-4

Belibasakis, G. N., & Manoil, D. (2021). Microbial community-driven Etiopathogenesis of Peri-Implantitis. *Journal of Dental Research.*, *100*(1), 21–28. https://doi.org/10.1177/0022034520949851

Berglundh, T., Armitage, G., Araujo, M. G., Avila-Ortiz, G., Blanco, J., Camargo, P. M., Chen, S., Cochran, D., Derks, J., Figuero, E., Hämmerle, C. H. F., Heitz-Mayfield, L. J. A., Huynh-Ba, G., Iacono, V., Koo, K. T., Lambert, F., McCauley, L., Quirynen, M., Renvert, S., … Zitzmann, N. (2018). Peri-implant diseases and conditions: Consensus report of workgroup 4 of the 2017 world workshop on the classification of periodontal and Peri-implant diseases and conditions. *Journal Clinical Periodontology.*, *45*(Suppl 20), S286–S291.

Edelhoff, D., Schweiger, J., Prandtner, O., Stimmelmayr, M., & Güth, J. F. (2019). Metal-free implant-supported single-tooth restorations. Part I: Abutments and cemented crowns. *Quintessence International.*, *50*(3), 176–184. https://doi.org/10.3290/j.qi.a41906

Fonseca, M., Molinero-Mourelle, P., Forrer, F. A., Schnider, N., Hicklin, S. P., Schimmel, M., & Brägger, U. (2021). Clinical performance of implant crowns with customized zirconia abutments: A prospective cohort study with a 4.5- to 8.8-year follow-up. *Clinical Oral Implants Research.*, *32*(7), 853–862. https://doi.org/10.1111/clr.13761

Fürst, M. M., Salvi, G. E., Lang, N. P., & Persson, G. R. (2007). Bacterial colonization immediately after installation on oral titanium implants. *Clinical Oral Implants Research.*, *18*(4), 501–508. https://doi.org/10.1111/j.1600-0501.2007.01381.x

Gracis, S., Michalakis, K., Vigolo, P., Vult von Steyern, P., Zwahlen, M., & Sailer, I. (2012). Internal vs. external connections for abutments/reconstructions: A systematic review. *Clinical Oral Implants Research.*, *23*(Suppl 6), 202–216. https://doi.org/10.1111/j.1600-0501.2012.02556.x

Hjalmarsson, L., Gheisarifar, M., & Jemt, T. (2016). A systematic review of survival of single implants as presented in longitudinal studies with a follow-up of at least 10 years. *European Journal of Oral Implantology.*, *9*(Suppl 1), S155–S162.

Jo, J. Y., Yang, D. S., Huh, J. B., Heo, J. C., Yun, M. J., & Jeong, C. M. (2014). Influence of abutment materials on the implant-abutment

joint stability in internal conical connection type implant systems. *Journal of Advance Prosthodontics.*, *6*(6), 491–497. https://doi.org/10.4047/jap.2014.6.6.491

Jung, R. E., Zembic, A., Pjetursson, B. E., Zwahlen, M., & Thoma, D. S. (2012). Systematic review of the survival rate and the incidence of biological, technical, and aesthetic complications of single crowns on implants reported in longitudinal studies with a mean follow-up of 5 years. *Clinical Oral Implants Research.*, *23*(Suppl 6), 2–21. https://doi.org/10.1111/j.1600-0501.2012.02547.x

Koutouzis, T. (2019). Implant-abutment connection as contributing factor to peri-implant diseases. *Periodontology 2000.*, *81*(1), 152–166. https://doi.org/10.1111/prd.12289

Lafaurie, G. I., Sabogal, M. A., Castillo, D. M., Rincón, M. V., Gómez, L. A., Lesmes, A., & Chambrone, L. (2017). Microbiome and microbial biofilm profiles of Peri-Implantitis: A systematic review. *Journal of Periodontology.*, *88*(10), 1066–1089. https://doi.org/10.1902/jop.2017.170123

Larrucea Verdugo, C., Jaramillo Núñez, G., Acevedo Avila, A., & Larrucea San Martín, C. (2014). Microleakage of the prosthetic abutment/implant interface with internal and external connection: In vitro study. *Clinical Oral Implants Research.*, *25*, 1078–1083. https://doi.org/10.1111/clr.12217

Lemos, C. A. A., Verri, F. R., Gomes, J. M. L., de Souza Batista, V. E., Cruz, R. S., Oliveira, H. F. F. E., & Pellizzer, E. P. (2019). Ceramic versus metal-ceramic implant-supported prostheses: A systematic review and meta-analysis. *Journal of Prosthetic Dentistry.*, *121*(6), 879–886. e4. https://doi.org/10.1016/j.prosdent.2018.09.016

Limones, A., Molinero-Mourelle, P., Azevedo, L., Romeo-Rubio, M., Correia, A., & Gómez-Polo, M. (2020). Zirconia-ceramic versus metal-ceramic posterior multiunit tooth-supported fixed dental prostheses: A systematic review and meta-analysis of randomized controlled trials. *Journal of American Dental Association.*, *151*(4), 230–238.e7. https://doi.org/10.1016/j.adaj.2019.12.013

Liu, Y., & Wang, J. (2017). Influences of microgap and micromotion of implant–abutment interface on marginal bone loss around implant neck. *Archives of Oral Biology.*, *83*, 153–160. https://doi.org/10.1016/j.archoralbio.2017.07.022

Martin-Gili, D., Molmeneu, M., Fernandez, M., Punset, M., Giner, L., Armengou, J., & Gil, F. J. (2015). Determination of fluid leakages in the different screw-retained implant-abutment connections in a mechanical artificial mouth. *Journal Material Science: Materials in Medicine.*, *26*, 21. https://doi.org/10.1007/s10856-015-5544-x

Mello, C. C., Lemos, C. A. A., de Luna Gomes, J. M., Verri, F. R., & Pellizzer, E. P. (2019). CAD/CAM vs conventional technique for fabrication of implant-supported frameworks: A systematic review and meta-analysis of in vitro studies. *International Journal of Prosthodontontics*, *32*(2), 182–192. https://doi.org/10.11607/ijp.5616

Mishra, S. K., Chowdhary, R., & Kumari, S. (2017). Microleakage at the different implant abutment Interface: A systematic review. *Journal of Clinical and Diagnostic Research.*, *11*(6), ZE10–ZE15. https://doi.org/10.7860/JCDR/2017/28951.10054

Molinero-Mourelle, P., Cascos-Sanchez, R., Yilmaz, B., Lam, W. Y. H., Pow, E. H. N., Del Río Highsmith, J., & Gómez-Polo, M. (2021). Effect of fabrication technique on the microgap of CAD/CAM cobalt-chrome and zirconia abutments on a conical connection implant: An in vitro study. *Materials (Basel).*, *14*(9), 2348. https://doi.org/10.3390/ma14092348

Morgan, E. F., Unnikrisnan, G. U., & Hussein, A. I. (2018). Bone mechanical properties in healthy and diseased states. *Annual Review of Biomedical Engineering.*, *20*, 119–143. https://doi.org/10.1146/annurev-bioeng-062117-121139

Naveau, A., Rignon-Bret, C., & Wulfman, C. (2019). Zirconia abutments in the anterior region: A systematic review of mechanical and esthetic outcomes. *Journal of Prosthetic Dentistry.*, *121*(5), 775–781. e1. https://doi.org/10.1016/j.prosdent.2018.08.005

Norton, M. R. (1997). An in vitro evaluation of the strength of an internal conical interface compared to a butt joint interface in implant design. *Clinical Oral Implants Research.*, *4*, 290–298. https://doi.org/10.1034/j.1600-0501.1997.080407.x

Ortega-Martínez, J., Delgado, L. M., Ortiz-Hernández, M., Punset, M., Cano-Batalla, J., Cayon, M. R., & Cabratosa-Termes, J. (2020). In vitro assessment of PEEK and titanium implant abutments: Screw loosening and microleakage evaluations under dynamic mechanical testing. *Journal of Prosthetic Dentistry*, *127*(3), 470–476. https://doi.org/10.1016/j.prosdent.2020.09.033

Pereira, L. M. S., Sordi, M. B., Magini, R. S., Calazans Duarte, A. R., & Souza, J. C. M. (2017). Abutment misfit in implant-supported prostheses manufactured by casting technique: An integrative review. *European Journal of Dentistry.*, *11*(4), 553–558. https://doi.org/10.4103/ejd.ejd_162_17

Rodrigo, D., Sanz-Sánchez, I., Figuero, E., Llodrá, J. C., Bravo, M., Caffesse, R. G., Vallcorba, N., Guerrero, A., & Herrera, D. (2018). Prevalence and risk indicators of peri-implant diseases in Spain. *Journal of Clinical Periodontology.*, *45*(12), 1510–1520. https://doi.org/10.1111/jcpe.13017

Salvi, G. E., Fürst, M. M., Lang, N. P., & Persson, G. R. (2008). One-year bacterial colonization patterns of *Staphylococcus aureus* and other bacteria at implants and adjacent teeth. *Clinical Oral Implants Research.*, *19*, 242–248. https://doi.org/10.1111/j.1600-0501.2007.01470.x

Sánchez, M. C., Marín, M. J., Figuero, E., Llama-Palacios, A., León, R., Blanc, V., Herrera, D., & Sanz, M. (2014). Quantitative real-time PCR combined with propidium monoazide for the selective quantification of viable periodontal pathogens in an in vitro subgingival biofilm model. *Journal of Periodontal Research*, *49*(1), 20–28. https://doi.org/10.1111/jre.12073

Şen, N., Şermet, I. B., & Gürler, N. (2019). Sealing capability and marginal fit of titanium versus zirconia abutments with different connection designs. *Journal of Advance Prosthodontics.*, *11*(2), 105–111. https://doi.org/10.4047/jap.2019.11.2.105

Socransky, S. S., & Haffajee, A. D. (2005). Periodontal microbial ecology. *Periodontology 2000*, *38*, 135–187. https://doi.org/10.1111/j.1600-0757.2005.00107.x

Socransky, S. S., Haffajee, A. D., Cugini, M. A., Smith, C., & Kent, R. L., Jr. (1998). Microbial complexes in subgingival plaque. *Journal of Clinical Periodontology.*, *25*(2), 134–144. https://doi.org/10.1111/j.1600-051x.1998.tb02419.x

Tallarico, M., Canullo, L., Caneva, M., & Özcan, M. (2017). Microbial colonization at the implant-abutment interface and its possible influence on periimplantitis: A systematic review and meta-analysis. *Journal of Prosthodontic Research*, *61*(3), 233–241. https://doi.org/10.1016/j.jpor.2017.03.001

Van Winkelhoff, A. J., Goené, R. J., Benschop, C., & Folmer, T. (2000). Early colonization of dental implants by putative periodontal pathogens in partially edentulous patients. *Clinical Oral Implants Research.*, *11*(6), 511–520. https://doi.org/10.1034/j.1600-0501.2000.011006511.x

Vélez, J., Peláez, J., López-Suárez, C., Agustín-Panadero, R., Tobar, C., & Suárez, M. J. (2020). Influence of implant connection, abutment design and screw insertion torque on implant-abutment misfit. *Journal of Clinical Medicine*, *9*, 2365. https://doi.org/10.3390/jcm9082365

How to cite this article: Molinero-Mourelle, P., Roccuzzo, A., Yilmaz, B., Lam, W. Y. H., Pow, E. H. N., Highsmith, J. D. R., & Gómez-Polo, M. (2022). Microleakage assessment of CAD-CAM Cobalt-Chrome and Zirconia abutments on a conical connection dental implant: A comparative in vitro study. *Clinical Oral Implants Research*, *33*, 945–952. https://doi.org/10.1111/clr.13973